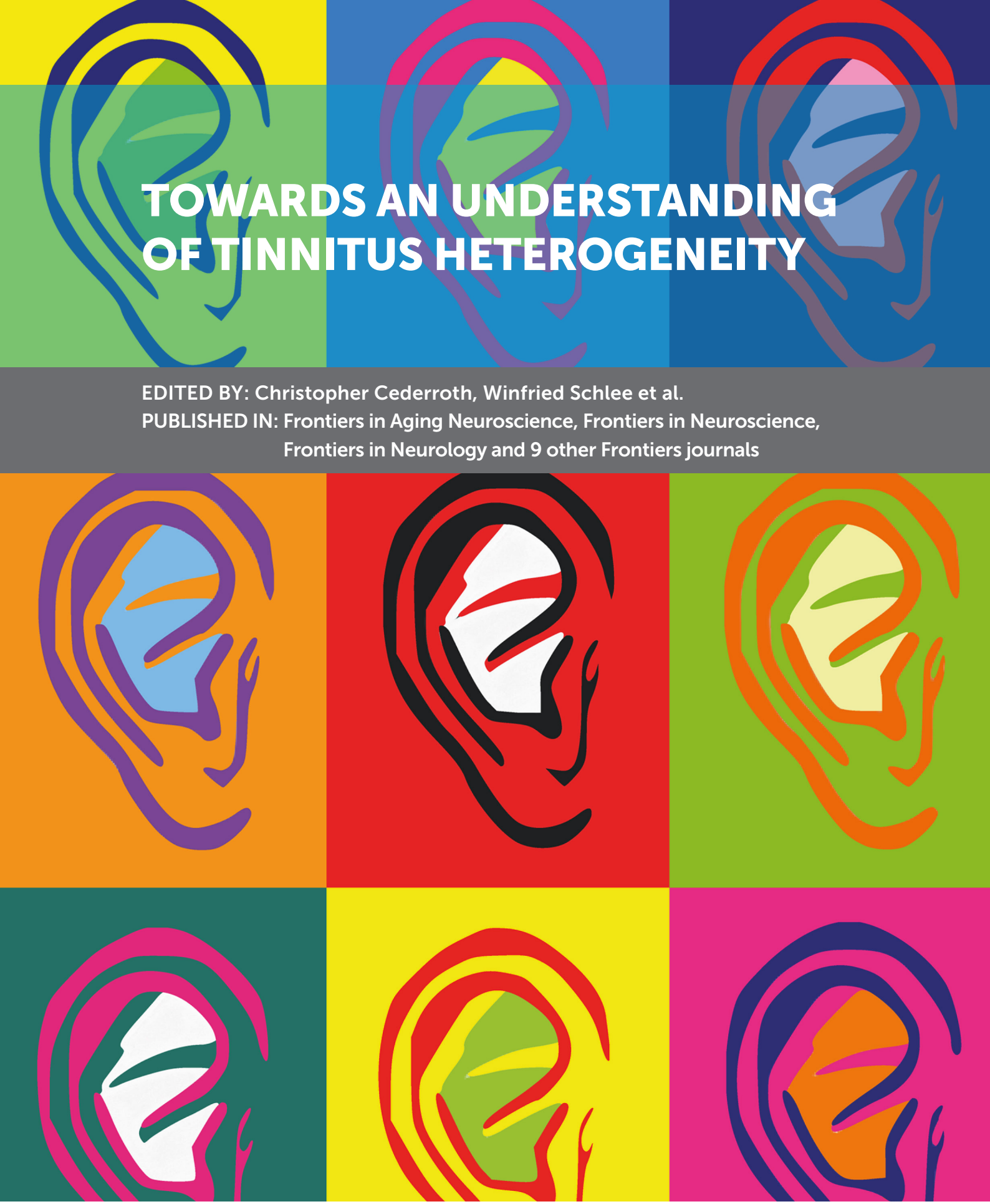

TOWARDS AN UNDERSTANDING OF TINNITUS HETEROGENEITY

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TOWARDS AN UNDERSTANDING OF TINNITUS HETEROGENEITY

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Image: "Heterogeneity of Tinnitus" by Winny Schlee

Tinnitus is the perception of a sound when no external sound is present. The severity of tinnitus varies but it can be debilitating for many patients. With more than 100 million people with chronic tinnitus worldwide, tinnitus is a disorder of high prevalence.

The increased knowledge in the neuroscience of tinnitus has led to the emergence of promising treatment approaches, but no uniformly effective treatment for tinnitus has been identified. The large patient heterogeneity is considered to be the major obstacle for the development of effective treatment strategies against tinnitus.

This eBook provides an inter- and multi-disciplinary collection of tinnitus research with the aim to better understand tinnitus heterogeneity and improve therapeutic outcomes.

The articles in this Research Topic appear in the following journals: *Frontiers in Aging Neuroscience*, *Frontiers in Neuroscience*, *Frontiers in Neurology*, *Frontiers in Psychology*, *Frontiers in Human Neuroscience*, *Frontiers in Behavioral Neuroscience*, *Frontiers in Psychiatry*, *Frontiers in Medicine*, *Frontiers in Genetics*, *Frontiers in Cellular Neuroscience*, *Frontiers in Pharmacology*, and *Frontiers for Young Minds*.

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frontiers in Aging Neuroscience

EDITORIAL
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Editorial: Towards an Understanding of Tinnitus Heterogeneity

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Editorial on the Research Topic

Towards an Understanding of Tinnitus Heterogeneity

Despite being a common condition that affects nearly 15% of the population, and despite much research progress made in the recent years, tinnitus remains a scientific and clinical enigma. Subjective tinnitus is defined as a phantom perception of a tone or noise in the absence of any physical source. It is known to be a heterogeneous condition, both in the way of manifestation and of generation. In general, "heterogeneity" describes the fact that there is a non-uniform appearance of a substance, organism, or disease. Whenever there is a non-uniformity in at least one quality, we can call it "heterogeneous." Tinnitus patients differ on at least four dimensions: First, tinnitus patients may present diverse clinical profiles with respect to the ***perception*** of tinnitus (e.g., laterality of tinnitus, tinnitus pitch, ringing, buzzing, hissing, or cricket sounds). Additionally, tinnitus can be occasional or permanent, acute or chronic, pulsatile, or constant. Second, while there are multiple ways of perceiving tinnitus, it is also associated with multiple ***causal risk factors***—hearing loss, temporomandibular joint disorder, and aging being among the most common ones. There are also numerous ***related comorbidities*** that add to the complex clinical picture of tinnitus (e.g., hyperacusis, depression, sleep disorders, headache, concentration problems). A third dimension is the associated tinnitus ***distress***, the psychological reaction to the ongoing tinnitus perception; it can differ largely among patients. Fourth, there is a large variation of ***treatment responses*** of the tinnitus patients.

With the heterogeneity in these four dimensions, we describe a diversity of observable qualities that can be investigated with the currently available research. The current interpretation of this observed heterogeneity is that several different tinnitus subtypes may exist and that these different subtypes may have different etiologies, different clinical profiles and different treatment responses. So far, the number of the potentially existing tinnitus subtypes is not known, nor the diagnostic criteria to identify them. The situation gets even more complex when we consider patients with a combination of several subtypes.

This heterogeneity culminates in the challenge for tinnitus treatment: A uniformly effective treatment for all tinnitus patients is unlikely. For each individual patient, a personalized treatment plan has to be developed, considering the tinnitus profile, the comorbidities, the psychological distress and the previous treatment experiences of the patient. To solve this clinical enigma, conceptual models of tinnitus are needed to develop innovative solutions for personalized medicine.

The aim of this research topic is to investigate the challenge of tinnitus heterogeneity by involving multiple disciplines ranging from neuroscience, neurology, genetics, audiology, otolaryngology, psychology, psychiatry, pharmacology, epidemiology, medical informatics, data mining, and statistics. The main idea is to move away from an abstract view of tinnitus toward an detailed understanding of what could constitute tinnitus subtypes in view of improving fundamental knowledge and ultimately lead to optimized therapeutic interventions. Within this topic, current knowledge is reviewed and new theories of tinnitus generation are proposed, animal models are used to understand the neural correlates better, audiological, and psychological aspects are explored, neuroimaging techniques investigate the involved brain networks, new questionnaire instruments are developed and others adapted to new languages, genome-wide associations are pioneered, mobile applications are used to explore tinnitus on new time-scales, and, finally, multiple therapeutic approaches are tested.

Statistics on this research topic: The research topic was open between February 18, 2016 and October 29, 2017. It received 96 submissions by 335 authors. 79 submissions were finally accepted after a rigorous and constructive reviewing process. During the phase in which the topic was active, it received over two million views.

Overview of this research topic: To cover these themes, this Frontiers research topic starts with several retrospective reviews of the current status of the field, before introducing a number of emerging hypotheses on how tinnitus is generated, and how it is experienced by individuals when it is persistent. The first chapter on the research performed on animals addresses the validity of existing models and improves the sensitivity of outcome measures. The second chapter addresses audiological and psychological aspects to tinnitus, as well as the accompanying co-morbidities. The third chapter concerns the instruments and technologies to assess tinnitus, ranging from questionnaires, neuroimaging methods, and mobile applications. The fourth chapter reviews existing clinical guidelines and describes the recent advances in tinnitus therapy including cognitive behavioral therapy (CBT), sound therapy, cochlear implants, electric stimulation, and repeated transcranial magnetic stimulation. Finally, the fifth chapter refers to advances on tinnitus therapies. These are briefly described below:

CHAPTER 1: HYPOTHESES AND THEORIES

There are many subtypes of tinnitus, and in at least one subtype, the tinnitus can be considered as a symptom of the aging ears and brain. Some theories of tinnitus take a gerontological perspective. Age-related hearing loss and tinnitus can go hand in hand and one of the most well-established theories considers tinnitus as the perceptual consequence of neuronal hyperactivity in the central auditory system; emerging after loss of normal input from the ear. Computational modeling can be one productive method for exploring the nature of the underlying neural signaling pathway based on certain assumptions about spatial and temporal dynamics of excitatory and inhibitory synaptic weighting and corresponding action potential activity. In their modeling approach, Krauss et al. examine stochastic resonance. This is an adaptive mechanism whereby weak sub-threshold signals can still be detected and transmitted upwards if internal noise is added. The authors present a thought-provoking hypothesis that neuronal hyperactivity is a "side effect" of stochastic resonance in a system whose main purpose is to optimize transmission of impoverished sound information.

Animal models provide a second productive approach to investigating the underlying neural signaling pathway in the aging brain. Ruan et al. treat new ground by considering the role of the cholinergic system. Their hypothesis that cholinergic innervation of various brain structures provides a link between tinnitus seen in age-related hearing loss and age-related cognitive impairment is supported by animal literature, as discussed in the paper.

One of the challenges in this field has been to provide a neuroscientific account of tinnitus that adequately explains the common perceptual experience (i.e., the conscious perception of a sound that does not have an external source), and yet at the same time has the flexibility to account for the heterogeneity in etiology, comorbidity, psychosocial impact, and such like. Ghodratitoostani et al. rose to this challenge. The neurofunctional tinnitus model described in this article has many elements in common with other contemporary models, and is applied by the authors to interpret clinical phenomena.

Of course, it is preferable to have a treatment whose therapeutic efficacy can be understood within the context of a theoretical model, but this is not a prerequisite for treatments to be clinically beneficial. Neurofeedback is an intriguing approach to modulate the tinnitus-related brain activity patterns. A review by Güntensperger et al. concisely summarizes the progress made in hypothesis testing, experimental design and neurofeedback algorithms. A clear presentation of the limitations sets out a challenge to provide insights on the variability in efficacy that is observed across individuals.

Despite the heterogeneity of tinnitus, somatic or somatosensory tinnitus is generally presented as if it were a distinct subtype associated with cross-modal brain connectivity. Haider et al. provided a great service to the community by systematically mapping out the literature on somatosensory tinnitus. This useful guide identified what is currently known with respect to its pathophysiology, diagnosis and treatment. What is particularly striking from the descriptions is in fact the bewildering variety of clinical presentations and potential treatment options of somatosensory tinnitus. It is therefore both relevant and timely that an international consensus among expert scientists and clinicians has been recently sought on what are the important diagnostic criteria for somatosensory tinnitus (Michiels et al., 2018). From this work, somatosensory tinnitus is not seen as a specific category of tinnitus, but more as a factor that can influence a patient's tinnitus to a greater or lesser degree. Based on more and more detailed knowledge about the involvement of the somatosensory system in tinnitus pathophysiology, a systematic review summarizes the influence of physical therapy on tinnitus (Michiels et al.).

A research topic provides the perfect channel for gathering different perspectives and for representing the true multi-disciplinarity of tinnitus research. In this regard, the original research article by Dauman et al. was particularly refreshing because it took a holistic psychosocial perspective on tinnitus, in stark contrast to the somewhat more conventional and reductionist methodology that characterized a majority of the papers in the collection. In this paper, the authors boldly delve deep into the personal experience of tinnitus to explore how each participant constructs his/her own meaning about what it is to live with a bothersome tinnitus. No previous study based on open-text responses has provided such a rich narrative about the issue of heterogeneity of the lived experience. For example, a recent review found that out of 86 studies (16,381 patients) which assessed the patient experience, only eight studies asked open questions (885 patients) (Hall et al., 2018). For the Editors, it brought the all-important emotional dimension of tinnitus under scientific scrutiny.

CHAPTER 2: INSIGHTS ON NEUROBIOLOGICAL MECHANISMS AND GENETICS: FROM ANIMALS TO HUMANS

Animal studies using micro-electrode recordings have shown that noise trauma (known to produce tinnitus in human subjects) is followed by neural hyperactivity at several stages of the auditory system, from the cochlear nucleus to the auditory cortex (Eggermont). Hesse et al. found in the inferior colliculus that the increased spontaneous firing caused by noise exposure was greater in CBA/Ca mice exposed to 100 dB (showing minimal hearing loss from ABRs or "hidden" hearing loss) compared to the 105 dB exposed group (showing small hearing loss), supporting the idea of a non-linear response between low and high threshold cochlear fibers to noise damage. When performing unilateral cochlear nerve section, Tighilet et al. found that this form of sensory deafferentation caused a dramatic reduction of the KCC2 co-transporter density in the cochlear nucleus suggesting that GABA may be less inhibitory (or even excitatory) after a cochlear lesion (Tighilet et al.) and suggesting a potential new target for pharmacological treatment of tinnitus. Using gas chromatography and mass spectrometry (GC/MS), He et al. describe the impact of noise trauma on the metabolite profiling in different brain areas, which could serve in characterizing animals with tinnitus.

The gap pre-pulse inhibition of the acoustic startle (GPIAS) is the method of choice for many animal studies on tinnitus. This approach does not require conditioning of the animals prior to the experiment and therefore saves time and resources. With the aim to further optimize this method, Schilling et al. developed a new statistical approach for the analysis of the GPIAS data in Mongolian gerbils, which is based on the fact that the amplitude ratios are approximately lognormally distributed. This allows a new statistical test that is independent from the number of repetitions of the measurement. Using GPIAS to reveal tinnitus, Longenecker et al. found that only 30% of CBA/CaJ mice exposed unilaterally to noise may present tinnitus although all of them showed an increase in spontaneous firing rate, and no change in bursting activity (Longenecker and Galazyuk). The authors suggest that neither neural hyperactivity nor bursting activity are strict neural correlates of tinnitus. However, the degree of inhibition during GPIAS in CBA mice is small and offers little dynamic range to infer the presence of tinnitus. Yu et al. found that the inhibition is more effective in C57BL/6J mice and can be improved even more when the delay between the gap and the startle stimulus is short. Using these improved parameters in combination with c-Fos activity mapping after the exposure to different sound stimuli, the neural circuits controlling GPIAS were found to differ from those regulating pre-pulse inhibition (Moreno-Paublete et al.).

Such refinements in GPIAS helped revealing a role of the glutamate aspartate transporter GLAST in salicylate-induced tinnitus (Yu et al.) This points at a first gene potentially involved in tinnitus using animal models. In this respect, greater knowledge of the genetic influences on tinnitus generation and persistence in humans may not only help developing a better understanding of the mechanisms and optimize the classification of patients, but could also contribute in the development of drugs to silence tinnitus (Lopez-Escamez et al.). In this respect, while the knowledge on the genetic basis of tinnitus is poorly understood (Vona et al.), a pilot genome-wide assocation study (GWAS) on 167 tinnitus subjects and 749 controls showed an enrichment in oxidative stress, endoplasmatic reticulum (ER) stress, and serotonin reception mediated signaling although it did not identify any significant association (Gilles et al.). A genotyping study on 78 individuals suggests that *GRM7* rs11928865 could be used as a biomarker for tinnitus severity (Haider et al.). The increasing evidence of genetic influences on tinnitus emphasizes the need of creating large biobanks in ENT

clinics and merge efforts to increase statistical power (Cederroth et al.).

CHAPTER 3: AUDITORY AND PSYCHOLOGICAL CHARACTERISTICS CONTRIBUTING TO TINNITUS HETEROGENEITY

Audiological factors contribute to tinnitus heterogeneity. Milloy et al. undertook a scoping review of the Auditory Brainstem Response (ABR) in tinnitus participants and found a large variation in measured latencies and amplitude of the earliest component of the ABR (wave I), likely due to a broad variation in methods, study population, sample size, and potentially the definitions of tinnitus. In a case report, Londero et al. monitored symptoms after a tinnitus-inducing acoustic shock and found abnormal tympanic membrane appearance suggestive of abnormal middle ear muscle activity causing local chronic inflammation. The authors suggest that the combination of local inflammation and neural response originating from this type of injury could result in otalgia and tinnitus, in absence of hearing loss. Ueberfuhr et al. found that low frequency sound exposure and Ménière's disease was not exclusively followed by low frequency or noise-like tinnitus, indicating a variation in the underlying pathology.

The availability of large databases generated new insights into the role of hearing in tinnitus generation. Indeed, using data from 37,661 patients, Gollnast et al. found that auditory thresholds were lower for young tinnitus patients than matched non-tinnitus patients. Thanks to data from the Tinnitus Research Initiative ($n = 2{,}838$ patients), eight types of hearing function could be identified using latent class analysis based on the audiogram of tinnitus patients (Langguth et al.). However, the authors recognized the limitations of the audiogram as a measure of hearing sensitivity, a factor contributing to increased interest in other measures, such as speech discrimination. In this regard, three studies found that tinnitus interferes with speech comprehension, consistent with dysfunctional central auditory processing in subjects with tinnitus (Gilles et al.; Ivansic et al.; Vielsmeier et al.). Reaction to external sounds, such as misophonia—the dislike of sound, also contributes to the heterogeneity of the tinnitus population (Palumbo et al.). A role for learning in annoyance and strong negative reactions to sound was emphasized as well as the similarity to synesthesia. Overall, these studies illustrate the wide variation in relationships between tinnitus and hearing loss as well as sensitivity to sound, contributing to the overall heterogeneity.

From a psychological perspective, it is of utmost importance to identify psychological features contributing to tinnitus and its heterogeneity. Several studies rose awareness for the core role of psychological features that have to be considered in tinnitus patients (e.g., stress, depression, alexithymia, cognition) in tinnitus (Alsalman et al.; Brüggemann et al.; Trevis et al.; Wielopolski et al.). Wallhausser-Franke et al. repeatedly collected information on tinnitus perception among incident tinnitus patients and found that only one in 10 patients had complete remission after 6 months, while voiced complaints were stable in the majority of patients, and tinnitus-related distress worsened in 30% of tinnitus patients with depression at onset. Analyzing a case series data from Germany, tinnitus patients with comorbid headache ($n = 193$) were found to have had a lower quality of life compared to those without comorbid headache ($n = 765$), and greater painful sensation to loud sounds, vertigo, pain, and depressive symptoms (Langguth et al.). Interestingly, cluster analyses using data from 1,783 patients revealed two cluster solutions with clearly different characteristics, however with poor stability, suggesting that the tested tinnitus population comprised a continuum rather than a number of clearly defined subgroups (van den Berge et al.).

CHAPTER 4: USE OF NEUROIMAGING, QUESTIONNAIRES AND MOBILE INSTRUMENTS IN UNDERSTANDING TINNITUS HETEROGENEITY

Neuroimaging studies of individuals with tinnitus published in this research topic further suggest that tinnitus is accompanied by both structural (Allan et al.) and functional (Chen et al.; Chen et al.) brain changes in a distributed network of auditory and non-auditory brain regions. Structurally, a decrease in cortical thickness for the tinnitus group in the left superior frontal gyrus and a decrease in cortical volume with hearing loss in left Heschl's gyrus was found, while no changes were observed in the subcallosal region (Allan et al.) Functionally, a meta-analysis including nine resting-state neuroimaging studies shows in tinnitus patients an increased brain activity in the insula, middle temporal gyrus, inferior frontal gyrus, parahippocampal gyrus, cerebellum posterior lobe, and right superior frontal gyrus, when compared to controls (Chen et al.). These structural and functional alterations go together with an aberrant brain network architecture featuring a disrupted connectivity in non-auditory regions, especially the prefrontal cortex (Chen et al.), which findings were further confirmed by sound-evoked fMRI (Davies et al.) and auditory steady state responses (Engel et al.). Brain alterations in the theta and gamma bands were found in the auditory regions of tinnitus patients by magnetoencephalography (Lau et al.) and electroencephalography (Song et al.). Interestingly, these effects were no longer observed when excluding subjects with mental health comorbidities, strongly supporting the psychological burden in such alterations (Lau et al.). On the other hand, after cochlear implantation in single-sided deafness, an overall decrease in cortical activity and functional connectivity was seen in patients with tinnitus, likely due to dynamic peripheral reafferentation rather than cortical plastic changes (Song et al.). Taken together, these findings imply that the neural mechanisms underlying different subtypes of tinnitus share similar features when compared to controls. Future studies comparing subtypes may identify signatures specific to these subtypes.

Evaluation of tinnitus pitch and loudness was tested using two different methods, namely Touchscreen and Stand-alone, which both rapidly provided reliable measures (Hébert and

Fournier). In the absence of any effective objective measures in humans, questionnaires have been supporting the assessment of tinnitus and its associated burden. Recent developments have prompted the translation and adaptation of several existing English language questionnaires into Swedish, Polish, and Italian (Müller et al.; Moschen et al.; Wrzosek et al.). New questionnaires to evaluate auditory processing deficits have also been created (Diges et al.). Each of these questionnaires assesses a particular aspect of tinnitus (e.g., tinnitus-associated distress, anxiety, stress, depression, and life quality among others). Moreover, in order to facilitate a more holistic clinical evaluation, a visualization tool consisting of radar plots has been proposed by Schlee et al.

This special issue accommodates four papers on the potential of mobile crowdsensing for the support and empowerment of tinnitus patients, three of which promote the use of active patient participation and report on findings acquired with Ecological Momentary Assessments (EMA) (Probst et al.; Probst et al.; Schlee et al.). The extent to which smart devices, as well as more conventional internet-based solutions, have been taken up clinically for tinnitus diagnostics, treatment and self-help solutions has been reviewed by Kalle et al. The amounts of data collected interactively and unobtrusively through the smart devices call for data mining solutions for knowledge extraction toward the medical researchers and for adaptive models that adjust to the within-day and across time variability of patients' symptoms and needs.

CHAPTER 5: THERAPY

The heterogeneity of tinnitus may explain why treatment outcomes are so variable. In most clinical trials in which a given therapeutic intervention is investigated, treatment results vary considerably across patients as well from trial to trial, even if the same treatment is investigated. This may be partly due to variability in the methodology for patient assessment and outcome measurement. In their paper, Londero and Hall stress the relevance of standards for the reporting of outcome measurements. This is also a precondition for the development of valid treatment guidelines since the current ones vary considerably (Fuller et al.)

Sound therapy is an umbrella term that includes any form of acoustic stimulation used to increase the engagement of auditory system to offer tinnitus relief. The existing research demonstrates that even when attempting to personalize sound therapy, the complex combination of factors tinnitus patients present with is overlooked, and instead focus is made on one aspect such as pitch, maskability, hearing loss, sound preference or psychosocial factors (Searchfield et al.) A randomized controlled trial using cross-over study design and mixed methods revealed the significance of broadband noise compared to nature sound administration for reducing tinnitus (Durai and Searchfield). Exploratory studies such as the one conducted by Neff et al. found amplitude modulated sounds presented near tinnitus pitch to be more effective in tinnitus suppression, something that was more evident immediately after the stimulus offset. The past two decades have witnessed the commercialization of novel sound-based technologies, one such example is Acoustic CR Neuromodulation. This is a safe, well tolerated research tool which results in reduction of tinnitus symptoms for some patients, likely due to desynchronizing effects (Wegger et al.) However, the evidence for this technique to become a clinical tool is yet insufficient. Haller and Hall recommended that controlled trials on Acoustic CR Neuromodulation should include a well-characterized placebo group. Two studies investigated the neurophysiological mechanism underlying the effectiveness of sound therapy. Krick et al. used fMRI to show that the Heidelberg Neuro-Music Therapy (HNMT) decreases tinnitus-related distress by increasing the brain's default-mode network (DMN) activity. HNMT shifts the attention of tinnitus patients from the auditory phantom percept toward visual cues, a process involving an increased activity in the angular gyrus (Krick et al.). The advancement in sound based technological options, participation of industry and community partners along with emphasis of exploring the mechanism of effect is taking this management tool toward exciting horizon.

Cochlear implantation is one of the most successful therapeutic approaches for the control of tinnitus. However, it is only applicable for patients with uni- or bilateral deafness. Two articles of this special issue confirm the positive influence of cochlear implantation on the subjectively perceived tinnitus loudness and the tinnitus associated psychological burden (Bruggemann et al.; Knopke et al.). The positive aspects of cochlear implantation on tinnitus suppression might be interpreted as an effect of electrical stimulation of the auditory nerve, something that was tested via the electrical stimulation of the cochlea through the external auditory canal. Another study on cochlear implantation performed a sensitive analysis on the tinnitus improvement after surgery: Servais et al. found differential improvements for the perceptive and the reactive aspects of tinnitus. Tinnitus loudness was significantly reduced following CI surgery, while reduction of tinnitus-related distress, depression and anxiety did not reach statistical significance (Servais et al.). Two articles by Mielczarek et al. show that non-invasive electrical stimulation of the outer ear canal is able to induce neuroplastic changes in the auditory system suggestive of tinnitus improvements (Mielczarek et al.; Mielczarek et al.).

Brain stimulation is also an area of active research as a potential therapeutic tool. Three studies aimed at improving the efficacy of repetitive transcranial magnetic stimulation (rTMS). Whereas peripheral stimulation as an add-on to cortical stimulation did not show beneficial effects (Vielsmeier et al.), the individualization of stimulation parameters has revealed relatively promising results (Kreuzer et al.). In a case study, the effects of combined treatment with rTMS and cognitive behavioral therapy (CBT) on tinnitus and insomnia symptoms were reported (Richter et al.). CBT was in fact shown to be effective in a multidisciplinary day clinic (Ivansic et al.). Transcranial direct current stimulation (tDCS) is another non-invasive method for inducing neuroplastic changes by applying electrical currency to the brain. The article of Rabau et al. presents different possibilities of cathode placement, however, without being able to evidence a superiority of a certain position over another.

There is still no pharmacological treatment for tinnitus available. A pilot study presented the benefits of the hormone oxytocin on tinnitus loudness (Azevedo et al.). Another study identified a potential modulator of Acetylcholine receptors (Ach), that could have an impact on tinnitus treatments that are mediated via Ach transmission such as vagus nerve therapy (Bojić et al.). Figueiredo et al. found a higher prevalence of arterial hypertension in subjects with tinnitus in comparison to non-tinnitus controls, and proposed that treatment against hypertension with diuretics, ACE inhibitors or calcium channels blockers, could have had a role on tinnitus pathophysiology.

SUMMARY: TINNITUS IS A COMPLEX CHRONIC DISORDER

Complex disorders are caused by a combination of genetic and environmental factors. Within this research topic we have accumulated evidence for the genetic and the environmental influence on the development of tinnitus. Studies on genetic factors in tinnitus have been published in this research topic and point toward a significant genetic component, at least in some subtypes of tinnitus. As an example, the heritability in men with bilateral tinnitus was estimated with 0.68, while the heritability of unilateral tinnitus is estimated much lower with 0.27 (Cederroth et al.; Maas et al., 2017). The most obvious environmental influence for the development of tinnitus is the exposure to loud sounds that can lead to damage of the hearing system and finally trigger tinnitus (Eggermont). Besides noise trauma, other examples for environmental influences are the exposure to neurotoxic drugs such as cisplatin (Eggermont) or injuries that can lead to Temporo-Mandibular Disorders, which can also cause tinnitus (Haider et al.). Furthermore, genetic contributions for the treatment response of the patient have been hypothesized in this research topic and possible scientific approaches are discussed (Lopez-Escamez et al.). The complex interaction between genetic and environmental factors for the generation of tinnitus are currently unknown. Tinnitus research has just begun to address this complex issue, which may then help in the classification of subtypes.

Because there is a lack of effective treatments, the tinnitus condition remains chronic in the majority of cases. Even though spontaneous remissions exist, the percentage of cases is low. A study by Wallhäusser-Franke et al. in this research topic focused on acute tinnitus patients and found that only 11% of them reported a complete remission of tinnitus after 6 months. The remaining 89% of patients were classified as chronic (Wallhausser-Franke et al.). But this chronic condition does not necessarily mean that patients are perceiving the tinnitus permanently at the same intensity at all times. After 6 months, the majority of patients report an intermittent tinnitus. This has been supported by other studies from this Topic using a smartphone app to investigate the moment-to-moment variability of tinnitus (Schlee et al.) and to assess the circadian variability of tinnitus loudness (Probst et al.). The underlying mechanisms for this variability in the tinnitus percept is currently being investigated, but it is likely that exogeneous as well as endogenous factors influence the perception of tinnitus. Using magnetoencephalography, even the tinnitus-associated brain activity in the resting state was found to be variable over time (Schlee et al., 2014). These temporal variations of tinnitus add a fourth dimension to the already complex disorder of tinnitus. A better understanding of the chronobiological mechanisms involved in tinnitus might help the development of effective treatments, as recent studies reveal the importance of the chronotype in response to specific treatments of neurological disorders (McCarthy et al., 2018).

The medical condition of tinnitus is a complex and chronic disorder that holds a lot of open and challenging question for future research. In order to unravel this complexity, the combined effort of multiple scientific disciplines is needed—as evidenced in this research topic. The intellectual challenge for future tinnitus research will be to master this inter-disciplinary approach, to bring insights, expertise, and techniques from a range of academic and clinical specialties. Therefore, new ways of formal education at the universities will be needed in order to build up a new generation of researchers equipped with the skills to perform such inter-disciplinary research and further dig in the heterogeneity of this complex disorder. New concepts for such education are already at the horizon and the European School for Interdisciplinary Tinnitus research (ESIT), one of the first EU-funded tinnitus research, published their training curriculum in this research topic (Schlee et al.).

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

Hall, D. A., Fackrell, K., Li, A. B., Thavayogan, R., Smith, S., Kennedy, V., et al. (2018). A narrative synthesis of research evidence for tinnitus-related complaints as reported by patients and their significant others. *Health Qual Life Outcomes* 16, 61. doi: 10.1186/s12955-018-0888-9

Maas, I. L., Bruggemann, P., Requena, T., Bulla, J., Edvall, N. K., Hjelmborg, J. V. B., et al. (2017). Genetic susceptibility to bilateral tinnitus in a Swedish twin cohort. *Genet. Med.* 19, 1007–1012. doi: 10.1038/gim.2017.4

McCarthy, M. J., Wei, H., Nievergelt, C. M., Stautland, A., Maihofer, A. X., Welsh, D. K., et al. (2018). Chronotype and cellular circadian

rhythms predict the clinical response to lithium maintenance treatment in patients with bipolar disorder. *Neuropsychopharmacology* 44, 620–628. doi: 10.1038/s41386-018-0273-8

Michiels, S., Ganz Sanchez, T., Oron, Y., Gilles, A., Haider, H. F., Erlandsson, S., et al. (2018). Diagnostic criteria for somatosensory tinnitus: a delphi process and face-to-face meeting to establish consensus. *Trends Hear.* 22:2331216518796403. doi: 10.1177/23312165 18796403

Schlee, W., Schecklmann, M., Lehner, A., Kreuzer, P. M., Vielsmeier, V., Poeppl, T. B., et al. (2014). Reduced variability of auditory alpha activity in chronic tinnitus. *Neural Plast.* 2014:436146. doi: 10.1155/2014/ 436146

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Stochastic Resonance Controlled Upregulation of Internal Noise after Hearing Loss as a Putative Cause of Tinnitus-Related Neuronal Hyperactivity

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Subjective tinnitus is generally assumed to be a consequence of hearing loss. In animal studies it has been demonstrated that acoustic trauma induced cochlear damage can lead to behavioral signs of tinnitus. In addition it was shown that noise trauma may lead to deafferentation of cochlear inner hair cells (IHC) even in the absence of elevated hearing thresholds, and it seems conceivable that such hidden hearing loss may be sufficient to cause tinnitus. Numerous studies have indicated that tinnitus is correlated with pathologically increased spontaneous firing rates and hyperactivity of neurons along the auditory pathway. It has been proposed that this hyperactivity is the consequence of a mechanism aiming to compensate for reduced input to the auditory system by increasing central neuronal gain, a mechanism referred to as homeostatic plasticity (HP), thereby maintaining mean firing rates over longer timescales for stabilization of neuronal processing. Here we propose an alternative, new interpretation of tinnitus-related development of neuronal hyperactivity in terms of information theory. In particular, we suggest that stochastic resonance (SR) plays a key role in both short- and long-term plasticity within the auditory system and that SR is the primary cause of neuronal hyperactivity and tinnitus. We argue that following hearing loss, SR serves to lift signals above the increased neuronal thresholds, thereby partly compensating for the hearing loss. In our model, the increased amount of internal noise—which is crucial for SR to work—corresponds to neuronal hyperactivity which subsequently causes neuronal plasticity along the auditory pathway and finally may lead to the development of a phantom percept, i.e., subjective tinnitus. We demonstrate the plausibility of our hypothesis using a computational model and provide exemplary findings in human patients that are consistent with that model. Finally we discuss the observed asymmetry in human tinnitus pitch distribution as a consequence of asymmetry of the distribution of auditory nerve type I fibers along the cochlea in the context of our model.

Keywords: dorsal cochlear nucleus, Zwicker tone, computational model, auditory nerve

INTRODUCTION

In western civilizations, 10–15% of the general population suffer from subjective tinnitus (Heller, 2003), the perception of a sound in the absence of any acoustic stimulus. In severe cases this phantom percept may lead to comorbidities like insomnia or psychological disorders like depression resulting in the inability to work or even suicide (Coles, 1984; Lewis et al., 1994; Langguth et al., 2011). Tinnitus is often accompanied by a hearing loss (Heller, 2003) and recent animal studies indicate that even relatively mild acoustic traumata which do not result in permanent elevation of hearing thresholds may lead to a massive loss of inner hair cell (IHC) synapses (synaptopathy) causing a so called hidden hearing loss (Liberman et al., 2015).

Despite the high prevalence and distress of affected patients, an effective cure for tinnitus still does not exist, since the exact mechanisms within the auditory system leading to the development of tinnitus are still unknown, and current models for tinnitus development are a matter of controversial debate (Gerken, 1996; Eggermont, 2003; Eggermont and Roberts, 2004; Engineer et al., 2011; Knipper et al., 2011; Schaette and McAlpine, 2011; Rüttiger et al., 2013). One class of such models proposes a mechanism within the central auditory system called homeostatic plasticity (HP) to be causative for tinnitus development. There, prolonged changes in the mean firing rates of the auditory nerve (AN), e.g., reduced AN activity caused by acoustic trauma are assumed to cause HP to readjust these mean firing rates to pre-trauma levels by means of increased neuronal gain, thereby compensating for the reduced cochlear input. This increased neuronal gain which is considered to emerge within long time scales (days to weeks) then leads to increased spontaneous firing rates within the auditory system which are hypothesized to be the correlate of the maladaptive hyperactivity observed in tinnitus (Knipper et al., 2011; Schaette and McAlpine, 2011). In support of current HP models it was argued that changes in firing rate, e.g., after a hearing loss event, may be detected by calcium-depended sensors that then regulate glutamate receptor trafficking (Turrigiano, 2008), thereby stabilization firing rates. This stabilization in turn is considered to be beneficial in terms of neuronal processing within these long time scales (Brenner et al., 2000; Dragoi et al., 2000; Schwartz and Simoncelli, 2001; Simoncelli and Olshausen, 2001; Dean et al., 2005, 2008; Dunn and Rieke, 2006; Robinson and McAlpine, 2009) but such a mechanism would not be helpful on short time scales for the detection of signals below the new threshold.

Here, inspired by information theory (Shannon, 1948), we propose an alternative and entirely new interpretation of tinnitus-related development of neuronal hyperactivity based on stochastic resonance (SR). SR refers to the phenomenon that weak signals that are sub-threshold for a given sensor (or synapse) still can be detected and transmitted by that sensor if noise is added to the sensor input (**Figure 1A**; Benzi et al., 1981; Collins et al., 1996; Levin and Miller, 1996; Gammaitoni et al., 1998). In that sense we follow the idea that any signal first has to be detected by a sensor before it could be amplified by increased neuronal gain. Or to use an analogy if the radio reception is bad due to a broken antenna it does not help to turn up the volume. Thus, the two main differences between our SR model and the HP models are, firstly that SR works prior of the detection threshold while HP influences processing after the detection threshold, and secondly the SR model assigns a functional role to spontaneous activity in the auditory system in contrast to the HP and other models, where increased spontaneous firing rates are simply a side-effect of the brain's attempt to cope with hearing loss.

SR has been found ubiquitously in nature covering a wide range of systems in physical and biological contexts (Wiesenfeld and Moss, 1995) and especially within the context of neuroscience (Douglass et al., 1993; Faisal et al., 2008; Mino, 2014). In addition, the existence of an optimal, non-zero intensity for the added noise has been demonstrated, allowing maximization of information transmission (Wiesenfeld and Moss, 1995). In self-adaptive signal detection systems based on SR, the optimum noise level is continuously adjusted via a feed-back loop, so that the system response in terms of information throughput remains optimal, even if the properties of the input signal change. For this processing principle the term adaptive SR has been coined (Mitaim and Kosko, 1998, 2004; Wenning and Obermayer, 2003). An objective function to quantify information content is the mutual information between the sensor input and output (Shannon, 1948). In the context of SR the mutual information is frequently used in theoretical approaches (Levin and Miller, 1996; Mitaim and Kosko, 2004; Moss et al., 2004). The choice of the mutual information is natural since the fundamental purpose of any transducer is to transmit information into a subsequent information processing system. It has been shown previously that the mutual information as a function of noise intensity has a maximum that indicates the optimal level of noise to be added to the input signal to achieve optimal information transmission by SR (Moss et al., 2004). However, a fundamental drawback of the mutual information is the impossibility of calculating it in any application of adaptive SR where the signal to be detected is unknown (Krauss et al., 2015). Furthermore, even if the underlying signal is known, the use of the mutual information still seems to be rather impractical within the context of neural network architectures, since calculating the mutual information requires evaluation of probability distributions, logarithms, products and fractions, i.e., operations that are hard to implement in neuronal networks. In a previous work (Krauss et al., 2015) we were able to show that this fundamental drawback can be overcome by another objective function, namely the autocorrelation of the sensor response. There we introduced the concept of the success probability and proved analytically and numerically that firstly, as a function of noise intensity, this quantity has a well-defined peak indicating the optimal level of noise for SR and secondly that mutual information and autocorrelation can be expressed as strictly monotonous functions of the success probability. Hence both, mutual information and autocorrelation, exhibit their maximum at the same level of noise and consequently, maximizing the output autocorrelation leads to similar or even identical estimates of optimal noise intensities for SR as the mutual information, yet with the decisive advantage that no knowledge of the input signal is required (Krauss et al., 2015). In contrast to the mutual

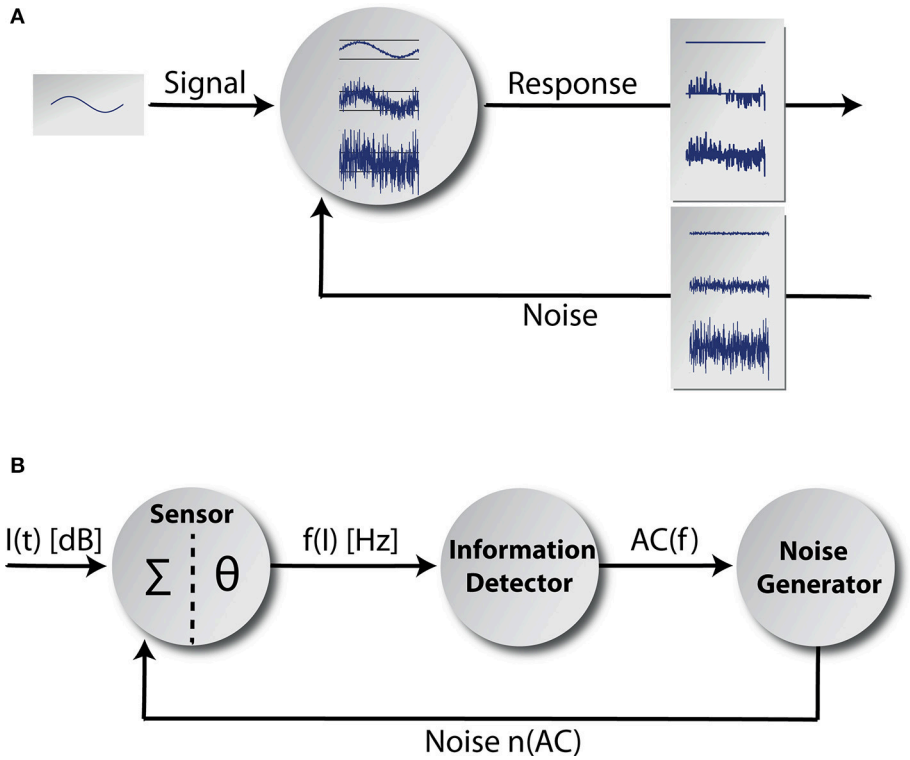

FIGURE 1 | Adaptive stochastic resonance. In **(A)** a sketch of the adaptive SR principle is shown. Some kind of sub-threshold signal serves as input to a detector. The detector response is shown for three different levels of noise intensity. If the amount of noise is too small, the signal cannot be transmitted and if the amount of noise is too large the signal vanishes within noise. In contrast for the optimal level of noise, signal transmission is best. The core model is shown in **(B)**. It mainly consists of three functional units and a feedback-loop. The information detector calculates the autocorrelation of the time course of AN firing rate *f(I)* originating at a sensor (with a summation function Σ and a threshold θ; Equation 7) reflecting its information content. The noise generator is controlled by the information detector and feeds noise back to the sensor via feedback connections (for details refer to the Discussion section).

information, the evaluation of autocorrelation functions may easily be implemented within neuronal networks using delay-lines and coincidence detectors (Licklider, 1951).

Taken together, we here propose that adaptive SR based on maximizing the output autocorrelation is a major adaptive principle in the auditory system that operates both on short and long time scales to maintain optimal information transmission in cases of changing statistics of the IHC output (= input to the auditory system), e.g., due to cochlear damage. In case of such reduced IHC output, first, the target neurons' autocorrelation decreases. As a consequence, second, the internal noise generated within the auditory system is increased and fed back to the sensor level to compensate for lost information transmission by means of SR. By that, this mechanism is able to at least partially restore hearing thresholds which have been elevated due to noise trauma. We here propose that in case of chronic cochlear damage this adaptively changed internal noise is increased permanently and hence a possible correlate of the hyperactivity often associated with subjective tinnitus. Note that in order to demonstrate the basic concept and principles of adaptive SR in the context of cochlear acoustic traumata and tinnitus development we here present a single frequency channel model approach and focus on within-channel mechanisms only. Putative cross-talk between neighboring frequency channels will be addressed in a follow up study. Furthermore, and in line with our model, we demonstrate in a cohort of over 39,000 patients that those with tinnitus have significantly improved hearing thresholds in the low frequency range compared to those without tinnitus. We discuss the possibility that the asymmetric distributions of the different type I AN fibers along the cochlea in combination with our SR model may explain the overrepresentation of high pitched tinnitus found in patients.

METHODS

We implemented a phenomenological single frequency channel model (**Figure 1B**) comprising the acoustic stimuli (function *I(t)* of the sound intensity levels), the first synapse from the IHC (cf. Discussion) to the AN (sensor with a summation function $\sum$ and a threshold θ), the AN responses *f(I)* and the effects of cochlear damage to AN responses. Furthermore, the model includes the adaptive SR principle based on the mean autocorrelation of the AN responses *AC(f)* calculated at the information detector whose output modulates the activity of a noise generator that feeds the noise *n(AC)* back into the sensor. We model coarse-grained functional units: to simplify matters, we focused on input-output mappings rather than on single neuron models or concrete neural

network architectures. Nevertheless, we emphasize that each part of the adaptive SR feedback-loop is highly biologically plausible and may be implemented as a neuronal network in a more fine-grained model. Possible candidate structures within the auditory system where the functional units of our model could be realized will be discussed in the Discussion section.

All parts of the model were implemented using the programming language C/C++.

Distribution of Sound Intensities of Acoustic Stimuli

Based on a computational model presented by Schaette and Kempter (2006) we assume the probability density function of the sound intensity levels I (in dB SPL) of the acoustic input (**Figure 2A**) to the model to be Gaussian (**Figure 2B**) with a mean value μ_I of 40 dB and standard deviation σ_I of 25 dB:

$$p_I(I) = \frac{1}{\sqrt{2\pi\sigma_I^2}} \exp\left(-\frac{(I-\mu_I)^2}{2\sigma_I^2}\right) \tag{1}$$

Note that this Gaussian distribution of sound intensity levels in dB corresponds to a log-normal distribution of the linear amplitudes of the sound stimuli. As input to the model we do not draw independent samples from this distribution during simulation. Instead, in order to generate an autocorrelated time series of sound intensities (**Figure 2C**), yet with identical mean value and standard deviation as in the uncorrelated case (**Figure 2B**), we implement an Ornstein-Uhlenbeck process (Uhlenbeck and Ornstein, 1930) that satisfies the stochastic differential equation:

$$dI_t = (\mu_I - I_t)\, dt + \sqrt{2\sigma_I^2}\, dW_t \tag{2}$$

where W_t is the Wiener process (Einstein, 1905).

It is plausible to assume sound intensities to be autocorrelated especially for meaningful acoustic stimuli like speech or music. We refer to this random walk through sound intensities as the standard acoustic environment.

Modeling of Auditory Nerve Responses

The firing rate *f(I)* of the AN at a sound intensity *I(t)* is modeled analogous to Schaette and Kempter (2006) with a threshold I_θ of 0 dB SPL, spontaneous firing rate f_{sp} of 50 Hz and maximum firing rate f_{max} of 250 Hz. The response function *f(I)* is assumed to be adapted to the distribution of sound intensities. This means that for $I > I_\theta$, *f(I)* is proportional to the normalized cumulative distribution function $\int_{I_\theta}^{I} p_I\left(I'\right) dI'$ of the sound intensities hence, according to the infomax principle, *f(I)* has maximum information on *I(t)* (Laughlin, 1981):

$$f(I) = \begin{cases} f_{sp} & \text{for } I < I_\theta \\ f_{sp} + \left(f_{max} - f_{sp}\right) \frac{\int_{I_\theta}^{I} p_I\left(I'\right) dI'}{1 - P_{sp}} & \text{for } I \geq I_\theta \end{cases} \tag{3}$$

with $P_{sp} = \int_{I_{-\infty}}^{I_\theta} p_I(I)\, dI$ the probability of occurrence of spontaneous activity.

Within the scope of this article we focus on changes of the threshold I_θ due to cochlear damage only and do not take into account changes of the spontaneous firing rate f_{sp} or the maximum firing rate f_{max}. In **Figure 3A** some example rate-intensity functions that are used in our computational model are shown for different thresholds. Note that with increasing threshold the fraction of sub-threshold sound intensities, i.e., the fraction where SR is effective, does also increase (**Figure 3B**).

Autocorrelation Function, Mean Autocorrelation, and Mutual Information

The autocorrelation function of a time-dependent variable *x(t)* is defined as:

$$AC(\tau) = \frac{\frac{1}{T}\sum_t (x(t) - \mu_X)(x(t+\tau) - \mu_X)}{\sigma_X^2} \tag{4}$$

where μ_X and σ_X are mean and standard deviation, respectively.

The mean autocorrelation is derived by averaging the autocorrelation function over all evaluated lag-times:

$$AC = \frac{1}{N}\sum_{\tau=1}^{N} AC(\tau) \tag{5}$$

The mutual information of input X and output Y is defined as:

$$MI(X;Y) = \int_Y \int_X p(x,y)\ log_2\left(\frac{p(x,y)}{p(x)\,p(y)}\right) dxdy \tag{6}$$

where $p(x)$, and $p(y)$ are the marginal probability density functions and $p(x, y)$ is the joint probability density function of X and Y.

Adaptive Stochastic Resonance Model

Our adaptive SR model mainly consists of three functional units and a feedback-loop (**Figure 1B**). The first unit, referred to as sensor (which we assume to be located within the cochlea, presumably at the post-synapse of the IHC, cf. Discussion), receives input from the environment, namely the time course of sound intensities *I(t)* which are generated using Equation (2). Its output, the AN firing rate *f(I),* is calculated according to Equation (3). The second unit receives its input from the sensor and calculates the autocorrelation of the time course of AN firing rates *AC(f)* according to Equation (5), reflecting their information content. We refer to this unit as the information detector. The third unit is controlled by the information detector and injects white noise n with constant zero mean $\mu_n = 0$ but tunable variance σ_n^2 back to the sensor via efferent connections. For this part of the system we use the term noise generator. We refer to σ_n^2 as the noise level. The model presumes that via feedback control the noise level for SR is set to a level where the autocorrelation function of the sensor output becomes maximal ($\sigma_{n,opt}^2(AC)$) which is equivalent to maximizing the

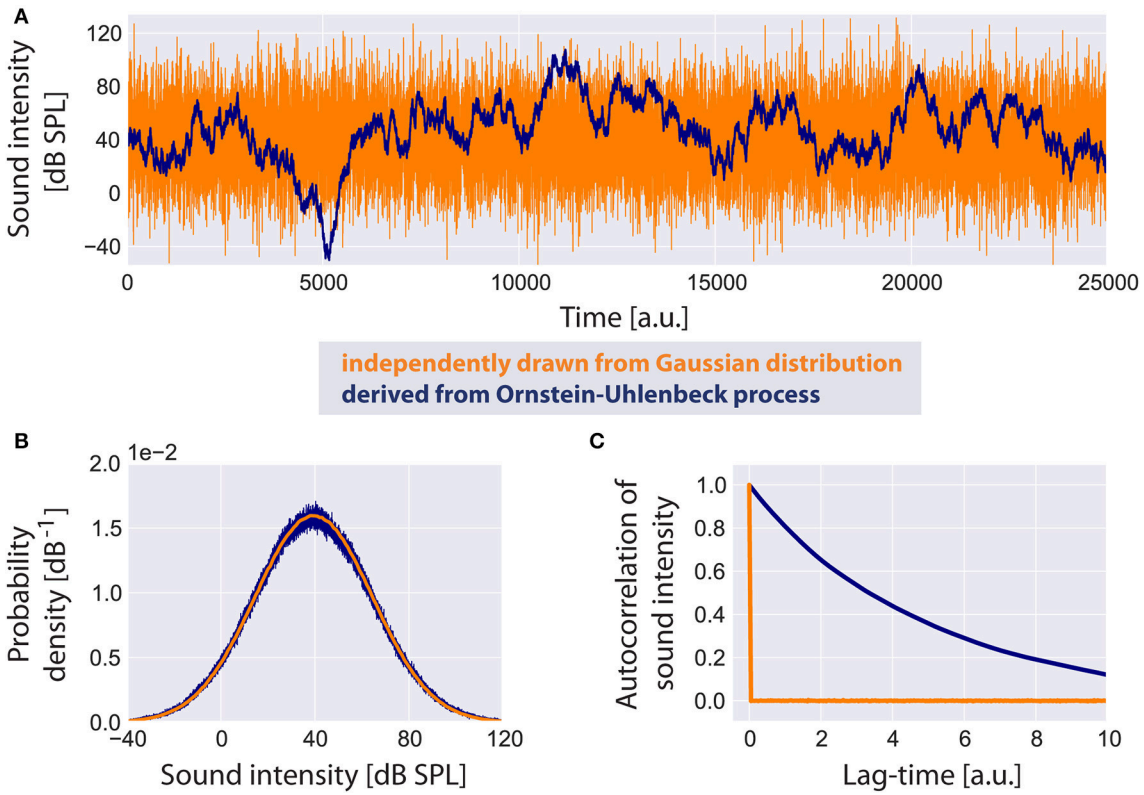

FIGURE 2 | Standard acoustic environment. Two sample time series of sound intensities are shown **(A)**: the uncorrelated time series (orange) has been generated by independently drawing values from a Gaussian distribution, whereas the correlated time series serving as input to the model (blue) is derived from an Ornstein-Uhlenbeck process. Although both time series look very different, their probability density functions **(B)** are identical. The autocorrelation function of the uncorrelated time series has a peak at lag-time zero. The autocorrelation of the Ornstein-Uhlenbeck process decreases exponentially with increasing lag-time **(C)**.

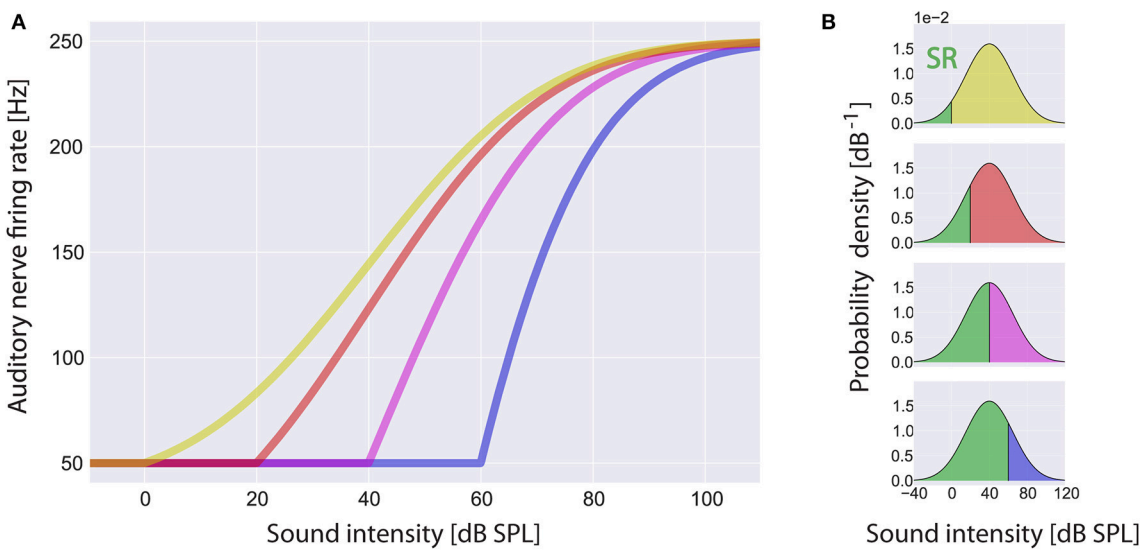

FIGURE 3 | Rate-intensity-functions used in the model. Shown are sample rate-intensity-functions **(A)** for different thresholds I_θ at 0 dB SPL (red), 20 dB SPL (green), 40 dB SPL (yellow) and 60 dB SPL (blue). In each case the spontaneous firing rate f_{sp} is 50 Hz and the maximum firing rate f_{max} is 250 Hz. In **(B)** the ratios of sub- and supra-threshold sound intensities are illustrated for increasing thresholds from top to bottom. Note that stochastic resonance (SR) only takes place in the range of sub-threshold sound intensities (green areas).

mutual information (**Figure 4A**; cf. Krauss et al., 2015). The optimal noise level depends on both the signal amplitude and the threshold. All further simulations presented in this report (**Figures 5–7**) use these previously evaluated optimal noise levels $\sigma^2_{n,opt}(AC)$. Hence, in case of SR the sensor receives two kinds of inputs, namely the sound intensities *I(t)* and white noise *n(AC)* with variance $\sigma^2_{n,opt}(AC)$. Both inputs are summed (Σ) before thresholding (θ) occurs. Thus, equation (3) is slightly modified to:

$$f\left(I + n(AC)\right) = \begin{cases} f_{sp} & for\ I + n(AC) < I_\theta \\ f_{sp} + \left(f_{max} - f_{sp}\right) \frac{\int_{I_\theta}^{I+n(AC)} p_I\left(I'\right) dI'}{1 - P_{sp}} & for\ I + n(AC) \geq I_\theta \end{cases} \quad (7)$$

The autocorrelation of the sensor output also does not only depend on the amount of injected noise $\sigma^2_{n,opt}(AC)$ but also on the hearing threshold, i.e., the larger the hearing threshold, the smaller is the autocorrelation at the optimal SR noise level (**Figures 4B,C**). Note that large hearing thresholds (**Figure 4B**) result in a rightward shift of the rising point of the autocorrelation as a function of noise intensity. As the noise intensity has to be higher to reach the threshold in the first place, the autocorrelation remains zero for lower noise intensities (**Figure 4B**). All resonance curves shown in **Figure 4** were computed using the standard acoustic environment as input, yet with different thresholds (cf. figure caption).

Patient Data

Anonymized audiometric data from patients who came to the ENT clinic in Erlangen for medical examination were used. Therefore, no declaration of consent was required by German law. 78,282 data sets of pure tone audiometries of both ears were collected in 39,141 patients between the years 2000 to 2015. Patients were not characterized by their gender, age [median (25, 75% quantil): 42 (21, 58)] or by former or current pathologies not affecting hearing thresholds but only by their report of percieving a tinnitus (group T) or not (group NT). Standardized audiometric testing instruments of an audiological clinic were used. Air conduction thresholds were measured by pure-tone audiometry (stimuli were presented from −10 to 130 dB in 5 dB steps) for both ears separately for every patient. Test frequencies were 125, 250, 500, 750, 1000, 1500, 2000, 3000, 4000, 6000, and 8000 Hz. Hearing thresholds were tested pairwise for every frequency with a Kolmogorow Smirnoff test for two samples.

RESULTS

The aim of this study was to present an alternative to existing models of tinnitus-related development of neuronal hyperactivity. To this end, we will demonstrate how adaptive SR based on maximizing the autocorrelation function of the sensor (IHC synapse) output after chronically reduced input into the auditory system, e.g., due to cochlear damage, may lead to permanently increased internal noise within the auditory system (as a possible correlate of tinnitus). We first show the effect of increased thresholds on the autocorrelation function of the time series of AN firing rates *f(t)* and subsequently the effect of SR on the autocorrelation function.

SR Improves Detection Probability after Hearing Loss

In **Figure 5** the main effects of the model on the auditory nerve firing rates are shown. For the standard acoustic environment (orange) the model's auditory nerve firing rate responses (left panels) and corresponding psychometric functions (detection probability as a function of sound intensity; right panels) are shown for different degrees of hearing loss (0, 20, 40 dB), both without SR (red) and with the optimum SR noise intensity (green). The hearing thresholds with the aid of SR are given by the sound level at 50% detection probability. As it turned out, the benefit of SR based on our model is about 4 dB (at 20 dB hearing loss) or 6 dB (at 40 db hearing loss), respectively.

Reduced Input Decreases the Mean Autocorrelation Function of the Sensor Output

Using our model we evaluated the autocorrelation function of the time series of simulated AN firing rates *f(I)* as defined in the Method section. In all simulations we used the standard acoustic environment as input to the sensor *I(t)*, namely a correlated random walk with normally distributed values with a mean value of 40 dB SPL and standard deviation of 25 dB (cf. Methods; **Figure 2**). In **Figure 6A**, autocorrelation functions for this input at different sensor thresholds are shown. For increased thresholds the values of the autocorrelation function systematically shift to smaller values. Accordingly, the mean autocorrelation obtained by averaging the autocorrelation function over all evaluated lag-times decreases monotonically with increasing threshold (**Figure 6B**). These data demonstrate, as could be expected, that the amount of information content transmitted by the sensor decreases with increasing sensor thresholds, that is, with increasing hearing thresholds.

Stochastic Resonance Improves Mean Autocorrelation and Increases Internal Noise after Hearing Loss

The effect of SR at the level of the sensor on the autocorrelation of the sensor output and its effect on hearing threshold is shown in **Figure 7**. Again the standard acoustic environment served as input. In **Figure 7A** sample autocorrelation functions for an exemplary threshold I_θ of 30 dB are shown with (green) and without (red) the effect of SR. Obviously, SR is able to increase the autocorrelation, that is, to improve information transmission. When averaging the autocorrelation over all lag times and plotting both functions (with and without SR) as a function of the threshold (**Figure 7B**) the benefit of SR on hearing threshold becomes obvious (arrows indicate threshold shift). In **Figure 7C** this benefit of SR in dB is plotted as a function of hearing loss, revealing maximal benefits of up to 6 dB.

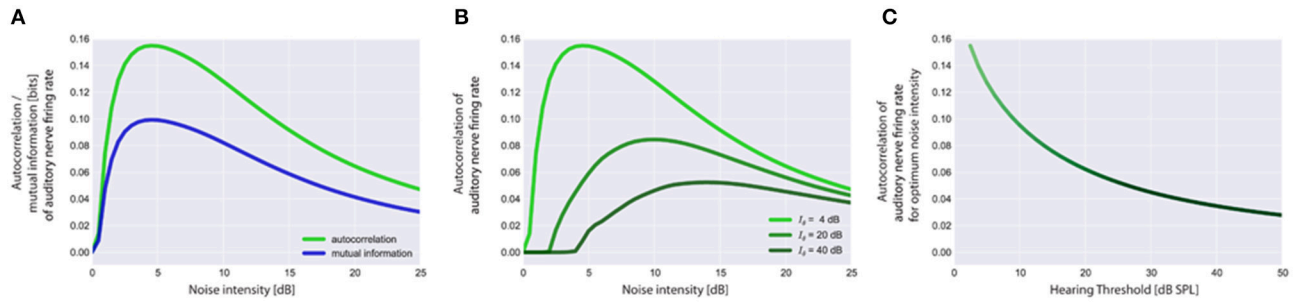

FIGURE 4 | Stochastic resonance and output autocorrelation. In (A) typical resonance curves for the standard acoustic environment (cf. Methods) with hearing threshold $I_\theta = 4$ dB are shown to illustrate the equivalence of the mean autocorrelation of the auditory nerve (AN) firing rates (output) and the mutual information between sound intensities (input) and AN firing rates (output). Both objective functions peak at the same noise intensity which is referred to as the optimum noise intensity. Resonance curves based on output autocorrelation are shown in **(B)** for the standard acoustic environment and three different hearing thresholds (4, 20, 40 dB). Note that with increasing hearing threshold the corresponding optimum noise intensity also increases whereas the maximum of output autocorrelation decreases **(B,C)**. Furthermore, the onset of the rise of the autocorrelation **(B)** is rightward shifted as the initial noise has to be increased more for larger values of the hearing threshold to produce effective SR.

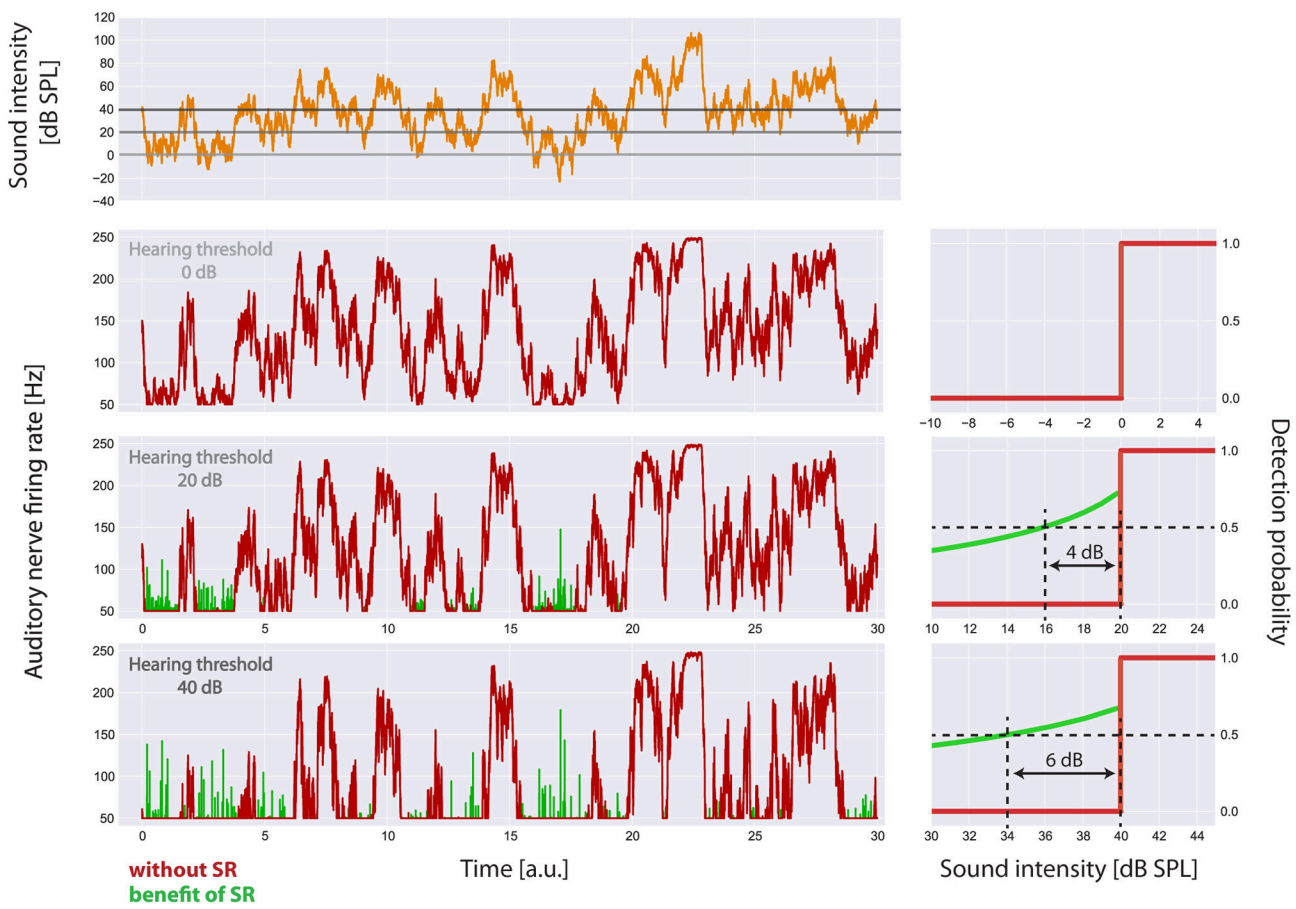

FIGURE 5 | Visualization of the main effects of the model. For the standard acoustic environment (orange) the auditory nerve firing rate responses (left panels) and corresponding psychometric functions (detection probability as a function of sound intensity, right panels) are shown for different degrees of hearing loss (0, 20, 40 dB), both without SR (red) and with the optimum SR noise intensity (green). The hearing thresholds with the aid of SR are given by the sound level at 50% detection probability. Hence the benefit of SR is about 4 dB (at 20 dB hearing loss) or 6 dB (at 40 dB hearing loss), respectively.

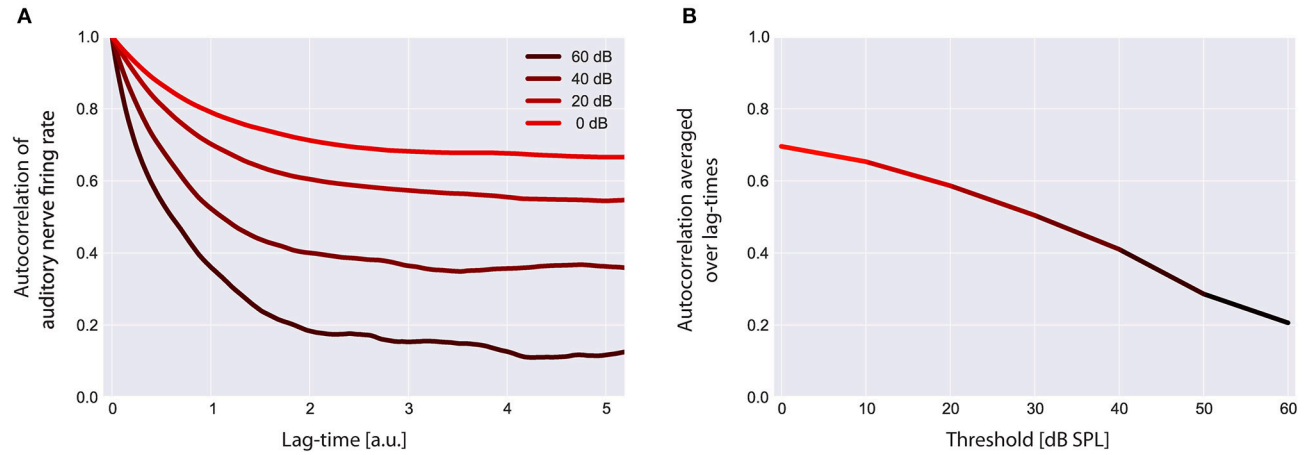

FIGURE 6 | Autocorrelation functions of the cochlear output. Shown are the autocorrelation functions for different thresholds **(A)**. For increased thresholds the values of the autocorrelation function systematically shift to smaller values, reflecting the decreased amount of information content transmitted by the cochlea. The mean autocorrelation obtained by averaging the autocorrelation function over all evaluated lag-times decreases monotonically with increasing threshold **(B)**.

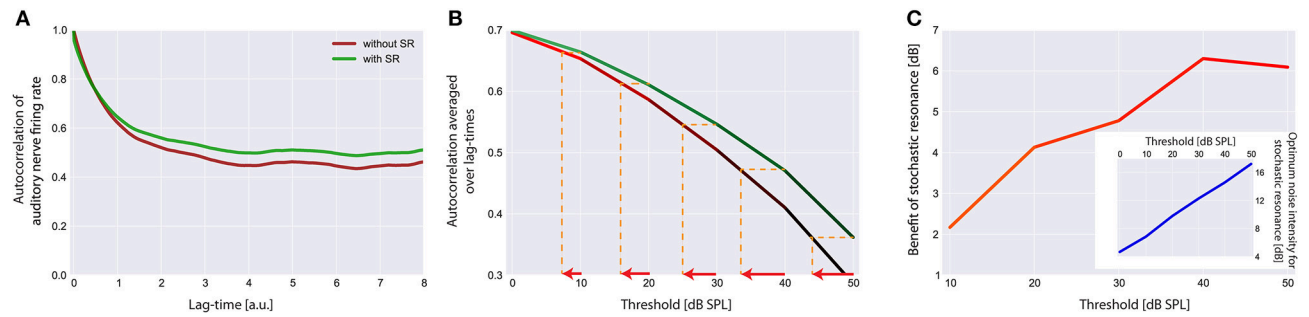

FIGURE 7 | Effect of stochastic resonance (SR). In **(A)** a sample autocorrelation function for a threshold I_θ of 30 dB is shown with (green) and without (red) the effect of SR. As can be clearly seen, SR is able to improve the autocorrelation. Note that an improvement of the autocorrelation is equivalent to improved hearing thresholds in our model. **(B)** Averaged autocorrelation as a function of the corresponding threshold. Horizontal iso-AC lines and arrows on the x-axis indicate the benefit of SR for different autocorrelation levels. **(C)** Benefit of SR on hearing threshold in dB for different threshold levels. Note that with increasing thresholds more noise is required to improve the autocorrelation (inset).

Remarkably, the benefit of SR (i.e., improvement of hearing thresholds) according to both, detection probability (**Figure 5**) and improvement of autocorrelation (**Figure 7**) exhibit nearly identical values (4 or 6 dB, respectively).

This improvement of hearing thresholds leads to a readjustment of the rate-intensity function *f(I)* equivalent to a leftward shift of the onset of the rise of the *f(I)* in **Figure 3A**. On the other hand, with increasing thresholds more noise is required to optimally improve the autocorrelation (**Figure 7C**, inset). We here propose that in case of chronically elevated thresholds, e.g., due to cochlear damage, this internal noise added for SR to compensate for the elevated thresholds has to be increased permanently and hence may be the correlate of tinnitus-related neuronal hyperactivity. If this would be the case then tinnitus could be viewed as a side-effect of a compensatory mechanism of the auditory system that aims to (at least partly) restore hearing thresholds after hearing loss by adding internal noise to the sensor level, thereby making use of SR.

Audiometric Patient Data in Support of Our Hypothesis

Finally, to scrutinize this hypothesis we analyzed the audiometric data of 39,141 patients from the ENT hospital Erlangen (**Figure 8A**). As it turned out and in line with our hypothesis, (tonal) tinnitus patients on average indeed had significantly better hearing thresholds than the non-tinnitus patients, namely in the frequency range below 3 kHz., i.e., the frequency range most important for speech processing (**Figure 8B**). This finding—at least for frequencies below 3 kHz—is consistent with the work of König et al. (2006) reporting better hearing thresholds for tinnitus patients compared to non-tinnitus patients in a range from 0.125 to 8 kHz. On the other hand, the majority of the patients (65.5%) reported tinnitus frequencies above 3 kHz (**Figure 8C**), i.e., in the frequency range where tinnitus patients on average had significantly higher hearing thresholds compared to the non-tinnitus patients.

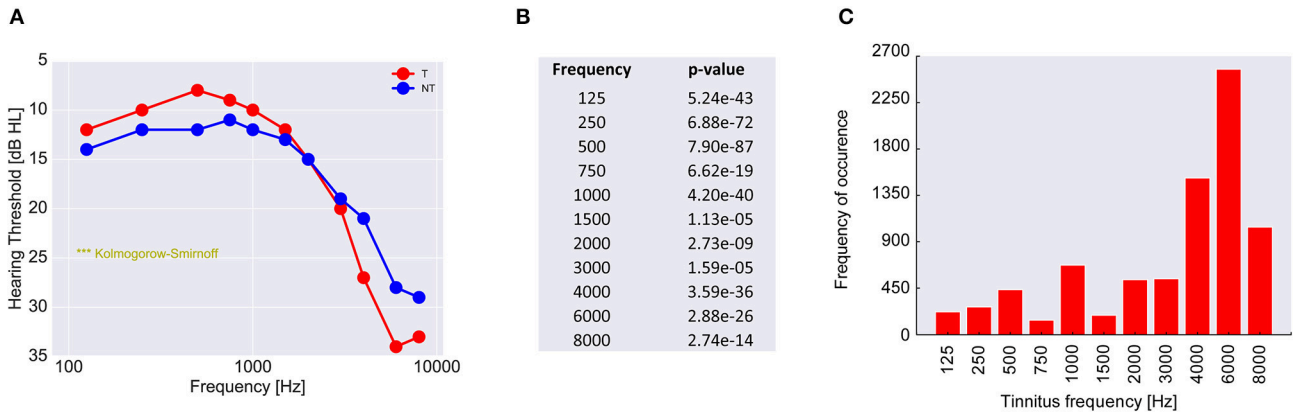

FIGURE 8 | Analysis of 39,141 patients with increased hearing thresholds (A). All patients are divided into two groups, namely those with (red) and those without (blue) tinnitus. Shown are the median hearing thresholds for each frequency. For lower frequencies the tinnitus patients have lower thresholds than the non-tinnitus patients, which is perfectly consistent with the prediction of our model. However, for higher frequencies tinnitus patients have higher hearing levels than non-tinnitus patients. Since the pitch of the perceived tinnitus mainly lies within the high-frequency region **(C)**, the tinnitus percept could cause a masking effect leading to secondarily increased hearing levels. In **(B)** the *p*-values of the statistical comparison of the data in **(A)** (two sample Kolmogorov-Smirnoff test) are summarized. For each tested frequency the distributions of hearing thresholds are significantly different for the two patient groups. Note that distributions still may be significantly different even if median values happen to be identical (as is the case at 3 kHz).

DISCUSSION

In this report we have demonstrated how an information processing system based on adaptive SR aiming to maximize the autocorrelation of a sensor's output may cause neuronal hyperactivity in the case of chronically reduced input to that sensor. In this context we view this neuronal hyperactivity as a side effect of the adaptive SR mechanism whose main purpose is to optimize information transmission and thereby partly restore lost sensitivity by improving thresholds in cases of sensor damage.

Plausibility of the Model in the Context of the Anatomy of the Auditory System

After phenomenologically describing the main concepts and mechanisms of our model we will now discuss how plausible the model may be in the context of the known anatomy of the auditory system. To this end we will try to identify candidate structures within the auditory system for the implementation of the model components as given in **Figure 1B**.

One candidate structure for the noise adding feedback loop predicted by our model is the efferent projection from the superior olivary complex to the IHC, the lateral olivocochlear bundle. For SR to be effective the internal noise fed back to the sensor must reach the post-synaptic site. Interestingly (and in contrast to efferent projections to the outer hair cells which directly contact the basolateral region of these cells via axosomatic synapses), the efferent projections from the superior olivary complex [especially the lateral olivocochlear bundle, a heterogeneous population of neurons utilizing several different neurotransmitter systems like acetylcholine, gamma-aminobutyric acid (GABA), glycine and dopamine Ruel et al., 2006, 2007; Dlugaiczyk et al., 2008] to the IHC form axodendritic synapses with the AN below the IHC (Groff and Liberman, 2003; Darrow et al., 2007) where they could modulate the response probability to sensory input by inhibition and excitation for SR, exactly at the site predicted by our SR model. The olivocochlear efferents are therefore able to modulate the AN post-synaptic membrane potential or—in terms of SR—feed noise into the sensor.

This prediction is in line with electrophysiological data from animal models (Dallos and Harris, 1978; Liberman and Kiang, 1978; Liberman and Dodds, 1984) where spontaneous rates of AN fibers after cochlear damage were either reduced or unchanged but never increased. In our model, the spontaneous rate would be initially decreased and then raised back to normal levels by the SR feedback.

Nevertheless, a recent study showed that most synaptic events were sufficient to trigger an action potential in spiral ganglion neurons (Rutherford et al., 2012). If they are responding to almost every bit of signal from the hair cell, it seems unlikely that noise injection into the spiral ganglion neurons could make them even more sensitive. Furthermore, it has been shown that one of the major functions of the lateral olivocochlear bundle feedback seems to be the regulation of responses to high sound intensities (Le Prell et al., 2003a), cochlear neuroprotection (Lendvai et al., 2011; Maison et al., 2013) and interaural balance (Darrow et al., 2006). Lesion studies showed that there was either no effect of lateral olivocochlear bundle lesions on hearing thresholds (Le Prell et al., 2003b; Darrow et al., 2007), or only a limited effect at high frequencies (Le Prell et al., 2005), but that evoked responses either decreased (Darrow et al., 2007) or increased (Le Prell et al., 2005) after removal of the lateral olivocochlear bundle feedback to the cochlea. These findings are not necessarily in opposition to our hypothesis, as we would rather predict an initial drop of spontaneous AN activity after a hearing loss with normalization over time (cf. below). Furthermore, and in support of our hypothesis it has been shown that the lateral olivocochlear

bundle could provide the required noise input to the AN, as disruption of lateral olivocochlear neurons with a dopaminergic neurotoxin depressed spontaneous auditory nerve activity (Le Prell et al., 2014) and efferent synapses of the lateral olivocochlear bundle re-innervate IHCs of the aged cochlea (Lauer et al., 2012).

In this context it is worth mentioning that hearing loss—at least in rodents—has been found to predominantly cause loss of low spontaneous rate ($^{lo}f_{sp}$) AN fibers (Furman et al., 2013). In contrast to high spontaneous rate ($^{hi}f_{sp}$) fibers with a low threshold ($^{lo}\theta$), these fibers have comparatively high thresholds ($^{hi}\theta$) (Bourien et al., 2014). Interestingly, whereas $^{lo}f_{sp}$ AN fibers are found across all frequency regions of the cochlea, $^{hi}f_{sp}$ AN fibers are predominately found in the frequency regions of the cochlea below 3–4 kHz (**Figure 9**; Ohlemiller and Echteler, 1990; Heil and Peterson, 2015). Based on these findings it seems obvious that noise trauma would affect the high and low frequency region of the cochlea differently: as $^{lo}f_{sp}$ AN fibers are more prone to get damaged by noise trauma than $^{hi}f_{sp}$ AN fibers, the high frequency region would be more affected by the trauma than the low frequency region as there the more resilient $^{hi}f_{sp}$ AN fibers are rare (cf. Figure 2 in Ohlemiller and Echteler, 1990). These findings are consistent with results from studies with human AN samples, describing a stronger loss of fibers in the basal compared to apical section of the cochlea (Zimmermann et al., 1995) in subjects with hidden and non-hidden hearing loss (Euteneuer and Praetorius, 2014). In the context of our model one may speculate that after trauma, SR may be more effective in the low frequency range below 3–4 kHz as there the number of $^{hi}f_{sp}$ AN fibers is high (**Figure 9**). Furthermore, as they have $^{lo}\theta$, for these fibers less internal noise would be needed to produce effective SR. By contrast, the few remaining $^{lo}f_{sp}$ AN fibers in the high frequency range show $^{hi}\theta$, so that much more internal noise would be needed for SR to be effective. This asymmetry of AN type I fiber innervation in the cochlea may therefore explain the asymmetry found in the patients' hearing threshold data. There, a threshold benefit was observed in tinnitus patients in the low frequency range only. If our model would be correct, this may further explain why tinnitus percepts are predominantly found in the high frequency range: there the system would aim to improve signal transmission by SR, but as thresholds of the remaining AN fibers are high, the high amount of internal noise added may be perceived as tinnitus which in turn could mask the possible threshold benefit introduced by SR, thereby finally resulting in overall elevated thresholds in tinnitus patients in that high frequency range (cf. below).

An alternative candidate structure for SR to be effective is the projection site of the AN at the dorsal cochlear nucleus (DCN): This hypothesis follows the basic idea that cochlear damage may result in reduced and therefore sub-threshold AN input to the DCN. This would be especially relevant in the context of light or hidden hearing loss, where the information from a diminished number of IHCs converge on DCN neurons and may not be sufficient to evoke a response there. In addition, the AN may also provide sub-threshold information of synchrony between different fibers (Young and Davis, 2002). As a source of noise background needed for SR—besides spontaneous activity

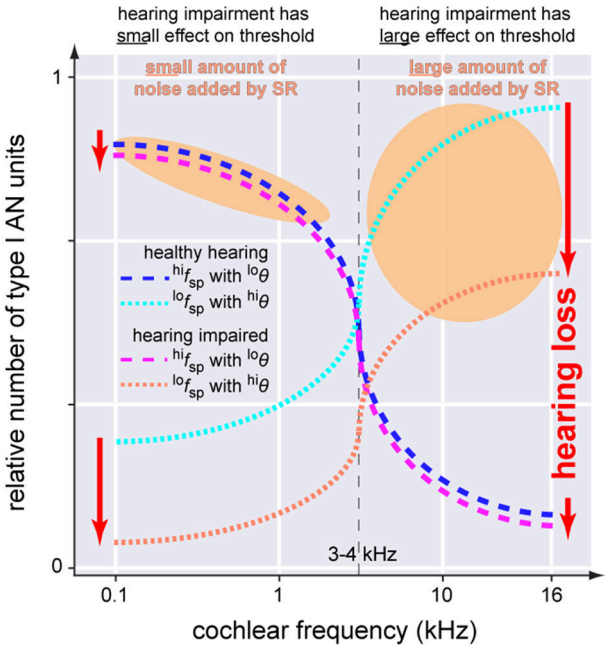

FIGURE 9 | Scheme of the anatomical asymmetry of threshold distributions of the AN type I units innervating the IHC. In the healthy cochlea neurons with high spontaneous rates and low thresholds (blue dashed line, $^{hi}f_{sp}$ with $^{lo}\theta$) are predominantly located below 3–4 kHz while units with low spontaneous rates and high thresholds (cyan dotted line, $^{lo}f_{sp}$ with $^{hi}\theta$) are found across the whole cochlea. Following hearing impairment these latter neurons are affected first and most severely.(red dotted line) while the first fibers are mostly spared (magenta dashed line). SR increases noise intensity to counteract hearing loss (orange areas). Note that the noise intensity in the higher frequency range has to be much higher which may make it more likely for tinnitus to be induced in those frequencies ranges.

generated within the DCN itself – also innervation from the somatosensory system seems conceivable (Dehmel et al., 2012) and may explain modulation of tinnitus sensation in patients, e.g., by jaw movements (Pinchoff et al., 1998).

In support of this view, in (animal) models of acoustic trauma induced tinnitus, increased spontaneous firing rates throughout the auditory system have been observed (Wang et al., 1997; Ahlf et al., 2012; Tziridis et al., 2015; Wu et al., 2016), and the DCN is the earliest processing stage in the auditory pathway in which acoustic trauma leads to tinnitus-related changes and increased spontaneous firing rates (Kaltenbach et al., 1998; Kaltenbach and Afman, 2000; Brozoski et al., 2002; Zacharek et al., 2002; Kaltenbach et al., 2004; Wu et al., 2016). The amount of this increase in spontaneous activity in the DCN has been shown to be correlated with the strength of the behavioral signs for tinnitus (Kaltenbach et al., 2004). Furthermore, this hyperactivity is only found in regions innervated by the damaged parts of the cochlear receptor epithelium (Kaltenbach et al., 2002) and is not brought back to normal levels even after cochlear ablation (Zacharek et al., 2002). Interestingly, Gao et al. recently described changes in DCN fusiform cell spontaneous activity after noise exposure that occur on short time scales (i.e., minutes). In contrast to HP mechanisms that work on long time scales (days or weeks), our

SR mechanism may easily explain such fast adaptive dynamics reported by Gao et al. (2016). In particular and consistent with our model, the time course of spontaneous rate changes shows an almost complete loss of spontaneous activity immediately after loud sound exposure (as no SR is needed due to stimulation that is well above threshold), followed by an overcompensation of sound induced spontaneous rate changes to levels well above pre-exposition rates where SR is now needed to compensate for acute hearing loss (Gao et al., 2016).

Another argument in favor of the DCN as plausible site for SR relates to the information detector (**Figure 1B**) within our model which computes the autocorrelation of the AN activity. As has been pointed out in the Introduction section already, the evaluation of autocorrelation functions may easily be implemented within neuronal networks using delay-lines and coincidence detectors (Licklider, 1951). A neuronal architecture resembling such delay-lines has been described in the DCN (Osen, 1988; Hackney et al., 1990; Baizer et al., 2012).

Finally, as mentioned above, besides the possibility of noise generation within the DCN, the somatosensory projections to the DCN (Ryugo et al., 2003; Shore and Zhou, 2006; Dehmel et al., 2012; Zeng et al., 2012) could correspond to the noise generator (**Figure 1B**) of the model. Especially the integration over many different sensory systems within the DCN in combination with the shaping of these inputs by inhibition (Ryugo et al., 2003) may provide the ideal anatomical basis for noise propagation and adjustment: SR in this view would be controlled by inhibition of the noise generators within the DCN or of the noise fed back to the DCN from the somatosensory system, where a downregulation of inhibition (as a consequence of reduced input and therefore reduced autocorrelation) would increase internal noise by disinhibition of the noise generators or inputs.

We therefore believe that the DCN is the most likely structure within the auditory pathway where the sensor, the information detector and the noise adjusting structure of our model may be implemented. Beyond these speculations the most likely candidate structures for noise generation and propagation has to be the subject of future studies.

Neuronal Hyperactivity within the Auditory System As a Possible Correlate for Auditory Phantom Percepts

We have shown in this report that SR at the sensor level may be a mechanism to partly restore and thus improve information transmission into the auditory system after damage to the receptor epithelium, e.g., due to noise trauma. We have further demonstrated that for this mechanism to be effective, internal noise has to be generated, probably in form of increased spontaneous activity at some level within the auditory pathway. This may lead to higher sensitivity for even suprathreshold stimuli resulting in hyperacusis. This idea is in line with observations by Hébert and colleagues who have reported that hyperacusis, i.e., increased auditory sensitivity, is a pervasive complaint of people with tinnitus, suggesting that both symptoms have a common origin (Hébert et al., 2013).

In technical and physical systems the term *suprathreshold SR* has been coined for such a phenomenon (Collins et al., 1995; Stocks, 2000, 2001; Stocks; Stocks et al., 2002; McDonnell et al., 2008). Furthermore, we propose that in cases of permanently increased spontaneous activity, this hyperactivity would be able to induce neuronal plasticity along the auditory pathway that subsequently leads to the development of subjective, central tinnitus.

From human studies it is known that the presentation of external noise simultaneously to pure tones is actually able to significantly improve the hearing thresholds for these pure tones (Zeng et al., 2000; Long et al., 2004; Ries, 2007), a finding that was also explained by SR. Also our finding that across almost 40,000 patients those with tinnitus have lower hearing thresholds in the low frequency range than those without tinnitus has cursory been observed previously (König et al., 2006). In that study, König and colleagues described significantly better hearing thresholds for patients with tone-like tinnitus percepts compared to patients with noise-like percepts or without any tinnitus percept.

Our observation that tinnitus patients had increased hearing thresholds in the high frequency range is still in line with our model (cf. speculation about the loss of $^{lo}f_{sp}$ *AN fibers*) but may need further inspection: As detailed above, the noise increase needed to produce effective SR in that high frequency range would have to be particularly large as thresholds there are disproportionately high and may therefore more likely induce tinnitus. In line with this rationale most tinnitus patients perceived their tonal tinnitus at high frequencies (**Figure 7C**). We therefore speculate that our finding of increased thresholds in high frequency ranges may result from masking of the hearing thresholds in the frequency range where tinnitus is perceived. The difference in mean audiogram observed in tinnitus patients compared to non-tinnitus patients may therefore be based on a combination of two effects: Primarily SR to restore hearing thresholds after hearing loss and subsequently masking of hearing thresholds by the SR-induced tinnitus. Whereas the former would be more effective in the low frequency range—due to the better survival of $^{hi}f_{sp}$ fibers in the AN, the latter was more effective in the high frequency range. If this view would be correct, than the auditory system would improve impaired hearing thresholds in the frequency range relevant for speech processing at the cost of further impairing hearing thresholds in the high frequency range. Especially the effect of tinnitus pitch described by König et al. where high pitched tinnitus worsened hearing thresholds compared to low pitched tinnitus percepts (Figure 1C in König et al., 2006), supports our view of a masking effect in the high frequency range that counteracts the beneficial threshold shift based on SR.

In this context, one could interprete the patient data presented in **Figure 7** as an extension of our single frequency channel model to a model with multiple independent frequency channels. The noise would be adjusted according to the local hearing threshold for the different frequency groups and not by a gain increase generalized to all frequency regions. This is consistent with the finding that in the DCN spontaneous activity has been shown to be correlated to the strength of the behavioral signs for tinnitus (Kaltenbach et al., 2004) and this hyperactivity is only found in

regions innervated by the damaged parts of the cochlear receptor epithelium (Kaltenbach et al., 2002).

Another aspect that supports our hypothesis is the so called Zwicker tone illusion. The term describes an intriguing auditory aftereffect. The typical sound evoking a Zwicker tone is a broadband noise containing a spectral gap, which is presented for several seconds. After the sound has been switched off, a faint, almost pure tone is audible for 1 up to 6 s. It is decaying and has a sharp pitch in the spectral gap where no stimulus was available (Zwicker, 1964; Lummis and Guttman, 1972). Both the localization of the Zwicker tone in the brain and its origin has been long-standing open problems. In terms of our model we would speculate the cause of this auditory illusion to be the autocorrelation controlled upregulated internal noise for SR in response to the missing input within a certain spectral region introduced by the Zwicker paradigm. This is consistent with the suggestion that gain adaptation enhances internal noise of a frequency band otherwise silent due to damage (Parra and Pearlmutter, 2007).

Another interesting fact is that during the Zwicker tone sensation, auditory sensitivity for tone pulses at frequencies adjacent to the Zwicker tone are improved by up to 13 dB (Wiegrebe et al., 1996). It is plausible that cross-talk between adjacent frequency channels plays a role here. The sound intensities or AN firing rates of neighboring channels may serve as some kind of reference. Note that this threshold improvement again supports our hypothesis that SR plays a major role within the hearing system.

Furthermore, it is known that complete sensory deprivation may in some individuals lead to hallucination like experiences that occur after several hours in such a state (Lilly, 1956) and can produce acoustic phantom percepts as complex as music (Kjellgren et al., 2008). Other studies described tinnitus-like phenomena already after a few minutes in a sound proof chamber in 75% of the test subjects (Heller and Bergman, 1953). Finally, our model also easily explains why plugging of the outer ear canals also leads to perceptual changes like measurable improvement of hearing thresholds after unplugging or a transient tinnitus percept that vanishes after restoration of normal hearing (Schaette et al., 2012; Fournier et al., 2014).

In summary, we provide evidence that temporary and chronic auditory phantom percepts (Zwicker tones and tinnitus, respectively) may result from SR effects in the auditory pathway which have been evolutionary developed to counteract hearing loss. This alternative view opens up new perspectives for understanding the development of subjective tinnitus that will hopefully result in advanced therapeutic approaches to treat the condition. In this context, both adding external noise to induce SR, thereby superseding the internally generated neuronal noise, as well as strategies to suppress SR in the high frequency range with considerable tinnitus perception are conceivable.

AUTHOR CONTRIBUTIONS

PK, AS, and CM performed the calculations for the models. PK, CM, KT, and HS discussed the theoretical background of the model. UH provided the human data. KT performed the analysis of the human data. PK, KT, and HS wrote the manuscript.

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REFERENCES

Ahlf, S., Tziridis, K., Korn, S., Strohmeyer, I., and Schulze, H. (2012). Predisposition for and prevention of subjective tinnitus development. *PLoS ONE* 7:e44519. doi: 10.1371/journal.pone.0044519

Baizer, J. S., Manohar, S., Paolone, N. A., Weinstock, N., and Salvi, R. J. (2012). Understanding tinnitus: the dorsal cochlear nucleus, organization and plasticity. *Brain Res.* 1485, 40–53. doi: 10.1016/j.brainres.2012.03.044

Benzi, R., Sutera, A., and Vulpiani, A. (1981). The mechanism of stochastic resonance. *J. Phys. A Math. Gen.* 14, L453–L457. doi: 10.1088/0305-4470/14/11/006

Bourien, J., Tang, Y., Batrel, C., Huet, A., Lenoir, M., Ladrech, S., et al. (2014). Contribution of auditory nerve fibers to compound action potential of the auditory nerve. *J. Neurophysiol.* 112, 1025–1039. doi: 10.1152/jn.00738.2013

Brenner, N., Bialek, W., and Van Steveninck, R. D. R. (2000). Adaptive rescaling maximizes information transmission. *Neuron* 26, 695–702. doi: 10.1016/S0896-6273(00)81205-2

Brozoski, T. J., Bauer, C. A., and Caspary, D. M. (2002). Elevated fusiform cell activity in the dorsal cochlear nucleus of chinchillas with psychophysical evidence of tinnitus. *J. Neurosci.* 22, 2383–2390.

Coles, R. R. (1984). Epidemiology of tinnitus: (1) prevalence. *J. Laryngol. Otol. Suppl.* 9, 7–15. doi: 10.1017/S1755146300090041

Collins, J. J., Chow, C. C., and Imhoff, T. T. (1995). Stochastic resonance without tuning. *Nature* 376, 236–238. doi: 10.1038/376236a0

Collins, J. J., Imhoff, T. T., and Grigg, P. (1996). Noise-enhanced information transmission in rat SA1 cutaneous mechanoreceptors via aperiodic stochastic resonance. *J. Neurophysiol.* 76, 642–645.

Dallos, P., and Harris, D. (1978). Properties of auditory nerve responses in absence of outer hair cells. *J. Neurophysiol.* 41, 365–383.

Darrow, K. N., Maison, S. F., and Liberman, M. C. (2006). Cochlear efferent feedback balances interaural sensitivity. *Nat. Neurosci.* 9, 1474–1476. doi: 10.1038/nn1807

Darrow, K. N., Maison, S. F., and Liberman, M. C. (2007). Selective removal of lateral olivocochlear efferents increases vulnerability to acute acoustic injury. *J. Neurophysiol.* 97, 1775–1785. doi: 10.1152/jn.00955.2006

Dean, I., Harper, N. S., and McAlpine, D. (2005). Neural population coding of sound level adapts to stimulus statistics. *Nat. Neurosci.* 8, 1684–1689. doi: 10.1038/nn1541

Dean, I., Robinson, B. L., Harper, N. S., and McAlpine, D. (2008). Rapid neural adaptation to sound level statistics. *J. Neurosci.* 28, 6430–6438. doi: 10.1523/JNEUROSCI.0470-08.2008

Dehmel, S., Pradhan, S., Koehler, S., Bledsoe, S., and Shore, S. (2012). Noise overexposure alters long-term somatosensory-auditory processing in the dorsal cochlear nucleus–possible basis for tinnitus-related hyperactivity? *J. Neurosci.* 32, 1660–1671. doi: 10.1523/JNEUROSCI.4608-11.2012

Dlugaiczyk, J., Singer, W., Schick, B., Iro, H., Becker, K., Becker, C. M., et al. (2008). Expression of glycine receptors and gephyrin in the rat cochlea. *Histochem. Cell Biol.* 129, 513–523. doi: 10.1007/s00418-008-0387-x

Douglass, J. K., Wilkens, L., Pantazelou, E., and Moss, F. (1993). Noise enhancement of information transfer in crayfish mechanoreceptors by stochastic resonance. *Nature* 365, 337–340. doi: 10.1038/365337a0

Dragoi, V., Sharma, J., and Sur, M. (2000). Adaptation-induced plasticity of orientation tuning in adult visual cortex. *Neuron* 28, 287–298. doi: 10.1016/S0896-6273(00)00103-3

Dunn, F. A., and Rieke, F. (2006). The impact of photoreceptor noise on retinal gain controls. *Curr. Opin. Neurobiol.* 16, 363–370. doi: 10.1016/j.conb.2006.06.013

Eggermont, J. J. (2003). Central tinnitus. *Auris Nasus Larynx* 30(Suppl.), S7–S12. doi: 10.1016/S0385-8146(02)00122-0

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Einstein, A. (1905). Über die von der molekularkinetischen Theorie der Wärme geforderte Bewegung von in ruhenden Flüssigkeiten suspendierten Teilchen. *Ann. Phys.* 322, 549–560. doi: 10.1002/andp.19053220806

Engineer, N. D., Riley, J. R., Seale, J. D., Vrana, W. A., Shetake, J. A., Sudanagunta, S. P., et al. (2011). Reversing pathological neural activity using targeted plasticity. *Nature* 470, 101–104. doi: 10.1038/nature09656

Euteneuer, S., and Praetorius, M. (2014). Hearing research news: from the periphery to the center. *HNO* 62, 88–92. doi: 10.1007/s00106-013-2807-z

Faisal, A. A., Selen, L. P., and Wolpert, D. M. (2008). Noise in the nervous system. *Nat. Rev. Neurosci.* 9, 292–303. doi: 10.1038/nrn2258

Fournier, P., Schonwiesner, M., and Hebert, S. (2014). Loudness modulation after transient and permanent hearing loss: implications for tinnitus and hyperacusis. *Neuroscience* 283, 64–77. doi: 10.1016/j.neuroscience.2014.08.007

Furman, A. C., Kujawa, S. G., and Liberman, M. C. (2013). Noise-induced cochlear neuropathy is selective for fibers with low spontaneous rates. *J. Neurophysiol.* 110, 577–586. doi: 10.1152/jn.00164.2013

Gammaitoni, L., Hänggi, P., Jung, P., and Marchesoni, F. (1998). Stochastic resonance. *Rev. Mod. Phys.* 70:223. doi: 10.1103/RevModPhys.70.223

Gao, Y., Manzoor, N., and Kaltenbach, J. A. (2016). Evidence of activity-dependent plasticity in the dorsal cochlear nucleus, *in vivo*, induced by brief sound exposure. *Hear. Res.* 341, 31–42. doi: 10.1016/j.heares.2016.07.011

Gerken, G. M. (1996). Central tinnitus and lateral inhibition: an auditory brainstem model. *Hear. Res.* 97, 75–83. doi: 10.1016/S0378-5955(96)80009-8

Groff, J. A., and Liberman, M. C. (2003). Modulation of cochlear afferent response by the lateral olivocochlear system: activation via electrical stimulation of the inferior colliculus. *J. Neurophysiol.* 90, 3178–3200. doi: 10.1152/jn.00537.2003

Hackney, C. M., Osen, K. K., and Kolston, J. (1990). Anatomy of the cochlear nuclear complex of guinea pig. *Anat. Embryol.* 182, 123–149. doi: 10.1007/BF00174013

Hébert, S., Fournier, P., and Noreña, A. (2013). The auditory sensitivity is increased in tinnitus ears. *J. Neurosci.* 33, 2356–2364. doi: 10.1523/JNEUROSCI.3461-12.2013

Heil, P., and Peterson, A. J. (2015). Basic response properties of auditory nerve fibers: a review. *Cell Tissue Res.* 361, 129–158. doi: 10.1007/s00441-015-2177-9

Heller, A. J. (2003). Classification and epidemiology of tinnitus. *Otolaryngol. Clin. North Am.* 36, 239–248. doi: 10.1016/S0030-6665(02)00160-3

Heller, M. F., and Bergman, M. (1953). Tinnitus aurium in normally hearing persons. *Ann. Otol. Rhinol. Laryngol.* 62, 73–83. doi: 10.1177/000348945306200107

Kaltenbach, J. A., and Afman, C. E. (2000). Hyperactivity in the dorsal cochlear nucleus after intense sound exposure and its resemblance to tone-evoked activity: a physiological model for tinnitus. *Hear. Res.* 140, 165–172. doi: 10.1016/S0378-5955(99)00197-5

Kaltenbach, J. A., Godfrey, D. A., Neumann, J. B., McCaslin, D. L., Afman, C. E., and Zhang, J. (1998). Changes in spontaneous neural activity in the dorsal cochlear nucleus following exposure to intense sound: relation to threshold shift. *Hear. Res.* 124, 78–84. doi: 10.1016/S0378-5955(98)00119-1

Kaltenbach, J. A., Rachel, J. D., Mathog, T. A., Zhang, J., Falzarano, P. R., and Lewandowski, M. (2002). Cisplatin-induced hyperactivity in the dorsal cochlear nucleus and its relation to outer hair cell loss: relevance to tinnitus. *J. Neurophysiol.* 88, 699–714.

Kaltenbach, J. A., Zacharek, M. A., Zhang, J., and Frederick, S. (2004). Activity in the dorsal cochlear nucleus of hamsters previously tested for tinnitus following intense tone exposure. *Neurosci. Lett.* 355, 121–125. doi: 10.1016/j.neulet.2003.10.038

Kjellgren, A., Lyden, F., and Norlander, T. (2008). Sensory isolation in flotation tanks: altered states of consciousness and effects on well-being. *Qual. Rep.* 13, 636–656.

Knipper, M., Ruettiger, L., Schick, B., and Dlugaiczyk, J. (2011). "Glycine receptor agonists for the treatment of phantom phenomena" in *Google Patents*. US20110077239 A1.

König, O., Schaette, R., Kempter, R., and Gross, M. (2006). Course of hearing loss and occurrence of tinnitus. *Hear. Res.* 221, 59–64. doi: 10.1016/j.heares.2006.07.007

Krauss, P., Metzner, C., Tziridis, K., and Schulze, H. (2015). Adaptive stochastic resonance based on output autocorrelations. arXiv:1504.05032.

Langguth, B., Landgrebe, M., Kleinjung, T., Sand, G. P., and Hajak, G. (2011). Tinnitus and depression. *World J. Biol. Psychiatry* 12, 489–500. doi: 10.3109/15622975.2011.575178

Lauer, A. M., Fuchs, P. A., Ryugo, D. K., and Francis, H. W. (2012). Efferent synapses return to inner hair cells in the aging cochlea. *Neurobiol. Aging* 33, 2892–2902. doi: 10.1016/j.neurobiolaging.2012.02.007

Laughlin, S. (1981). A simple coding procedure enhances a neuron's information capacity. *Z. Naturforsch. C* 36, 910–912.

Lendvai, B., Halmos, G. B., Polony, G., Kapocsi, J., Horváth, T., Aller, M., et al. (2011). Chemical neuroprotection in the cochlea: the modulation of dopamine release from lateral olivocochlear efferents. *Neurochem. Int.* 59, 150–158. doi: 10.1016/j.neuint.2011.05.015

Levin, J. E., and Miller, J. P. (1996). Broadband neural encoding in the cricket cercal sensory system enhanced by stochastic resonance. *Nature* 380, 165–168. doi: 10.1038/380165a0

Le Prell, C. G., Dolan, D. F., Schacht, J., Miller, J. M., Lomax, M. I., and Altschuler, R. A. (2003a). Pathways for protection from noise induced hearing loss. *Noise Health* 5, 1–17.

Le Prell, C. G., Halsey, K., Hughes, L. F., Dolan, D. F., and Bledsoe, S. C. Jr. (2005). Disruption of lateral olivocochlear neurons via a dopaminergic neurotoxin depresses sound-evoked auditory nerve activity. *J. Associ. Res. Otolaryngol.* 6, 48–62. doi: 10.1007/s10162-004-5009-2

Le Prell, C. G., Hughes, L. F., and Bledsoe, S. C. (2014). Dynorphin release by the lateral olivocochlear efferents may inhibit auditory nerve activity: a cochlear drug delivery study. *Neurosci. Lett.* 571, 17–22. doi: 10.1016/j.neulet.2014.04.024

Le Prell, C. G., Shore, S. E., Hughes, L. F., and Bledsoe, S. C. Jr. (2003b). Disruption of lateral efferent pathways: functional changes in auditory evoked responses. *J. Assoc. Res. Otolaryngol.* 4, 276–290. doi: 10.1007/s10162-002-3018-6

Lewis, J. E., Stephens, S. D., and McKenna, L. (1994). Tinnitus and suicide. *Clin. Otolaryngol. Allied Sci.* 19, 50–54. doi: 10.1111/j.1365-2273.1994.tb01147.x

Liberman, L. D., Suzuki, J., and Liberman, M. C. (2015). Dynamics of cochlear synaptopathy after acoustic overexposure. *J. Assoc. Res. Otolaryngol.* 16, 205–219. doi: 10.1007/s10162-015-0510-3

Liberman, M. C., and Dodds, L. W. (1984). Single-neuron labeling and chronic cochlear pathology. II. Stereocilia damage and alterations of spontaneous discharge rates. *Hear. Res.* 16, 43–53. doi: 10.1016/0378-5955(84)90024-8

Liberman, M. C., and Kiang, N. Y. (1978). Acoustic trauma in cats. Cochlear pathology and auditory-nerve activity. *Acta Otolaryngol. Suppl.* 358, 1–63.

Licklider, J. C. R. (1951). A duplex theory of pitch perception. *J. Acoust. Soc. Am.* 23, 147–147. doi: 10.1121/1.1917296

Lilly, J. C. (1956). Mental effects of reduction of ordinary levels of physical stimuli on intact, healthy persons. *Psychiatric Res. Rep.* 5, 1–9. discussion: 10–28.

Long, Z. C., Shao, F., Zhang, Y. P., and Qin, Y. G. (2004). Noise-enhanced hearing sensitivity. *Phys. Lett. A* 323, 434–438. doi: 10.1016/j.physleta.2004.02.019

Lummis, R. C., and Guttman, N. (1972). Exploratory studies of Zwicker's "negative afterimage" in hearing. *J. Acoust. Soc. Am.* 51, 1930–1944. doi: 10.1121/1.1913052

Maison, S. F., Usubuchi, H., and Liberman, M. C. (2013). Efferent feedback minimizes cochlear neuropathy from moderate noise exposure. *J. Neurosci.* 33, 5542–5552. doi: 10.1523/JNEUROSCI.5027-12.2013

McDonnell, M. D., Stocks, N. G., Pearce, C. E. M., and Abbott, D. (2008). *Stochastic Resonance: From Suprathreshold Stochastic Resonance to Stochastic Signal Quantization*. Cambridge: Cambridge University Press.

Mino, H. (2014). The effects of spontaneous random activity on information transmission in an auditory brain stem neuron model. *Entropy* 16, 6654–6666. doi: 10.3390/e16126654

Mitaim, S., and Kosko, B. (1998). Adaptive stochastic resonance. *Proc. IEEE* 86, 2152–2183. doi: 10.1109/5.726785

Mitaim, S., and Kosko, B. (2004). Adaptive stochastic resonance in noisy neurons based on mutual information. *Neural Netw. IEEE Transact.* 15, 1526–1540. doi: 10.1109/TNN.2004.826218

Moss, F., Ward, L. M., and Sannita, W. G. (2004). Stochastic resonance and sensory information processing: a tutorial and review of application. *Clin. Neurophysiol.* 115, 267–281. doi: 10.1016/j.clinph.2003.09.014

Ohlemiller, K. K., and Echteler, S. M. (1990). Functional correlates of characteristic frequency in single cochlear nerve fibers of the Mongolian gerbil. *J. Comp. Physiol. A* 167, 329–338. doi: 10.1007/BF00192568

Osen, K. K. (1988). "Anatomy of the mammalian cochlear nuclei; a review," in *Auditory Pathway*, eds J. Syka and R. Bruce Masterton (New York, NY: Springer), 65–75.

Parra, L. C., and Pearlmutter, B. A. (2007). Illusory percepts from auditory adaptation. *J. Acoust. Soc. Am.* 121, 1632–1641. doi: 10.1121/1.2431346

Pinchoff, R. J., Burkard, R. F., Salvi, R. J., Coad, M. L., and Lockwood, A. H. (1998). Modulation of tinnitus by voluntary jaw movements. *Otol. Neurotol.* 19, 785–789.

Ries, D. T. (2007). The influence of noise type and level upon stochastic resonance in human audition. *Hear. Res.* 228, 136–143. doi: 10.1016/j.heares.2007.01.027

Robinson, B. L., and McAlpine, D. (2009). Gain control mechanisms in the auditory pathway. *Curr. Opin. Neurobiol.* 19, 402–407. doi: 10.1016/j.conb.2009.07.006

Ruel, J., Wang, J., Demêmes, D., Gobaille, S., Puel, J. L., and Rebillard, G. (2006). Dopamine transporter is essential for the maintenance of spontaneous activity of auditory nerve neurones and their responsiveness to sound stimulation. *J. Neurochem.* 97, 190–200. doi: 10.1111/j.1471-4159.2006.03722.x

Ruel, J., Wang, J., Rebillard, G., Eybalin, M., Lloyd, R., Pujol, R., et al. (2007). Physiology, pharmacology and plasticity at the inner hair cell synaptic complex. *Hear. Res.* 227, 19–27. doi: 10.1016/j.heares.2006.08.017

Rutherford, M. A., Chapochnikov, N. M., and Moser, T. (2012). Spike encoding of neurotransmitter release timing by spiral ganglion neurons of the cochlea. *J. Neurosci.* 32, 4773–4789. doi: 10.1523/JNEUROSCI.4511-11.2012

Rüttiger, L., Singer, W., Panford-Walsh, R., Matsumoto, M., Lee, S. C., Zuccotti, A., et al. (2013). The reduced cochlear output and the failure to adapt the central auditory response causes tinnitus in noise exposed rats. *PLoS ONE* 8:e57247. doi: 10.1371/journal.pone.0057247

Ryugo, D. K., Haenggeli, C. A., and Doucet, J. R. (2003). Multimodal inputs to the granule cell domain of the cochlear nucleus. *Exp. Brain Res.* 153, 477–485. doi: 10.1007/s00221-003-1605-3

Schaette, R., and Kempter, R. (2006). Development of tinnitus-related neuronal hyperactivity through homeostatic plasticity after hearing loss: a computational model. *Eur. J. Neurosci.* 23, 3124–3138. doi: 10.1111/j.1460-9568.2006.04774.x

Schaette, R., and McAlpine, D. (2011). Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J. Neurosci.* 31, 13452–13457. doi: 10.1523/JNEUROSCI.2156-11.2011

Schaette, R., Turtle, C., and Munro, K. J. (2012). Reversible induction of phantom auditory sensations through simulated unilateral hearing loss. *PLoS ONE* 7:e35238. doi: 10.1371/journal.pone.0035238

Schwartz, O., and Simoncelli, E. P. (2001). Natural signal statistics and sensory gain control. *Nat. Neurosci.* 4, 819–825. doi: 10.1038/90526

Shannon, C. E. (1948). Bell system tech. j. 27 (1948) 379; ce shannon. *Bell Syst. Tech. J.* 27, 623. doi: 10.1002/j.1538-7305.1948.tb01338.x

Shore, S. E., and Zhou, J. (2006). Somatosensory influence on the cochlear nucleus and beyond. *Hear. Res.* 216, 90–99. doi: 10.1016/j.heares.2006.01.006

Simoncelli, E. P., and Olshausen, B. A. (2001). Natural image statistics and neural representation. *Annu. Rev. Neurosci.* 24, 1193–1216. doi: 10.1146/annurev.neuro.24.1.1193

Stocks, N. G. (2000). Suprathreshold stochastic resonance in multilevel threshold systems. *Phys. Rev. Lett.* 84:2310. doi: 10.1103/PhysRevLett.84.2310

Stocks, N. G. (2001). Information transmission in parallel threshold arrays: suprathreshold stochastic resonance. *Phys. Rev. E* 63:041114. doi: 10.1103/PhysRevE.63.041114

Stocks, N. G., Allingham, D., and Morse, R. P. (2002). The application of suprathreshold stochastic resonance to cochlear implant coding. *Fluct. Noise Lett.* 2, L169–L181. doi: 10.1142/S0219477502000774

Turrigiano, G. G. (2008). The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell* 135, 422–435. doi: 10.1016/j.cell.2008.10.008

Tziridis, K., Ahlf, S., Jeschke, M., Happel, M. F. K., Ohl, F. W., and Schulze, H. (2015). Noise trauma induced neural plasticity throughout the auditory system of Mongolian gerbils: differences between tinnitus developing and non-developing animals. *Front. Neurol.* 6:22. doi: 10.3389/fneur.2015.00022

Uhlenbeck, G. E., and Ornstein, L. S. (1930). On the theory of the Brownian motion. *Phys Rev.* 36:823. doi: 10.1103/physrev.36.823

Wang, J., Powers, N. L., Hofstetter, P., Trautwein, P., Ding, D., and Salvi, R. (1997). Effects of selective inner hair cell loss on auditory nerve fiber threshold, tuning and spontaneous and driven discharge rate. *Hear. Res.* 107, 67–82. doi: 10.1016/S0378-5955(97)00020-8

Wenning, G., and Obermayer, K. (2003). Activity driven adaptive stochastic resonance. *Phys. Rev. Lett.* 90:120602. doi: 10.1103/PhysRevLett.90.120602

Wiegrebe, L., Koessl, M., and Schmidt, S. (1996). Auditory enhancement at the absolute threshold of hearing and its relationship to the Zwicker tone. *Hear. Res.* 100, 171–180. doi: 10.1016/0378-5955(96)00111-6

Wiesenfeld, K., and Moss, F. (1995). Stochastic resonance and the benefits of noise: from ice ages to crayfish and SQUIDs. *Nature* 373, 33–36. doi: 10.1038/373033a0

Wu, C., Stefanescu, R. A., Martel, D. T., and Shore, S. E. (2016). Tinnitus: maladaptive auditory–somatosensory plasticity. *Hear. Res.* 334, 20–29. doi: 10.1016/j.heares.2015.06.005

Young, E. D., and Davis, K. A. (2002). "Circuitry and function of the dorsal cochlear nucleus," in *Integrative Functions in the Mammalian Auditory Pathway*, eds D. Oertel, R. R. Fay, and A. N. Popper (New York, NY: Springer), 160–206.

Zacharek, M. A., Kaltenbach, J. A., Mathog, T. A., and Zhang, J. (2002). Effects of cochlear ablation on noise induced hyperactivity in the hamster dorsal cochlear nucleus: implications for the origin of noise induced tinnitus. *Hear. Res.* 172, 137–144. doi: 10.1016/S0378-5955(02)00575-0

Zeng, C., Yang, Z., Shreve, L., Bledsoe, S., and Shore, S. (2012). Somatosensory projections to cochlear nucleus are upregulated after unilateral deafness. *J. Neurosci.* 32, 15791–15801. doi: 10.1523/JNEUROSCI.2598-12.2012

Zeng, F. G., Fu, Q. J., and Morse, R. (2000). Human hearing enhanced by noise. *Brain Res.* 869, 251–255. doi: 10.1016/S0006-8993(00)02475-6

Zimmermann, C. E., Burgess, B. J., and Nadol, J. B. Jr. (1995). Patterns of degeneration in the human cochlear nerve. *Hear. Res.* 90, 192–201. doi: 10.1016/0378-5955(95)00165-1

Zwicker, E. (1964). "Negative afterimage" in hearing. *J. Acoust. Soc. Am.* 36, 2413–2415. doi: 10.1121/1.1919373

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HYPOTHESIS AND THEORY
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Cholinergic Hypofunction in Presbycusis-Related Tinnitus With Cognitive Function Impairment: Emerging Hypotheses

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Presbycusis (age-related hearing loss) is a potential risk factor for tinnitus and cognitive deterioration, which result in poor life quality. Presbycusis-related tinnitus with cognitive impairment is a common phenotype in the elderly population. In these individuals, the central auditory system shows similar pathophysiological alterations as those observed in Alzheimer's disease (AD), including cholinergic hypofunction, epileptiform-like network synchronization, chronic inflammation, and reduced GABAergic inhibition and neural plasticity. Observations from experimental rodent models indicate that recovery of cholinergic function can improve memory and other cognitive functions via acetylcholine-mediated GABAergic inhibition enhancement, nicotinic acetylcholine receptor (nAChR)-mediated anti-inflammation, glial activation inhibition and neurovascular protection. The loss of cholinergic innervation of various brain structures may provide a common link between tinnitus seen in presbycusis-related tinnitus and age-related cognitive impairment. We hypothesize a key component of the condition is the withdrawal of cholinergic input to a subtype of GABAergic inhibitory interneuron, neuropeptide Y (NPY) neurogliaform cells. Cholinergic denervation might not only cause the degeneration of NPY neurogliaform cells, but may also result in decreased AChR activation in GABAergic inhibitory interneurons. This, in turn, would lead to reduced GABA release and inhibitory regulation of neural networks. Reduced nAChR-mediated anti-inflammation due to the loss of nicotinic innervation might lead to the transformation of glial cells and release of inflammatory mediators, lowering the buffering of extracellular potassium and glutamate metabolism. Further research will provide evidence for the recovery of cholinergic function with the use of cholinergic input enhancement alone or in combination with other rehabilitative interventions to reestablish inhibitory regulation mechanisms of involved neural networks for presbycusis-related tinnitus with cognitive impairment.

Keywords: presbycusis, tinnitus, cognitive impairment, cholinergic hypofunction, glial cell, neurogliaform cell

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INTRODUCTION

Subjective tinnitus, mainly induced by hearing loss and emotional states, is heterogeneous, affecting the development of effective intervention strategies. Presbycusis, commonly referred to as age-related hearing impairment, is a potential risk factor for tinnitus (Shargorodsky et al., 2010; Knipper et al., 2013) and cognitive impairment, including Alzheimer's disease (AD) and non-AD dementia (Lin et al., 2011, 2013; Bakhos et al., 2015; Panza et al., 2015a,b; Taljaard et al., 2016; Thomson et al., 2017). Thus, presbycusis-related tinnitus and cognitive impairment often appear stimulaneously within a subset of the elderly population.

Epidemiological studies have shown that the prevalence of both presbycusis and dementia increases with age. Approximately one-third of individuals over 65 years of age experience hearing loss greater than 40 dB (averaged across 0.5–4 kHz), more than 10% experience dementia, and more than 90% of individuals with dementia have hearing abnormalities (Marti et al., 2014). Presbycusis is associated with cognitive decline and late-life cognitive disorders due to peripheral hearing impairment (Gates and Mills, 2005; Wallhagen et al., 2008; Gallacher et al., 2012; Lin et al., 2013; Behrman et al., 2014; Deal et al., 2017; Loughrey et al., 2018) or central auditory processing dysfunction (Gennis et al., 1991; Gates et al., 2002, 2011). A prospective epidemiological cohort study showed that observed hearing loss was associated with a greater risk of incident dementia in a multiethnic population (n = 1881) followed up over a mean of 7.3 ± 4.4 years (Golub et al., 2017). Moreover, case-control and population-based studies have shown that patients with mild cognitive impairment (MCI), dementia, and AD also have central auditory processing dysfunction and topographically specific neurodegeneration resulting from amyloid senile plaques (SP) and neurofibrillary tangles (NFTs; Sinha et al., 1993; reviewed by Panza et al., 2015a,b).

It is difficult to establish a causal relationship between presbycusis and age-related cognitive decline. Nonetheless, hearing loss could be an early symptom of cognitive decline in elderly individuals, and therefore an appropriate component of screening tools for preclinical diagnosis (Wong et al., 2014). Presbycusis also could be seen as a modifiable factor for preventing cognitive impairment (Lin, 2011; Lin et al., 2011; Gurgel et al., 2014; Marti et al., 2014; Panza et al., 2015a,b). Indeed, timely hearing rehabilitation at the preclinical stage of cognitive decline, including hearing aids and/or cochlear implants, may act to suppress tinnitus and protect cognition by reducing social isolation and depression, reversing maladaptive neuronal plasticity, and improving neurotrophic support and working memory (Acar et al., 2011; Langguth et al., 2013; Marti et al., 2014; Panza et al., 2015a,b; Shore et al., 2016). A whole body of literature indicates that there is no causal relationship between hearing loss and general cognitive loss. Presentation of two age-related disorders together could purely reflect the fact that both conditions are more common in elderly individuals.

Epidemiological studies have also reported that the prevalence of tinnitus increases with age and is highest in elderly individuals aged 60 and 69 years (Adams et al., 1999; Ahmad and Seidman, 2004). The most common symptom of tinnitus is cognitive deficits (Andersson et al., 1999; Hallam et al., 2004; Andersson and McKenna, 2006; Pierce et al., 2012), including working memory and processing speeds on neurocognitive testing (Rossiter et al., 2006), cognitive efficiency (Hallam et al., 2004) and attention control (Stevens et al., 2007). The prevalence of cognitive deficits in patients with tinnitus is higher than would be expected by chance. Approximately 70% of patients with tinnitus had self-reported difficulty concentrating (Andersson et al., 1999). Compared with healthy controls and those with acquired hearing loss, patients with tinnitus also report a greater number of cognitive impairments (Hallam et al., 2004). However, individuals with normal-hearing and tinnitus report similar cognitive performance with individuals with normal hearing without tinnitus (Waechter and Brännström, 2015).

Presbycusis-related tinnitus and cognitive impairment are associated with aging. The former may reflect an independent pathological process that shares some etiologies and pathophysiological alterations with cognitive decline (Marti et al., 2014). The ApoE ε4 allele is a genetic risk factor for both age-related hearing loss (Kurniawan et al., 2012) and AD (Hollands et al., 2017). Cholinergic hypofunction, chronic inflammation and vascular factors are probably linked to the pathogenesis of both presbycusis-related tinnitus and age-related cognitive impairment (Benzing et al., 1993; Emre et al., 1993; Shulman et al., 2008; Daulatzai, 2010; Haase et al., 2011; Fortunato et al., 2016; Wu and Chiu, 2016; Panza et al., 2017). Particularly, cholinergic hypofunction related to aging can aggravate functional deficits of GABAergic interneurons, NFTs, chronic systemic inflammation, age-related blood-brain barrier dysfunction and maladaptive plasticity resulting in an increased spontaneous firing rate, synchronized epileptic-like neuronal activity and excitotoxicity (Knipper et al., 2013; Shore et al., 2016).

While the majority of studies that we refer to are based on animal models, age-related degeneration of synapses and neural anatomy in the peripheral and central nervous system (CNS) may represent a common neurophysiological basis of presbycusis-related tinnitus and age-related cognitive impairment. We hypothesize that age-related loss of cholinergic innervation of various brain structures may be a common link between tinnitus seen in presbycusis-related tinnitus and age-related cognitive impairment. Recovery of cholinergic function may be useful to treat presbycusis-related tinnitus with cognitive impairment by affecting multiple shared pathophysiological targets.

Declining Cholinergic Function in Humans With Presbycusis-Related Tinnitus and Age-Related Cognitive Impairment

Aging and neurodegenerative diseases are the major causes of declining cholinergic function. Aging leads to cholinergic hypofunction of the basal forebrain cholinergic complex, which is the main cholinergic projection to the cerebral cortex and hippocampus. Gradual age-related loss of cholinergic function results from decreased trophic support from nerve

growth factor (NGF) and degeneration of dendritic, axonal and synaptic structures, which cause brain function decline, including cognitive impairment (Daulatzai, 2010; Schliebs and Arendt, 2011).

As in normal aging, patients with MCI and early-stage AD only exhibit declining cholinergic function without cholinergic neurodegeneration. Such changes include an imbalance in the expression of NGF, pro-NGF, the high NGF receptor, trkA and low NGF neurotrophin p75 receptor, as well as changes in acetylcholine release and choline uptake (Cohen et al., 1995; Schliebs and Arendt, 2011). The advanced stages of early-onset and late-onset AD and psychiatric disorders (e.g., Parkinson's disease and Lewy body dementia) are characterized by a severe loss of NGF receptor positive cholinergic cells in the basal forebrain (Mufson and Kordower, 1989; Perry, 1990). NGF receptors play a role in cholinergic neuron death. Decreased expression of NGF receptors was also observed on among striatal cholinergic neurons in the AD brain (Boissière et al., 1996). Furthermore, encapsulated cell implants releasing NGF bilaterally to the basal forebrain of patients with AD across 12 months significantly enhanced cerebrospinal fluid levels of the cholinergic biomarker choline acetyltransferase (ChAT; Karami et al., 2015). Age-related loss of the calcium-binding protein, calbindin-D28K, in basal forebrain cholinergic neurons has been related to the full range of tau pathology of AD (Ahmadian et al., 2015).

Cholinergic hypofunction also involves changes in the presynaptic synthetic enzyme, ChAT and acetylcholine receptor (AChR) expression. In patients with AD compared with age-matched healthy controls, there is a 50%–90% decline in activity of presynaptic ChAT (Perry et al., 1978; Davies, 1979). Moreover, significant declines in enzyme activity that result in cholinergic dysfunction do not occur until a relatively late stage (Davies et al., 1999; Tiraboschi et al., 2000). In contrast, loss of ChAT activity in patients with Lewy bodies was present in the earliest stage (Tiraboschi et al., 2002). In the frontal cortex of individuals with AD, different alterations have been observed in muscarinic (M) subtypes, with diminished M1 and M2 but increased M4 immunoreactivity, and normal M1, decreased M2 and increased M4 numbers of binding sites (Flynn et al., 1995). Cholinergic deficits are associated with the loss or derangement of nicotinic acetylcholine receptors (nAChRs) in the brains of those with AD and Down syndrome (Engidawork et al., 2001), with significantly decreased alpha 7 and significantly increased alpha 3 receptors in the frontal cortex in AD. Autopsy brain tissue (Guan et al., 2000; Lee et al., 2000) and *in vivo* evaluations (Nordberg et al., 1997) of patients with AD have consistently shown decreased nAChR levels. Moreover, after blockade of muscarinic receptors with scopolamine, young healthy individuals have a similar pattern of memory and cognitive decline as aged individuals with cholinergic dysfunction (Drachman et al., 1980). Nicotinic cholinergic blockade with mecamylamine in elderly healthy individuals resulted in AD-like cognitive deficits and specific blood flow abnormalities in the parieto-temporal cortex (Gitelman and Prohovnik, 1992). Therefore, tacrine and nicotine, which stimulate the cholinergic system, could significantly improve attentional function associated with basal forebrain cholinergic innervation of the cortex and other brain regions in patients with AD (Lawrence and Sahakian, 1995).

Degeneration of the basal forebrain cholinergic system due to aging and AD causes impairment of thalamo-cortical function, reduced connectivity between the thalamo-cortical system, hippocampus, and other key brain regions, and decreased cerebral blood flow (CBF), which has been associated with cognitive disturbances and age-related sensory loss (Daulatzai, 2010). The amygdala is a component of the limbic system involved in emotion, attention and memory. Differences have also been observed between the aging human brain and AD in the loss of cholinergic innervation of the amygdaloid complex (Benzing et al., 1993; Emre et al., 1993). Compared with middle-aged controls, no decline in cholinergic input of the amygdale was observed in immunohistological specimens from aging participants (Emre et al., 1993). Another study reported that individuals without dementia but with high rates of SP showed highly dystrophic neurites, but no significant loss of fiber innervations (Benzing et al., 1993). However, there does appear to be a severe and regionally selective loss of cholinergic innervations in the amygdaloid complex of patients with AD.

Cholinergic hypofunction results in impairments of the auditory pathway, as well as impaired cortico-cortical interactions between auditory and other sensory regions. In patients with mild to moderate AD, dysfunction is observed in the primary auditory pathway and ascending reticular activating system, which have cortical cholinergic innervation. Furthermore, significant delays in I∼V interpeak latency of brain auditory evoked responses and dysfunction in the generation of primary auditory cortex evoked potentials, as well as reduced neuronal activity in the ascending reticular activating system are observed in AD (O'Mahony et al., 1994). There is a progressive decline in the attenuation of subsequent auditory evoked potentials by a visual stimulus from the young to the healthy elderly to individuals with MCI and AD (Golob et al., 2015). However, in the human cochlear nucleus, nAchR beta 2 immunostaining was unchanged from birth to 90 years (Sharma et al., 2014). Based on observations from human studies, the loss of cholinergic innervation to various brain structures may provide a link between tinnitus seen in presbycusis-related tinnitus and age-related cognitive impairment. Recovery of cholinergic function during an optimal time window before the loss of cholinergic neurons may therefore lead to better outcomes.

Declining Cholinergic Function May Contribute to the Accumulation of Beta-Amyloid Oligomers and NFTs in Age-Related Cognitive and Hearing Impairments

The neuropathological hallmarks of AD, including amyloid deposits and tau-immunoreactive NFTs, are also present in the healthy aging brain. An immunohistological study of serial sections from 105 autopsy brains of cognitively normal patients (age range: 40–104 years) showed that NFTs appear earlier than

amyloid plaques during normal aging. All cases from people over 48 years old displayed at least a few NFTs (more frequently in the entorhinal than in the transentorhinal cortex), which was preceded by tau pathology in these areas rather than in the brainstem (Tsartsalis et al., 2018). In the auditory system of individuals with AD, the ventral nucleus of the medial geniculate body and central nucleus of the inferior colliculus show SP and NFT distributions with a topographically specific and consistent pattern of degeneration (Sinha et al., 1993). Significant age-related reductions in calcium binding proteins has been observed in later decades in the ventral cochlear nucleus, which is similar to results for cholinergic neurons of the basal forebrain in patients with AD, and might be related to tau pathology (Sharma et al., 2014; Ahmadian et al., 2015).

Noise exposure is a common cause of tinnitus and hearing impairment. Animal research shows that exposure to moderate intensity white noise (80 dB SPL, 2 h/day) can impair learning and memory in mice (Cheng et al., 2011). Moreover, it has been demonstrated that the hippocampus is more susceptible to noise than is the auditory cortex (Cheng et al., 2016). Indeed, significant increases in peroxidation and tau hyperphosphorylation in the hippocampus have been observed after a week of noise exposure, but there were no increases in the auditory cortex 3 weeks after exposure. Chronic white noise (100 dB SPL, 4 h/day × 14 day) persistently increased tau hyperphosphorylation at the same sites that are typically phosphorylated in the AD brain and glycogen synthase kinase 3β (GSK3β), as well as increased the formation of pathological NFT tau in the hippocampus and prefrontal cortex (Cui et al., 2012). Such changes in the frontal cortex also play an important role in the pathogenesis of frontal dementia, while changes in the frontal acoustic cortex are seen in the early onset of communication deficiency (Baloyannis et al., 2001).

Tau hyperphosphorylation sequesters normal tau and microtubule-associated proteins into insoluble NFTs and inhibits microtubule assembly (Iqbal et al., 2013). Tau reduction prevents cognitive decline, synaptic transmission and plasticity, and spontaneous epileptiform activity in AD model mice that overexpress Aβ, without changing the expression of Aβ (Ittner et al., 2010). Furthermore, tau-deficient AD models have demonstrated a reversal in the Aβ induced imbalance of excitation/inhibition, NMDA receptor dysfunction, and excitotoxicity in both transgenic and wild type mice (Roberson et al., 2007, 2011).

Loss of cholinergic innervations may play important roles in both AD and hearing impairment during aging. The AChE inhibitor donepezil can protect against Aβ induced neurotoxicity by enhancing protein phosphatase 2A (PP2A) activity and inhibiting GSK3β activity via the activation of nAChRs, which reduces tau-induced neuronal toxicity and neurodegeneration (Bitner et al., 2009; Noh et al., 2009). In the brain, mAChRs may mediate cognitive function and neuropsychiatric symptoms and they are also considered potential targets in AD and schizophrenia (Clader and Wang, 2005; Poulin et al., 2010; Foster et al., 2014). M1 type mAChRs, mainly present in the striatum, hippocampus and neocortex, are activated by M1 specific agonists doses without adverse effects. Such activation could improve learning, memory, synaptic plasticity, and cognitive functions via the activation of extracellular signal-regulated kinases (Berkeley et al., 2001; Ragozzino et al., 2012). In A7KO-APP AD transgenic mice, the absence of alpha-7 nAChRs leads to Aβ accumulation and oligomerization, exacerbating early-stage cognitive decline and septohippocampal pathology (Hernandez et al., 2010).

Cholinergic Denervation of NPY Neurogliaform Cells May Be Involved in Presbycusis-Related Tinnitus With Cognitive Impairment

Reduced functional connectivity in the brains of patients with AD or MCI, as well as the elderly with cognitive complaints or cognitively normal ApoEε4 carriers, reflects activity changes within the default-mode network, which is most active at rest and deactivated during cognitive tasks (Ruan et al., 2016). The loss of cholinergic innervations and reduced GABAergic inhibition might play important roles in such changes.

Distinct GABAergic cell types project to the surface of pyramidal cells in the cortex and hippocampus, forming neural circuits for inhibitory control of brain function and plasticity. Functional remodeling of GABAergic neurotransmission has been observed in the human brain with AD (Limon et al., 2012). Moreover, GABA currents in the temporal cortex of the AD brain show age-related reductions, which were associated with reduced mRNA and protein for the main GABA receptor subunits. In the ADbrain compared with controls, α1 and γ2 transcription shows down-regulation, while but α2, β1 and γ1 transcription shows up-regulation. In patients with AD and/or epilepsy, deficits of GABAergic interneurons are associated with aberrant network activity, including hyperexcitability, clusters of hyperactive and hypoactive neurons, and network/spontaneous epileptiform activity (Olney, 1995; Nägerl et al., 2000; Snider et al., 2005; Palop and Mucke, 2009).

Patients with tinnitus show alterations in global brain networks, including decreased default-mode network activity, and increased activation of the auditory cortex and amygdala (Schlee et al., 2009; Elgoyhen et al., 2015). These alterations may result from decreased fuctional connectivity from peripheral and other brain regions. Tinnitus may be a consequence of maladaptive plasticity-induced disturbances of excitation-inhibition homeostasis with net down-regulation of inhibitory neurotransmission in the central auditory pathway. Subsequently, the central auditory system compensates for decreased input by up-regulating network activity among central circuits (Salvi et al., 2000; Knipper et al., 2013; Shore et al., 2016). Decreased peripheral input induced by auditory trauma and aging leads to altered cortical activity patterns, including increased spontaneous firing rates, synchronized epileptic-like neuronal activity, and basal excitatory postsynaptic potentials (for a review, see Knipper et al., 2013). Plastic tinnitus-related changes include loss of glycinergic inhibition in

the adult dorsal cochlear nucleus and/or loss of GABAergic inhibition in the inferior colliculus and higher centers, resulting in aberrant cortical activity patterns (Wang et al., 2011).

Although cholinergic drugs can temporarily suppress tinnitus in some patients, these interventions cannot eliminate the pathological neural activity. Mounting evidence from clinical trials suggests that vagus nerve stimulation (VNS)-based targeted plasticity therapies are effective in patients with neurological diseases (Hays, 2016). VNS in combination with auditory stimulation can reverse pathological neuroplastic changes of the auditory cortex toward physiological neural activity and synchronicity via M cholinergic neuromodulation (Engineer et al., 2013; Bojić et al., 2017; Tyler et al., 2017). Based on these studies in humans, GABAergic interneuron deficits in the auditory cortex and limbic system may play a key role in presbycusis-related tinnitus with cognitive impairment.

Loss of Cholinergic Innervation and Reduced Inhibition of NPY Neurogliaform Cells in Age-Related Cognitive Impairment

Animals studies have shown that GABAergic interneuron deficits result in aberrant excitatory neuronal activity in mouse AD models (Palop et al., 2007; Roberson et al., 2007, 2011; Verret et al., 2012; Iaccarino et al., 2016). Both nAChRs (Buhler and Dunwiddie, 2002; Maloku et al., 2011; Zappettini et al., 2011) and mAChRs (Pitler and Alger, 1992; Zhong et al., 2003; González et al., 2011; Yi et al., 2014) are expressed in GABAergic interneurons and mediate GABA release from these neurons. Neuropeptide Y (NPY)-neurogliaform (Faust et al., 2015), somatostatin (Faust et al., 2015; Muñoz et al., 2017) and parvalbumin (Yi et al., 2014) subtype interneurons express AChRs and receive cholinergic excitatory input. NPY-neurogliaform cells primarily reside within both the stratum radiatum and lacunosum-moleculare of the hippocampus, as well as the superficial and deep layers of the neocortex, which are significantly decreased in the hippocampus of animal models with AD or seizures (Mazarati and Wasterlain, 2002; Faust et al., 2015). However, optogenetic stimulation of cholinergic fibers in transgenic mice expressing the human ApoE ε4 allele has been shown to abolish partial neuronal loss in the entorhinal cortex induced by abnormal hyperactivity in dentate networks (Bott et al., 2016).

The activation of both the α(7) nAChR and α4β2 nAChR subtypes could enhance GABA release in hippocampal synaptosomes (Zappettini et al., 2011). Furthermore, α(4)β(2) nAChR agonists may control epigenetic alterations induced by glutamic acid decarboxylase 67 (GAD 67) increases in GABAergic neurons better in schizophrenia than do α(7) nAChR agonists (Maloku et al., 2011). M1 mAChRs in parvalbumin interneurons could improve GABAergic transmission in hippocampal and prefrontal cortical pyramidal neurons (Yi et al., 2014). Moreover, activation of M1–M5 mAChRs in rat hippocampal neurons *in vitro* increases GABAergic inhibitory transmission (González et al., 2011). Treatment with Huperzine A leads to robust and sustained seizure resistance in genetic epilepsy models with voltage-gated sodium channel mutation via the activation of mAChRs and $GABA_A$ receptors (Wong et al., 2014). However, nAChR-mediated GABAergic cortical inhibition in rats, related to increased high gamma frequency visible on electroencephalogram, might also be involved in the Huperzine A anticonvulsant mechanisms (Gersner et al., 2015). Thus, solely based on the animal models, the loss of cholinergic innervation of NPY-neurogliaform cells in various brain structures contributes to aberrant excitatory neuronal activity in age-related cognitive impairment.

Cholinergic Denervation of NPY Neurogliaform Cells in the Central Auditory System in Presbycusis With Tinnitus

In animal studies, changes in inhibitory properties that are induced by aging and acoustic trauma, similar to deafferentation plasticity changes in other mammalian sensory systems, have been observed from the cochlear nuclei to the auditory system. The cochlear nuclei of aged rats have lower glycine levels and altered glycine receptor subunit compositions compared with young rats (Banay-Schwartz et al., 1989). However, in the inferior colliculus of rats, age-related loss of GABAergic inhibition caused by the loss of the biosynthetic enzyme GAD, as well as reduced GABA levels and GABA release, may be involved in the abnormal perception of signals in noise and the deterioration of speech discrimination (Milbrandt et al., 1994, 2000; Raza et al., 1994).

Age-related decreases in GAD have been observed in the primary auditory cortex, parietal cortex and hippocampus, with more significant reductions observed in the auditory cortex of rats (Stanley and Shetty, 2004; Ling et al., 2005). Age-related alterations in GABA receptor subunit composition have also been observed in the inferior colliculus and primary auditory cortex of aged rats, such that there are changes to the wild-type receptor proportions (Caspary et al., 2013). These presynaptic and postsynaptic changes may contribute to increased spontaneous activity in neurons of the inferior colliculus and layer-specific increases in the spontaneous activity of the primary auditory cortex (Ling et al., 2005). Following sound exposure in rats with tinnitus, single units within the medial geniculate body of rats exhibited enhanced spontaneous firing, altered burst properties, and increased rate-level function slopes, which acts to alter sensory gating and enhance the gain of neuronal networks in the auditory cortex and limbic centers (Kalappa et al., 2014).

Inhibitory transmission and survival of NPY-neurogliaform cells in the hippocampus and prefrontal cortex is mainly under cholinergic regulation in experimental rodents (Mazarati and Wasterlain, 2002; Faust et al., 2015; Overstreet-Wadiche and McBain, 2015; Bott et al., 2016). Therefore, we hypothesized that withdrawal of nicotinic cholinergic input to NPY neurogliaform cells is a key component of the pathological mechanism underlying presbycusis with tinnitus and cognitive impairment, solely based on animal models. The enhancement of GABA release from NPY-neurogliaform

cells and the reversal of the imbalance between excitation and inhibition in the central auditory system following the recovery of cholinergic function may provide an important target for interventions to treat presbycusis with tinnitus (**Figure 1**).

Beyond the central auditory system, axosomatic synapses between the medial olivocochlear efferent system and outer hair cells are cholinergic. A feedback system eliciting efferent suppression via alpha-9/alpha-10 nAChRs can improve the detection of signals in background noise, enable selective attention to particular signals, and protect the periphery from damage caused by overly loud sounds (Maison et al., 2002; Elgoyhen et al., 2009). Our previous animal studies have shown that aging and ototoxic drugs exacerbate the degeneration of the mouse medial olivocochlear efferent system (Ruan et al., 2014a,b,c). Furthermore, histopathological studies of the human cochlear have shown that those with presbycusis and tinnitus had a significantly greater loss of outer hair cells in the basal and upper middle turns, and greater atrophy of the stria vascularis in the basal turn compared with those with presbycusis without tinnitus (Terao et al., 2011). Therefore, nAChR activation in the peripheral medial olivocochlear efferent system may also play a role in the suppression of presbycusis with tinnitus.

Nicotinic Denervation Induced Immuno-Dysregulation May Involved in Presbycusis-Related Tinnitus With Cognitive Impairment

Observations from clinical studies indicate that, glial cell activation and chronic systemic inflammation during normal and pathologic brain aging are related to poor cognitive performance and a risk of cognitive decline in dementia, vascular dementia, and AD (Schmidt et al., 2002; Weaver et al., 2002; Engelhart et al., 2004; Yaffe et al., 2004). Inflammation plays a critical role in the fluctuation of non-cognitive neuropsychiatric symptoms (Kat et al., 2008; van Gool et al., 2010). Indeed, free radical-induced oxidative damage and chronic inflammation play important roles in the development of dysfunctional connections between the central cortex and the inner ear in hearing disorders (Haase et al., 2011).

Age-related increase in GFAP positive glial cells have been observed in the cochlear nucleus (Sharma et al., 2014). In a cross-sectional cohort of 360 community-dwelling individuals aged 60 years and over, increased inflammatory markers and white blood cell count were associated with worsening presbycusis, with the strongest positive correlation seen in those over 75 years (Verschuur et al., 2014). Furthermore, the inflammatory cytokine TNF-α (rs1800630) and the TNF receptor superfamily 1B (rs1061624) have been related to an increased risk of hearing damage in a population-based cohort study of elderly Japanese individuals (Uchida et al., 2014).

Chronic inflammation also leads to blood brain barrier (BBB) vulnerability and brain hypoperfusion. Increased release of neurotoxic and inflammatory mediators has been observed in the brain microvessels of patients with AD (Grammas, 2011). Further, chronic inflammation causes BBB dysfunction and increased vascular permeability during aging, as well as in AD and other neurodegenerative disorders (Farrall and Wardlaw, 2009; Erdő et al., 2017). Moreover, the loss of cholinergic innervation to the basal forebrain results in decreased CBF (Martin et al., 1991; Daulatzai, 2010). Compared with neurologically healthy individuals without the ApoE ε4 allele, those with the ApoE ε4 allele show greater regional CBF reductions in the brain, making it vulnerable to pathological alterations in AD (Thambisetty et al., 2010; Hollands et al., 2017) and presbycusis (Kurniawan et al., 2012).

These results suggest that chronic inflammation and hypoperfusion play important roles in the pathogenesis of presbucusis-related tinnitus with cognitive impairment. Recovery of cholinergic function with AChE inhibitors, including donepezil, tacrine, pyridostigmine, galantamine, rivastigmine and Huperzine A shows potential disease-modifying benefits in the treatment of neuropsychiatric symptoms in patients with AD (Linton, 2005; Rafii et al., 2011) and dementia (Freund-Levi et al., 2014), as well as for the musical hallucinations that occur with hearing loss (Ukai et al., 2007; Zilles et al., 2012; Blom et al., 2014, 2015) or hearing loss with tinnitus (Strauss and Gertz, 2009). However, there is no mechanistic explanation for the relationship between cholinergic hypofunction and chronic inflammation alterations in presbucusis-related tinnitus with cognitive impairment.

Loss of Cholinergic Innervation and Chronic Systemic Inflammation in Age-Related Cognitive Impairment

Observations from experimental rodent models indicate that anticholinergic activity might initiate and/or accelerate AD pathology in the tauopathy mouse model by enhancing neuroinflammation, including microglial activation. The recovery of lost cholinergic innervation or function by the cholinesterase inhibitor donepezil or Huperzine A could alleviate tau pathology as well as age- and AD-related chronic neuroinflammation (Yoshiyama et al., 2015), and D-galactose-induced neurovascular damage (Ruan et al., 2014d). Moreover, chronic inflammation induced cognitive decline in rats with cerebral hypoperfusion (Wang et al., 2010).

The mechanisms underlying cholinergic anti-inflammation were first observed in human immune cells (Wang et al., 2003). The observations suggested that nicotinic activation of α7nAChR in human macrophages or monocytes is necessary to attenuate the systemic inflammatory response and inhibit the production of proinflammatory mediators by suppression of I-κB phosphorylation and nuclear factor-κB transcriptional activity (Wang et al., 2003; Yoshikawa et al., 2006).

Subsequently, a similar anti-inflammatory mechanism was also observed in rat CNS. Increased brain ACh induced by Huperzine A activates cholinergic-mediated suppression of nuclear translocation of NF-κB, as well as inducing oxidative stress, glial cell activation, and neuroinflammation in rats with ischemia (Wang et al., 2008). Huperzine A combines tetrameric AChE (G4) and indirectly activates both muscarinic and nicotinic types of AChRs (Wang et al., 2010). Moreover, the obvious overlap of tetrameric AChE and α7nAChRs in

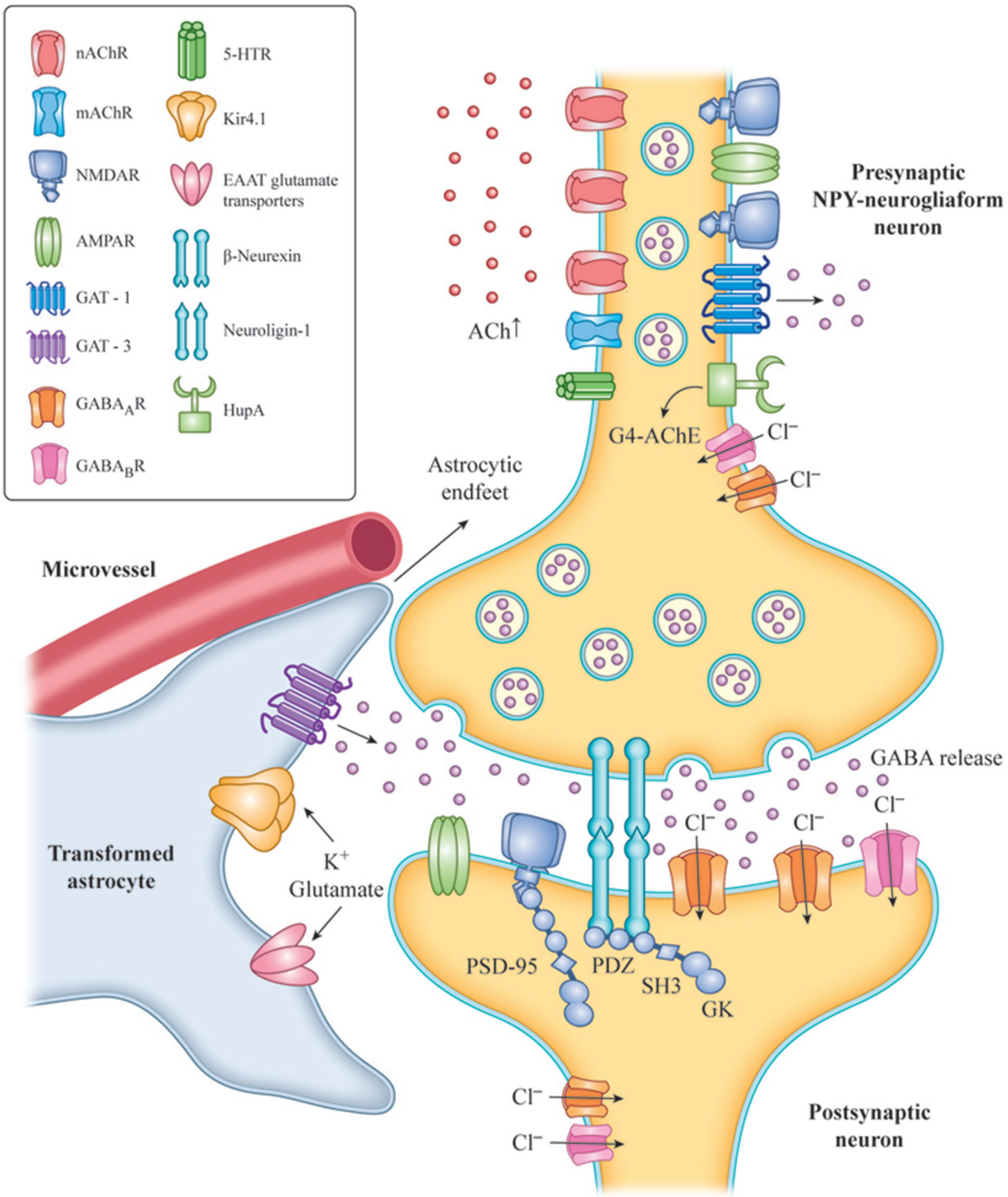

FIGURE 1 | Increased level of Ach with HupA inhibition of AChE activation or VNS, resulting in activation of AChRs, in NPY-neurogliaform neurons.The increased GABAergic signaling may regulate inhibitory tone and network activity by phasic or synaptic transmission, tonic activation and volume transmission. The adhesion complex of Neuroligin-1 (NL1) and β-neurexin is involved in the maintenance of synapses. The C-terminal fragment of NL-1 and NMDA receptors interacts with the PDZ domains of PSD-95 in the postsynaptic region of neurons, and mediates excitatory synaptic efficacy and plasticity. Transformed astrocytes contribute to neuron hypersynchronicity and excitability, which mainly occurs by reduced expression of potassium inward-rectifying channels (Kir4.1), reduced gap junctions, impaired glutamate metabolism and increased release of inflammatory mediators. VNS, vagus nerve stimulation; NPY, neuropeptide Y; G4-ACh, tetrameric acetylcholinesterase; AChR, acetylcholine receptor; EAAT, glutamate transporter of astrocytes; and GAT, GABA transporter.

the hypothalamus, hippocampus, amygdale, cerebral cortex and midbrain of humans and rats (reviewed by Damar et al., 2017) indicates that cholinergic anti-inflammatory effects occur mainly via α7nAChRs in glial and neuronal cells (Pavlov and Tracey, 2006; Wang et al., 2008).

The activation of α7nAChRs in neural cells suppresses central inflammatory responses in mice with Parkinson disease (Stuckenholz et al., 2013), stroke (Han et al., 2014), or traumatic brain injury (Kelso and Oestreich, 2012), and also suppresses glutamate-induced neurotoxicity *in vitro* (Shimohama et al., 1998; Iwamoto et al., 2013). Futhermore, the activation of α7nAChRs in astrocytes down-regulates Aβ1–42-induced increases in NF-κB in *in vitro* (Xie et al., 2016), and improves neurotrophic cytokine S100B secretion, which is decreased in the cerebrospinal fluid in rat models of dementia (Lunardi et al., 2013). Moreover, the upregulation of α7nAChR expression induced by neuregulin in microglial cells suppresses neuroinflammation *in vitro* (Mencel et al., 2013). Based on the above results from clinical and animal studies, loss of cholinergic innervations results in reduced cholinergic anti-inflammatory effects and glial activation, which further aggravates the loss of GABAergic interneurons. Therefore, we hypothesize that the withdrawal of nicotinic cholinergic input induces chronic inflammation, acting as another key step in the pathological mechanism underlying presbycusis with tinnitus and cognitive impairment.

Induction of Immuno-Dysregulation by Nicotinic Denervation in the Central Auditory System May Contribute to Presbycusis-Related Tinnitus With Cognitive Impairment

Animal research suggests that auditory cortical cholinergic inputs from the basal forebrain in adult ferrets contribute to cognitive functions related to the processing of auditory stimuli, including normal auditory perception and adaption to changes in spatial cues (Leach et al., 2013). Furthermore, the central auditory pathway, including the inferior colliculus and nuclei of the lateral lemniscus, but not the cochlear nucleus, show significantly reduced ChAT activity in aged Fischer-344 rats (Raza et al., 1994). A significant decrease in muscarinic receptors, but not ChAT activity, in the dorsal hippocampi of aged rats has also been observed (Lippa et al., 1980). Moreover, noise-induced hyperactivity in fusiform cells of the dorsal cochlear nucleus of adult male Syrian golden hamsters has been shown to be inhibited by the cholinergic agonist carbachol (Manzoor et al., 2013). There is also evidence in experimental animals that chronic inflammation contributes to the dysfunction of auditory pathways (Haase et al., 2011; Menardo et al., 2012; Tan et al., 2016). Acute and chronic noise exposure in C57BL/6 mice (Tan et al., 2016) and senescence-accelerated mouse prone 8 mice (Menardo et al., 2012) also results in increased inflammatory responses in the cochlea.

Chronic inflammation leads to BBB dysfunction and increased vascular permeability during aging, as well as in AD and other neurodegenerative disorders (Zlokovic, 2011; Takeda et al., 2014; Erdő et al., 2017). Increased vascular permeability facilitates the spread of peripheral inflammation into the brain and causes more severe non-cognitive symptoms in AD animal models (Takeda et al., 2013), as well as brain hypoperfusion (Zlokovic, 2011; Takeda et al., 2013). A prominent alteration following BBB breakdown is the decrease in the levels of tight junction proteins, which has been observed in an aging animal model and dementia-related diseases (Zlokovic, 2008; Kalaria, 2010; Ruan et al., 2014d).

Loss of cholinergic input during aging and neurodegenerative diseases causes decreased ACh release and brain hypoperfusion. Reduced sensory input can also lead to decreased ACh release in the neocortex and hippocampus (Penschuck et al., 2002), and decreased hippocampal blood flow (Cao et al., 1992). Hypoxia and ischemia clearly contribute to the pathogenesis of sensorineural tinnitus, and some agents can effectively suppress tinnitus by improving the blood supply and inhibiting chronic inflammatory damage in the acute stage (Mazurek et al., 2006). CBF reductions and hypoxia may not only result in the accumulation of hyperphosphorylated tau and filament formation in experimental animals (Gordon-Krajcer et al., 2007), but also cause increased β-secretase transcription (Zhang et al., 2007), decreased Aβ clearance due to loss or oxidization of lipoprotein receptors in endothelial cells and astrocytes (Bell et al., 2009; Owen et al., 2010), Reduced glutamate reuptake by astrocytes (Boycott et al., 2007), and the accumulation of oxidative damage in the vascular endothelium and high metabolic neurons (Fernández-Checa et al., 2010; **Figure 1**).

Based on animal research, we hypothesize that the cholinergic anti-inflammation mediated by α7nAChR may be one potential mechanism by which hearing loss occurs with tinnitus or cognitive impairment. AChE inhibitors might suppress presbycusis accompanied by tinnitus and may indirectly protect auditory and cognitive function by activating α7nAChR-mediated anti-inflammatory effects in various cells of the brain's neural vascular unit. This might include the suppression of glial and endothelial activation, neuroinflammation, tau-induced neurotoxicity and decreased gap junctions, as well as improved glutamate and extracellular potassium reuptake by astrocytes. These effects inhibit network hyperexcitability and excitotoxicity in the auditory pathway (**Figure 1**).

CONCLUSION

Presbycusis is a risk factor for tinnitus and cognitive decline. Cholinergic hypofunction might be a major contributor to presbycusis-related tinnitus and age-related cognitive impairment. Cholinergic denervation in the CNS, might lead to the reduction of both inhibition by NPY neurogliaform cells and cholinergic anti-inflammatory effects on the neural vascular unit mediated by nAChRs, as well as suppression of GSK3β activity and tau-induced neurodegeneration.

Implementing VNS and AChE inhibitors alone or in combination with other hearing rehabilitative interventions during the optimal time window may lead to greater disease-modifying benefits in the treatment of presbycusis-related tinnitus with cognitive impairment. However, in the evidence reviewed here, data have mainly been obtained from animal

experiments. Age-related hearing loss and AD in humans become apparent very slowly, and are associated with a long preclinical period. Therefore, animal models with a life expectancy of approximately 3 years are not really comparable to humans with these disorders. Further studies are required to elucidate the roles played by M or N cholinergic neuromodulation and distinct GABAergic cell types in the pathophysiological process. Furthermore, it must be investigated whether mechanisms underlying peripheral and central cholinergic regulation are the same.

The potential relationship between tinnitus and depressive systems or affective disorders, and the mechanisms underlying this, should also be investigated in rodents. In addition, dynamic changes in CNS-derived biomarkers of cholinergic hypofunction and neuronal impairment in peripheral body fluids should be investigated as possible screening tools for preclinical or early stage disease, predictors of diagnosis, predictors of intervention outcomes. Finally, innovative, specific and selective neuromodulatory methods and multi-center longitudinal cohort studies are also urgently needed.

AUTHOR CONTRIBUTIONS

QR and ZY designed the study and analyzed the data. QR, ZY, WZ, JR, CL and RZ provided a consensus agreement on the final hypotheses and drafted the initial version of the manuscript. WZ, JR and QR collected the data. All authors contributed to the final version of the manuscript.

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REFERENCES

Acar, B., Yurekli, M. F., Babademez, M. A., Karabulut, H., and Karasen, R. M. (2011). Effects of hearing aids on cognitive functions and depressive signs in elderly people. *Arch. Gerontol. Geriatr.* 52, 250–252. doi: 10.1016/j.archger.2010.04.013

Adams, P. F., Hendershot, G. E., and Marano, M. A. (1999). Current estimates from the National Health Interview Survey, 1996. *Vital Health Stat.* 10, 1–203.

Ahmad, N., and Seidman, M. (2004). Tinnitus in the older adult: epidemiology, pathophysiology and treatment options. *Drugs Aging* 21, 297–305. doi: 10.2165/00002512-200421050-00002

Ahmadian, S. S., Rezvanian, A., Peterson, M., Weintraub, S., Bigio, E. H., Mesulam, M. M., et al. (2015). Loss of calbindin-D28K is associated with the full range of tangle pathology within basal forebrain cholinergic neurons in Alzheimer's disease. *Neurobiol. Aging* 36, 3163–3170. doi: 10.1016/j.neurobiolaging.2015.09.001

Andersson, G., Lyttkens, L., and Larsen, H. C. (1999). Distinguishing levels of tinnitus distress. *Clin. Otolaryngol. Allied Sci.* 24, 404–410. doi: 10.1046/j.1365-2273.1999.00278.x

Andersson, G., and McKenna, L. (2006). The role of cognition in tinnitus. *Acta Otolaryngol. Suppl.* 556, 39–43. doi: 10.1080/03655230600895226

Bakhos, D., Villeuneuve, A., Kim, S., Hammoudi, K., and Hommet, C. (2015). Hearing loss and Alzheimer's disease. *Geriatr. Psychol. Neuropsychiatr. Vieil.* 13, 195–204. doi: 10.1684/pnv.2015.0539

Baloyannis, S. J., Manolidis, S. L., and Manolidis, L. S. (2001). The acoustic cortex in frontal dementia. *Acta Otolaryngol.* 121, 289–292. doi: 10.1080/000164801300043884

Banay-Schwartz, M., Lajtha, A., and Palkovits, M. (1989). Changes with aging in the levels of amino acids in rat CNS. structural elements. II. Taurine and small neutral amino acids. *Neurochem. Res.* 14, 563–570. doi: 10.1007/bf00964919

Behrman, S., Chouliaras, L., and Ebmeier, K. P. (2014). Considering the senses in the diagnosis and management of dementia. *Maturitas* 77, 305–310. doi: 10.1016/j.maturitas.2014.01.003

Bell, R. D., Deane, R., Chow, N., Long, X., Sagare, A., Singh, I., et al. (2009). SRF and myocardin regulate LRPmediated amyloidβ clearance in brain vascular cells. *Nat. Cell Biol.* 11, 143–153. doi: 10.1038/ncb1819

Benzing, W. C., Mufson, E. J., and Armstrong, D. M. (1993). Immunocytochemical distribution of peptidergic and cholinergic fibers in the human amygdala: their depletion in Alzheimer's disease and morphologic alteration in non-demented elderly with numerous senile plaques. *Brain Res.* 625, 125–138. doi: 10.1016/0006-8993(93)90145-d

Berkeley, J. L., Gomeza, J., Wess, J., Hamilton, S. E., Nathanson, N. M., and Levey, A. I. (2001). M1 muscarinic acetylcholine receptors activate extracellular signal-regulated kinase in CA1 pyramidal neurons in mouse hippocampal slices. *Mol. Cell. Neurosci.* 18, 512–524. doi: 10.1006/mcne.2001.1042

Bitner, R. S., Nikkel, A. L., Markosyan, S., Otte, S., Puttfarken, P., and Gopalakrishnan, M. (2009). Selective α7 nicotinic acetylcholine receptor activation regulates glycogen synthase kinase3β and decreases tau phosphorylation *in vivo*. *Brain Res.* 1265, 65–74. doi: 10.1016/j.brainres.2009.01.069

Blom, J. D., Coebergh, J. A., Lauw, R., and Sommer, I. E. (2015). Musical hallucinations treated with acetylcholinesterase inhibitors. *Front. Psychiatry* 6:46. doi: 10.3389/fpsyt.2015.00046

Blom, J. D., Sommer, I. E., Koops, S., and Sacks, O. W. (2014). Prosopometamorphopsia and facial hallucinations. *Lancet* 384:1998. doi: 10.1016/s0140-6736(14)61690-1

Boissière, F., Lehéricy, S., Strada, O., Agid, Y., and Hirsch, E. C. (1996). Neurotrophin receptors and selective loss of cholinergic neurons in Alzheimer disease. *Mol. Chem. Neuropathol.* 28, 219–223. doi: 10.1007/bf02815225

Bojić, T., Perović, V. R., Senćanski, M., and Glišić, S. (2017). Identification of candidate allosteric modulators of the M1 muscarinic acetylcholine receptor which may improve vagus nerve stimulation in chronic tinnitus. *Front. Neurosci.* 11:636. doi: 10.3389/fnins.2017.00636

Bott, J. B., Héraud, C., Cosquer, B., Herbeaux, K., Aubert, J., Sartori, M., et al. (2016). Apoe-sensitive cholinergic sprouting compensates for hippocampal dysfunctions due to reduced entorhinal input. *J. Neurosci.* 36, 10472–10486. doi: 10.1523/JNEUROSCI.1174-16.2016

Boycott, H. E., Dallas, M., Boyle, J. P., Pearson, H. A., and Peers, C. (2007). Hypoxia suppresses astrocyte glutamate transport independently of amyloid formation. *Biochem. Biophys. Res. Commun.* 364, 100–104. doi: 10.1016/j.bbrc.2007.09.102

Buhler, A. V., and Dunwiddie, T. V. (2002). α7 nicotinic acetylcholine receptors on GABAergic interneurons evoke dendritic and somatic inhibition of hippocampal neurons. *J. Neurophysiol.* 87, 548–557. doi: 10.1152/jn.00316.2001

Cao, W. H., Sato, A., Sato, Y., and Zhou, W. (1992). Somatosensory regulation of regional hippocampal blood flow in anesthetized rats. *Jap. J. Physiol.* 42, 731–740. doi: 10.2170/jjphysiol.42.731

Caspary, D. M., Hughes, L. F., and Ling, L. L. (2013). Age-related $GABA_A$ receptor changes in rat auditory cortex. *Neurobiol. Aging* 34, 1486–1496. doi: 10.1016/j.neurobiolaging.2012.11.009

Cheng, L., Wang, S. H., Chen, Q. C., and Liao, X. M. (2011). Moderate noise induced cognition impairment of mice and its underlying mechanisms. *Physiol. Behav.* 104, 981–988. doi: 10.1016/j.physbeh.2011.06.018

Cheng, L., Wang, S. H., Huang, Y., and Liao, X. M. (2016). The hippocampus may be more susceptible to environmental noise than the auditory cortex. *Hear. Res.* 333, 93–97. doi: 10.1016/j.heares.2016.01.001

Clader, J. W., and Wang, Y. (2005). Muscarinic receptor agonists and antagonists in the treatment of Alzheimer's disease. *Curr. Pharm. Des.* 11, 3353–3361. doi: 10.2174/138161205774370762

Cohen, B. M., Renshaw, P. F., Stoll, A. L., Wurtman, R. J., Yurgelun-Todd, D., and Babb, S. M. (1995). Decreased brain choline uptake in older adults. An

in vitro proton magnetic resonance spectroscopy study. *JAMA* 274, 902–907. doi: 10.1001/jama.1995.03530110064037

Cui, B., Zhu, L., She, X., Wu, M., Ma, Q., Wang, T., et al. (2012). Chronic noise exposure causes persistence of tauhyperphosphorylation and formation of NFT tau in the rat hippocampus and prefrontal cortex. *Exp. Neurol.* 238, 122–129. doi: 10.1016/j.expneurol.2012.08.028

Damar, U., Gersner, R., Johnstone, J. T., Schachter, S., and Rotenberg, A. (2017). Huperzine A: a promising anticonvulsant, disease modifying, and memory enhancing treatment option in Alzheimer's disease. *Med. Hypotheses* 99, 57–62. doi: 10.1016/j.mehy.2016.12.006

Daulatzai, M. A. (2010). Early stages of pathogenesis in memory impairment during normal senescence and Alzheimer's Disease. *J. Alzheimers Dis.* 20, 355–367. doi: 10.3233/JAD-2010-1374

Davies, P. (1979). Neurotransmitter-related enzymes in senile dementia of the Alzheimer type. *Brain Res.* 171, 319–327. doi: 10.1016/0006-8993(79)90336-6

Davies, K. L., Mohs, R. C., Marin, D., Purohit, D. P., Perl, D. P., Lantz, M., et al. (1999). Cholinergic markers in the elderly patients with early signs of Alzheimer's disease. *JAMA* 281, 1401–1406. doi: 10.1001/jama.281.15.1401

Deal, J. A., Betz, J., Yaffe, K., Harris, T., Purchase-Helzner, E., Satterfield, S., et al. (2017). Hearing impairment and incident dementia and cognitive decline in older adults: the health ABC study. *J. Gerontol. A Biol. Sci. Med. Sci.* 72, 703–709. doi: 10.1093/gerona/glw069

Drachman, D. A., Noffsinger, D., Sahakian, B. J., Kurdziel, S., and Fleming, P. (1980). Aging, memory, and the cholinergic system: a study of dichotic listening. *Neurobiol. Aging* 1, 39–43. doi: 10.1016/0197-4580(80)90022-6

Elgoyhen, A. B., Katz, E., and Fuchs, P. A. (2009). The nicotinic receptor of cochlear hair cells: a possible pharmacotherapeutic target? *Biochem. Pharmacol.* 78, 712–719. doi: 10.1016/j.bcp.2009.05.023

Elgoyhen, A. B., Langguth, B., De Ridder, D., and Vanneste, S. (2015). Tinnitus: perspectives from human neuroimaging. *Nat. Rev. Neurosci.* 16, 632–642. doi: 10.1038/nrn4003

Emre, M., Heckers, S., Mash, D. C., Geula, C., and Mesulam, M. M. (1993). Cholinergic innervation of the amygdaloid complex in the human brain and its alterations in old age and Alzheimer's disease. *J. Comp. Neurol.* 336, 117–134. doi: 10.1002/cne.903360110

Engelhart, M. J., Geerlings, M. I., Meijer, J., Kiliaan, A., Ruitenberg, A., van Swieten, J. C., et al. (2004). Inflammatory proteins in plasma and the risk of dementia: the Rotterdam study. *Arch. Neurol.* 61, 668–672. doi: 10.1001/archneur.61.5.668

Engidawork, E., Gulesserian, T., Balic, N., Cairns, N., and Lubec, G. (2001). Changes in nicotinic acetylcholine receptor subunits expression in brain of patients with down syndrome and Alzheimer's disease. *J. Neural. Transm. Suppl.* 61, 211–222. doi: 10.1007/978-3-7091-6262-0_17

Engineer, N. D., Møller, A. R., and Kilgard, M. P. (2013). Directing neural plasticity to understand treat tinnitus. *Hear. Res.* 295, 58–66. doi: 10.1016/j.heares.2012.10.001

Erdő, F., Denes, L., and de Lange, E. (2017). Age-associated physiological and pathological changes at the blood-brain barrier: a review. *J. Cereb. Blood Flow Metab.* 37, 4–24. doi: 10.1177/0271678x16679420

Farrall, A. J., and Wardlaw, J. M. (2009). Blood-brain barrier: ageing and microvascular disease—systematic review and meta-analysis. *Neurobiol. Aging* 30, 337–352. doi: 10.1016/j.jns.2009.02.089

Faust, T. W., Assous, M., Shah, F., Tepper, J. M., and Koos, T. (2015). Novel fast adapting interneurons mediate cholinergicinduced fast $GABA_A$ inhibitory postsynaptic currents in striatal spiny neurons. *Eur. J. Neurosci.* 42, 1764–1774. doi: 10.1111/ejn.12915

Fernández-Checa, J. C., Fernández, A., Morales, A., Marí, M., García-Ruiz, C., and Colell, A. (2010). Oxidative stress and altered mitochondrial function in neurodegenerative diseases: lessons from mouse models. *CNS Neurol. Disord. Drug Targets.* 9, 439–454. doi: 10.2174/187152710791556113

Flynn, D. D., Ferrari-DiLeo, G., Levey, A. I., and Mash, D. C. (1995). Differential alterations in muscarinic receptor subtypes in Alzheimer's disease: implications for cholinergic-based therapies. *Life Sci.* 56, 869–876. doi: 10.1016/0024-3205(95)00022-x

Fortunato, S., Forli, F., Guglielmi, V., De Corso, E., Paludetti, G., Berrettini, S., et al. (2016). A review of new insights on the association between hearing loss and cognitive decline in ageing. *Acta Otorhinolaryngol. Ital.* 36, 155–166. doi: 10.14639/0392-100X-993

Foster, D. J., Choi, D. L., Conn, P. J., and Rook, J. M. (2014). Activation of M1 and M4 muscarinic receptors as potential treatments for Alzheimer's disease and schizophrenia. *Neuropsychiatr. Dis. Treat.* 10, 183–191. doi: 10.2147/NDT.s55104

Freund-Levi, Y., Jedenius, E., Tysen-Bäckström, A. C., Lärksäter, M., Wahlund, L. O., Eriksdotter, M., et al. (2014). Galantamine versus risperidone treatment of neuropsychiatric symptoms in patients with probable dementia: an open randomized trial. *Am. J. Geriatr. Psychiatry* 22, 341–348. doi: 10.1016/j.jagp.2013.05.005

Gallacher, J., Ilubaera, V., Ben-Shlomo, Y., Bayer, A., Fish, M., Babisch, W., et al. (2012). Auditory threshold, phonologic demand incident dementia. *Neurology* 79, 1583–1590. doi: 10.1212/WNL.0b013e31826e263d

Gates, G. A., Anderson, M. L., McCurry, S. M., Feeney, M. P., and Larson, E. B. (2011). Central auditory dysfunction as a harbinger of Alzheimer dementia. *Arch. Otolaryngol. Head Neck Surg.* 137, 390–395. doi: 10.1001/archoto.2011.28

Gates, G. A., Beiser, A., Rees, T. S., D'Agostino, R. B., and Wolf, P. A. (2002). Central auditory dysfunction may precede the onset of clinical dementia in people with probable Alzheimer's disease. *J. Am. Geriatr. Soc.* 50, 482–488. doi: 10.1046/j.1532-5415.2002.50114.x

Gates, G. A., and Mills, J. H. (2005). Presbycusis. *Lancet* 366, 1111–1120. doi: 10.1016/S0140-6736(05)67423-5

Gennis, V., Garry, P. J., Haaland, K. Y., Yeo, R. A., and Goodwin, J. S. (1991). Hearing and cognition in the elderly. New findings and a review of the literature. *Arch. Intern. Med.* 151, 2259–2264. doi: 10.1001/archinte.151.11.2259

Gersner, R., Ekstein, D., Dhamne, S. C., Schachter, S. C., and Rotenberg, A. (2015). Huperzine a prophylaxis against pentylenetetrazole-induced seizures in rats is associated with increased cortical inhibition. *Epilepsy Res.* 117, 97–103. doi: 10.1016/j.eplepsyres.2015.08.012

Gitelman, D. R., and Prohovnik, I. (1992). Muscarinic and nicotinic contributions to cognitive function and cortical blood flow. *Neurobiol. Aging* 13, 313–318. doi: 10.1016/0197-4580(92)90044-x

Golob, E. J., Miranda, G. G., Johnson, J. K., and Starr, A. (2015). Sensory cortical interactions in aging, mild cognitive impairment, and Alzheimer's disease. *Neurobiol. Aging* 22, 755–763. doi: 10.1016/s0197-4580(01)00244-5

Golub, J. S., Luchsinger, J. A., Manly, J. J., Stern, Y., Mayeux, R., and Schupf, N. (2017). Observed hearing loss and incident dementia in a multiethnic cohort. *J. Am. Geriatr. Soc.* 65, 1691–1697. doi: 10.1111/jgs.14848

González, J. C., Albiñana, E., Baldelli, P., García, A. G., and Hernández-Guijo, J. M. (2011). Presynaptic muscarinic receptor subtypes involved in the enhancement of spontaneous GABAergic postsynaptic currents in hippocampal neurons. *Eur. J. Neurosci.* 33, 69–81. doi: 10.1111/j.1460-9568.2010.07475.x

Gordon-Krajcer, W., Kozniewska, E., Lazarewicz, J. W., and Ksiezak-Reding, H. (2007). Differential changes in phosphorylation of tau at PHF1 and 12E8 epitopes during brain ischemia and reperfusion in gerbils. *Neurochem. Res.* 32, 729–737. doi: 10.1007/s11064-006-9199-3

Grammas, P. (2011). Neurovascular dysfunction, inflammation and endothelial activation: implications for the pathogenesis of Alzheimer's disease. *J. Neuroinflammation* 8:26. doi: 10.1186/1742-2094-8-26

Guan, Z. Z., Zhang, X., Ravid, R., and Nordberg, A. (2000). Decreased protein levels of nicotinic receptor subunits in the hippocampus and temporal cortex of patients with Alzheimer's disease. *J. Neurochem.* 74, 237–243. doi: 10.1046/j.1471-4159.2000.0740237.x

Gurgel, R. K., Ward, P. D., Schwartz, S., Norton, M. C., Foster, N. L., and Tschanz, J. T. (2014). Relationship of hearing loss and dementia: a prospective, population-based study. *Otol. Neurotol.* 35, 775–781. doi: 10.1097/MAO.0000000000000313

Haase, G. M., Prasad, K. N., Cole, W. C., Baggett-Strehlau, J. M., and Wyatt, S. E. (2011). Antioxidant micronutrient impact on hearing disorders: concept, rationale, and evidence. *Am. J. Otolaryngol.* 32, 55–61. doi: 10.1016/j.amjoto.2009.09.002

Hallam, R. S., McKenna, L., and Shurlock, L. (2004). Tinnitus impairs cognitive efficiency. *Int. J. Audiol.* 43, 218–226. doi: 10.1080/14992020400050030

Han, Z., Li, L., Wang, L., Degos, V., Maze, M., and Su, H. (2014). α-7 nicotinic acetylcholine receptor agonist treatment reduces neuroinflammation, oxidative stress, and brain injury in mice with ischemic stroke and bone fracture. *J. Neurochem.* 131, 498–508. doi: 10.1111/jnc.12817

Hays, S. A. (2016). Enhancing rehabilitative therapies with vagus nerve stimulation. *Neurotherapeutics* 13, 382–394. doi: 10.1007/s13311-015-0417-z

Hernandez, C. M., Kayed, R., Zheng, H., Sweatt, J. D., and Dineley, K. T. (2010). Loss of α7 nicotinic receptors enhances β-amyloid oligomer accumulation, exacerbating early-stage cognitive decline and septo-hippocampal pathology in a mouse model of Alzheimer's disease. *J. Neurosci.* 30, 2442–2453. doi: 10.1523/JNEUROSCI.5038-09.2010

Hollands, S., Lim, Y. Y., Laws, S. M., Villemagne, V. L., Pietrzak, R. H., Harrington, K., et al. (2017). APOEε4 genotype, amyloid, and clinical disease progression in cognitively normal older adults. *J. Alzheimers Dis.* 57, 411–422. doi: 10.3233/JAD-161019

Iaccarino, H. F., Singer, A. C., Martorell, A. J., Rudenko, A., Gao, F., Gillingham, T. Z., et al. (2016). γ frequency entrainment attenuates amyloid load and modifies microglia. *Nature* 540, 230–235. doi: 10.1038/nature20587

Iqbal, K., Gong, C. X., and Liu, F. (2013). Hyperphosphorylation-induced tau oligomers. *Front Neurol.* 4:112. doi: 10.3389/fneur.2013.00112

Ittner, L. M., Ke, Y. D., Delerue, F., Bi, M., Gladbach, A., van Eersel, J., et al. (2010). Dendritic function of tau mediates amyloid-β toxicity in Alzheimer's disease mouse models. *Cell* 142, 387–397. doi: 10.1016/j.cell.2010.06.036

Iwamoto, K., Mata, D., Linn, D. M., and Linn, C. L. (2013). Neuroprotection of rat retinal ganglion cells mediated through α7 nicotinic acetylcholine receptors. *Neuroscience* 237, 184–198. doi: 10.1016/j.neuroscience.2013.02.003

Kalappa, B. I., Brozoski, T. J., Turner, J. G., and Caspary, D. M. (2014). Single unit hyperactivity and bursting in the auditory thalamus of awake rats directly correlates with behavioural evidence of tinnitus. *J. Physiol.* 592, 5065–5078. doi: 10.1113/jphysiol.2014.278572

Kalaria, R. N. (2010). Vascular basis for brain degeneration: faltering controls and risk factors for dementia. *Nutr. Rev.* 68, S74–S87. doi: 10.1111/j.1753-4887.2010.00352.x

Karami, A., Eyjolfsdottir, H., Vijayaraghavan, S., Lind, G., Almqvist, P., Kadir, A., et al. (2015). Changes in CSF cholinergic biomarkers in response to cell therapy with ngf in patients with Alzheimer's disease. *Alzheimers Dement.* 11, 1316–1328. doi: 10.1016/j.jalz.2014.11.008

Kat, M. G., Vreeswijik, R., de Jonghe, J. F., van der Ploeg, T., van Gool, W. A., Eikelenboom, P., et al. (2008). Long-term cognitive outcome of delirium in elderly hip surgery patients. A prospective matched controlled study over two and a half years. *Dement. Geriatr. Cogn. Disord.* 26, 1–8. doi: 10.1159/000140611

Kelso, M. L., and Oestreich, J. H. (2012). Traumatic brain injury: central and peripheral role of α7 nicotinic acetylcholine receptors. *Curr. Drug Targets* 13, 631–636. doi: 10.2174/138945012800398964

Knipper, M., Van Dijk, P., Nunes, I., Rüttiger, L., and Zimmermann, U. (2013). Advances in the neurobiology of hearing disorders: recent developments regarding the basis of tinnitus and hyperacusis. *Prog. Neurobiol.* 111, 17–33. doi: 10.1016/j.pneurobio.2013.08.002

Kurniawan, C., Westendorp, R. G., de Craen, A. J., Gussekloo, J., de Laat, J., and van Exel, E. (2012). Gene dose of apolipoprotein E and age-related hearing loss. *Neurobiol. Aging* 33, 2230.e7–2230.e12. doi: 10.1016/j.neurobiolaging.2012.04.001

Langguth, B., Kreuzer, P. M., Kleinjung, T., and De Ridder, D. (2013). Tinnitus: causes and clinical management. *Lancet Neurol.* 12, 920–930. doi: 10.1016/S1474-4422(13)70160-1

Lawrence, A. D., and Sahakian, B. J. (1995). Alzheimer disease, attention, and the cholinergic system. *Alzheimer Dis. Assoc. Disord.* 9, 43–49. doi: 10.1097/00002093-199501002-00008

Leach, N. D., Nodal, F. R., Cordery, P. M., King, A. J., and Bajo, V. M. (2013). Cortical cholinergic input is required for normal auditory perception and experience-dependent plasticity in adult ferrets. *J. Neurosci.* 33, 6659–6671. doi: 10.1523/JNEUROSCI.5039-12.2013

Lee, D. H., Dandrea, M. R., Plata-Salaman, C. R., and Wang, H. Y. (2000). Decreased a7 nicotinic acetylcholine receptor protein levels in sporadic Alzheimers disease hippocampus. *Alzheimer Rep.* 3, 217–220.

Limon, A., Reyes-Ruiz, J. M., and Miledi, R. (2012). Loss of functional $GABA_A$ receptors in the Alzheimer diseased brain. *Proc. Natl. Acad. Sci. U S A* 109, 10071–10076. doi: 10.1073/pnas.1204606109

Lin, F. R. (2011). Hearing loss and cognition among older adults in the United States. *J. Gerontol. A Biol. Sci. Med. Sci.* 66, 1131–1136. doi: 10.1093/gerona/glr115

Lin, F. R., Thorpe, R., Gordonsalant, S., and Ferrucci, L. (2011). Hearing loss prevalence and risk factors among older adults in the united states. *J. Gerontol. A Biol. Sci. Med. Sci.* 66, 582–590. doi: 10.1093/gerona/glr002

Lin, F. R., Yaffe, K., Xia, J., Xue, Q. L., Harris, T. B., Purchase-Helzner, E., et al. (2013). Hearing loss and cognitive decline in older adults. *JAMA Intern. Med.* 173, 293–299. doi: 10.1001/jamainternmed.2013.1868

Ling, L. L., Hughes, L. F., and Caspary, D. M. (2005). Age-related loss of the GABA synthetic enzyme glutamic acid decarboxylase in rat primary auditory cortex. *Neuroscience* 132, 1103–1113. doi: 10.1016/j.neuroscience.2004.12.043

Linton, A. (2005). The benefits of cholinesterase inhibitors managing the behavioral and neuropsychiatric symptom of Alzheimer's disease. *J. Gerontol. Nurs.* 31, 4–10. doi: 10.3928/0098-9134-20051201-04

Lippa, A. S., Pelham, R. W., Beer, B., Critchett, D. J., Dean, R. L., and Bartus, R. T. (1980). Brain cholinergic dysfunction and memory in aged rats. *Neurobiol. Aging* 1, 13–19. doi: 10.1016/0197-4580(80)90019-6

Loughrey, D. G., Kelly, M. E., Kelley, G. A., Brennan, S., and Lawlor, B. A. (2018). Association of age-related hearing loss with cognitive function, cognitive impairment, and dementia: a systematic review and meta-analysis. *JAMA Otolaryngol. Head Neck Surg.* 144, 115–126. doi: 10.1001/jamaoto.2017.2513

Lunardi, P., Nardin, P., Guerra, M. C., Abib, R., Leite, M. C., and Gonçalves, C. A. (2013). Huperzine A, but not tacrine, stimulates S100B secretion in astrocyte cultures. *Life Sci.* 92, 701–707. doi: 10.1016/j.lfs.2013.01.029

Maison, S. F., Luebke, A. E., Liberman, M. C., and Zuo, J. (2002). Efferent protection from acoustic injury is mediated via alpha9 nicotinic acetylcholine receptors on outer hair cells. *J. Neurosci.* 22, 10838–10846.

Maloku, E., Kadriu, B., Zhubi, A., Dong, E., Pibiri, F., Satta, R., et al. (2011). Selective α4β2 nicotinic acetylcholine receptor agonists target epigenetic mechanisms in cortical GABAergic neurons. *Neuropsychopharmacology* 36, 1366–1374. doi: 10.1038/npp.2011.21

Manzoor, N. F., Chen, G., and Kaltenbach, J. A. (2013). Suppression of noise-induced hyperactivity in the dorsal cochlear nucleus following application of the cholinergic agonist, carbachol. *Brain Res.* 1523, 28–36. doi: 10.1016/j.brainres.2013.05.025

Marti, A., Castiglione, A., Bovo, R., Vallesi, A., and Gabelli, C. (2014). Aging, cognitive load, dementia and hearing loss. *Audiol. Neurotol.* 19, 2–5. doi: 10.1159/000371593

Martin, A. J., Friston, K. J., Colebatch, J. G., and Frackowiak, R. S. (1991). Decreases in regional cerebral blood flow with normal aging. *J. Cereb. Blood Flow Metab.* 11, 684–689. doi: 10.1038/jcbfm.1991.121

Mazarati, A., and Wasterlain, C. G. (2002). Anticonvulsant effects of four neuropeptides in the rat hippocampus during self-sustaining status epilepticus. *Neurosci. Lett.* 331, 123–127. doi: 10.1016/s0304-3940(02)00847-9

Mazurek, B., Haupt, H., and Gross, J. (2006). Pharmacotherapy in acute tinnitis. The special role of hypoxia and ischemia in the pathogenesis of tinnitus. *HNO* 54, 9–15. doi: 10.1007/s00106-005-1292-4

Menardo, J., Tang, Y., Ladrech, S., Lenoir, M., Casas, F., Michel, C., et al. (2012). Oxidative stress, inflammation, and autophagic stress as the key mechanisms of premature age-related hearing loss in SAMP8 mouse Cochlea. *Antioxid. Redox Signal.* 16, 263–274. doi: 10.1089/ars.2011.4037

Mencel, M., Nash, M., and Jacobson, C. (2013). Neuregulin upregulates microglial α7 nicotinic acetylcholine receptor expression in immortalized cell lines: implications for regulating neuroinflammation. *PLoS One* 8:e70338. doi: 10.1371/journal.pone.0070338

Milbrandt, J. C., Albin, R. L., and Caspary, D. M. (1994). Age-related decrease in GABAB receptor binding in the Fischer 344 rat inferior colliculus. *Neurobiol. Aging* 15, 699–703. doi: 10.1016/0197-4580(94)90051-5

Milbrandt, J. C., Holder, T. M., Wilson, M. C., Salvi, R. J., and Caspary, D. M. (2000). GAD levels and muscimol binding in rat inferior colliculus following acoustic trauma. *Hear. Res.* 147, 251–260. doi: 10.1016/s0378-5955(00)00135-0

Mufson, E. J., and Kordower, J. H. (1989). Nerve growth factor receptor expressing human basal forebrain neurons: pathologic alterations in Alzheimer's and Parkinson's disease. *Prog. Clin. Biol. Res.* 317, 401–414.

Muñoz, W., Tremblay, R., Levenstein, D., and Rudy, B. (2017). Layer-specific modulation of neocortical dendritic inhibition during active wakefulness. *Science* 355, 954–959. doi: 10.1126/science.aag2599

Nägerl, U. V., Mody, I., Jeub, M., Lie, A. A., Elger, C. E., and Beck, H. (2000). Surviving granule cells of the sclerotic human hippocampus have reduced Ca^{2+} influx because of a loss of calbindin-D28K in temporal lobe epilepsy. *J. Neurosci.* 20, 1831–1836.

Noh, M. Y., Koh, S. H., Kim, Y., Kim, H. Y., Cho, G. W., and Kim, S. H. (2009). Neuroprotective effects of donepezil against Aβ42-induced neuronal toxicity are mediated through not only enhancing PP2A activity but also regulating GSK-3β and nAChRs activity. *J. Neurochem.* 108, 1116–1125. doi: 10.1111/j.1471-4159.2008.05837.x

Nordberg, A., Lundkvist, H., Hartvig, P., Andersson, J., Johansson, M., Hellstrom-Lindahl, E., et al. (1997). Imaging of nicotinic and muscarinic receptors in Alzheimer's disease: effect of tacrine treatment. *Dement. Geriatr. Cogn. Disord.* 8, 78–84. doi: 10.1159/000106611

O'Mahony, D., Rowan, M., Feely, J., Walsh, J. B., and Coakley, D. (1994). Primary auditory pathway and reticular activating system dysfunction in Alzheimer's disease. *Neurology* 44, 2089–2094. doi: 10.1212/WNL.44.11.2089

Olney, J. W. (1995). NMDA receptor hypofunction, excitotoxicity, and Alzheimer's disease. *Neurobiol. Aging* 16, 459–461. doi: 10.1016/0197-4580(94)00185-4

Overstreet-Wadiche, L., and McBain, C. J. (2015). Neurogliaform cells in cortical circuits. *Nat. Rev. Neurosci.* 16, 458–468. doi: 10.1038/nrn3969

Owen, J. B., Sultana, R., Aluise, C. D., Erickson, M. A., Price, T. O., Bu, G., et al. (2010). Oxidative modification to LDL receptor-related protein 1 in hippocampus from subjects with Alzheimer disease: implications for Aβ accumulation in AD brain. *Free Radic. Biol. Med.* 49, 1798–1803. doi: 10.1016/j.freeradbiomed.2010.09.013

Palop, J. J., Chin, J., Roberson, E. D., Wang, J., Thwin, M. T., Bien-Ly, N., et al. (2007). Aberrant excitatory neuronal activity and compensatory remodeling of inhibitory hippocampal circuits in mouse models of Alzheimer's disease. *Neuron* 55, 697–711. doi: 10.1016/j.neuron.2007.07.025

Palop, J. J., and Mucke, L. (2009). Epilepsy and cognitive impairments in Alzheimer disease. *Arch. Neurol.* 66, 435–440. doi: 10.1001/archneurol.2009.15

Panza, F., Solfrizzi, V., and Logroscino, G. (2015a). Age-related hearing impairment-a risk factor and frailty marker for dementia and AD. *Nat. Rev. Neurol.* 11, 166–175. doi: 10.1038/nrneurol.2015.12

Panza, F., Solfrizzi, V., Seripa, D., Imbimbo, B. P., Capozzo, R., Quaranta, N., et al. (2015b). Age-related hearing impairment and frailty in Alzheimer's disease: interconnected associations and mechanisms. *Front. Aging Neurosci.* 7:113. doi: 10.3389/fnagi.2015.00113

Panza, F., Quaranta, N., and Logroscino, G. (2017). Sensory changes and the hearing loss-cognition link: the cognitive ear. *JAMA Otolaryngol. Head Neck Surg.* 144, 127–128. doi: 10.1001/jamaoto.2017.2514

Pavlov, V. A., and Tracey, K. J. (2006). Controlling inflammation: the cholinergic anti-inflammatory pathway. *Biochem. Soc. Trans.* 34, 1037–1040. doi: 10.1042/bst0341037

Penschuck, S., Chen-Bee, C. H., Prakash, N., and Frostig, R. D. (2002). *In vivo* modulation of a cortical functional sensory representation shortly after topical cholinergic agent application. *J. Comp. Neurol.* 452, 38–50. doi: 10.1002/cne.10361

Perry, E. K. (1990). Nerve growth factor and the basal forebrain cholinergic system: a link in the etiopathology of neurodegenerative dementias? *Alzheimer Dis. Assoc. Disord.* 4, 1–13. doi: 10.1097/00002093-199040100-00001

Perry, E. K., Tomlinson, B. E., Blessed, G., Bergman, K., Gibson, P. H., and Perry, R. H. (1978). Correlation of cholinergic abnormalities with senile plaques and mental test scores in senile dementia. *Br. Med. J.* 2, 1457–1459. doi: 10.1136/bmj.2.6150.1457

Pierce, K. J., Kallogjeri, D., Piccirillo, J. F., Garcia, K. S., Nicklaus, J. E., and Burton, H. (2012). Effects of severe bothersome tinnitus on cognitive function measured with standardized tests. *J. Clin. Exp. Neuropsychol.* 34, 126–134. doi: 10.1080/13803395.2011.623120

Pitler, T. A., and Alger, B. E. (1992). Cholinergic excitation of GABAergic interneurons in the rat hippocampal slice. *J. Physiol.* 450, 127–142. doi: 10.1113/jphysiol.1992.sp019119

Poulin, B., Butcher, A., McWilliams, P., Bourgognon, J. M., Pawlak, R., Kong, K. C., et al. (2010). The M3-muscarinic receptor regulates learning and memory in a receptor phosphorylation/arrestin-dependent manner. *Proc. Natl. Acad. Sci. U S A* 107, 9440–9445. doi: 10.1073/pnas.0914801107

Rafii, M. S., Walsh, S., Little, J. T., Behan, K., Reynolds, B., Ward, C., et al. (2011). A phase II trial of huperzine A in mild to moderate Alzheimer disease. *Neurology* 76, 1389–1394. doi: 10.1212/WNL.0b013e318216eb7b

Ragozzino, M. E., Artis, S., Singh, A., Twose, T. M., Beck, J. E., and Messer, W. S. Jr. (2012). The selective M1 muscarinic cholinergic agonist CDD-0102A enhances working memory and cognitive flexibility. *J. Pharmacol. Exp. Ther.* 340, 588–594. doi: 10.1124/jpet.111.187625

Raza, A., Milbrandt, J. C., Arneric, S. P., and Caspary, D. M. (1994). Age-related changes in brainstem auditory neurotransmitters: measures of GABA and acetylcholine function. *Hear. Res.* 77, 221–230. doi: 10.1016/0378-5955(94)90270-4

Roberson, E. D., Halabisky, B., Yoo, J. W., Yao, J., Chin, J., Yan, F., et al. (2011). Amyloid-β/Fyn-induced synaptic, network, and cognitive impairments depend on Tau levels in multiple mouse models of Alzheimer's disease. *J. Neurosci.* 31, 700–711. doi: 10.1523/JNEUROSCI.4152-10.2011

Roberson, E. D., Scearce-Levie, K., Palop, J. J., Yan, F., Cheng, I. H., Wu, T., et al. (2007). Reducing endogenous tau ameliorates amyloid β-induced deficits in an Alzheimer's disease mouse model. *Science* 316, 750–754. doi: 10.1126/science.1141736

Rossiter, S., Stevens, C., and Walker, G. (2006). Tinnitus and its effect on working memory and attention. *J. Speech Lang. Hear. Res.* 49, 150–160. doi: 10.1044/1092-4388(2006/012)

Ruan, Q., Ao, H., He, J., Chen, Z., Yu, Z., Zhang, R., et al. (2014a). Topographic and quantitative evaluation of gentamicin-induced damage to peripheral innervation of mouse cochleae. *Neurotoxicology* 40, 86–96. doi: 10.1016/j.neuro.2013.11.002

Ruan, Q., Zeng, S., Liu, A., Chen, Z., Yu, Z., Zhang, R., et al. (2014b). Overexpression of X-Linked Inhibitor of Apoptotic Protein (XIAP) reduces age-related neuronal degeneration in the mouse cochlea. *Gene Ther.* 21, 967–974. doi: 10.1038/gt.2014.77

Ruan, Q., Ma, C., Zhang, R., and Yu, Z. (2014c). Current status of auditory aging and anti-aging research. *Geriatr. Gerontol. Int.* 14, 40–53. doi: 10.1111/ggi.12124

Ruan, Q., Hu, X., Ao, H., Ma, H., Gao, Z., Liu, F., et al. (2014d). The neurovascular protective effects of huperzine A on D-galactose-induced inflammatory damage in the rat hippocampus. *Gerontology* 60, 424–439. doi: 10.1159/000358235

Ruan, Q., D'Onofrio, G., Sancarlo, D., Bao, Z., Greco, A., and Yu, Z. (2016). Potential neuroimaging biomarkers of pathologic brain changes in Mild Cognitive Impairment and Alzheimer's disease: a systematic review. *BMC Geriatr.* 16:104. doi: 10.1186/s12877-016-0281-7

Salvi, R. J., Wang, J., and Ding, D. (2000). Auditory plasticity and hyperactivity following cochlear damage. *Hear. Res.* 147, 261–274. doi: 10.1016/s0378-5955(00)00136-2

Schlee, W., Mueller, N., Hartmann, T., Keil, J., Lorenz, I., and Weisz, N. (2009). Mapping cortical hubs in tinnitus. *BMC Biol.* 7:80. doi: 10.1186/1741-7007-7-80

Schliebs, R., and Arendt, T. (2011). The cholinergic system in aging and neuronal degeneration. *Behav. Brain Res.* 221, 555–563. doi: 10.1016/j.bbr.2010.11.058

Schmidt, R., Schmidlt, H., Curb, J. D., Masaki, K., White, L. R., and Launer, L. J. (2002). Early inflammation and dementia: a 25-year follow-up of the Honolulu-asia aging study. *Ann. Neurol.* 52, 168–174. doi: 10.1002/ana.10265

Shargorodsky, J., Curhan, G. C., and Farwell, W. R. (2010). Prevalence and characteristics of tinnitus among US adults. *Am. J. Med.* 123, 711–718. doi: 10.1016/j.amjmed.2010.02.015

Sharma, S., Nag, T. C., Thakar, A., Bhardwaj, D. N., and Roy, T. S. (2014). The aging human cochlear nucleus: changes in the glial fibrillary acidic protein, intracellular calcium regulatory proteins, gaba neurotransmitter and cholinergic receptor. *J. Chem. Neuroanat.* 56, 1–12. doi: 10.1016/j.jchemneu.2013.12.001

Shimohama, S., Greenwald, D. L., Shafron, D. H., Akaika, A., Maeda, T., Kaneko, S., et al. (1998). Nicotinic α 7 receptors protect against glutamate

neurotoxicity and neuronal ischemic damage. *Brain Res.* 779, 359–363. doi: 10.1016/s0006-8993(97)00194-7

Shore, S. E., Roberts, L. E., and Langguth, B. (2016). Maladaptive plasticity in tinnitus—triggers, mechanisms and treatment. *Nat. Rev. Neurol.* 12, 150–160. doi: 10.1038/nrneurol.2016.12

Shulman, A., Goldstein, B., and Strashun, A. M. (2008). Central nervous system neurodegeneration and tinnitus: a clinical experience. Part II: translational neurovascular theory of neurodegenerative CNS disease and tinnitus. *Int. Tinnitus J.* 14, 43–51.

Sinha, U. K., Hollen, K. M., Rodriguez, R., and Miller, C. A. (1993). Auditory system degeneration in Alzheimer's disease. *Neurology* 43, 779–785. doi: 10.1212/WNL.43.4.779

Snider, B. J., Norton, J., Coats, M. A., Chakraverty, S., Hou, C. E., Jervis, R., et al. (2005). Novel presenilin 1 mutation (S170F) causing Alzheimer disease with Lewy bodies in the third decade of life. *Arch. Neurol.* 62, 1821–1830. doi: 10.1001/archneur.62.12.1821

Stanley, D. P., and Shetty, A. K. (2004). Aging in the rat hippocampus is associated with widespread reductions in the number of glutamate decarboxylase-67 positive interneurons but not interneuron degeneration. *J. Neurochem.* 89, 204–216. doi: 10.1111/j.1471-4159.2004.02318.x

Stevens, C., Walker, G., Boyer, M., and Gallagher, M. (2007). Severe tinnitus and its effect on selective and divided attention. *Int. J. Audiol.* 46, 208–216. doi: 10.1080/14992020601102329

Strauss, M., and Gertz, H. J. (2009). Treatment of musical hallucinations with acetylcholinesterase inhibitors. *J. Neurol. Neurosurg. Psychiatry* 80, 1298–1299. doi: 10.1136/jnnp.2008.160978

Stuckenholz, V., Bacher, M., Balzer-Geldsetzer, M., Alvarez-Fischer, D., Oertel, W. H., Dodel, R. C., et al. (2013). The α7 nAChR agonist PNU-282987 reduces inflammation and MPTP-induced nigral dopaminergic cell loss in mice. *J. Parkinsons Dis.* 3, 161–172. doi: 10.3233/JPD-120157

Takeda, S., Sato, N., Ikimura, K., Nishino, H., Rakugi, H., and Morishita, R. (2013). Increased blood-brain barrier vulnerability to systemic inflammation in an Alzheimer's disease mouse model. *Neurobiol. Aging* 34, 2064–2070. doi: 10.1016/j.neurobiolaging.2013.02.010

Takeda, S., Sato, N., and Morishita, R. (2014). Systemic inflammation, blood-brain barrier vulnerability and congnitive/non-cognitive symptoms in Alzheimer's disease: relevance to pathogenesis and therapy. *Front. Aging Neurosci.* 6:171. doi: 10.3389/fnagi.2014.00171

Taljaard, D. S., Olaithe, M., Brennan-Jones, C. G., Eikelboom, R. H., and Bucks, R. S. (2016). The relationship between hearing impairment and cognitive function: a meta-analysis in adults. *Clin. Otolaryngol.* 41, 718–729. doi: 10.1111/coa.12607

Tan, W. J. T., Thorne, P. R., and Vlajkovic, S. M. (2016). Characterisation of cochlear inflammation in mice following acute and chronic noise exposure. *Histochem. Cell Biol.* 146, 219–230. doi: 10.1007/s00418-016-1436-5

Terao, K., Cureoglu, S., Schachern, P. A., Morita, N., Nomiya, S., Deroee, A. F., et al. (2011). Cochlear changes in presbycusis with tinnitus. *Am. J. Otolaryngol.* 32, 215–220. doi: 10.1016/j.amjoto.2010.02.001

Thambisetty, M., Beason-Held, L., An, Y., Kraut, M. A., and Resnick, S. M. (2010). APOE ε4 genotype and longitudinal changes in cerebral blood flow in normal aging. *Arch. Neurol.* 67, 93–98. doi: 10.1001/archneurol.2009.913

Thomson, R. S., Auduong, P., Miller, A. T., and Gurgel, R. K. (2017). Hearing loss as a risk factor for dementia: a systematic review. *Laryngoscope Investig. Otolaryngol.* 2, 69–79. doi: 10.1002/lio2.65

Tiraboschi, P., Hansen, L. A., Alford, M., Masliah, E., Thal, L. J., and Corey-Bloom, J. (2000). The decline in synapses and cholinergic activity is asynchronous in Alzheimer's disease. *Neurology* 55, 1278–1283. doi: 10.1212/WNL.55.9.1278

Tiraboschi, P., Hansen, L. A., Alford, M., Merdes, A., Masliah, E., Thal, L. J., et al. (2002). Early and widespread cholinergic losses differentiate dementia with lewy bodies from Alzheimer disease. *Arch. Gen. Psychiatry* 59, 946–951. doi: 10.1001/archpsyc.59.10.946

Tsartsalis, S., Xekardaki, A., Hof, P. R., Kövari, E., and Bouras, C. (2018). Early Alzheimer-type lesions in cognitively normal subjects. *Neurobiol. Aging* 62, 34–44. doi: 10.1016/j.neurobiolaging.2017.10.002

Tyler, R., Cacace, A., Stocking, C., Tarver, B., Engineer, N., Martin, J., et al. (2017). Vagus nerve stimulation paired with tones for the treatment of tinnitus: a prospectiverandomized double-blind controlled pilot study in humans. *Sci. Rep.* 7:11960. doi: 10.1038/s41598-017-12178-w

Uchida, Y., Sugiura, S., Ueda, H., Nakashima, T., Ando, F., and Shimokata, H. (2014). The association between hearing impairment and polymorphisms of genes encoding inflammatory mediators in Japanese aged population. *Immun. Ageing* 11:18. doi: 10.1186/s12979-014-0018-4

Ukai, S., Yamamoto, M., Tanaka, M., Shinosaki, K., and Takeda, M. (2007). Donezepil in the treatment of musical hallucinations. *Psychiatry Clin. Neurosci.* 61, 190–192. doi: 10.1111/j.1440-1819.2007.01636.x

van Gool, W. A., van de Beek, D., and Eikelenboom, P. (2010). Systemic infection and delirium: when cytokines and acetylcholine collide. *Lancet* 375, 773–775. doi: 10.1016/S0140-6736(09)61158-2

Verret, L., Mann, E. O., Hang, G. B., Barth, A. M., Cobos, I., Ho, K., et al. (2012). Inhibitory interneuron deficit links altered network activity and cognitive dysfunction in Alzheimer model. *Cell* 149, 708–721. doi: 10.1016/j.cell.2012.02.046

Verschuur, C., Agyemangprempeh, A., and Newman, T. A. (2014). Inflammation is associated with a worsening of presbycusis: evidence from the MRC national study of hearing. *Int. J. Audiol.* 53, 469–475. doi: 10.3109/14992027.2014.891057

Waechter, S., and Brännström, K. J. (2015). The impact of tinnitus on cognitive performance in normal-hearing individuals. *Int. J. Audiol.* 54, 845–851. doi: 10.3109/14992027.2015.1055836

Wallhagen, M. I., Strawbridge, W. J., and Shema, S. J. (2008). The relationship between hearing impairment and cognitive function: a 5-year longitudinal study. *Res. Gerontol. Nurs.* 1, 80–86. doi: 10.3928/19404921-20080401-08

Wang, Z. F., Wang, J., Zhang, H. Y., and Tang, X. C. (2008). Huperzine A exhibits anti-inflammatory and neuroprotective effects in a rat model of transient focal cerebral ischemia. *J. Neurochem.* 106, 1594–1603. doi: 10.1111/j.1471-4159.2008.05504.x

Wang, Y., Wei, Y., Oguntayo, S., Jensen, N., Doctor, B. P., and Nambiar, M. P. (2011). [+]-Huperzine A protects against soman toxicity in guinea pigs. *Neurochem. Res.* 36, 2381–2390. doi: 10.1007/s11064-011-0564-5

Wang, H., Yu, M., Ochani, M., Amella, C. A., Tanovic, M., Susarla, S., et al. (2003). Nicotinic acetylcholine receptor α7 subunit is an essential regulator of inflammation. *Nature* 421, 384–388. doi: 10.1038/nature01339

Wang, J., Zhang, H. Y., and Tang, X. C. (2010). Huperzine a improves chronic inflammation and cognitive decline in rats with cerebral hypoperfusion. *J. Neurosci. Res.* 88, 807–815. doi: 10.1002/jnr.22237

Weaver, J. D., Huang, M. H., Albert, M., Harris, T., Rowe, J. W., and Seeman, T. E. (2002). Interleukin-6 and risk of cognitive decline: MacArthur studies of successful aging. *Neurology* 59, 371–378. doi: 10.1212/wnl.59.3.371

Wong, L. L. N., Yu, J. K. Y., Chan, S. S., and Tong, M. C. F. (2014). Screening of cognitive function and hearing impairment in older adults: a preliminary study. *Biomed Res. Int.* 2014:867852. doi: 10.1155/2014/867852

Wu, S. T., and Chiu, C. J. (2016). Age-related trajectories of memory function in middle-aged and older adults with and without hearing impairment. *Neuroepidemiology* 46, 282–289. doi: 10.1159/000445378

Xie, L., Jiang, C., Wang, Z., Yi, X., Gong, Y., Chen, Y., et al. (2016). Effect of Huperzine A on Aβ-induced p65 of astrocyte *in vitro*. *Biosci. Biotechnol. Biochem.* 80, 2334–2337. doi: 10.1080/09168451.2016.1222265

Yaffe, K., Kanaya, A., Lindquist, K., Simonsick, E. M., Harris, T., Shorr, R. I., et al. (2004). The metabolic syndrome, inflammation and risk of cognitive decline. *JAMA* 292, 2237–2242. doi: 10.1001/jama.292.18.2237

Yi, F., Ball, J., Stoll, K. E., Satpute, V. C., Mitchell, S. M., Pauli, J. L., et al. (2014). Direct excitation of parvalbumin-positive interneurons by M1 muscarinic acetylcholine receptors: roles in cellular excitability, inhibitory transmission and cognition. *J. Physiol.* 592, 3463–3494. doi: 10.1113/jphysiol.2014.275453

Yoshiyama, Y., Kojima, A., Itoh, K., Isose, S., Koide, M., Hori, K., et al. (2015). Does anticholinergic activity affect neuropathology implication of neuroinflammation in Alzheimer's disease. *Neurodegener. Dis.* 15, 140–148. doi: 10.1159/000381484

Yoshikawa, H., Kurokawa, M., Ozaki, N., Nara, K., Atou, K., Takada, E., et al. (2006). Nicotine inhibits the production of proinflammatory mediators in human monocytes by suppression of i-κb phosphorylation and nuclear factor-κb transcriptional activity through nicotinic acetylcholine

receptor α7. *Clin. Exp. Immunol.* 146, 116–123. doi: 10.1111/j.1365-2249.2006.03169.x

Zappettini, S., Grilli, M., Lagomarsino, F., Cavallero, A., Fedele, E., and Marchi, M. (2011). Presynaptic nicotinic α7 and non-α7 receptors stimulate endogenous GABA release from rat hippocampal synaptosomes through two mechanisms of action. *PLoS One* 6:e16911. doi: 10.1371/journal.pone.0016911

Zhang, X., Zhou, K., Wang, R., Cui, J., Lipton, S. A., Liao, F. F., et al. (2007). Hypoxia-inducible factor 1α (HIF1α)-mediated hypoxia increases BACE1 expression and β-amyloid generation. *J. Biol. Chem.* 282, 10873–10880. doi: 10.1074/jbc.M608856200

Zhong, P., Gu, Z., Wang, X., Jiang, H., Feng, J., and Yan, Z. (2003). Impaired modulation of GABAergic transmission by muscarinic receptors in a mouse transgenic model of Alzheimer's disease. *J. Biol. Chem.* 278, 26888–26896. doi: 10.1074/jbc.M302789200

Zilles, D., Zerr, I., and Wedekind, D. (2012). Successful treatment of musical hallucinations with the acetylcholinesterase inhibitor donezepil. *J. Clin. Psychopharmacol.* 32, 422–424. doi: 10.1097/JCP.0b013e318253a086

Zlokovic, B. V. (2008). The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron* 57, 178–201. doi: 10.1016/j.neuron.2008.01.003

Zlokovic, B. V. (2011). Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. *Nat. Rev. Neurosci.* 12, 723–738. doi: 10.1038/nrn3114

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Theoretical Tinnitus Framework: A Neurofunctional Model

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Subjective tinnitus is the conscious (attended) awareness perception of sound in the absence of an external source and can be classified as an auditory phantom perception. Earlier literature establishes three distinct states of conscious perception as unattended, attended, and attended awareness conscious perception. The current tinnitus development models depend on the role of external events congruently paired with the causal physical events that precipitate the phantom perception. We propose a novel Neurofunctional Tinnitus Model to indicate that the conscious (attended) awareness perception of phantom sound is essential in activating the cognitive-emotional value. The cognitive-emotional value plays a crucial role in governing attention allocation as well as developing annoyance within tinnitus clinical distress. Structurally, the Neurofunctional Tinnitus Model includes the peripheral auditory system, the thalamus, the limbic system, brainstem, basal ganglia, striatum, and the auditory along with prefrontal cortices. Functionally, we assume the model includes presence of continuous or intermittent abnormal signals at the peripheral auditory system or midbrain auditory paths. Depending on the availability of attentional resources, the signals may or may not be perceived. The cognitive valuation process strengthens the lateral-inhibition and noise canceling mechanisms in the mid-brain, which leads to the cessation of sound perception and renders the signal evaluation irrelevant. However, the "sourceless" sound is eventually perceived and can be cognitively interpreted as suspicious or an indication of a disease in which the cortical top-down processes weaken the noise canceling effects. This results in an increase in cognitive and emotional negative reactions such as depression and anxiety. The negative or positive cognitive-emotional feedbacks within the top-down approach may have no relation to the previous experience of the patients. They can also be associated with aversive stimuli similar to abnormal neural activity in generating the phantom sound. Cognitive and emotional reactions depend on general personality biases toward evaluative conditioning combined with a cognitive-emotional negative appraisal of stimuli such as the case of people with present hypochondria. We acknowledge that the projected Neurofunctional Tinnitus Model does not cover all tinnitus variations and patients. To support our model, we present evidence from several studies using neuroimaging, electrophysiology, brain lesion, and behavioral techniques.

Keywords: tinnitus modeling, cognitive processes in tinnitus, attention role in tinnitus, tinnitus brain network, evaluation learning role in tinnitus

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INTRODUCTION

Tinnitus has been described as the conscious perception of sounds, usually hissing or ringing, in the absence of an external sound source. It can be a significant annoyance and can noticeably decrease the quality of life. Statistically, most of the people who suffer from tinnitus tend to live with the condition without seeking any treatment. Tinnitus affects 30% of the general population, mostly affecting the elderly population. 6% have debilitating symptoms (Heller, 2003), and an equal 6% prevalence has also been found in children (Mills et al., 1986; Coelho et al., 2007; Savastano, 2007).

In many cases, tinnitus is a serious condition that becomes a chronic problem and is often reported as "annoying" and "severely affecting quality of life." It has been demonstrated that 60% of tinnitus patients suffered from lifetime and 55% suffered from current psychiatric disorders, while depression and anxiety were the most common types of comorbidity disorders (Malakouti et al., 2011). Over the past few decades, several hypotheses have attempted to explain tinnitus. A few of them have reached animal and human trials and successfully received acceptance by the academic and clinical communities and reached diagnosis and/or rehabilitation product stages.

Several animal models have attempted to explain tinnitus by revealing its physiological characteristics in different processing centers of the auditory system. In the dorsal cochlear nucleus (DCN), enhanced firing rates were distinguished after intense acoustic exposure (Kaltenbach et al., 1998; Brozoski et al., 2002; Chang et al., 2002). In the inferior colliculus, elevated firing rates were distinguished after large doses of salicylate induction (Jastreboff and Sasaki, 1986; Chen and Jastreboff, 1995). In addition, it has been established that noise trauma generates hyperactivity in the auditory cortex (Eggermont and Komiya, 2000; Seki and Eggermont, 2003; Zhang et al., 2011). Other tinnitus-related animal studies include the following, tonotopic reorganization in auditory cortex (Eggermont and Roberts, 2004; Eggermont, 2006; Stolzberg et al., 2011), increase in spontaneous activity in DCN and the inferior colliculus (Kaltenbach and McCaslin, 1996; Zhang and Kaltenbach, 1998; Noreña and Eggermont, 2003), magnification in auditory central gain (Sun et al., 2009; Zeng, 2013; Auerbach et al., 2014), and synchronization of neuronal activities (Strauss et al., 2005, 2008; Dominguez et al., 2006; Lorenz et al., 2009). Moreover, a recent imaging study also demonstrated that different inhibitory and excitatory neurotransmitters modulate the tinnitus-dependent hyperactivity (Middleton et al., 2011).

Furthermore, neuroanatomical and activation alteration of the auditory pathways were correlated with abnormal activities in the non-auditory brain areas in tinnitus patients vs. control volunteers, specifically by means of Magnetic Resonance Imaging (MRI) (Crönlein et al., 2007; Langguth et al., 2007; Landgrebe et al., 2009; Schneider et al., 2009; Husain et al., 2011; Boyen et al., 2013), Functional MRI (Smits et al., 2007; Lanting et al., 2008), Positron Emission Tomography (PET) (Arnold et al., 1996; Lockwood et al., 1998; Andersson et al., 2000; Langguth et al., 2006; Plewnia et al., 2007), Single-Photon Emission Computerized Tomography (SPECT), (Gardner et al., 2002; Marcondes et al., 2006), and Magnetoencephalography (MEG) (Mühlnickel et al., 1998).

Integrating information about tinnitus from different studies can provide general tinnitus models (Jastreboff et al., 1988b; Jastreboff and Hazell, 2004; Tyler et al., 2008; Rauschecker et al., 2010). Jastreboff et al. (1988a) proposed the neurophysiological model of tinnitus (NM), which resulted in a tinnitus management procedure known as Tinnitus Retraining Therapy. NM differs radically from previous models due to the following postulations: (a) Tinnitus is a phantom auditory perception (Jastreboff, 1990); (b) Tinnitus occurs due to the interaction of different brain networks with auditory pathways that result in the conscious perception of the phantom sound. Principally, the limbic system is responsible for growth of tinnitus annoyance (Jastreboff, 1990); (c) Perception of tinnitus is not necessarily the key element that causes tinnitus to be problematic, and it is possible to have reactions to the tinnitus signal without perceiving it (Jastreboff and Jastreboff, 2006); (d) Sustained over-activation of the sympathetic autonomic nervous system is largely responsible for the behavioral manifestation of tinnitus-induced problems (Jastreboff and Jastreboff, 2006); and (e) Once habituation of reactions is sufficiently advanced and the tinnitus signal becomes neutral and unimportant, the habituation of perception follows automatically (Jastreboff and Jastreboff, 2006).

NM has considered the involvement of several areas of the central nervous system as well as the autonomous nervous system. The flow of information commonly starts with sound wave stimulation of the peripheral auditory system. The next stage is a two-way connection with the "Auditory Subconscious" regions. The following stage, via a two-way connection, is the "Auditory and Other Cortical Areas" process perception and evaluation of sound and includes functions such as consciousness, memory, and attention. The last neuroanatomical and functional component in NM is the "Limbic System," which is illustrated in **Figure 1**. The "Limbic System" is connected via a two-way link to components of "Auditory Subconscious" and "Auditory & Other Cortical Areas." It is also connected to the "Autonomic Nervous System," influencing neuroendocrine and autonomic reactions, such as respiratory, circulatory, digestive and hormonal. The "Autonomic Nervous System" sends inputs to the "Auditory Subconscious" component as well as sends and receives inputs from the "Auditory & Other Cortical Areas." The sixth and the last component is termed "Reactions", which refers to clinical observations such as annoyance, anxiety, panic, sleep, and concentration disturbances. These "Reactions" have two-way connections to the "Limbic System," "Autonomic Nervous System," and the "Auditory and Other Cortical Areas."

NM hypothesizes prediction of tinnitus perception in subjects who do not have any clinical symptoms, where the limbic and autonomic nervous systems are not activated, and no reactions can be observed; abnormal neuronal activities are processed as sourceless soundwaves by the peripheral auditory system, which originate in the auditory periphery and move through the auditory pathways to the primary auditory cortex and other cortical areas. Conscious perception of the sound wave only occurs during the final cortical stages.

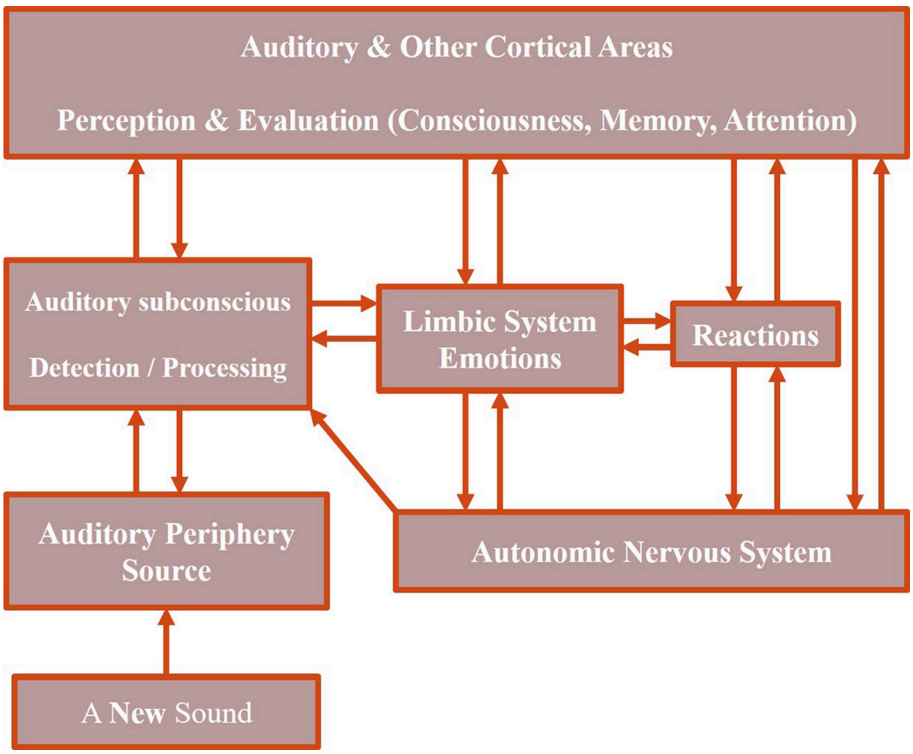

FIGURE 1 | Neurophysiological model of tinnitus. Adapted from Jastreboff and Jastreboff (2006).

Additionally, tinnitus develops by the generation of abnormal neural activities in the auditory pathways. When detected by the upper-stream components of the auditory pathways, it is processed at the subconscious levels. Auditory and other cortical areas are activated and sound is consciously perceived. This conscious perception, evoked by abnormal neural activity, does not elicit any emotional or behavioral reactions other than the mere perception of sound.

Furthermore, the abnormal neural activities are evaluated subconsciously and consciously. If they are evaluated as representing a neutral event, they will not be perceived consciously. However, if the neural activities are evaluated negatively or as unknown, they will be classified as potentially unpleasant and/or dangerous which activate the limbic and autonomic nervous systems and subsequently generate negative reactions such as annoyance. Future perception of similar neural activities will receive more attention than usual and become evaluated. To create a condition of "reflex arc," it is sufficient to experience tinnitus when at a high-level of negative emotional/autonomic state. The initial reflex arc will be created automatically. This reflex has a strong tendency to become stronger as both the signal (tinnitus) and reinforcement (reactions of the limbic and autonomic nervous systems) are contiguously present, corresponding to continuous learning that enhances the strength of the reflex.

Rauschecker et al. (2010) proposed another tinnitus model based on noise cancelation mechanism in which efferent projections from the subcallosal area are involved in the suppression of tinnitus signal as a sensory input at the thalamic level of brain processes. Functionally, the nucleus accumbens (NAc) and its correlated paralimbic circuitry were considered in the ventromedial prefrontal cortex (vmPFC), exhibiting a pivotal performance in long-term habituation to continuous unpleasant sounds.

It was revealed that, in order to be perceived consciously, sound-evoked neural activity is passed from the auditory periphery through the brainstem and thalamus (MGN: medial geniculate nucleus) to the auditory cortex. For emotional content evaluation of the sound, the same signal is conducted in parallel over the amygdala to the subcallosal area (which includes the NAc region of the ventral, "limbic" striatum, and the vmPFC). The thalamic reticular nucleus (TRN) receives excitatory feedback projections from the subcallosal area. TRN consecutively applies selective inhibition at the sections of the MGN corresponding to the unpleasant sound frequencies. It was also suggested this gain-control mechanism results in a highly specific filtering ("tuning out") of repetitive unwanted noises, which do not reach conscious perception in the auditory cortex as exhibited in **Figure 2**. It was recommended that if the abnormal neural activity in the peripheral system originated the sourceless tinnitus, the sound signal was being filtered out at thalamic MGN and would not be relayed to the auditory cortex in normal tuning out process. NAc-system weakening may no longer result in the tinnitus signal cancelation

at the thalamic level and lead to tinnitus perception and long-term reorganization of auditory cortex, resulting in the tinnitus being carried out to the chronic phenomena. It was also implied that intermittent tinnitus might arise during the developing damage to the subcallosal area, which could strongly justify the fluctuating activity (and corresponding neurotransmitter) levels and transient filtering of the tinnitus signal.

The integrative model of the auditory phantom perception is a recent proposal, which conceptualizes that "tinnitus core" subnetworks incorporate neurophysiological model and noise canceling process. The discussion theorizes that minimal brain areas (auditory cortex, inferior parietal area, and ventromedial prefrontal/frontopolar cortex) jointly activate to achieve the conscious perception of tinnitus. The hypothesis assumes that separable tinnitus characteristics can be extracted by evaluating resting-state magnetic and electrical studies toward the evaluation of specific characteristics and control of other parameters. Furthermore, the combination of functional neuroimaging with neuromodulation studies could provide some causal relationship between the acquired correlated networks. It was proposed that the tinnitus could be perceived as an emergent aspect of several dynamic overlapping subnetworks with different spontaneous oscillatory patterns and functional connectivity arrangement. It was theorized that communications within different subnetworks would take place in hubs, which are defined as brain regions that simultaneously participate in various brain networks and can be involved in distinct subnetworks at discrete oscillatory frequencies (De Ridder et al., 2014). The integrative model conceptualizes that the tinnitus core is comprised of the neural correlation of auditory pitch awareness and memory. Furthermore, it was hypothesized that the tinnitus core connects to other subnetworks via hubs and leads to bothersome effects such as mood disorders, distress, and lateralization. Integrative tinnitus model is illustrated in **Figure 3**.

It is important to note that all the aforementioned studies are limited in their scope as they have focused only on functional tinnitus-related activities of isolated regions. These studies have concentrated their efforts on structural dimensions, solo behavioral, and absolute clinical investigations. They are abstract in their descriptions and are only supported by minimal empirical evidence.

Therefore, the objective of this study is to describe a neurofunctional model of tinnitus, which can predict future results, while considering a testable framework of the structural, functional, behavioral, and clinical empirical evidence.

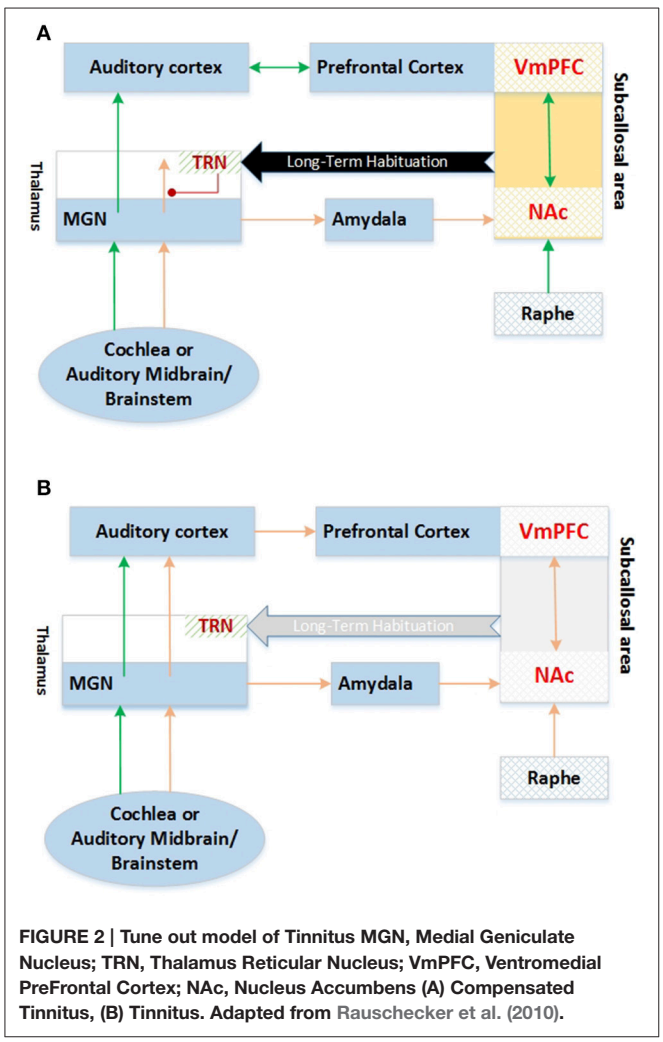

FIGURE 2 | Tune out model of Tinnitus MGN, Medial Geniculate Nucleus; TRN, Thalamus Reticular Nucleus; VmPFC, Ventromedial PreFrontal Cortex; NAc, Nucleus Accumbens (A) Compensated Tinnitus, (B) Tinnitus. Adapted from Rauschecker et al. (2010).

FUNDAMENTAL IDEAS AND POSTULATIONS OF NEUROFUNCTIONAL TINNITUS MODEL

By considering previous functional and structural neuroimaging techniques, quantitative electroencephalography (qEEG),

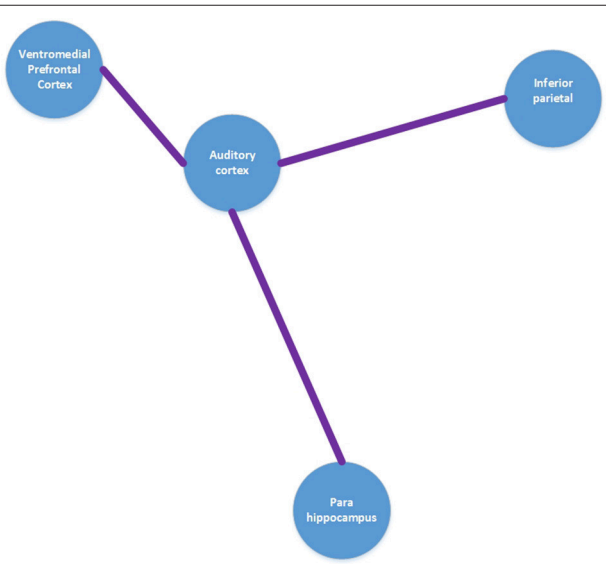

FIGURE 3 | Tinnitus core model. Adapted from De Ridder et al. (2014).

magnetoencephalography (MEG), and animal lesion studies, distinct brain areas have been implicated in tinnitus. These areas are as follows: the peripheral auditory system, the thalamus (reticular, medial geniculate and dorsal nuclei), auditory cortex, the limbic system (anterior cingulate cortex, amygdala), brainstem (raphe nucleus), subcallosal and paralimbic areas, which include basal ganglia (ventral palladium), striatum (nucleus accumbens), and vmPFC (Ghodratitoostani et al., 2016). **Figure 3** provides a schematic tinnitus network overview of different brain areas as composed by integrating data from SPECT, PET, fMRI and MEG studies research in tinnitus.

Authors agree that the continuous or intermittent abnormal neuronal activity at the peripheral (Kaltenbach et al., 1998; Brozoski et al., 2002; Chang et al., 2002), midbrain (Jastreboff and Sasaki, 1986; Chen and Jastreboff, 1995), auditory paths (Kaltenbach and McCaslin, 1996; Zhang and Kaltenbach, 1998; Eggermont and Komiya, 2000; Noreña and Eggermont, 2003; Seki and Eggermont, 2003; Zhang et al., 2011)or associative cortices such as limbic area (Zikopoulos and Barbas, 2007; Yu et al., 2009; Kaping et al., 2011; Weinberger, 2011; Kuchinke et al., 2015) can cause phantom sound generation. This aberrant neural activity can be generated by any type of acoustic traumas, aging, brain lesions, and medicine. The Neurofunctional Tinnitus Model hypothesizes that the perception of sound fundamentally depends on the allocation of attentional resources via frontal cortex, which in turn, depends on the cognitive-emotional value and the relevance of the phantom stimulus to the context. Small value of stimuli has less chance to allocate attentional resources. Noise-canceling mechanisms at the thalamic MGN level, which are governed by TRN inhibitory projections, maintain the weakness of the upward irrelevant signal. However, high cognitive-emotional value of sensory stimuli may allocate adequate attentional resources and trigger a top-down suppression process that acts on the thalamus noise-canceling mechanism and may lead to the awareness perception of the tinnitus phantom sound. Initially, the phantom sound is considered neutral, which we have defined as the "Neutral stage" within the Neurofunctional Tinnitus Model as illustrated in **Figure 4A**.

Authors hypothesize that the malfunction of the noise canceling mechanism reinforces higher rates of the phantom sound that reaches the auditory cortex and consequently facilitates the abnormal neural plasticity in auditory cortex and tonotopic reorganization coined as "Central tinnitus." Centralization can develop during both neutral and clinical stages of the Neurofunctional Tinnitus Model**.**

The patient's general suspicion can result in negative cognitive interpretation. This negative appraisal and evaluative conditioning (EC) learning mechanism, jointly enhance the associated cognitive-emotional value and the persistence of the sound conscious (attended) awareness perception. Therefore, the probability of limbic system involvement and the appearance of negative cognitive-emotional reactions increase (such as attention deficit, anxiousness, insomnia, and phobias). If the cognitive-emotional value remains at low levels, tinnitus will cease or be perceived as the failure of noise canceling mechanism and suppression at the thalamic level. This condition is identified as "Clinical Distress stage" of Neurofunctional Tinnitus Model as exhibited in **Figure 4B.**

It is postulated that rapidly encoded tinnitus, such as acoustic trauma tinnitus-induced, relates to temporary interactions between the hippocampus and auditory neocortical processing regions. Due to complexity reduction, we ignore this extraordinary transient hippocampus involvement in Neurofunctional Tinnitus Model.

Model Compartments

Auditory Peripheral and Cochlear

This includes external-middle ears organs and cochlea, which receive acoustical pressure and sound as input and propagate electrical signals from auditory nerves (Ghodratitoostani et al., 2016).

Auditory Midbrain/Brainstem

This includes cochlear nucleus, superior olivary complex (SOC), inferior colliculus auditory pathway, and raphe nucleus. Cochlear nucleus sends projection to SOC and inferior colliculus and receives input from, auditory nerve which encompasses cochlea (Kraus and Canlon, 2012; Ghodratitoostani et al., 2016). The SOC receives inputs from the cochlear nucleus and relays the signal to inferior colliculus (Kraus and Canlon, 2012; Ghodratitoostani et al., 2016). We have ignored the internal parts of the auditory midbrain/brainstem compartment to decrease the complexity of the model.

Raphe Nucleus

The serotonin originates in the raphe nucleus of the cerebellum. Its non-equilibrium can disturb normal sleep (Ghodratitoostani et al., 2016).

Thalamic Area

It is located between cerebral cortex and midbrain. Despite the several sections of the thalamic area, we have proposed that medial geniculate nucleus (MGN), mediodorsal nucleus (MDN), and TRN play crucial roles in tinnitus generation. The **MGN** acts as a thalamus relay in the auditory system that receives excitatory inputs from auditory midbrain (IC), auditory cortex, and inhibitory inputs from TRN. In return, it sends excitatory projections into TRN, auditory cortex, and amygdala. The **MDN** sends excitatory output to TRN and vmPFC and receives excitatory projections from vmPFC and ventral palladium of the subcallosal area. **TRN** receives excitatory inputs from MGN, auditory cortex, MDN, vmPFC and amygdale. It sends inhibitory projections to thalamus MGN and MDN (Ghodratitoostani et al., 2016).

Auditory Cortex

It includes primary and secondary and associative auditory cortices, which receive excitatory inputs from MGN and send excitatory projections to MGN, TRN, prefrontal cortex, and amygdala (Ghodratitoostani et al., 2016).

Subcallosal Area

It includes nucleus accumbens (**NAc**), ventral palladium (**VP**), and **vmPFC**. NAc receives excitatory inputs from the amygdala.

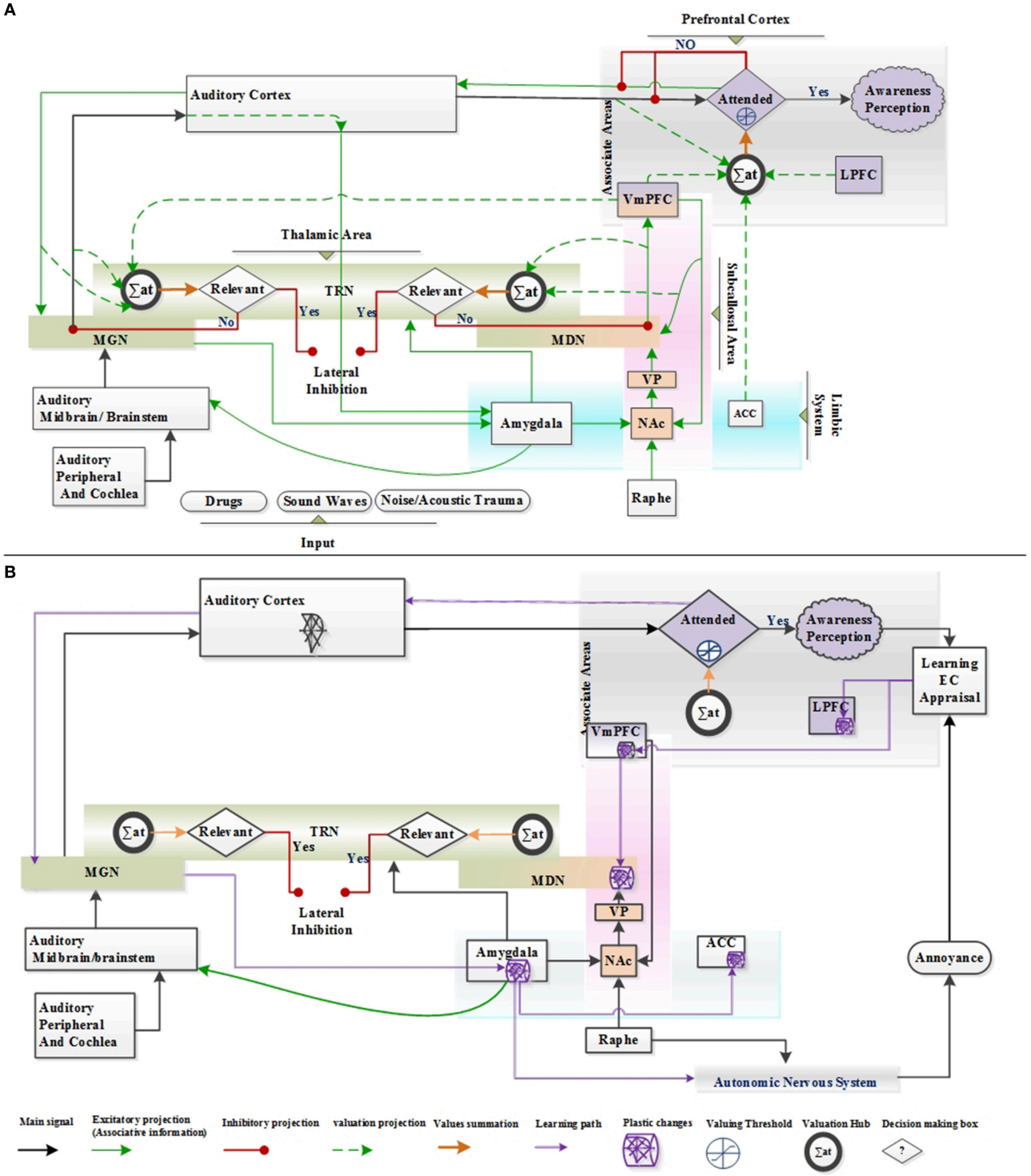

FIGURE 4 | Anatomical structure of the proposed Neurofunctional Tinnitus Model; LPFC, Lateral prefrontal cortex; vmPFC, ventromedial prefrontal cortex; NAc, nucleus accumbens; ACC, anterior cingulate cortex; MGN, medial geniculate nucleus; TRN, thalamic reticular nucleus; VP, ventral palladium; MDN, medial dorsal nucleus; Basal ganglia include VP and NAc; EC, Evaluation Conditioning. (A) Phantom sound perception without negative reaction is defined as "Neutral stage" within the Neurofunctional Tinnitus Model; **(B)** The evaluation conditioning and appraisal learning procedure and anatomical part of plastic change in tinnitus brain network, is identified as "Clinical Distress stage" of the Neurofunctional Tinnitus Model.

The vmPFC accepts serotonergic inputs from raphe nucleus, which trigger VP via unidirectional excitatory projection. VP receives excitatory input from NAc and sends excitatory projection to MDN (Ghodratitoostani et al., 2016).

Prefrontal Cortex

It includes lateral prefrontal cortex (**lPFC**), ventromedial prefrontal cortex (vmPFC, and associative cortex in which lPFC and vmPFC send excitatory outputs into the valuation hub of attentional allocation as a cognitive-emotional value. The vmPFC sends excitatory projections to TRN, MGN, MDN, and NAc (Ghodratitoostani et al., 2016).

Limbic System

It includes amygdala and anterior cingulate cortex (**ACC**). The ACC sends output to the valuation hub as a purely emotional value of familiar known audio signals. The amygdala sends excitatory projections into TRN, midbrain (IC) and NAc. The NAc projection may trigger vmPFC via VP of the subcallosal area. Amygdala receives excitatory projections from MGN and auditory cortex (Ghodratitoostani et al., 2016).

Cognitive Processes Involved In Initiating Tinnitus "Neutral Stage," Noise Canceling Mechanism, and Lateral Inhibition Circuitry

The bottom-up selective attention processes support the suppression of irrelevant stimuli, which may occur at the early stages by the TRN along the "lateral inhibition mechanism" (Kiang et al., 1970; Tyler, 2006; Zikopoulos and Barbas, 2012). The thalamocortical neurons, carrying the relevant stimuli up from thalamus to cortex via TRN, excite the adjacent TRN neurons which in turn inhibit the irrelevant thalamic cortical neuron carriers. These adjacent TRN neurons, inhibit the TRN neurons connected to the relevant thalamocortical carriers leading to dis-inhibition of the relevant signal carriers (Pinault and Deschênes, 1998). This mechanism of lateral inhibition ideally suppresses the noise originated from distracters and facilitates the processing of important stimuli. The amygdala, posterior orbito frontal cortex (pOFC), and mediodorsal (MD) thalamus ending at the TRN may suppress the signal of distracting stimuli at sensorial cortices (Zikopoulos and Barbas, 2007). TRN can perform gain–control function of the thalamocortical transmission in a highly localized manner. Due to serotonergic neurons in the subcallosal area, TRN inputs vigorously inhibit MGN neurons in the conscious state and anesthetic trials in a specific high frequency manner (Yu et al., 2009). Thalamus projections to TRN can modulate transmission from the sensory periphery and brainstem to the cerebral cortex (Yu et al., 2009).

Furthermore, voxel-based morphometric tinnitus patient studies revealed the reduction in gray matter in vmPFC that resulted in the decline of the vmPFC inhibitory output, leading to increased activity of NAc (Schlee et al., 2009). While auditory cortical activity is essential for conscious perception of phantom sound, NAc-TRN in Neurofunctional Tinnitus Model is postulated as noise canceling mechanism for preventing the unpleasant permanent sound to reach auditory cortices. This mechanism is in partial agreement with the tuned out model of tinnitus (Rauschecker et al., 2010).

The amygdala is a crucial component of noise canceling circuitry for processing sensory inputs with emotional value, which is related to both the mediodorsal thalamus nucleus (MD) and the orbitofrontal cortex (Ghashghaei et al., 2007).

Valuation Process

Selective attention prioritizes the processing of behaviorally relevant stimuli at the expense of processing of irrelevant stimuli (Tsotsos, 2011). Relevance of stimulus requires an active associated neuronal network to indicate its related value or its reward outcome in a special context (Kaping et al., 2011). Recent evidence proposes that the brain network regularly determines and processes the values related to the stimuli that effectively bias the attentional stimulus selection against the more valuable stimuli in the peripherals (Shuler and Bear, 2006; Seitz et al., 2009; Anderson et al., 2011). Neural clusters related to valuations within the vmPFC were disassociated from the top-down goals network in spatial attentions. Behavioral analysis suggested that shifting attention to less important stimuli required specific mechanism to overcome a motivational bias of attending to the more important stimuli (Anderson et al., 2011).

In a recent paper, the visual valuation hub demonstrated that the value-selection response correlated with the activity of neurons located across the medial to lateral extent of the PFC (vmPFC), the anterior cingulate cortex (ACC), and the lateral PFC (Kaping et al., 2011). The highly valued stimuli suppression in human and related macaque studies have been conceptualized as a "self-control" process (Kaping et al., 2011), which is associated with the alterations in neural activity level of the dorsolateral PFC and the rostral ACC in human subjects (Hare et al., 2009). Together, these findings propose that the valuation hub plays a similar role across different sensory modalities and that the same process within the auditory stimuli can drive attentional resources to hear tinnitus in the Neurofunctional Tinnitus Model.

Conscious Awareness Perception Processes and Attention

The ability to consciously report sensory inputs is theorized as the perception process. We propose to add more details to the model of distinction between awareness and attention (Lamme, 2003; Watanabe et al., 2011). Conscious inputs originate from triggers in sensory pathways or can be retrieved from cortices and memory.

The proposed Conscious perception process (CPP) conceptual model provides logical solutions as depicted in **Figure 5**. It discriminates the inputs in conscious and unconscious states in the initial stages. In this approach, the consciousness level alters from deep sleep and reaches wakefulness. The wakefulness levels dynamically fluctuate during awakeness.

The triggered inputs, coming through sensory pathways, are evaluated via automated bottom-up valuation processes. The relevant and novel signals along with pulled memory signals reach the top-down valuation process stage. The top-down

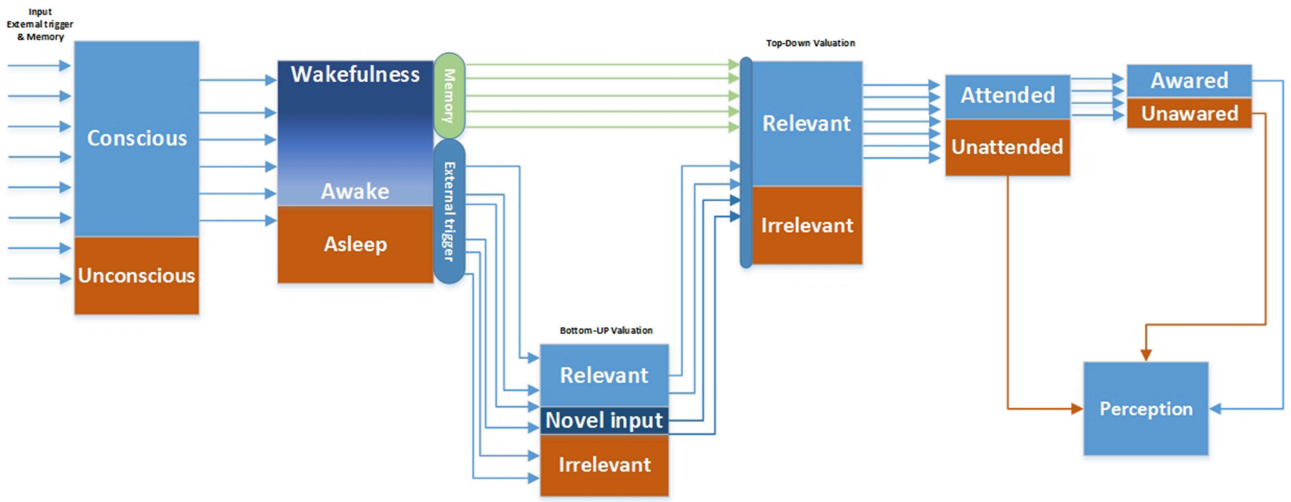

FIGURE 5 | Conscious perception process model.

relevant (cognitive emotional valued) signal is permitted to reach the attentive processes whereby the signal can become aware or unaware (Graziano and Webb, 2015).

According to the CPP conceptual framework, conscious perception can be characterized into unattended, attended, and attended awareness. Attention represents a cognitive mechanism that allows certain information to be processed more intensively. Attention facilitates the transmission of the selected information across the cortex in comparison to the non-attended information (Cohen et al., 2012). Conscious awareness requires attention because it permits information to remain on-line long enough to be completely processed by an expanded network of cortical associations (Cohen et al., 2012). Structurally, the lateral prefrontal cortex (LPFC) is associated with attention and inhibitory control (Sasaki et al., 1989; MacDonald et al., 2000; Ploner et al., 2005), particularly in auditory gating (Woods and Knight, 1986; Knight et al., 1989).

By considering the Neurofunctional Tinnitus Model and the CPP, we proposed that the clinical distress tinnitus could only be developed and maintained during conscious (attended) awareness perception of neutral tinnitus. Several studies have reported no tinnitus-related neural activity in patients during coma, anesthetics, vegetative states, restless eye movement (REM) sleep, and dreams (De Ridder et al., 2014).

Furthermore, the top-down attention and conscious awareness processes suggest that the triggered inputs and phantom sound cannot be perceived without attended awareness. This finding is in agreement with the unconditional sensitization model, which proposed that the highly complex auditory tinnitus stimulus pattern is controlled by attention (Zenner et al., 2006).

Cognitive Processes Involved in the Centralization of Tinnitus

Acoustical Memory for Repeated Items

It was recently proposed that the memory systems should not be classified in association with the conscious states as the conscious states would not influence the encoding or retrieval of information (Henke, 2010). The critical factors seemed to be the quantity of elements required to encode the number and the duration of information presentations.

Rapidly encoded novel associations are postulated to relate interactions between the hippocampus and certain neocortical processing sites. In temporally extended learning state, the hippocampus might temporarily improve learning performance, but this is not essential for successful information retention, consolidation or retrieval. The memory system relies heavily on the interactions between neocortical structures, the basal ganglia, the cerebellum, and the parahippocampal gyrus (Henke, 2010; Hannula and Greene, 2012).

Considering the Henke model, abnormal neural activity or sound perception best fit the temporally extended learning system as related to tinnitus characteristic. The hippocampus structure is not necessarily involved in tinnitus generation; however, the paralimbic is involved in the generation of tinnitus. This proposal is supported by the results of a recent resting-state connectivity network fMRI study (Maudoux et al., 2012) which found no correlation between the hippocampus and resting-state network activity. However, it found a significant correlation between typical tinnitus connectivity networks and the parahippocampal, cerebellum, basal ganglia, subcallosal region, thalamic areas, and amygdala regions. It also exhibited a significant correlation between the anterior cingulate, auditory and prefrontal cortices (Maudoux et al., 2012).

These two studies suggest that clinical distress tinnitus is more prone to emerge from a gradual learning procedure rather than a single exposure to an external stimulus.

Cognitive Processes in Clinical Distress Stage

Cognitive-Emotional Appraisal

The cognitive-emotional appraisal is a mechanism that emerges as a reaction to the differences between the information stored

in memory and the actual information. Generally, patients have no intuitive understanding of tinnitus signal; thus, the risk of negative appraisal will increase. One of the crucial causes of appraisal is the patient's hypochondriac impression of neutral phenomena (de Maddalena and Pfrang, 1993a,b; Marciano et al., 2003). Since, the individual's response to illness is mainly shaped by their understanding of the illness, the personal disorder concept becomes an important factor (Zenner et al., 2006). Examples for such personality disorders include the potential triggers of tinnitus and available tinnitus managements and treatments leading to appraisals such as "tinnitus is detrimental to my health" or " now, I am becoming deaf" (Zenner et al., 2006).

Evaluation Conditioning (EC)

The Neurofunctional Tinnitus Model hypothesizes that negative symptoms related to tinnitus emerge in the neutral tinnitus stage together with the EC learning procedure. When presented in association with a negative or positive unconditioned stimulus (US), EC points to the change in the valence of the cognitive-emotionally neutral conditioned stimulus (CS). This change in the valence is the retained response to the previously neutral stimulus (De Houwer et al., 2001; Bar-Anan et al., 2010). Recently, studies have demonstrated that attention as the stimulus focus does not cause EC; rather, to promote the acquisition of contingency awareness, the attention to the contingencies between stimuli seem to be crucial in EC (Stahl et al., 2009; Kattner, 2012; Hütter and Sweldens, 2013). Furthermore, the ERP-EEG source localization indicated a role of medial-frontal brain regions as the likely origin of early valence discrimination signals (Kuchinke et al., 2015). The prevailing tinnitus models emphasize classical conditioning learning via pairing of the external events with the causal physical events.

We project that the Clinical Distress tinnitus stage is developed and maintained through the EC learning only in conscious (attended) awareness perception of CS (neutral tinnitus) and US (comorbidities reaction) contingency. EC encodes the memory through the learning pathway, **Figure 4B**. Encoding memory initiates plasticity in VMPFC and LPFC areas of frontal cortex which result in plasticity in several regions of the Limbic and Auditory systems via the subcallosal area and the corticothalamus auditory pathway.

In the subcallosal area pathway, the EC encoded memory consolidates in MDN to bias tinnitus valence of the automated bottom-up valuation process. Intermittent occurrences of EC learning reinforce the tinnitus valence. In the corticothalamus auditory pathway, the EC encoded memory leads to the plasticity of the MGN and lateral nucleus of amygdala (LA). LA connects to the central nucleus of the amygdala (CE) directly and via other amygdala regions. The output of the CE creates plasticity in ACC and controls the expression of the distress responses (such as fear), the related autonomic nervous system (e.g., blood pressure and heart rate), and endocrine (pituitary adrenal hormones) responses (Weinberger, 2011).

Pavlovian conditioning (PC) learning occurrence needs adequate frequency of pairing of the CS and US to encode the memory. Violation in synchrony of CS and US weakens the encoded memory. In return, EC learning (valence) only happens when pairing of the US and CS also incorporates awareness of contingency. Furthermore, unpaired occurrences of CS or US have no effects on valence. Therefore, we argue that EC learning is the only well-known mechanism which can explain tinnitus clinical distress development and maintenance.

Model Function and Information Flow

In this Neurofunctional Tinnitus Model, we proposed that in parallel with centralization, during the two stages of tinnitus development, phantom sound can lead to clinical symptoms. Initially, continuous or intermittent abnormal neuronal activities are developed in the peripheral or midbrain auditory paths or associative cortices (such as the limbic area). Several other factors such as different types of lesions, acoustic trauma, and drugs can also cause temporary abnormal activity in the auditory cortices. Depending on the availability of attentional resources, the phantom sound may be suppressed or perceived. Weaker cognitive emotional value of this potential phantom sound lowers the chances of its conscious (attended) awareness perception.

Irrelevance of the cognitive-emotional evaluations biases low valuation scores. This biasing of cognitive-emotional valuation strengthens the suppressive effects of both the lateral-inhibition of the bottom-up selective attention and the noise canceling mechanisms at the MGN thalamic nucleus. The noise canceling mechanism, governed by TRN inhibitory projections, reduces the frequency of phantom sound reaching the auditory cortex and maintains irrelevancy of the signal evaluation.

Based on the EC learning procedure and cognitive appraisal, phantom sound perception during Neutral tinnitus stage, gradually strengthens the negative valence of perceived tinnitus, and interprets it as suspicious and/or indicative of a disease. The cortical top-down processes weaken the noise canceling effects (Rauschecker et al., 2010). The phantom sound is considered a relevant stimulus which results in gradual formation of a vigilant auditory expectation of neutral tinnitus perception. The consequences are engendering the sense of cognitive and emotional reactions, which usually leads to negative reactions such as stress and depression (Halford and Anderson, 1991; Robinson, 2007; Robinson et al., 2007), anxiousness (Halford and Anderson, 1991; Langguth, 2011), hypochondriasis (Marciano et al., 2003), phobias (Zenner et al., 2006; Adjamian et al., 2009), and insomnia. These cognitive and emotional reactions, and related comorbidities, may cause tinnitus or tinnitus may cause these conditions. Ultimately, via the EC learning procedure, clinical distress tinnitus can be caused by the contingent relationship between the perceived relevant sound and negative valance of cognitive and emotional reactions.

Failure of lateral inhibition, caused by the effects of drugs, somatosensory modulation or other external negative events creates neuronal disturbances within the GABAergic pathways. These disturbances can cause abnormal activity where sourceless sounds may frequently reach the auditory cortex. However, regardless of the development of Clinical Distress stage from the conscious (attended) awareness perception of the neutral phantom sound, the neuroplasticity and long-term associative

memory consolidation forms in the auditory cortices. Depending on the availability of attentional resources, the phantom sound may be perceived. From this point on, we name the conscious perception of phantom sound as the neutral/clinical distress tinnitus. Furthermore, memory consolidation during centralization can bypass the noise canceling procedure, which strengthens the irrelevant cognitive-emotional value of the bottom-up attention. This leads to hyperactivity of noise canceling procedure, which is in agreement with the reduction of subcallosal gray matter volume. Correspondingly, the failure of noise canceling process is recognized in the amygdala evaluation procedure. The basolateral part of the amygdala receives excitatory projections from both MGN and auditory cortex which can trigger TRN to apply more inhibitory force for irrelevant stimuli. Persistence of abnormal activity in auditory peripheral is not necessary to the perception of tinnitus; however, it can reinforce neuroplasticity and associative memory.

NEUROFUNCTIONAL TINNITUS MODEL PREDICTIONS AND DISCUSSIONS

We hypothesize that conscious (attended) awareness perception is necessary for neutral tinnitus to turn to clinical distress tinnitus. This concept has not been disclosed in prior tinnitus models. In our opinion, unattended phantom sound or neutral tinnitus cannot cause bothersome or distress symptoms. The attended awareness to sourceless sound, allows information to remain on-line long enough to be thoroughly processed by a distributed network of cortical circuits, the limbic and autonomous nervous system.

Zenner et al. (2006) proposed a role for auditory attention in establishing the neural changes underlying tinnitus, although a specific mechanism for attention and the circumstances leading to its engagement were not described (Zenner et al., 2006). The Neurofunctional Tinnitus Model asserts that in order to perceive tinnitus, the valuation process in frontal cortex plays a crucial role in the prioritization of sensory inputs in attention resource allocation and lead to clinical distress tinnitus development. The three stages involved are: tinnitus generation, maintenance, and clinical distress development.

1. Tinnitus Generation: Our hypotheses regarding noise canceling mechanism failure is in agreement with the Winkler et al. (2009) model, which argues that, attention is considered to be a factor in modulating the detection of prediction failure (detection of deviance) and in promoting the stimulus-driven binding of sensory attributes to create new auditory objects in dynamic auditory environments. We further propose that such failure results in the perception of neutral phantom sound, which is not mentioned in Rauschecker's tuning out noise model (Winkler et al., 2009; Rauschecker et al., 2010; Roberts et al., 2013).
2. Tinnitus Maintenance: We argue that the auditory attention involvement (Roberts et al., 2013) and the discrepancy between top-down and bottom-up attentional processes can bias the cognitive-emotional value of the neutral phantom sound. We also hypothesize that the valuation hub in pre-frontal cortex continuously regulates the persistence of tinnitus perception and compares the value of intermittent attended awareness perceived tinnitus (phantom sound) with all sensory and auditory inputs. During conscious (attended) awareness of tinnitus, according to the patient's emotional stability, appraisal magnifies the cognitive-emotional value of tinnitus and results in increased duration of the perception. Furthermore, independent of patient perception, the continuous, and repetitive abnormal neural activities reaching the auditory cortex, form plasticity in auditory pathways and lead to the auditory memory of tinnitus "centralized tinnitus." This is in agreement with the Henke memory model (Henke, 2010; Roberts et al., 2013).
3. Tinnitus Clinical Distress: We propose that cognitive-emotional cognitive value of tinnitus increases via the negative appraisal and evaluative conditional learning mechanisms during conscious (attended) awareness perception of tinnitus. The tinnitus signal links with the limbic system and actuates annoyance, leading to clinical distress. We also agree that the conscious awareness of tinnitus can be suppressed and substantially modulated when the patients engage in cognitively demanding tasks (Searchfield et al., 2007; Searchfield and Kobayashi, 2012).

Due to the lack of contingency and pairing CS and US, the EC learning vs. conventional Pavlovian conditioning (PC) can strongly legitimize the emergence of various tinnitus symptoms in patients. However, to develop learning, both PC and EC are needed to simultaneously perceive CS (tinnitus) and US. This seems to be in violation of assumptions of the neurophysiological tinnitus model (Jastreboff, 1990). We propose that, not only conscious perception of tinnitus, but also conscious (attended) awareness perception of tinnitus contingency and US (distress symptom causes) are needed to develop, maintain, and rehabilitate tinnitus.

According to the Neurofunctional Tinnitus Model, the patients can be categorized into two general groups of neutral and clinical distress as summarized in **Table 1**. In the neutral stage, we have divided the patients into those who only perceive tinnitus (Type NT-I), those who perceive tinnitus with auditory cortex plasticity (Type NT-II), and those who do not perceive tinnitus and have no auditory cortex plasticity (Type NT-X). Since, biasing of their negative valence via EC may support future prevention programs, type X patients, who no longer perceive tinnitus, can become potential future tinnitus patients. The patients in the clinical stage can be further divided into two groups: those who perceive tinnitus and only have limbic system plasticity (Type CL-I) and those who have Type CL-I symptoms and auditory cortex plastic changes (Type CL-2). This novel view may help us investigate corresponding diagnostic assessment results in correct classifications and lead to the selection of the most appropriate rehabilitative methodologies.

Further experimental and clinical studies on tinnitus brain mapping, brain imaging, neuromodulation, cognitive behavioral therapy, and head modeling to evaluate and validate the following Neurofunctional Tinnitus Model predictions and suggested validation methodologies:

TABLE 1 | Tinnitus patient categorization accordance to the Neurofunctional Tinnitus Model development stage; Y, Yes and N, No.

Tinnitus Stage	Type	Phantom sound perception	Negative reaction	Plasticity in Auditory cortex	Plasticity in Limbic system
Neutral	NT-I	Y	N	N	N
	NT-II	Y	N	Y	N
	NT-X	N	N	N	N
Clinical Distress	CL-I	Y	Y	N	Y
	CL-II	Y	Y	Y	Y

- In cognitive rehabilitation approaches, rehabilitation cannot happen without conscious (attended) awareness perception of tinnitus
  - Examining changes in clinical distress symptoms during unconscious vs. conscious and conscious attended cognitive intervention. Recently, Probst et al. (2016) described a model examining emotional states, the perceived loudness of tinnitus, and tinnitus distress in patients who are in the clinical distress stage and not in the developing stage (Probst et al., 2016). However, if we consider the clinical distress as a dynamic spectrum to maintain tinnitus bothersome, the results support our hypothesis that, consciousness is necessary for neutral tinnitus to turn to clinical distress tinnitus. In addition, the tinnitus bothersome (distress) fluctuations occur during conscious (attended) awareness perception [ability to cognitively report scale of his/her tinnitus]. Furthermore, in the Probst et al. (2016) study, the patients scaled their stress levels [awareness of contingency with unconditional stimulus]. We agree that the awareness of contingency and conditional stimuli is necessary for EC learning procedure but not in classical conditioning learning.
- Tinnitus psychoacoustic specifications encode to memory in auditory cortex during centralization of tinnitus
  - Use resting-state fMRI-EEG analysis to investigate the brain network activity and connectivity in correlation with loudness and pitch before and after electrical neuromodulation stimulation trials in auditory cortex.
- Continuous evaluation of Tinnitus valence is performed in PFC
  - Use electrical neuromodulation stimulation on dorsolateral prefrontal cortex to indicate decrease in bothersome and negative valence.
- Change in tinnitus valence leads to change in attentional allocation and duration of conscious perception
  - Perform cognitive behavioral therapy to prove that decreasing the tinnitus negative valence can decrease frequency of tinnitus perception and bothersome.
  - Perform cognitive counseling trials to improve patient knowledge of tinnitus, which can decrease cognitive-emotional appraisal, lead to decrease in negative valence, and decrease frequency of tinnitus perception and bothersome.
- Cognitive disorders (like insomnia, stress) cannot generate tinnitus but, they can develop tinnitus negative valence and bothersome
  - Perform clinical trials and meta-analysis on patients with cognitive disorders.
- Conscious pairing of adequate pleasant audio-visual stimulus with tinnitus can decrease tinnitus negative valence
  - Perform pleasant multi-modality virtual reality application in future trials.

AUTHOR CONTRIBUTIONS

IG, Principle investigator. YZ, Cognitive Neuroscience adviser. AD, information flow adviser. SS, Audiology adviser. HE, Neuroscience adviser. TS, Otolaryngology and clinical adviser.

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REFERENCES

Adjamian, P., Sereda, M., and Hall, D. A. (2009). The mechanisms of tinnitus: perspectives from human functional neuroimaging. *Hear. Res.* 253, 15–31. doi: 10.1016/j.heares.2009.04.001

Anderson, B. A., Laurent, P. A., and Yantis, S. (2011). Value-driven attentional capture. *Proc. Natl. Acad. Sci. U.S.A.* 108, 10367–10371. doi: 10.1073/pnas.1104047108

Andersson, G., Lyttkens, L., Hirvelä, C., Furmark, T., Tillfors, M., and Fredrikson, M. (2000). Regional cerebral blood flow during tinnitus: a PET case study with lidocaine and auditory stimulation. *Acta Otolaryngol.* 120, 967–972. doi: 10.1080/00016480050218717

Arnold, W., Bartenstein, P., Oestreicher, E., Romer, W., and Schwaiger, M. (1996). Focal metabolic activation in the predominant left auditory cortex in patients suffering from tinnitus: a PET study with [18F]deoxyglucose. *ORL J. Otorhinolaryngol. Relat. Spec.* 58, 195–199. doi: 10.1159/000276835

Auerbach, B. D., Rodrigues, P. V., and Salvi, R. J. (2014). Central gain control in tinnitus and hyperacusis. *Front. Neurol.* 5:206. doi: 10.3389/fneur.2014.00206

Bar-Anan, Y., De Houwer, J., and Nosek, B. A. (2010). Evaluative conditioning and conscious knowledge of contingencies: a correlational investigation with large samples. *Q. J. Exp. Psychol. (Hove)* 63, 2313–2335. doi: 10.1080/17470211003802442

Boyen, K., Langers, D. R. M., de Kleine, E., and van Dijk, P. (2013). Gray matter in the brain: differences associated with tinnitus and hearing loss. *Hear. Res.* 295, 67–78. doi: 10.1016/j.heares.2012.02.010

Brozoski, T. J., Bauer, C. A., and Caspary, D. M. (2002). Elevated fusiform cell activity in the dorsal cochlear nucleus of chinchillas with psychophysical evidence of tinnitus. *J. Neurosci.* 22, 2383–2390.

Chang, H., Chen, K., Kaltenbach, J. A., Zhang, J., and Godfrey, D. A. (2002). Effects of acoustic trauma on dorsal cochlear nucleus neuron activity in slices. *Hear. Res.* 164, 59–68. doi: 10.1016/S0378-5955(01)00410-5

Chen, G. D., and Jastreboff, P. J. (1995). Salicylate-induced abnormal activity in the inferior colliculus of rats. *Hear. Res.* 82, 158–178. doi: 10.1016/0378-5955(94)00174-O

Coelho, C. B., Sanchez, T. G., and Tyler, R. S. (2007). Tinnitus in children and associated risk factors. *Prog. Brain Res.* 166, 179–191. doi: 10.1016/S0079-6123(07)66016-6

Cohen, M. A., Cavanagh, P., Chun, M. M., and Nakayama, K. (2012). The attentional requirements of consciousness. *Trends Cogn. Sci.* 16, 411–417. doi: 10.1016/j.tics.2012.06.013

Crönlein, T., Langguth, B., Geisler, P., and Hajak, G. (2007). Tinnitus and insomnia. *Prog. Brain Res.* 166, 227–233. doi: 10.1016/S0079-6123(07)66021-X

De Houwer, J., Thomas, S., and Baeyens, F. (2001). Associative learning of likes and dislikes: a review of 25 years of research on human evaluative conditioning. *Psychol. Bull.* 127, 853–869. doi: 10.1037/0033-2909.127.6.853

de Maddalena, H., and Pfrang, H. (1993a). Improvement of communication behavior of laryngectomized and voice-rehabilitated patients by a psychological training program. *HNO* 41, 289–295.

de Maddalena, H., and Pfrang, H. (1993b). Subjective attitudes of laryngectomized patients of the cause of the tumor disease. Correlation with psychosocial adjustment and pre- and postoperative alcohol and tobacco consumption. *HNO* 41, 198–205.

De Ridder, D., Vanneste, S., Weisz, N., Londero, A., Schlee, W., Elgoyhen, A. B., et al. (2014). An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci. Biobehav. Rev.* 44, 16–32. doi: 10.1016/j.neubiorev.2013.03.021

Dominguez, M., Becker, S., Bruce, I., and Read, H. (2006). A spiking neuron model of cortical correlates of sensorineural hearing loss: spontaneous firing, synchrony, and tinnitus. *Neural Comput.* 18, 2942–2958. doi: 10.1162/neco.2006.18.12.2942

Eggermont, J. J. (2006). Cortical tonotopic map reorganization and its implications for treatment of tinnitus. *Acta Otolaryngol. Suppl.* 556, 9–12. doi: 10.1080/03655230600895259

Eggermont, J. J., and Komiya, H. (2000). Moderate noise trauma in juvenile cats results in profound cortical topographic map changes in adulthood. *Hear. Res.* 142, 89–101. doi: 10.1016/S0378-5955(00)00024-1

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Gardner, A., Pagani, M., Jacobsson, H., Lindberg, G., Larsson, S. A., Wägner, A., et al. (2002). Differences in resting state regional cerebral blood flow assessed with 99mTc-HMPAO SPECT and brain atlas matching between depressed patients with and without tinnitus. *Nucl. Med. Commun.* 23, 429–439. doi: 10.1097/00006231-200205000-00002

Ghashghaei, H. T., Hilgetag, C. C., and Barbas, H. (2007). Sequence of information processing for emotions based on the anatomic dialogue between prefrontal cortex and amygdala. *Neuroimage* 34, 905–923. doi: 10.1016/j.neuroimage.2006.09.046

Ghodratitoostani, I., Delbem, A. C. B., Torabi-Nami, M., Makkiabadi, B., Jalilvand, H., and Sanchez, T. G. (2016). Theoretical tinnitus multimodality framework: a neurofunctional model. *J. Adv. Med. Sci. Appl. Technol.* 2, 181–189.

Graziano, M. S., and Webb, T. W. (2015). The attention schema theory: a mechanistic account of subjective awareness. *Front. Psychol.* 6:500. doi: 10.3389/fpsyg.2015.00500

Halford, J. B., and Anderson, S. D. (1991). Anxiety and depression in tinnitus sufferers. *J. Psychosom. Res.* 35, 383–390. doi: 10.1016/0022-3999(91)90033-K

Hannula, D. E., and Greene, A. J. (2012). The hippocampus reevaluated in unconscious learning and memory: at a tipping point? *Front. Hum. Neurosci.* 6:80. doi: 10.3389/fnhum.2012.00080

Hare, T. A., Camerer, C. F., and Rangel, A. (2009). Self-control in decision-making involves modulation of the vmPFC valuation system. *Science* 324, 646–648. doi: 10.1126/science.1168450

Heller, A. J. (2003). Classification and epidemiology of tinnitus. *Otolaryngol. Clin. North Am.* 36, 239–248. doi: 10.1016/S0030-6665(02)00160-3

Henke, K. (2010). A model for memory systems based on processing modes rather than consciousness. *Nat. Rev. Neurosci.* 11, 523–532. doi: 10.1038/nrn2850

Husain, F. T., Pajor, N. M., Smith, J. F., Kim, H. J., Rudy, S., Horwitz, B., et al. (2011). Discrimination task reveals differences in neural bases of tinnitus and hearing impairment. *PLoS ONE* 6:e26639. doi: 10.1371/journal.pone.0026639

Hütter, M., and Sweldens, S. (2013). Implicit misattribution of evaluative responses: contingency-unaware evaluative conditioning requires simultaneous stimulus presentations. *J. Exp. Psychol. Gen.* 142, 638–643. doi: 10.1037/a0029989

Jastreboff, P. J. (1990). Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci. Res.* 8, 221–254. doi: 10.1016/0168-0102(90)90031-9

Jastreboff, P. J., Brennan, J. F., Coleman, J. K., and Sasaki, C. T. (1988b). Phantom auditory sensation in rats: an animal model for tinnitus. *Behav. Neurosci.* 102, 811–822. doi: 10.1037/0735-7044.102.6.811

Jastreboff, P. J., and Hazell, J. W. (2004). *Tinnitus Retraining Therapy: Implementing the Neurophysiological Model*. New York, NY: Cambridge university press.

Jastreboff, P. J., and Jastreboff, M. M. (2006). Tinnitus retraining therapy: a different view on tinnitus. *ORL J. Otorhinolaryngol. Relat. Spec.* 68, 23–29. doi: 10.1159/000090487

Jastreboff, P. J., and Sasaki, C. T. (1986). Salicylate-induced changes in spontaneous activity of single units in the inferior colliculus of the guinea pig. *J. Acoust. Soc. Am.* 80, 1384–1391. doi: 10.1121/1.394391

Jastreboff, P. J., Sasaki, C. T., and Brennan, J. F. (1988a). An animal model for tinnitus. *Laryngoscope* 98, 280–286. doi: 10.1288/00005537-198803000-0000

Kaltenbach, J. A., and McCaslin, D. L. (1996). Increases in spontaneous activity in the dorsal cochlear nucleus following exposure to high intensity sound: a possible neural correlate of tinnitus. *Aud. Neurosci.* 3, 57–78.

Kaltenbach, J. A., Godfrey, D. A., Neumann, J. B., McCaslin, D. L., Afman, C. E., and Zhang, J. (1998). Changes in spontaneous neural activity in the dorsal cochlear nucleus following exposure to intense sound: relation to threshold shift. *Hear. Res.* 124, 78–84. doi: 10.1016/S0378-5955(98)00119-1

Kaping, D., Vinck, M., Hutchison, R. M., Everling, S., and Womelsdorf, T. (2011). Specific contributions of ventromedial, anterior cingulate, and lateral prefrontal cortex for attentional selection and stimulus valuation. *PLoS Biol.* 9:e1001224. doi: 10.1371/journal.pbio.1001224

Kattner, F. (2012). Revisiting the relation between contingency awareness and attention: evaluative conditioning relies on a contingency focus. *Cogn. Emot.* 26, 166–175. doi: 10.1080/02699931.2011.565036

Kiang, N. Y. S., Moxon, E. C., and Levine, R. A. (1970). "Auditory-nerve activity in cats with normal and abnormal cochleas," in *Ciba Foundation Symposium-Sensorineural Hearing Loss* (John Wiley & Sons, Ltd.), 241–273.

Knight, R. T., Scabini, D., and Woods, D. L. (1989). Prefrontal cortex gating of auditory transmission in humans. *Brain Res.* 504, 338–342. doi: 10.1016/0006-8993(89)91381-4

Kraus, K. S., and Canlon, B. (2012). Neuronal connectivity and interactions between the auditory and limbic systems. Effects of noise and tinnitus. *Hear. Res.* 288, 34–46. doi: 10.1016/j.heares.2012.02.009

Kuchinke, L., Fritsch, N., and Müller, C. J. (2015). Evaluative conditioning of positive and negative valence affects P1 and N1 in verbal processing. *Brain Res.* 1624, 405–413. doi: 10.1016/j.brainres.2015.07.059

Lamme, V. A. (2003). Why visual attention and awareness are different. *Trends Cogn. Sci.* 7, 12–18. doi: 10.1016/S1364-6613(02)00013-X

Landgrebe, M., Langguth, B., Rosengarth, K., Braun, S., Koch, A., Kleinjung, T., et al. (2009). Structural brain changes in tinnitus: grey matter decrease in auditory and non-auditory brain areas. *Neuroimage* 46, 213–218. doi: 10.1016/j.neuroimage.2009.01.069

Langguth, B. (2011). A review of tinnitus symptoms beyond 'ringing in the ears': a call to action. *Curr. Med. Res. Opin.* 27, 1635–1643. doi: 10.1185/03007995.2011.595781

Langguth, B., Eichhammer, P., Kreutzer, A., Maenner, P., Marienhagen, J., Kleinjung, T., et al. (2006). The impact of auditory cortex activity on characterizing and treating patients with chronic tinnitus–first results from a PET study. *Acta Otolaryngol. Suppl. 556,* 84–88. doi: 10.1080/03655230600895317

Langguth, B., Kleinjung, T., Fischer, B., Hajak, G., Eichhammer, P., and Sand, P. G. (2007). Tinnitus severity, depression, and the big five personality traits. *Prog. Brain Res.* 166, 221–225. doi: 10.1016/S0079-6123(07)66020-8

Lanting, C. P., De Kleine, E., Bartels, H., and Van Dijk, P. (2008). Functional imaging of unilateral tinnitus using fMRI. *Acta Otolaryngol.* 128, 415–421. doi: 10.1080/00016480701793743

Lockwood, A. H., Salvi, R. J., Coad, M. L., Towsley, M. L., Wack, D. S., and Murphy, B. W. (1998). The functional neuroanatomy of tinnitus: evidence for limbic system links and neural plasticity. *Neurology* 50, 114–120. doi: 10.1212/WNL.50.1.114

Lorenz, I., Müller, N., Schlee, W., Hartmann, T., and Weisz, N. (2009). Loss of alpha power is related to increased gamma synchronization—a marker of reduced inhibition in tinnitus? *Neurosci. Lett.* 453, 225–228. doi: 10.1016/j.neulet.2009.02.028

MacDonald, A. W. III, Cohen, J. D., Stenger, V. A., and Carter, C. S. (2000). Dissociating the role of the dorsolateral prefrontal and anterior cingulate cortex in cognitive control. *Science* 288, 1835–1838. doi: 10.1126/science.288.5472.1835

Malakouti, S., Mahmoudian, M., Alifattahi, N., and Salehi, M. (2011). Comorbidity of chronic tinnitus and mental disorders. *Int. Tinnitus J.* 16, 118–122.

Marciano, E., Carrabba, L., Giannini, P., Sementina, C., Verde, P., Bruno, C., et al. (2003). Psychiatric comorbidity in a population of outpatients affected by tinnitus. *Int. J. Audiol.* 42, 400000–400009. doi: 10.3109/14992020309056079

Marcondes, R., Fregni, F., and Pascual-Leone, A. (2006). Tinnitus and brain activation: insights from transcranial magnetic stimulation. *Ear Nose Throat J.* 85, 236–238.

Maudoux, A., Lefebvre, Ph., Cabay, J.-E., Demertzi, A., Vanhaudenhuyse, A., Laureys, S., et al. (2012). Connectivity graph analysis of the auditory resting state network in tinnitus. *Brain Res.* 1485, 10–21. doi: 10.1016/j.brainres.2012.05.006

Middleton, J. W., Kiritani, T., Pedersen, C., Turner, J. G., Shepherd, G. M., and Tzounopoulos, T. (2011). Mice with behavioral evidence of tinnitus exhibit dorsal cochlear nucleus hyperactivity because of decreased GABAergic inhibition. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7601–7606. doi: 10.1073/pnas.1100223108

Mills, R. P., Albert, D. M., and Brain, C. E. (1986). Tinnitus in childhood. *Clin. Otolaryngol. Allied Sci.* 11, 431–434. doi: 10.1111/j.1365-2273.1986.tb00147.x

Mühlnickel, W., Elbert, T., Taub, E., and Flor, H. (1998). Reorganization of auditory cortex in tinnitus. *Proc. Natl. Acad. Sci. U.S.A.* 95, 10340–10343. doi: 10.1073/pnas.95.17.10340

Noreña, A. J., and Eggermont, J. J. (2003). Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. *Hear. Res.* 183, 137–153. doi: 10.1016/S0378-5955(03)00225-9

Pinault, D., and Deschênes, M. (1998). Anatomical evidence for a mechanism of lateral inhibition in the rat thalamus. *Eur. J. Neurosci.* 10, 3462–3469. doi: 10.1046/j.1460-9568.1998.00362.x

Plewnia, C., Reimold, M., Najib, A., Brehm, B., Reischl, G., Plontke, S., et al. (2007). Dose-dependent attenuation of auditory phantom perception (tinnitus) by PET-guided repetitive transcranial magnetic stimulation. *Hum. Brain Mapp.* 28, 238–246. doi: 10.1002/hbm.20270

Ploner, C. J., Gaymard, B. M., Rivaud-Péchoux, S., and Pierrot-Deseilligny, C. (2005). The prefrontal substrate of reflexive saccade inhibition in humans. *Biol. Psychiatry* 57, 1159–1165. doi: 10.1016/j.biopsych.2005.02.017

Probst, T., Pryss, R., Langguth, B., and Schlee, W. (2016). Emotional states as mediators between tinnitus loudness and tinnitus distress in daily life: results from the track your tinnitus application. *Sci. Rep.* 6:20382. doi: 10.1038/srep20382

Rauschecker, J. P., Leaver, A., M., and Mühlau, M. (2010). Tuning out the noise: limbic-auditory interactions in tinnitus. *Neuron* 66, 819–826. doi: 10.1016/j.neuron.2010.04.032

Roberts, L. E., Husain, F. T., and Eggermont, J. J. (2013). Role of attention in the generation and modulation of tinnitus. *Neurosci. Biobehav. Rev.* 37, 1754–1773. doi: 10.1016/j.neubiorev.2013.07.007

Robinson, S. (2007). Antidepressants for treatment of tinnitus. *Prog. Brain Res.* 166, 263–271. doi: 10.1016/S0079-6123(07)66024-5

Robinson, S. K., Viirre, E. S., and Stein, M. B. (2007). Antidepressant therapy in tinnitus. *Hear. Res.* 226, 221–231. doi: 10.1016/j.heares.2006.08.004

Sasaki, K., Gemba, H., and Tsujimoto, T. (1989). Suppression of visually initiated hand movement by stimulation of the prefrontal cortex in the monkey. *Brain Res.* 495, 100–107. doi: 10.1016/0006-8993(89)91222-5

Savastano, M. (2007). Characteristics of tinnitus in childhood. *Eur. J. Pediatr.* 166, 797–801. doi: 10.1007/s00431-006-0320-z

Schlee, W., Mueller, N., Hartmann, T., Keil, J., Lorenz, I., and Weisz, N. (2009). Mapping cortical hubs in tinnitus. *BMC Biol.* 7:80. doi: 10.1186/1741-7007-7-80

Schneider, P., Andermann, M., Wengenroth, M., Goebel, R., Flor, H., Rupp, A., et al. (2009). Reduced volume of Heschl's gyrus in tinnitus. *Neuroimage* 45, 927–939. doi: 10.1016/j.neuroimage.2008.12.045

Searchfield, G. D., and Kobayashi, K. (2012). "Game training of tinnitus," in *6th International TRI Tinnitus Conference* (Brugge).

Searchfield, G. D., Morrison-Low, J., and Wise, K. (2007). Object identification and attention training for treating tinnitus. *Prog. Brain Res.* 166, 441–460. doi: 10.1016/S0079-6123(07)66043-9

Seitz, A. R., Kim, D., and Watanabe, T. (2009). Rewards evoke learning of unconsciously processed visual stimuli in adult humans. *Neuron* 61, 700–707. doi: 10.1016/j.neuron.2009.01.016

Seki, S., and Eggermont, J. J. (2003). Changes in spontaneous firing rate and neural synchrony in cat primary auditory cortex after localized tone-induced hearing loss. *Hear. Res.* 180, 28–38. doi: 10.1016/S0378-5955(03)00074-1

Shuler, M. G., and Bear, M. F. (2006). Reward timing in the primary visual cortex. *Science* 311, 1606–1609. doi: 10.1126/science.1123513

Smits, M., Kovacs, S., de Ridder, D., Peeters, R. R., van Hecke, P., and Sunaert, S. (2007). Lateralization of functional magnetic resonance imaging (fMRI) activation in the auditory pathway of patients with lateralized tinnitus. *Neuroradiology* 49, 669–679. doi: 10.1007/s00234-007-0231-3

Stahl, C., Unkelbach, C., and Corneille, O. (2009). On the respective contributions of awareness of unconditioned stimulus valence and unconditioned stimulus identity in attitude formation through evaluative conditioning. *J. Pers. Soc. Psychol.* 97, 404–420. doi: 10.1037/a0016196

Stolzberg, D., Chen, G. D., Allman, B. L., and Salvi, R. J. (2011). Salicylate-induced peripheral auditory changes and tonotopic reorganization of auditory cortex. *Neuroscience* 180, 157–164. doi: 10.1016/j.neuroscience.2011.02.005

Strauss, D. J., Delb, W., D'Amelio, R., and Falkai, P. (2005). "Neural synchronization stability in the tinnitus decompensation," in *Conference Proceedings 2nd International IEEE EMBS Conference on Neural Engineering* (Washington, DC: IEEE).

Strauss, D. J., Delb, W., D'Amelio, R., Low, Y. F., and Falkai, P. (2008). Objective quantification of the tinnitus decompensation by synchronization measures of auditory evoked single sweeps. *IEEE Trans. Neural Syst. Rehabil. Eng.* 16, 74–81. doi: 10.1109/TNSRE.2007.911086

Sun, W., Lu, J., Stolzberg, D., Gray, L., Deng, A., Lobarinas, E., et al. (2009). Salicylate increases the gain of the central auditory system. *Neuroscience* 159, 325–334. doi: 10.1016/j.neuroscience.2008.12.024

Tsotsos, J. K. (2011). *A Computational Perspective on Visual Attention*. Cambridge, MA: MIT Press.

Tyler, R., Coelho, C., Tao, P., Ji, H., Noble, W., Gehringer, A., et al. (2008). Identifying tinnitus subgroups with cluster analysis. *Am. J. Audiol.* 17, S176–S184. doi: 10.1044/1059-0889(2008/07-0044)

Tyler, R. S. (2006). *Tinnitus Treatment: Clinical Protocols*. New York, NY: Thieme.

Watanabe, M., Cheng, K., Murayama, Y., Ueno, K., Asamizuya, T., Tanaka, K., et al. (2011). Attention but not awareness modulates the BOLD signal in the human V1 during binocular suppression. *Science* 334, 829–831. doi: 10.1126/science.1203161

Weinberger, N. M. (2011). The medial geniculate, not the amygdala, as the root of auditory fear conditioning. *Hear. Res.* 274, 61–74. doi: 10.1016/j.heares.2010.03.093

Winkler, I., Denham, S. L., and Nelken, I. (2009). Modeling the auditory scene: predictive regularity representations and perceptual objects. *Trends Cogn. Sci.* 13, 532–540. doi: 10.1016/j.tics.2009.09.003

Woods, D. L., and Knight, R. T. (1986). Electrophysiologic evidence of increased distractibility after dorsolateral prefrontal lesions. *Neurology* 36, 212–216. doi: 10.1212/WNL.36.2.212

Yu, X. J., Xu, X. X., Chen, X., He, S., and He, J. (2009). Slow recovery from excitation of thalamic reticular nucleus neurons. *J. Neurophysiol.* 101, 980–987. doi: 10.1152/jn.91130.2008

Zeng, F.-G. (2013). An active loudness model suggesting tinnitus as increased central noise and hyperacusis as increased nonlinear gain. *Hear. Res.* 295, 172–179. doi: 10.1016/j.heares.2012.05.009

Zenner, H. P., Pfister, M., and Birbaumer, N. (2006). Tinnitus sensitization: sensory and psychophysiological aspects of a new pathway of acquired centralization of chronic tinnitus. *Oto. Neurotol.* 27, 1054–1063. doi: 10.1097/01.mao.0000231604.64079.77

Zhang, J. S., and Kaltenbach, J. A. (1998). Increases in spontaneous activity in the dorsal cochlear nucleus of the rat following exposure to high-intensity sound. *Neurosci. Lett.* 250, 197–200. doi: 10.1016/S0304-3940(98)00482-0

Zhang, X., Yang, P., Cao, Y., Qin, L., and Sato, Y. (2011). Salicylate induced neural changes in the primary auditory cortex of awake cats. *Neuroscience* 172, 232–245. doi: 10.1016/j.neuroscience.2010.10.073

Zikopoulos, B., and Barbas, H. (2007). Circuits formultisensory integration and attentional modulation through the prefrontal cortex and the thalamic reticular nucleus in primates. *Rev. Neurosci.* 18, 417–438. doi: 10.1515/REVNEURO.2007.18.6.417

Zikopoulos, B., and Barbas, H. (2012). Pathways for emotions and attention converge on the thalamic reticular nucleus in primates. *J. Neurosci.* 32, 5338–5350. doi: 10.1523/JNEUROSCI.4793-11.2012

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REVIEW
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Neurofeedback for Tinnitus Treatment – Review and Current Concepts

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An effective treatment to completely alleviate chronic tinnitus symptoms has not yet been discovered. However, recent developments suggest that neurofeedback (NFB), a method already popular in the treatment of other psychological and neurological disorders, may provide a suitable alternative. NFB is a non-invasive method generally based on electrophysiological recordings and visualizing of certain aspects of brain activity as positive or negative feedback that enables patients to voluntarily control their brain activity and thus triggers them to unlearn typical neural activity patterns related to tinnitus. The purpose of this review is to summarize and discuss previous findings of neurofeedback treatment studies in the field of chronic tinnitus. In doing so, also an overview about the underlying theories of tinnitus emergence is presented and results of resting-state EEG and MEG studies summarized and critically discussed. To date, neurofeedback as well as electrophysiological tinnitus studies lack general guidelines that are crucial to produce more comparable and consistent results. Even though neurofeedback has already shown promising results for chronic tinnitus treatment, further research is needed in order to develop more sophisticated protocols that are able to tackle the individual needs of tinnitus patients more specifically.

Keywords: tinnitus, phantom perception, EEG, plasticity, heterogeneity, neurofeedback, frequency bands, alpha band

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INTRODUCTION

Subjective tinnitus has been described as the constant perception of an auditory sensation that does not correlate to any external acoustic stimulus (Stouffer and Tyler, 1990). It can be perceived as either pitch or noise-like sound and its perception may be unilateral, bilateral or spread out in the whole head (De Ridder et al., 2014b). In industrialized countries, roughly 10% of the population is affected by this stressful condition and many people suffer from sleeping or concentration problems, affected social interactions and psychological distress that can also lead to severe depression or anxiety impairments (Heller, 2003; Henry et al., 2005). The relatively large percentage of affected people, recently developed neuropsychological models, and the fact that, to date, no satisfactory potent treatment has been discovered may explain the increasing interest in tinnitus research. New findings on the pathophysiology of tinnitus have led to the development of several promising neuromodulatory techniques that have been shown to relieve symptoms of the chronic acoustic sensation and significantly increase quality of life for tinnitus sufferers (e.g.,

Eggermont and Roberts, 2004; Weisz et al., 2007a). One of them is neurofeedback, an already well-established form of neuropsychological treatment that recently enjoys great popularity due to its non-invasive nature, its long-lasting effects, its easy-handling and relatively low cost, as well as its rapid technological improvements. The purpose of this review is to summarize and discuss findings of neurofeedback studies for the treatment of chronic tinnitus. The focus is hereby laid on neurofeedback based on electrophysiological recordings with electroencephalography (EEG) or magnetoencephalography (MEG) but also a short summary of new innovative methods (e.g., real-time functional Magnetic Resonance Imaging, rt-fMRI) will be given. In a first step, an overview about popular models of tinnitus genesis will be provided, and studies investigating chronic tinnitus with EEG or MEG will be presented and critically discussed. Next, the development and history of neurofeedback will be briefly introduced and the different neurofeedback protocols used in tinnitus treatment summarized and evaluated. Finally, limitations of existing treatment studies will be discussed, and implications for future studies will be given.

TINNITUS MODELS AND ELECTROPHYSIOLOGICAL STUDIES

Tinnitus was first assumed to be solely generated in the ear or by a dysfunction of the auditory nerve (Møller, 1984; Eggermont, 1990), but the focus of attention quickly shifted to the human brain after Jastreboff (1990) proposed what is nowadays known as the *neurophysiological model of tinnitus*. Even though some form of inner ear damage indeed seems to be a necessary prerequisite, Jastreboff (1990) suggested central processes in the auditory cortex, the limbic system, and prefrontal areas to be crucial for tinnitus genesis. Later models picked up this idea and tried to specify the neuroplastic alterations emerging after auditory deafferentation. In this context, an increase in central gain in subcortical structures of the auditory pathway (Noreña, 2011), reorganization of tonotopic maps in the primary auditory cortex (Mühlnickel et al., 1998), a thalamocortical dysrhythmia (Llinás et al., 1998, 1999, 2005; Weisz et al., 2007a) and changes in neural synchrony (Noreña and Eggermont, 2003; Seki and Eggermont, 2003; Eggermont and Roberts, 2004; Weisz et al., 2005), or a failing top-down noise-canceling mechanism (Rauschecker et al., 2010, 2015) have been discussed. Furthermore, global workspace models emphasize the importance of networks beyond the auditory system (De Ridder et al., 2014b), and frameworks of filling-in missing auditory information have been suggested in a Bayesian way (De Ridder et al., 2006, 2011, 2014a) or based on predictive coding (Sedley et al., 2016).

First Wave of Electrophysiological Studies

Apart from animal experiments, brain imaging and morphometry studies, the investigation of resting-state brain activity with electrophysiological methods, such as EEG or MEG, enjoys great popularity in tinnitus research (Adjamian, 2014). In order to pinpoint neural correlates of the ongoing tinnitus sensation, first studies compared spontaneous brain activity of tinnitus patients at rest with the one of healthy controls. In this context, most investigations focused on the analysis of neuronal oscillations separated into distinct frequency bands: delta (0.5–4 Hz), theta (4.5–8 Hz), alpha (8.5–12 Hz), beta (12.5–35 Hz), and gamma (35.5–80 Hz). Following this approach, early studies (Weisz et al., 2005, 2007b; Ashton et al., 2007; Kahlbrock and Weisz, 2008; Lorenz et al., 2009) found a relatively consistent pattern of enhanced activity in delta- and gamma frequencies, alongside with reduced amounts of alpha oscillations over temporal areas of tinnitus patients (for a review, see Schlee et al., 2008; Adjamian et al., 2009). These findings have been interpreted in the framework of the *thalamocortical dysrhythmia model* (TCD), originally proposed by Llinás et al.'s (1998, 1999, 2005) and later significantly refined by Weisz et al. (2007a) to the *synchronization by loss of inhibition modulation* (SLIM) model. Both models aim at sketching tinnitus genesis as the result of an imbalance between inhibition and excitation in thalamocortical circuits. Loss of sensory input (deafferentation) gives raise to low frequent self-oscillations of thalamic cells which activate the auditory cortex and can thus be measured as oscillations in a slow delta rhythm on the scalp. At the same time, input deprivation also leads to a downregulation of inhibitory mechanisms which is reflected in alpha desynchronization in the resting-state EEG or MEG. This decrease of inhibition is then proposed to lead to spontaneous synchronization of firing reflected in increasing activity in fast gamma oscillations. This pattern of increased resting-state delta and gamma and decreased alpha has thus been termed the *neural signature of tinnitus,* and gamma has been interpreted as the neuronal substrate of the sound percept itself.

Limitations of the Early Studies

One of the major flaws of these early studies, however, was that they did not consider that chronic tinnitus is a very heterogeneous phenomenon and can differ substantially between individuals. It has clearly been shown that the subjective experience of the chronic sound (intensity, pitch, location) as well as the related distress and comorbid symptoms vary considerably among sufferers (Landgrebe et al., 2010; Langguth et al., 2013; Weidt et al., 2016; van den Berge et al., 2017). In addition, the underlying neuroanatomical and neurophysiological alterations may be far from homogenous in the population of tinnitus patients. Instead of comparing tinnitus patients with healthy controls, more recent studies thus focused on differences *within* the tinnitus sample with the ultimate goal of identifying distinct subtypes of tinnitus and finding different forms of treatment for each of these subtypes.

Another issue that the earlier studies had to deal with is the fact that electrophysiological methods suffer from rather poor spatial resolution. In terms of neuroscience, the *inverse problem* describes the fact that signal as measured by electrodes or magnetometers on the scalp could be generated by infinite combinations of neuronal sources (Scherg and Berg, 1991). The described pattern of tinnitus-specific oscillations found in the earlier studies, even though measured over temporal areas, could therefore have been generated in (or significantly altered

by) cell assemblies outside of the primary auditory cortex. Different *source estimation algorithms* have been developed in the recent past to solve this inverse problem as well as possible by applying different *a priori* assumptions. With these algorithms the source of a measured signal can be estimated and spatial resolution of resting-state EEG and MEG measurements significantly increased (Michel et al., 2004). Standardized Low Resolution Electromagnetic Tomography (sLORETA) (Pascual-Marqui, 2002) or beamformer algorithms (van Veen et al., 1997; Hillebrand et al., 2005; Grosse-Wentrup et al., 2009) are examples of fairly precise and therefore relatively popular source estimation techniques.

The new focus on differences within the tinnitus population and the improvements in electrophysiological analysis methods have led to a veritable boom of resting-state tinnitus studies. Some investigations have confirmed the neuronal tinnitus code and auditory gamma as its major brain correlate by applying sLORETA (van der Loo et al., 2009; Moazami-Goudarzi et al., 2010; Vanneste et al., 2011a) or beamformer (Ortmann et al., 2011) source estimations to the measured signal, reporting correlations between tinnitus loudness and auditory gamma (van der Loo et al., 2009) or by performing intervention studies with acoustic coordinated reset (Tass et al., 2012; Adamchic et al., 2014a,b, 2017). Schlee et al. (2014), on the other hand, found decreased power (and variability) only for the lower (8–10 Hz) but not for the upper alpha band (10–12 Hz) and other studies failed completely to find the expected pattern in the auditory areas (Vanneste et al., 2011b, 2012; Song et al., 2013; Meyer et al., 2014; Zobay and Adjamian, 2015). Furthermore, two studies (Sedley et al., 2012; Sedley and Cunningham, 2013) discussed the possibility that auditory gamma oscillations could emerge as an attempt of the brain to suppress the tinnitus percept rather than causing it.

Tinnitus Network(s) and Areas Beyond the Auditory Cortex

In neuroscience, the gamma frequency range has also been debated as a binding medium connecting activity of various circuits to form a unified percept (Singer, 1993). Already Schlee et al. (2009) reported gamma-related abnormalities in a network with core regions in prefrontal, orbitofrontal, and parieto-occipital areas. Later the different parallel networks that may differentially contribute to the various tinnitus symptoms were described in more detail (De Ridder et al., 2011, 2014b; Vanneste and De Ridder, 2012). A *tinnitus core network* was proposed to generate the sound *per se* and code its intensity and location (holocranial, uni- or bilateral). Other networks were introduced as modulating the sound type (sine wave tone, hissing, ringing) as well as aversive states and feelings (e.g., distress or mood) of tinnitus (De Ridder et al., 2014b). An increased and persisting amount of gamma oscillations and coupling with slow-waves could thus suggest that activity of these widely-distributed brain networks is constantly *bound together* (synchronized), and a unified tinnitus percept is formed with its very own characteristics for each individual coded in the relevant sub-networks. In order to capture the tinnitus phenomenon in its entirety, areas outside of the central auditory regions therefore have to be considered. Furthermore, the specificity of the measured EEG-patterns has to be carefully validated as related disorders might produce similar findings (e.g., Joos et al., 2012; Meyer et al., 2017). These considerations are also relevant with regard to the development of neurofeedback protocols.

Apart from investigations comparing brain networks of tinnitus patients and healthy controls based on analyses with graph theory or machine learning algorithms (Mohan et al., 2016a,b, 2017a,b), a multitude of recent electrophysiological studies attempt to find specific correlates in neural networks for the different aspects of tinnitus (Adjamian, 2014; De Ridder et al., 2015; Eggermont, 2015; Elgoyhen et al., 2015). These studies mainly investigated tinnitus-related distress or loudness, but also covered tinnitus type, pitch, location/laterality, duration, age of onset, day-time awareness, or related problems such as hearing loss, hyperacusis, depression, or general quality of life (a detailed summary is provided in the Supplementary Materials). The most consistent findings are reported for tinnitus-related distress, which seems to be represented in a network ranging from structures of the limbic system (e.g., anterior cingulate cortex and amygdala) to prefrontal areas (e.g., dorsolateral prefrontal cortex), and also includes the insula. Altogether, however, the results of these studies are rather heterogeneous, and attempts of replication are scarce and partly fail to confirm previous findings (Pierzycki et al., 2015; Meyer et al., 2017). This can partially be explained by different EEG or MEG hardware used for resting-state recordings, different paradigms during the measurement [e.g., length of measurement, operationalization of tinnitus symptoms, or condition of resting-state (eyes open/closed) used for the analysis], different source estimation algorithms and data analysis procedures. To resolve this issue, scholars of the European research network *TINNET*[1] are channeling their efforts to establishing general guidelines for (electrophysiological) tinnitus studies and collecting comparable data in a large database[2]. In order to tackle the problem of tinnitus heterogeneity, it is thus of utmost importance that future studies take these guidelines into consideration, report also null- or conflicting results and further also extend their focus to replicating previous findings.

NEUROFEEDBACK

Applying neurophysiological methods, neurofeedback is a non-invasive neuromodulation technique which records a subject's neuronal activity, extracts relevant aspects of brain processes by means of real time signal processing and returns feedback to the subject as visual or auditory stimuli. The aim of neurofeedback is to change behavioral traits or medical conditions associated with altered neural activity as demonstrated for chronic tinnitus in the previous section. This is generally done by means of operant conditioning (i.e., rewarding of wanted,

[1] http://tinnet.tinnitusresearch.net/

[2] https://www.tinnitus-database.de/

inhibiting of unwanted changes) whereby the subjects learn to voluntarily change their own brain activity in the desired direction.

A Brief History of Neurofeedback

In the early 1930's and 1940's, human studies already suggested the capability of the central nervous system to alter neural activity patterns by means of conditioning methods (Loomis et al., 1936; Jasper and Shagass, 1941). Later, Wyrwicka and Sterman (1968) were able to train cats to change their brain activity in a specific direction, and, shortly after that, the first study with human subjects in this context was published (Sterman and Friar, 1972). In the following years, neurofeedback was intensively tested and showed promising results mainly in treatment studies with epilepsy and attention deficit hyperactivity disorder (ADHD) (Lubar and Bahler, 1976; Lubar and Lubar, 1984). For ADHD, neurofeedback already found acceptance as alternative to established medication based treatment, due to its non-invasive character, the almost complete absence of any side-effects and high self-efficacy experienced by the subjects (Lubar et al., 1995; Lévesque et al., 2006; Arns et al., 2009; Gevensleben et al., 2009; Strehl et al., 2017). Apart from that, effectiveness and feasibility of neurofeedback are more and more investigated in the context of many other psychological disorders and neurological conditions ranging from the treatment of depression (Kelley et al., 2017), anxiety (Mennella et al., 2017), or autism (Datko et al., 2017) to stroke patients (Kober et al., 2017) and prevention of Alzheimer's disease (Jiang et al., 2017). Today, quality control is an important aspect in the neurofeedback field. The Biofeedback Certification International Alliance (BCIA)[3] certifies bio- and neurofeedback practitioners who meet certain requirements and the Association for Applied Psychophysiology and Biofeedback (AAPB)[4] recently released the 3rd edition of *Evidence-Based Practice in Biofeedback and Neurofeedback*, a document that summarizes treatment efficacy for various disorders (Tan et al., 2016).

Common Neurofeedback Paradigms

Neurofeedback training of classical definitions of distinct frequency bands (i.e., delta, theta, alpha, beta, and gamma) are the most commonly used protocols in the current literature. The main field of frequency band neurofeedback is the treatment of ADHD, where often a combination of different frequencies is trained (Lofthouse et al., 2012). However, classic frequency band training has also been adapted for other disorders, most prominently anxiety or affective problems (Hammond, 2005). Importantly, neurofeedback training based on this paradigm ultimately depends on findings of fundamental research about disorder-specific neural alterations and can even be used to confirm or disprove these findings.

Sensorimotor rhythms (SMR) are defined as EEG oscillations in the lower beta range (12 – 20 Hz). They are generally measured over the sensorimotor cortex and proposed to originate from the ventrobasal nucleus in the thalamus (Howe and Sterman, 1972, 1973). Neurofeedback training based on SMR mainly found application in the treatment of epilepsy (Sterman and Egner, 2006) or ADHD (Monastra et al., 2002; Fuchs et al., 2003).

Slow cortical potentials (SCP's) describe very slow oscillations in a range of 0.3–1.5 Hz. They describe slow, discrete, and continuous shifts (up to seconds) of the overall cortical distribution of electrical activity representing increased or decreased excitability of underlying neuronal structures. SCP's are usually recorded with a single electrode in a central position (Cz) and are proposed to reflect cognitive or motor preparation (Hammond, 2011). Initially, SCP training was exclusively applied in trials with patients suffering from epilepsy (Rockstroh et al., 1993) but later also found application in the treatment of ADHD (Strehl et al., 2017).

Infra-low neurofeedback (ILN) relies on training of even slower brain oscillations, ranging from 0.001 to 1.5 Hz (Vanhatalo et al., 2004). Infra-low oscillations were shown to correlate with other frequency bands as well (Monastra et al., 2002). There is an overlap with SCP-based neurofeedback, which mainly differs in the recording of SCP's with a single central electrode and thus a training of a more summarized potential over the whole head. Positive effects of ILN on different neurological conditions were reported in case reports (Legarda et al., 2011).

In *z*-score neurofeedback, the training protocol for an individual patient is based on previous recordings of EEG data and comparison to a healthy age-matched normative database (Thatcher, 2010). During the neurofeedback training, patients try to normalize their EEG patterns and minimize deviations from this control group. This NFB alternative is a rather data-driven technique, and some studies report successful treatment of various disorders (e.g., schizophrenia, addiction, ADHD, or personality, anxiety, and affective disorders) with *z*-score neurofeedback (Surmeli and Ertem, 2009; Surmeli et al., 2012; Simkin et al., 2014).

Functional magnetic resonance imaging (fMRI) was introduced to the field of neurofeedback to obtain a better spatial resolution. Real-time acquisition of blood oxygenation level dependent (BOLD) signals demonstrates increased neural activity according to higher oxygen supply to active neurons (Ogawa et al., 1990). Although newer to the field, a large quantity of clinical treatment studies already focused on the use of real-time fMRI neurofeedback (Sulzer et al., 2013). The higher spatial resolution of fMRI neurofeedback, however, does not come without limitations. Increased blood oxygenation can be measured only after a delay of several seconds and is an indirect correlate of underlying neuronal processes. Compared to electrophysiological methods, the temporal resolution of fMRI is thus rather poor, and fast fluctuations cannot be captured accordingly and used for the feedback. Additionally, it is questionable if an MRI-scanner is a favorable setting to perform neurofeedback because of the limited space and the loud constant background noise. For tinnitus patients, this is a huge drawback, in particular in those individuals suffering from additional hyperacusis.

To address the poor spatial resolution of single- or multi-electrode EEG and MEG recordings, neurofeedback techniques have also been combined with source estimation algorithms.

[3]http://www.bcia.org

[4]https://www.aapb.org

Congedo et al. (2004) introduced the first tomographic neurofeedback protocol based on the inverse solution technique *LORETA* (Pascual-Marqui et al., 1994). This approach has subsequently been intensely tested mainly in the context of ADHD treatment (Cannon et al., 2006, 2007, 2009, 2014; Koberda et al., 2012, 2013) and has recently been further refined (Congedo, 2006; Pllana and Bauer, 2011; Kopřivová et al., 2013; Bauer and Pllana, 2014; White et al., 2014).

Neurofeedback and Tinnitus: Existing Studies

Presently, only a handful of studies investigated the efficacy of neurofeedback in the treatment of chronic tinnitus according to standard searching tools such as *PubMed*[5]. An overview is provided in **Table 1**.

In the first study in this context published by Gosepath et al. (2001), 40 patients suffering from chronic tinnitus and 15 control subjects underwent neurofeedback training. The training protocol included alpha training (8–13 Hz) alongside with a reduction of beta oscillations (14–30 Hz). While one group of patients ($n = 24$) was able to only increase their alpha activity, the effects of the other group ($n = 16$) were limited to the decrease of beta oscillations. All patients, however, reported to be less disturbed by their tinnitus after the training, indicated by significant decrement in scores of the tinnitus questionnaire (TQ) (Goebel and Hiller, 1994). Control subjects underwent identical trainings but without real-time feedback and did thus not show any changes in alpha or beta activity. Schenk et al. (2005) aimed at replicating the findings from Gosepath et al. (2001) with the aforementioned protocol. Before assigning them to different study groups, participants underwent baseline EEG-recordings at rest and during a stress test. Participants ($n = 40$) were assigned to three different groups according to their results. Twenty-three subjects showing decreased alpha activity under stress were allocated to a first group and set to train alpha activity (8–13 Hz) in the subsequent neurofeedback training. The second group consisted of 13 patients with increased beta activity in the stress condition and their treatment protocol thus aimed at the decreasing of beta oscillations (14–30 Hz). Four patients could not be assigned to either of the aforementioned groups according to their spontaneous brain activity and hence were allocated in a third group that had to increase alpha and decrease beta activity simultaneously. Subjects of the first group were able to increase their alpha activity, whereas subjects of the second group failed to significantly decrease their amount of beta oscillations. Surprisingly, also subjects of the second group showed increases in alpha activity even though it was not intended with the feedback. Reduced subjective tinnitus distress in terms of a reduction of TQ scores was reported for both groups. The third group was excluded from data analysis due to its small size.

A third rather explorative study shall briefly be mentioned. In a case report, Weiler et al. (2002) used z-score neurofeedback for one patient with bilateral tinnitus. The feedback protocol was based on EEG recordings prior to the training where decreased delta, theta, alpha and beta activities compared to 20 control subjects had been observed. The results indicated a normalization of depressive and anxiety symptoms and the patient reported that tinnitus was only occasionally present. However, no comparisons of pre–post changes in EEG patterns have been drawn in this study.

Even though these three first attempts to treat tinnitus with neurofeedback seemed to be promising, they should not be over-interpreted. First, the training-protocols were chosen rather arbitrarily and not based on previous findings of tinnitus-specific neural abnormalities. Moreover, the fact that patients of all groups reported significant improvements in tinnitus-related distress, regardless of their actual alterations of neural activity, speaks in favor of unspecific effects of the neurofeedback training. Especially the unintended increase of alpha activity in the second group of the study by Schenk et al. (2005) suggests that a general relaxation effect might have had a bigger impact than the actual neurofeedback protocol. In general, these first three studies rather aimed at helping their patients relax and reduce their general level of stress, and it is thus not surprising that reduced distress was reported after the training. However, since knowledge about the origins of tinnitus was still rare at this time, these studies can clearly be seen as pioneering works in the treatment of tinnitus with neurofeedback.

The TCD-model by Llinás et al. (1999, 2005) and the proposition of the *neural signature of tinnitus* (Weisz et al., 2007a) gave rise to new and potentially more appropriate neurofeedback protocols. Dohrmann et al. (2007a,b) developed their neurofeedback protocols by reference to these findings and aimed at an increasing of alpha and a decreasing of delta activity. Twenty-one patients suffering from chronic tinnitus were included into their study and further assigned to three different treatment groups (see **Table 1**). For the neurofeedback application 4 fronto-central electrodes (F3, F4, Fc1, and Fc2) were chosen because the recorded signal is most likely generated in the auditory cortex according to the authors. For a forth group of tinnitus patients ($n = 27$) frequency discrimination training (FDT) was applied aiming at a change of hearing-loss induced cortical map reorganization. Data analysis showed a significantly increased ratio between alpha and delta activity for the three neurofeedback groups suggesting an increase of alpha alongside with a decrease of delta over temporal auditory regions. These alterations were also correlated with a significant decline of tinnitus loudness for tinnitus patients. Subjects who were able to modify both bands simultaneously in the desired way showed the strongest relief from tinnitus compared to other groups (i.e., subgroups of patients with only alpha-, only delta-, or no change). Furthermore, the training generally resulted in a reduction of tinnitus related distress that was still notable even 6 months after the termination of the training. No statistically meaningful effects regarding tinnitus loudness or distress were found in the FDT group. In order to replicate these findings, Crocetti et al. (2011) conducted a study with 15 normal hearing tinnitus patients and tried to train them in decreasing delta and increasing alpha frequency bands. Even though no significant differences between pre- and post-training EEG patterns have been found, the results suggested an obvious trend toward an

[5]https://www.ncbi.nlm.nih.gov/pubmed/

TABLE 1 | Summary of studies investigating neurofeedback for treatment of tinnitus.

Authors	Tinnitus patients	Neurofeedback	Electrodes/Sources	Feedback	Behavioral findings	Neuronal findings
Crocetti et al., 2011	$N = 15$	α↑ δ↓ 12 sessions	F3, F4, Fc1, Fc2	Plane moving up and down (with audio-visual reinforcement)	Distress ↓ Loudness ↓	α/δ-ratio ↑ (not all participants were able to manipulate α and δ successfully)
Dohrmann et al., 2007a,b	Group 1 ($n = 11$) Group 2 ($n = 5$) Group 3 ($n = 5$) Controls ($n = 27$)	Group 1: α↑ δ↓ Group 2: α↑ Group 3: δ↓ Control: FDT 10 sessions	F3, F4, Fc1, Fc2	Fish moving up and down	All groups: Distress ↓ Loudness↓ Group 1: strongest relief Controls: no reduction	All groups: α↑ and δ↓ Correlation with decrease in loudness
Gosepath et al., 2001	$N = 40$ Controls ($n = 15$)	α↑ β↓ 15 sessions	P4	Auditory and visual (not further explained)	Distress ↓	Group 1 ($n = 24$): α↑ Group 2 ($n = 16$): β↓ Controls: no effect
Hartmann et al., 2013	$N = 8$ Controls ($n = 9$)	α↑ 10 sessions Controls: rTMS	Source space projection on two temporal sources	Smiley	Distress ↓ Controls: no reductions	α↑ estimated over r PAC
Schenk et al., 2005	Group 1 ($n = 23$) Group 2 ($n = 13$)	Group 1: α↑ Group 2: β↓ Group 3: α↑ β↓	Group 1: P4 Group 2: C3	Floating ball and melody	Distress ↓	Both groups: α↑
Vanneste et al., 2016	Group 1 ($n = 23$) Controls 1 ($n = 17$) Controls 2 ($n = 22$)	Group 1: α↑ β↓ γ↓ 15 sessions Controls 1: α↑ β↓ γ↓ Controls 2: passive	sLORETA Group 1: PCC Controls 1: LG	Green bar moving up and down	Group 1: distress ↓ Controls: no reduction	No alterations in target areas for α, β and γ Changes in functional and effectivity connectivity
Weiler et al., 2002	$N = 1$	α↑ β↑ δ↑ θ↑	19 electrodes	Varying	Depression ↓ Anxiety ↓ Tinnitus ↓	No analysis

↑, *increase;* ↓, *decrease; r PAC, right primary auditory cortex; PCC, posterior cingulate cortex; LG, lingual gyrus.*

increasing alpha/delta ratio. In addition, scores evaluated with the Tinnitus Handicap Questionnaire (THI) (Newman et al., 1996) indicated significant improvements, which were maintained after the end of the training period.

All in all, these two studies suggested the protocol of upregulating alpha and downregulating delta to be a highly promising approach in tinnitus treatment. However, the surface-based nature of the neurofeedback application by simply using four electrodes on the scalp could not ensure that the brain activity used for the feedback indeed originated in the auditory areas. To address this problem, Hartmann et al. (2013) used a 32-channel EEG system and projected the recorded activity on the surface to eight regional dipole-sources, of which two were situated in the temporal cortex. Eight subjects of this investigation received neurofeedback treatment to train an increase of alpha power and nine subjects were treated with repetitive transcranial magnetic stimulation (rTMS). With the completion of the training, only patients of the neurofeedback group showed improved tinnitus distress scores. In comparison to the control group with rTMS treatment, they achieved significantly ameliorated scores in the TQ. Additionally, a comparison of MEG resting-state activity before and after treatment combined with spatial filtering based on a LCMV beamformer algorithm (van Veen et al., 1997) revealed a significant increase of alpha activity over the right primary auditory cortex. According to Hartmann et al. (2013) this proves that alpha activity can be systematically altered in the primary auditory cortex which helps restore the disturbed excitatory–inhibitory balance of tinnitus patients.

Finally, two recently published neurofeedback studies shall be mentioned. Milner et al. (2015) used SCP neurofeedback training in a case report and could show decreased tinnitus pitch and loudness as well as a reduction of delta and theta frequencies over left hemispheric fronto-temporal and temporo-occipital electrodes which they interpret as a normalization of tinnitus-specific activity. Vanneste et al. (2016) applied neurofeedback combined with sLORETA source estimation to a group of 58 tinnitus patients. A first group ($n = 23$) of this study received alpha-up training, and beta- and gamma-down training whereby the feedback was limited on the activity that was estimated to originate over the posterior cingulate cortex (PCC). A second group of 17 tinnitus patients received the same training but for activity over the lingual gyrus and a third group ($n = 18$) did not receive any treatment at all. Decreased tinnitus distress was only found for the PCC-group but no significant changes in any frequency bands were found in the trained areas. However, decreased cross-frequency coupling (i.e., alpha to beta and alpha to gamma power nesting) in the PCC and changes in functional and effective connectivity between PCC and different areas of the distress network suggest a specific effect of this training.

Finally, even though this review mainly focuses on neurofeedback based on electrophysiological recordings, it shall be noted that also real-time fMRI protocols are currently being developed and tested for tinnitus treatment with promising results (Haller et al., 2010, 2013; Emmert et al., 2017). In their investigations, the auditory cortex of tinnitus patients is first precisely localized thanks to the good spatial resolution of fMRI, and, subsequently, neurofeedback training aiming at reducing auditory BOLD activity provided. Even though this protocol leads to the intended neuronal alterations, no significant effects on tinnitus symptoms have been reported (Emmert et al., 2017).

Limitations of Neurofeedback Training Studies

Currently, the AAPB rates the efficacy of chronic tinnitus treatment with neurofeedback as *possibly efficacious* (level 2) (Tan et al., 2016). Although various neurofeedback training protocols showed promising results in treatment of several neurological disorders, there still remain limitations and open issues which need to be addressed. In particular, EEG- and MEG-based neurofeedback studies are often criticized about the low spatial resolution of electrophysiological recordings. Despite more refined source estimation algorithms, an uncertainty about the precision of the estimation remains, which is especially important when changes in frequency bands are considered as primary outcome measures. Studies that are able to verify specific effects in the brain areas of interest are still scarce and successful improvements of certain symptoms are thus often criticized to be the mere result of unspecific placebo effects (Thibault et al., 2016, 2017). Expectations of researcher and participant, the treatment condition in general (e.g., taking time off from a busy work schedule) and interactions with the practitioner (such as, the simple meeting with a clinician) can contribute greatly to the improvement of psychological symptoms. This problem is especially predominant in the context of chronic tinnitus therapy where most participants turn to neurofeedback hopefully after repeatedly being told by their doctors that nothing can be done to treat tinnitus and having undergone a wide variety of (sometimes rather questionable) treatments on their own.

One way to resolve this issue is to improve study designs and conduct double-blind trials with control groups using a form of sham neurofeedback. In this context, Thibault et al. (2016) suggest the use of prerecorded feedback of other participants, feedback of another disease-unrelated brain area, or inverse feedback protocols that reward unwanted and inhibit wanted changes of brain activity. The use of sham-control is, however, difficult to establish in clinical neurofeedback trials because of several reasons. First, participation in neurofeedback treatment studies requires considerable investments in time and energy on the part of participants as they generally have to attend multiple training sessions over the course of several weeks. Furthermore, in sham-controlled clinical studies, participants always enter a trial with some form of expectation and hope to be part of the treatment group. Absent success after the first training sessions may lead to a misleading belief that they instead have been assigned to the control group which negatively affects their motivation and further success in the training process (Strehl et al., 2017). These drawbacks of placebo-controlled trials have to be considered and alleviated with appropriate designs, such as a cross-over approach where one group of participants receives sham training first while the other starts with verum treatment. In a second step the protocols are swapped so that both groups undergo sham- as well as verum-neurofeedback. In this context several authors point to the importance of a

systematic investigation of non-specific factors in neurofeedback studies (Friedrich et al., 2014; Sitaram et al., 2017; Thibault et al., 2017). Appropriate knowledge about the factors favoring and the ones hindering success in neurofeedback treatment can indeed lead to a better understanding of the actual mode of action of neurofeedback as well as help improve the treatment setting in order to optimize therapy outcomes for patients.

A major flaw of previous neurofeedback studies is that most of them settle for reporting positive effects of their trained protocol. It is known, however, that there is a wide variability among the efficacy of neurofeedback treatment for different subjects. While some are able to successfully self-regulate their neural activity in the desired way and show improvements of corresponding symptoms (responders), others fail to do so (non-responders) (Friedrich et al., 2014). This issue was described as *neurofeedback inefficacy* by Alkoby et al. (2017) who provide a thorough review about this currently existing topic. In their publication, they chose 20 papers published after 2010 at random and found that only two of them reported the actual number of responders and non-responders in their studies. This, of course, hampers a proper evaluation of the feasibility of a given neurofeedback protocol for the treatment of a certain disorder. For one thing, positive effects of the training might be concealed or confounded by the negative results of non-responders in the clinical trial. Furthermore, information provided about responder and non-responder groups helps define and analyze factors for success or failure of the protocol. That is, by means of a thorough investigation of the attributes of responders and non-responders, predictors for (un-) successful neurofeedback can be identified, which can be used to improve training protocols for future patients.

Another issue in this context is the high heterogeneity among outcome measures and definitions to appropriately measure success or failure used in previous neurofeedback studies. On the one hand, it can be useful to use a wide variety of outcome measures in a clinical study in order to account for changes which might not be anticipated in the first place. For instance, it can be important to measure the general level of stress of tinnitus patients as the positive effects of neurofeedback could also be explained by a decrease of the general stress condition of the patient. However, guidelines need to be established which suggest the use of certain questionnaires or tests for a given field of interest to which scholars can relate when planning an investigation [substantial work in the tinnitus field is currently being done by Hall et al. (2016) in this context]. This will limit the amount of different outcome measures in clinical trials, promote the use of well-established and validated questionnaires, and foster direct comparability between findings of different investigations. Additionally, guidelines in the context of neurofeedback treatment need to answer the question as to what can be regarded as successful or unsuccessful training and how to distinguish responders from non-responders. Is it already sufficient that a given symptom simply changes over the course of a training in a positive way or does it have to improve by a certain amount (e.g., an increase by certain points in a questionnaire score)? What, on the other hand, needs to happen to and in between brain circuits? How and how much does neural activity have to be altered by the neurofeedback treatment so that an individual can be labeled as a responder? Even though some publications already tried to postulate criteria or guidelines (Gruzelier, 2014; Rogala et al., 2016; Enriquez-Geppert et al., 2017), many open issues remain in this regard.

CONCLUSION

In this review, we summarized and discussed the current state of electrophysiological brain research in the field of chronic tinnitus as well as recent advances of neurofeedback treatment. Up to date, only a handful of studies exist that investigated feasibility of neurofeedback protocols for chronic tinnitus patients. While the first studies in this context rather focused on creating a general state of relaxation for the subject, later trials considered tinnitus-specific alterations in brain activity based on comparisons of EEG or MEG resting-state recordings between tinnitus patients and healthy controls. The main region of interest in these studies was the auditory cortex, and fairly good results have been achieved following this approach. With the newer developments in tinnitus research and the numerous investigations dealing with differences within the tinnitus population, which take into account the substantial amount of heterogeneity amongst tinnitus sufferers, also other potential tinnitus-related brain areas can be targeted in future neurofeedback studies. A good example in this regard is the recent publication by Vanneste et al. (2016) where the posterior cingulate cortex as part of the tinnitus distress network has been targeted. Furthermore, this investigation is the only neurofeedback study in the context of chronic tinnitus treatment to date that included a control group with training of a tinnitus-irrelevant brain area in its design.

To sum up, even though often criticized in the recent past, results of current studies suggest that neurofeedback seems to be a promising method for efficient tinnitus treatment and may enjoy great popularity in the future. The ultimate goal may be to develop different neurofeedback alternatives for a given subgroup of tinnitus sufferers or even establish neurofeedback on an individualized basis for each patient. In this context, multi-location and multi-frequency neurofeedback protocols with adequate source estimation algorithms, which are able to train multiple brain networks in power and maybe even connectivity changes simultaneously, can be seen as the gold standard for future neurofeedback protocols. At the moment, however, there still exist several challenges that need to be overcome. A general issue are technological aspects of electrophysiological measurements (e.g., the limited spatial precision of resting-state EEG recordings) and neurofeedback applications (e.g., the implementation of connectivity-based neurofeedback protocols) that need to be improved. Regarding the treatment of chronic tinnitus in particular, results of existing fundamental studies are still too heterogeneous in order to suffice for the development of more sophisticated neurofeedback protocols. One possibility to resolve this latter issue is by means of the establishment of general guidelines about adequate symptom assessment, measurement paradigms, and analysis methods. In this way, more coherent and comparable results should be published in order to lead

to a better understanding of tinnitus heterogeneity and its underlying alterations in brain networks that could be tackled by future neurofeedback protocols. Additionally, this urgent need for guidelines has been shown to be an open issue in the field of clinical neurofeedback research in general. Clarity is needed about how to separate responders from non-responders, and which outcome domains and measurements are best suited to do so. Furthermore, also non-specific effects of the training have to be taken into account and systematic investigations about the most (or least) favorable neurofeedback settings and treatment conditions are needed.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Adamchic, I., Langguth, B., Hauptmann, C., and Tass, P. A. (2014a). Abnormal cross-frequency coupling in the tinnitus network. *Front. Neurosci.* 8:284. doi: 10.3389/fnins.2014.00284

Adamchic, I., Toth, T., Hauptmann, C., and Tass, P. A. (2014b). Reversing pathologically increased EEG power by acoustic coordinated reset neuromodulation. *Hum. Brain Mapp.* 35, 2099–2118. doi: 10.1002/hbm.22314

Adamchic, I., Toth, T., Hauptmann, C., Walger, M., Langguth, B., Klingmann, I., et al. (2017). Acute effects and after-effects of acoustic coordinated reset neuromodulation in patients with chronic subjective tinnitus. *Neuroimage Clin.* 15, 541–558. doi: 10.1016/j.nicl.2017.05.017

Adjamian, P. (2014). The application of electro- and magneto-encephalography in tinnitus research - methods and interpretations. *Front. Neurol.* 5:228. doi: 10.3389/fneur.2014.00228

Adjamian, P., Sereda, M., and Hall, D. A. (2009). The mechanisms of tinnitus. Perspectives from human functional neuroimaging. *Hear. Res.* 253, 15–31. doi: 10.1016/j.heares.2009.04.001

Alkoby, O., Abu-Rmileh, A., Shriki, O., and Todder, D. (2017). Can we predict who will respond to neurofeedback? A review of the inefficacy problem and existing predictors for successful EEG neurofeedback learning. *Neuroscience* doi: 10.1016/j.neuroscience.2016.12.050 [Epub ahead of print].

Arns, M., De Ridder, S., Strehl, U., Breteler, M., and Coenen, A. (2009). Efficacy of neurofeedback treatment in ADHD: the effects on inattention, impulsivity and hyperactivity: a meta-analysis. *Clin. EEG Neurosci.* 40, 180–189. doi: 10.1177/155005940904000311

Ashton, H., Reid, K., Marsh, R., Johnson, I., Alter, K., and Griffiths, T. D. (2007). High frequency localised "hot spots" in temporal lobes of patients with intractable tinnitus: a quantitative electroencephalographic (QEEG) study. *Neurosci. Lett.* 426, 23–28. doi: 10.1016/j.neulet.2007.08.034

Bauer, H., and Pllana, A. (2014). EEG-based local brain activity feedback training - tomographic neurofeedback. *Front. Hum. Neurosci.* 8:1005. doi: 10.3389/fnhum.2014.01005

Cannon, R. L., Baldwin, D. R., Diloreto, D. J., Phillips, S. T., Shaw, T. L., and Levy, J. J. (2014). LORETA neurofeedback in the precuneus: operant conditioning in basic mechanisms of self-regulation. *Clin EEG Neurosci.* 45, 238–248. doi: 10.1177/1550059413512796

Cannon, R. L., Congedo, M., Lubar, J. F., and Hutchens, T. (2009). Differentiating a network of executive attention: loreta neurofeedback in anterior cingulate and dorsolateral prefrontal cortices. *Int. J. Neurosci.* 119, 404–441. doi: 10.1080/00207450802480325

Cannon, R. L., Lubar, J. F., Congedo, M., Thornton, K., Towler, K., and Hutchens, T. (2007). The effects of neurofeedback training in the cognitive division of the anterior cingulate gyrus. *Int. J. Neurosci.* 117, 337–357. doi: 10.1080/00207450500514003

Cannon, R. L., Lubar, J. F., Gerke, A., Thornton, K., Hutchens, T., and McCammon, V. (2006). EEG spectral-power and coherence: LORETA neurofeedback training in the anterior cingulate gyrus. *J. Neurother.* 10, 5–31. doi: 10.1300/J184v10n01_02

Congedo, M. (2006). Subspace projection filters for real-time brain electromagnetic imaging. *IEEE Trans. Biomed. Eng.* 53, 1624–1634. doi: 10.1109/TBME.2006.878055

Congedo, M., Lubar, J. F., and Joffe, D. (2004). Low-resolution electromagnetic tomography neurofeedback. *IEEE Trans. Neural Syst. Rehabil. Eng.* 12, 387–397. doi: 10.1109/TNSRE.2004.840492

Crocetti, A., Forti, S., and Del Bo, L. (2011). Neurofeedback for subjective tinnitus patients. *Auris Nasus Larynx* 38, 735–738. doi: 10.1016/j.anl.2011.02.003

Datko, M., Pineda, J. A., and Muller, R.-A. (2017). Positive effects of neurofeedback on autism symptoms correlate with brain activation during imitation and observation. *Eur. J. Neurosci.* doi: 10.1111/ejn.13551 [Epub ahead of print].

De Ridder, D., Elgoyhen, A. B., Romo, R., and Langguth, B. (2011). Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8075–8080. doi: 10.1073/pnas.1018466108

De Ridder, D., Fransen, H., Francois, O., Sunaert, S., Kovacs, S., and van de Heyning, P. (2006). Amygdalohippocampal involvement in tinnitus and auditory memory. *Acta Otolaryngol.* 126, 50–53. doi: 10.1080/03655230600895580

De Ridder, D., Vanneste, S., and Freeman, W. (2014a). The Bayesian brain: phantom percepts resolve sensory uncertainty. *Neurosci. Biobehav. Rev.* 44, 4–15. doi: 10.1016/j.neubiorev.2012.04.001

De Ridder, D., Vanneste, S., Weisz, N., Londero, A., Schlee, W., Elgoyhen, A. B., et al. (2014b). An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci. Biobehav. Rev.* 44, 16–32. doi: 10.1016/j.neubiorev.2013.03.021

De Ridder, D., Vanneste, S., Langguth, B., and Llinás, R. R. (2015). Thalamocortical dysrhythmia: a theoretical update in tinnitus. *Front. Neurol.* 6:124. doi: 10.3389/fneur.2015.00124

Dohrmann, K., Elbert, T., Schlee, W., and Weisz, N. (2007a). Tuning the tinnitus percept by modification of synchronous brain activity. *Restor. Neurol. Neurosci.* 25, 371–378.

Dohrmann, K., Weisz, N., Schlee, W., Hartmann, T., and Elbert, T. (2007b). Neurofeedback for treating tinnitus. *Prog. Brain Res.* 166, 473–485. doi: 10.1016/S0079-6123(07)66046-4

Eggermont, J. J. (1990). On the pathophysiology of tinnitus; a review and a peripheral model. *Hear. Res.* 48, 111–123. doi: 10.1016/0378-5955(90)90202-Z

Eggermont, J. J. (2015). The auditory cortex and tinnitus – a review of animal and human studies. *Eur. J. Neurosci.* 41, 665–676. doi: 10.1111/ejn.12759

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Elgoyhen, A. B., Langguth, B., De Ridder, D., and Vanneste, S. (2015). Tinnitus: perspectives from human neuroimaging. *Nat. Rev. Neurosci.* 16, 632–642. doi: 10.1038/nrn4003

Emmert, K., Kopel, R., Koush, Y., Maire, R., Senn, P., van de Ville, D., et al. (2017). Continuous vs. intermittent neurofeedback to regulate auditory cortex activity of tinnitus patients using real-time fMRI - A pilot study. *Neuroimage. Clin.* 14, 97–104. doi: 10.1016/j.nicl.2016.12.023

Enriquez-Geppert, S., Huster, R. J., and Herrmann, C. S. (2017). EEG-Neurofeedback as a tool to modulate cognition and behavior: a review tutorial. *Front. Hum. Neurosci.* 11:51. doi: 10.3389/fnhum.2017.00051

Friedrich, E. V. C., Wood, G., Scherer, R., and Neuper, C. (2014). Mind over brain, brain over mind: cognitive causes and consequences of controlling brain activity. *Front. Hum. Neurosci.* 8:348. doi: 10.3389/fnhum.2014.00348

Fuchs, T., Birbaumer, N., Lutzenberger, W., Gruzelier, J. H., and Kaiser, J. (2003). Neurofeedback treatment for attention-deficit/hyperactivity disorder in children: a comparison with methylphenidate. *Appl. Psychophysiol. Biofeedback* 28, 1–12. doi: 10.1023/A:1022353731579

Gevensleben, H., Holl, B., Albrecht, B., Vogel, C., Schlamp, D., Kratz, O., et al. (2009). Is neurofeedback an efficacious treatment for ADHD? A randomised controlled clinical trial. *J. Child Psychol. Psychiatry* 50, 780–789. doi: 10.1111/j.1469-7610.2008.02033.x

Goebel, G., and Hiller, W. (1994). Tinnitus-Fragebogen (TF). Standardinstrument zur Graduierung des Tinnitusschweregrades. Ergebnisse einer Multicenterstudie mit dem Tinnitus-Fragebogen (TF) [The tinnitus questionnaire. A standard instrument for grading the degree of tinnitus. Results of a multicenter study with the tinnitus questionnaire]. *HNO* 42, 166–172. doi: 10.1007/s00106-013-2798-9

Gosepath, K., Nafe, B., Ziegler, E., and Mann, W. J. (2001). Neurofeedback in der Therapie des Tinnitus [Neurofeedback in therapy of tinnitus]. *HNO* 49, 29–35. doi: 10.1007/s001060050704

Grosse-Wentrup, M., Liefhold, C., Gramann, K., and Buss, M. (2009). Beamforming in noninvasive brain-computer interfaces. *IEEE Trans. Biomed. Eng.* 56, 1209–1219. doi: 10.1109/TBME.2008.2009768

Gruzelier, J. H. (2014). EEG-neurofeedback for optimising performance. III. A review of methodological and theoretical considerations. *Neurosci. Biobehav. Rev.* 44, 159–182. doi: 10.1016/j.neubiorev.2014.03.015

Hall, D. A., Haider, H., Szczepek, A. J., Lau, P., Rabau, S., Jones-Diette, J., et al. (2016). Systematic review of outcome domains and instruments used in clinical trials of tinnitus treatments in adults. *Trials* 17, 270. doi: 10.1186/s13063-016-1399-9

Haller, S., Birbaumer, N., and Veit, R. (2010). Real-time fMRI feedback training may improve chronic tinnitus. *Eur. Radiol.* 20, 696–703. doi: 10.1007/s00330-009-1595-z

Haller, S., Kopel, R., Jhooti, P., Haas, T., Scharnowski, F., Lovblad, K.-O., et al. (2013). Dynamic reconfiguration of human brain functional networks through neurofeedback. *Neuroimage* 81, 243–252. doi: 10.1016/j.neuroimage.2013.05.019

Hammond, D. C. (2005). Neurofeedback with anxiety and affective disorders. *Child Adolesc. Psychiatr. Clin. N. Am.* 14, 105. doi: 10.1016/j.chc.2004.07.008

Hammond, D. C. (2011). What is Neurofeedback. An Update. *J. Neurother.* 15, 305–336. doi: 10.1080/10874208.2011.623090

Hartmann, T., Lorenz, I., Müller, N., Langguth, B., and Weisz, N. (2013). The effects of neurofeedback on oscillatory processes related to tinnitus. *Brain Topogr.* 27, 149–157. doi: 10.1007/s10548-013-0295-9

Heller, A. J. (2003). Classification and epidemiology of tinnitus. *Otolaryngol. Clin. N. Am.* 36, 239–248. doi: 10.1016/S0030-6665(02)00160-3

Henry, J. A., Dennis, K. C., and Schechter, M. A. (2005). General review of tinnitus: prevalence, mechanisms, effects, and management. *J. Speech Lang. Hear. Res.* 48, 1204–1235. doi: 10.1044/1092-4388(2005/084)

Hillebrand, A., Singh, K. D., Holliday, I. E., Furlong, P. L., and Barnes, G. R. (2005). A new approach to neuroimaging with magnetoencephalography. *Hum. Brain Mapp.* 25, 199–211. doi: 10.1002/hbm.20102

Howe, R. C., and Sterman, M. B. (1972). Cortical-subcortical EEG correlates of suppressed motor behavior during sleep and waking in the cat. *Electroencephalogr. Clin. Neurophysiol.* 32, 681–695. doi: 10.1016/0013-4694(72)90104-6

Howe, R. C., and Sterman, M. B. (1973). Somatosensory system evoked potentials during waking behavior and sleep in the cat. *Electroencephalogr. Clin. Neurophysiol.* 34, 605–618. doi: 10.1016/0013-4694(73)90006-0

Jasper, H., and Shagass, C. (1941). Conditioning of the occipital alpha rhythm in man. *J. Exp. Psychol.* 28, 373–388. doi: 10.1037/h0056139

Jastreboff, P. J. (1990). Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci. Res.* 8, 221–254. doi: 10.1016/0168-0102(90)90031-9

Jiang, Y., Abiri, R., and Zhao, X. (2017). Tuning up the old brain with new tricks: attention training via neurofeedback. *Front. Aging Neurosci.* 9:52. doi: 10.3389/fnagi.2017.00052

Joos, K., Vanneste, S., and De Ridder, D. (2012). Disentangling depression and distress networks in the tinnitus brain. *PLOS ONE* 7:e40544. doi: 10.1371/journal.pone.0040544.g001

Kahlbrock, N., and Weisz, N. (2008). Transient reduction of tinnitus intensity is marked by concomitant reductions of delta band power. *BMC Biol.* 6:4. doi: 10.1186/1741-7007-6-4

Kelley, N. J., Hortensius, R., Schutter, D. J. L. G., and Harmon-Jones, E. (2017). The relationship of approach/avoidance motivation and asymmetric frontal cortical activity. A review of studies manipulating frontal asymmetry. *Int. J. Psychophysiol.* 119, 19–30. doi: 10.1016/j.ijpsycho.2017.03.001

Kober, S. E., Schweiger, D., Reichert, J. L., Neuper, C., and Wood, G. (2017). Upper Alpha based neurofeedback training in chronic stroke: brain plasticity processes and cognitive effects. *Appl. Psychophysiol. Biofeedback* 42, 69–83. doi: 10.1007/s10484-017-9353-5

Koberda, J. L., Koberda, P., Bienkiewicz, A. A., Moses, A., and Koberda, L. (2013). Pain management using 19-Electrode Z-Score LORETA neurofeedback. *J. Neurother.* 17, 179–190. doi: 10.1080/10874208.2013.813204

Koberda, J. L., Moses, A., Koberda, L., and Koberda, P. (2012). Cognitive enhancement using 19-Electrode Z-Score neurofeedback. *J. Neurother.* 16, 224–230. doi: 10.1080/10874208.2012.705769

Kopřivová, J., Congedo, M., Razska, M., Praško, J., Brunovský, M., and Horáček, J. (2013). Prediction of treatment response and the effect of independent component neurofeedback in obsessive-compulsive disorder: a randomized, sham-controlled, and double-blind study. *Neuropsychobiology* 67, 210–223. doi: 10.1159/000347087

Landgrebe, M., Zeman, F., Koller, M., Eberl, Y., Mohr, M., Reiter, J., et al. (2010). The Tinnitus Research Initiative (TRI) database: a new approach for delineation of tinnitus subtypes and generation of predictors for treatment outcome. *BMC Med. Inform. Decis. Mak.* 10:42. doi: 10.1186/1472-6947-10-42

Langguth, B., Kreuzer, P. M., Kleinjung, T., and De Ridder, D. (2013). Tinnitus. Causes and clinical management. *Lancet Neurol.* 12, 920–930. doi: 10.1016/S1474-4422(13)70160-1

Legarda, S. B., McMahon, D., Othmer, S., and Othmer, S. (2011). Clinical neurofeedback. Case studies, proposed mechanism, and implications for pediatric neurology practice. *J. Child Neurol.* 26, 1045–1051. doi: 10.1177/0883073811405052

Lévesque, J., Beauregard, M., and Mensour, B. (2006). Effect of neurofeedback training on the neural substrates of selective attention in children with attention-deficit/hyperactivity disorder: a functional magnetic resonance

imaging study. *Neurosci. Lett.* 394, 216–221. doi: 10.1016/j.neulet.2005.10.100

Llinás, R. R., Ribary, U., Contreras, D., and Pedroarena, C. (1998). The neuronal basis for consciousness. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 353, 1841–1849. doi: 10.1098/rstb.1998.0336

Llinás, R. R., Ribary, U., Jeanmonod, D., Kronberg, E., and Mitra, P. P. (1999). Thalamocortical dysrhythmia: a neurological and neuropsychiatric syndrome characterized by magnetoencephalography. *Proc. Natl. Acad. Sci. U.S.A.* 96, 15222–15227. doi: 10.1073/pnas.96.26.15222

Llinás, R. R., Urbano, F. J., Leznik, E., Ramírez, R. R., and van Marle, H. J. F. (2005). Rhythmic and dysrhythmic thalamocortical dynamics: GABA systems and the edge effect. *Trends Neurosci.* 28, 325–333. doi: 10.1016/j.tins.2005.04.006

Lofthouse, N., Arnold, L. E., Hersch, S., Hurt, E., and DeBeus, R. (2012). A review of neurofeedback treatment for pediatric ADHD. *J. Atten. Disord.* 16, 351–372. doi: 10.1177/1087054711427530

Loomis, A. L., Harvey, E. N., and Hobart, G. (1936). Electrical potentials of the human brain. *J. Exp. Psychol.* 19, 249–279. doi: 10.1037/h0062089

Lorenz, I., Müller, N., Schlee, W., Hartmann, T., and Weisz, N. (2009). Loss of alpha power is related to increased gamma synchronization—A marker of reduced inhibition in tinnitus? *Neurosci. Lett.* 453, 225–228. doi: 10.1016/j.neulet.2009.02.028

Lubar, J. F., and Bahler, W. W. (1976). Behavioral management of epileptic seizures following EEG biofeedback training of the sensorimotor rhythm. *Biofeedback Self Regul.* 1, 77–104. doi: 10.1007/BF00998692

Lubar, J. F., Swartwood, M. O., Swartwood, J. N., and O'Donnell, P. H. (1995). Evaluation of the effectiveness of EEG neurofeedback training for ADHD in a clinical setting as measured by changes in T.O.V.A. scores, behavioral ratings, and WISC-R performance. *Biofeedback Self Regul.* 20, 83–99. doi: 10.1007/BF01712768

Lubar, J. O., and Lubar, J. F. (1984). Electroencephalographic biofeedback of SMR and beta for treatment of attention deficit disorders in a clinical setting. *Biofeedback Self Regul.* 9, 1–23. doi: 10.1007/BF00998842

Mennella, R., Patron, E., and Palomba, D. (2017). Frontal alpha asymmetry neurofeedback for the reduction of negative affect and anxiety. *Behav. Res. Ther.* 92, 32–40. doi: 10.1016/j.brat.2017.02.002

Meyer, M., Luethi, M. S., Neff, P., Langer, N., and Büchi, S. (2014). Disentangling tinnitus distress and tinnitus presence by means of EEG power analysis. *Neural Plast.* 2014, 1–13. doi: 10.1155/2014/468546

Meyer, M., Neff, P., Grest, A., Hemsley, C., Weidt, S., and Kleinjung, T. (2017). EEG oscillatory power dissociates between distress- and depression-related psychopathology in subjective tinnitus. *Brain Res.* 1663, 194–204. doi: 10.1016/j.brainres.2017.03.007

Michel, C. M., Murray, M. M., Lantz, G., Gonzalez, S., Spinelli, L., and Grave de Peralta, R. (2004). EEG source imaging. *Clin. Neurophysiol.* 115, 2195–2222. doi: 10.1016/j.clinph.2004.06.001

Milner, R., Lewandowska, M., Ganc, M., Cieśla, K., Niedziałek, I., and Skarżyński, H. (2015). Slow cortical potential neurofeedback in chronic tinnitus therapy: a case report. *Appl. Psychophysiol. Biofeedback* 41, 225–249. doi: 10.1007/s10484-015-9318-5

Moazami-Goudarzi, M., Michels, L., Weisz, N., and Jeanmonod, D. (2010). Temporo-insular enhancement of EEG low and high frequencies in patients with chronic tinnitus. *BMC Neurosci.* 11:40. doi: 10.1186/1471-2202-11-40

Mohan, A., De Ridder, D., and Vanneste, S. (2016a). Emerging hubs in phantom perception connectomics. *Neuroimage Clin.* 11, 181–194. doi: 10.1016/j.nicl.2016.01.022

Mohan, A., De Ridder, D., and Vanneste, S. (2016b). Graph theoretical analysis of brain connectivity in phantom sound perception. *Sci. Rep.* 6:19683. doi: 10.1038/srep19683

Mohan, A., De Ridder, D., and Vanneste, S. (2017a). Robustness and dynamicity of functional networks in phantom sound. *Neuroimage* 146, 171–187. doi: 10.1016/j.neuroimage.2016.04.033

Mohan, A., Moreno, N., Song, J.-J., De Ridder, D., and Vanneste, S. (2017b). Evidence for behaviorally segregated, spatiotemporally overlapping subnetworks in phantom sound perception. *Brain Connect.* 7, 197–210. doi: 10.1089/brain.2016.0459

Møller, A. R. (1984). Pathophysiology of tinnitus. *Otolaryngol. Clin. N. Am.* 36, 249–266.

Monastra, V. J., Monastra, D. M., and George, S. (2002). The effects of stimulant therapy, EEG biofeedback, and parenting style on the primary symptoms of attention-deficit/hyperactivity disorder. *Appl. Psychophysiol. Biofeedback* 27, 231–249. doi: 10.1023/A:1021018700609

Mühlnickel, W., Elbert, T., Taub, E., and Flor, H. (1998). Reorganization of auditory cortex in tinnitus. *Proc. Natl. Acad. Sci. U.S.A.* 95, 10340–10343. doi: 10.1073/pnas.95.17.10340

Newman, C. W., Jacobson, G. P., and Spitzer, J. B. (1996). Development of the tinnitus handicap inventory. *Arch. Otolaryngol. Head Neck Surg.* 122, 143–148. doi: 10.1001/archotol.1996.01890140029007

Noreña, A. J. (2011). An integrative model of tinnitus based on a central gain controlling neural sensitivity. *Neurosci. Biobehav. Rev.* 35, 1089–1109. doi: 10.1016/j.neubiorev.2010.11.003

Noreña, A. J., and Eggermont, J. J. (2003). Changes in spontaneous neural activity immediately after an acoustic trauma. Implications for neural correlates of tinnitus. *Hear. Res.* 183, 137–153. doi: 10.1016/S0378-5955(03)00225-9

Ogawa, S., Lee, T. M., Kay, A. R., and Tank, D. W. (1990). Brain magnetic resonance imaging with contrast dependent on blood oxygenation. *Proc. Natl. Acad. Sci. U.S.A.* 87, 9868–9872. doi: 10.1073/pnas.87.24.9868

Ortmann, M., Müller, N., Schlee, W., and Weisz, N. (2011). Rapid increases of gamma power in the auditory cortex following noise trauma in humans. *Eur. J. Neurosci.* 33, 568–575. doi: 10.1111/j.1460-9568.2010.07542.x

Pascual-Marqui, R. D. (2002). Standardized low-resolution brain electromagnetic tomography (sLORETA): technical details. *Methods Find. Exp. Clin. Pharmacol.* 24, 5–12.

Pascual-Marqui, R. D., Michel, C. M., and Lehmann, D. (1994). Low resolution electromagnetic tomography: a new method for localizing electrical activity in the brain. *Int. J. Psychophysiol.* 18, 49–65. doi: 10.1016/0167-8760(84)90014-X

Pierzycki, R. H., McNamara, A. J., Hoare, D. J., and Hall, D. A. (2015). Whole scalp resting state EEG of oscillatory brain activity shows no parametric relationship with psychoacoustic and psychosocial assessment of tinnitus: a repeated measures study. *Hear. Res.* 331, 101–108. doi: 10.1016/j.heares.2015.11.003

Pllana, A., and Bauer, H. (2011). BEM-based SMS-LORETA – an advanced method to localize multiple simultaneously active sources in the cerebral cortex. arXiv:1106.2679

Rauschecker, J. P., Leaver, A. M., and Mühlau, M. (2010). Tuning out the noise: limbic-auditory interactions in tinnitus. *Neuron* 66, 819–826. doi: 10.1016/j.neuron.2010.04.032

Rauschecker, J. P., May, E. S., Maudoux, A., and Ploner, M. (2015). Frontostriatal gating of tinnitus and chronic pain. *Trends Cogn. Sci.* 19, 567–578. doi: 10.1016/j.tics.2015.08.002

Rockstroh, B., Elbert, T., Birbaumer, N., Wolf, P., Düchting-Röth, A., Reker, M., et al. (1993). Cortical self-regulation in patients with epilepsies. *Epilepsy Res.* 14, 63–72. doi: 10.1016/0920-1211(93)90075-I

Rogala, J., Jurewicz, K., Paluch, K., Kublik, E., Cetnarski, R., and Wrobel, A. (2016). The Do's and Don'ts of neurofeedback training: a review of the controlled studies using healthy adults. *Front. Hum. Neurosci.* 10:301. doi: 10.3389/fnhum.2016.00301

Schenk, S., Lamm, K., Gündel, H., and Ladwig, K.-H. (2005). Neurofeedbackgestütztes EEG-alpha- und EEG-beta-training. Wirksamkeit in der Therapie des chronisch-dekompensierten Tinnitus [Neurofeedback-based EEG alpha and EEG beta training. Effectiveness in patients with chronically decompensated tinnitus]. *HNO* 53, 29–37. doi: 10.1007/s00106-004-1066-4

Scherg, M., and Berg, P. (1991). Use of prior knowledge in brain electromagnetic source analysis. *Brain Topogr.* 4, 143–150. doi: 10.1007/BF01132771

Schlee, W., Dohrmann, K., Hartmann, T., Lorenz, I., Müller, N., Elbert, T., et al. (2008). Assessment and modification of the tinnitus-related cortical network. *Semin. Hear.* 29, 270–287. doi: 10.1055/s-0028-1082033

Schlee, W., Müller, N., Hartmann, T., Keil, J., Lorenz, I., and Weisz, N. (2009). Mapping cortical hubs in tinnitus. *BMC Biol.* 7:80. doi: 10.1186/1741-7007-7-80

Schlee, W., Schecklmann, M., Lehner, A., Kreuzer, P. M., Vielsmeier, V., Poeppl, T. B., et al. (2014). Reduced variability of auditory alpha activity in chronic tinnitus. *Neural Plast.* 2014:436146. doi: 10.1155/2014/436146

Sedley, W., and Cunningham, M. O. (2013). Do cortical gamma oscillations promote or suppress perception? An under-asked question with an over-assumed answer. *Front. Hum. Neurosci.* 7:595. doi: 10.3389/fnhum.2013.00595

Sedley, W., Friston, K. J., Gander, P. E., Kumar, S., and Griffiths, T. D. (2016). An integrative tinnitus model based on sensory precision. *Trends Neurosci.* 39, 799–812. doi: 10.1016/j.tins.2016.10.004

Sedley, W., Teki, S., Kumar, S., Barnes, G. R., Bamiou, D.-E., and Griffiths, T. D. (2012). Single-subject oscillatory gamma responses in tinnitus. *Brain* 135, 3089–3100. doi: 10.1093/brain/aws220

Seki, S., and Eggermont, J. J. (2003). Changes in spontaneous firing rate and neural synchrony in cat primary auditory cortex after localized tone-induced hearing loss. *Hear. Res.* 180, 28–38. doi: 10.1016/S0378-5955(03)00074-1

Simkin, D. R., Thatcher, R. W., and Lubar, J. F. (2014). Quantitative EEG and neurofeedback in children and adolescents: anxiety disorders, depressive disorders, comorbid addiction and attention-deficit/hyperactivity disorder, and brain injury. *Child Adolesc. Psychiatr. Clin. N. Am.* 23, 427–464. doi: 10.1016/j.chc.2014.03.001

Singer, W. (1993). Synchronization of cortical activity and its putative role in information processing and learning. *Annu. Rev. Physiol.* 55, 349–374. doi: 10.1146/annurev.physiol.55.1.349

Sitaram, R., Ros, T., Stoeckel, L., Haller, S., Scharnowski, F., Lewis-Peacock, J., et al. (2017). Closed-loop brain training: the science of neurofeedback. *Nat. Rev. Neurosci.* 18, 86–100. doi: 10.1038/nrn.2016.164

Song, J.-J., Punte, A. K., De Ridder, D., Vanneste, S., and van de Heyning, P. (2013). Neural substrates predicting improvement of tinnitus after cochlear implantation in patients with single-sided deafness. *Hear. Res.* 299, 1–9. doi: 10.1016/j.heares.2013.02.001

Sterman, M. B., and Egner, T. (2006). Foundation and practice of neurofeedback for the treatment of epilepsy. *Appl. Psychophysiol. Biofeedback* 31, 21–35. doi: 10.1007/s10484-006-9002-x

Sterman, M. B., and Friar, L. (1972). Suppression of seizures in an epileptic following sensorimotor EEG feedback training. *Electroencephalogr. Clin. Neurophysiol.* 33, 89–95. doi: 10.1016/0013-4694(72)90028-4

Stouffer, J. L., and Tyler, R. S. (1990). Characterization of tinnitus by tinnitus patients. *J. Speech Hear. Disord.* 55, 439–453. doi: 10.1044/jshd.5503.439

Strehl, U., Aggensteiner, P., Wachtlin, D., Brandeis, D., Albrecht, B., Arana, M., et al. (2017). Neurofeedback of slow cortical potentials in children with attention-deficit/hyperactivity disorder: a multicenter randomized trial controlling for unspecific effects. *Front. Hum. Neurosci.* 11:135. doi: 10.3389/fnhum.2017.00135

Sulzer, J., Haller, S., Scharnowski, F., Weiskopf, N., Birbaumer, N., Blefari, M., et al. (2013). Real-time fMRI neurofeedback: progress and challenges. *Neuroimage* 76, 386–399. doi: 10.1016/j.neuroimage.2013.03.033

Surmeli, T., and Ertem, A. (2009). QEEG guided neurofeedback therapy in personality disorders: 13 case studies. *Clin. EEG Neurosci.* 40, 5–10. doi: 10.1177/155005940904000107

Surmeli, T., Ertem, A., Eralp, E., and Kos, I. H. (2012). Schizophrenia and the efficacy of qEEG-guided neurofeedback treatment: a clinical case series. *Clin. EEG Neurosci.* 43, 133–144. doi: 10.1177/1550059411429531

Tan, G., Shaffer, F., Lyle, R., and Teo, I. (2016). *Evidence-Based Practice in Biofeedback and Neurofeedback*, 3rd Edn. Wheat Ridge, CO: AAPB.

Tass, P. A., Adamchic, I., Freund, H.-J., von Stackelber, T., and Hauptmann, C. (2012). Counteracting tinnitus by acoustic coordinated reset neuromodulation. *Restor. Neurol. Neurosci.* 30, 137–159. doi: 10.3233/RNN-2012-110218

Thatcher, R. W. (2010). Neuropsychiatry and quantitative electroencephalography (qEEG) in the 21st Century. *Neuropsychiatry* 1, 495–514. doi: 10.2217/npy.11.45

Thibault, R. T., Lifshitz, M., and Raz, A. (2016). The self-regulating brain and neurofeedback. Experimental science and clinical promise. *Cortex* 74, 247–261. doi: 10.1016/j.cortex.2015.10.024

Thibault, R. T., Lifshitz, M., and Raz, A. (2017). Neurofeedback or neuroplacebo? *Brain* 140, 862–864. doi: 10.1093/brain/awx033

van den Berge, M. J. C., Free, R. H., Arnold, R., De Kleine, E., Hofman, R., van Dijk, J., et al. (2017). Cluster analysis to identify possible subgroups in tinnitus patients. *Front. Neurol.* 8:115. doi: 10.3389/fneur.2017.00115

van der Loo, E., Gais, S., Congedo, M., Vanneste, S., Plazier, M., Menovsky, T., et al. (2009). Tinnitus intensity dependent gamma oscillations of the contralateral auditory cortex. *PLOS ONE* 4:e7396. doi: 10.1371/journal.pone.0007396

van Veen, B. D., van Drongelen, W., Yuchtman, M., and Suzuki, A. (1997). Localization of brain electrical activity via linearly constrained minimum variance spatial filtering. *IEEE Trans. Biomed. Eng.* 44, 867–880. doi: 10.1109/10.623056

Vanhatalo, S., Palva, J. M., Holmes, M. D., Miller, J. W., Voipio, J., and Kaila, K. (2004). Infraslow oscillations modulate excitability and interictal epileptic activity in the human cortex during sleep. *Proc. Natl. Acad. Sci. U.S.A.* 101, 5053–5057. doi: 10.1073/pnas.0305375101

Vanneste, S., and De Ridder, D. (2012). The auditory and non-auditory brain areas involved in tinnitus. An emergent property of multiple parallel overlapping subnetworks. *Front. Syst. Neurosci.* 6:31. doi: 10.3389/fnsys.2012.00031

Vanneste, S., Joos, K., and De Ridder, D. (2012). Prefrontal cortex based sex differences in tinnitus perception. Same tinnitus intensity, same tinnitus distress, different mood. *PLOS ONE* 7:e31182. doi: 10.1371/journal.pone.0031182

Vanneste, S., Joos, K., Ost, J., and De Ridder, D. (2016). Influencing connectivity and cross-frequency coupling by real-time source localized neurofeedback of the posterior cingulate cortex reduces tinnitus related distress. *Neurobiol. Stress* (in press). doi: 10.1016/j.ynstr.2016.11.003

Vanneste, S., van de Heyning, P., and de Ridder, D. (2011a). Contralateral parahippocampal gamma-band activity determines noise-like tinnitus laterality: a region of interest analysis. *J. Neurosci.* 199, 481–490. doi: 10.1016/j.neuroscience.2011.07.067

Vanneste, S., van de Heyning, P., and De Ridder, D. (2011b). The neural network of phantom sound changes over time. A comparison between recent-onset and chronic tinnitus patients. *Eur. J. Neurosci.* 34, 718–731. doi: 10.1111/j.1460-9568.2011.07793.x

Weidt, S., Delsignore, A., Meyer, M., Rufer, M., Peter, N., Drabe, N., et al. (2016). Which tinnitus-related characteristics affect current health-related quality of life and depression? A cross-sectional cohort study. *Psychiatry Res.* 237, 114–121. doi: 10.1016/j.psychres.2016.01.065

Weiler, E. W., Brill, K., Tachiki, K. H., and Schneider, D. (2002). Neurofeedback and quantitative electroencephalography. *Int. Tinnitus J.* 8, 87–93.

Weisz, N., Dohrmann, K., and Elbert, T. (2007a). The relevance of spontaneous activity for the coding of the tinnitus sensation. *Prog. Brain Res.* 166, 61–70. doi: 10.1016/S0079-6123(07)66006-3

Weisz, N., Müller, S., Schlee, W., Dohrmann, K., Hartmann, T., and Elbert, T. (2007b). The neural code of auditory phantom perception. *J. Neurosci.* 27, 1479–1484. doi: 10.1523/JNEUROSCI.3711-06.2007

Weisz, N., Moratti, S., Meinzer, M., Dohrmann, K., and Elbert, T. (2005). Tinnitus perception and distress is related to abnormal spontaneous brain activity as measured by magnetoencephalography. *PLOS Med.* 2:e153. doi: 10.1371/journal.pmed.0020153

White, D. J., Congedo, M., and Ciorciari, J. (2014). Source-based neurofeedback methods using EEG recordings: training altered brain activity in a functional brain source derived from blind source separation. *Front. Behav. Neurosci.* 8:373. doi: 10.3389/fnbeh.2014.00373

Wyrwicka, W., and Sterman, M. B. (1968). Instrumental conditioning of sensorimotor cortex EEG spindles in the waking cat. *Physiol. Behav.* 3, 703–707. doi: 10.1016/0031-9384(68)90139-X

Zobay, O., and Adjamian, P. (2015). Source-space cross-frequency amplitude-amplitude coupling in tinnitus. *Biomed. Res. Int.* 2015:489619. doi: 10.1155/2015/489619

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Pathophysiology, Diagnosis and Treatment of Somatosensory Tinnitus: A Scoping Review

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Somatosensory tinnitus is a generally agreed subtype of tinnitus that is associated with activation of the somatosensory, somatomotor, and visual-motor systems. A key characteristic of somatosensory tinnitus is that is modulated by physical contact or movement. Although it seems common, its pathophysiology, assessment and treatment are not well defined. We present a scoping review on the pathophysiology, diagnosis, and treatment of somatosensory tinnitus, and identify priority directions for further research.

Methods: Literature searches were conducted in Google Scholar, PubMed, and EMBASE databases. Additional broad hand searches were conducted with the additional terms etiology, diagnose, treatment.

Results: Most evidence on the pathophysiology of somatosensory tinnitus suggests that somatic modulations are the result of altered or cross-modal synaptic activity within the dorsal cochlear nucleus or between the auditory nervous system and other sensory subsystems of central nervous system (e.g., visual or tactile). Presentations of somatosensory tinnitus are varied and evidence for the various approaches to treatment promising but limited.

Discussion and Conclusions: Despite the apparent prevalence of somatosensory tinnitus its underlying neural processes are still not well understood. Necessary involvement of multidisciplinary teams in its diagnosis and treatment has led to a large heterogeneity of approaches whereby tinnitus improvement is often only a secondary effect. Hence there are no evidence-based clinical guidelines, and patient care is empirical rather than research-evidence-based. Somatic testing should receive further attention considering the breath of evidence on the ability of patients to modulate their tinnitus through manouvers. Specific questions for further research and review are indicated.

Keywords: somatosensation, somatosensory, tinnitus, physical therapy, physiotherapy, cross modal

INTRODUCTION

Tinnitus is defined as the conscious perception and reaction to a sound in the absence of a matching external acoustic stimulus, commonly described as a *phantom* perception. It is considered a symptom rather than a disease *per se* (Jastreboff and Hazell, 1993; Bürgers et al., 2013). Tinnitus is present in more than 10% (11.9–30.3%) of the adult population (McCormack et al., 2016), although only 0.5–3% refers to it as a problem that decreases quality of life (Coles, 1984; Swain et al., 2016).

Although tinnitus has been the subject of much research, its pathophysiology remains poorly understood. It is well-accepted that many social factors, such as poor education, lower income, or occupational and recreational activity associated with high noise exposure, influences the prevalence and risk of tinnitus (Hoffman and Reed, 2004). Moreover, it is regularly associated with hearing loss, trauma, or ototoxic medication triggering cochlear damage, with sustained neural changes in the central auditory system that succeeds such lesions (Møller, 2011a; Langguth et al., 2013). Tinnitus prevalence is believed to increase with age up to 65 years, where after it decreases (Hoffman and Reed, 2004; Shargorodsky et al., 2010). It is also a widespread symptom among children with hearing loss (Coelho et al., 2007) and many causes of hearing loss and tinnitus are thought to be the same (Crummer and Hassan, 2004).

Recent neuroimaging and animal model studies suggest that tinnitus-related neural activity may involve complex interactions between several sensory modalities, sensorimotor, somatomotor, and visual-motor systems, neuro-cognitive, and neuronal-emotional networks (Cacace, 2003; Sanchez and Rocha, 2011a,c; Ostermann et al., 2016). Signs of interactions between the auditory system and the somatosensory system include gaze-evoked tinnitus (Cacace et al., 1994; Pinchoff et al., 1998; Lockwood et al., 2001), cutaneous-evoked tinnitus (Cacace et al., 1999a,b), motor manipulation or forceful muscle contractions of head, neck and limbs that induce or suppress tinnitus, or affect tinnitus loudness (Sanchez et al., 2002, 2007; Simmons et al., 2008). Pressure on myofascial trigger points (Travell, 1960; Wyant, 1979; Fricton et al., 1985; Bjorne, 1993; Rocha et al., 2006, 2008; Rocha and Sanchez, 2007), electrical stimulation of the median nerve and hand (Moller and Rollins, 2002), finger movements (Cullington, 2001), orofacial movements (Pinchoff et al., 1998), and pressure applied to the temporomandibular joint (i.e., Bjorne, 1993) are also observed to modulate tinnitus in some people. Such "somatosensory tinnitus" is supposed to be a prevalent tinnitus subtype (for review see Ralli et al., 2016) and prevalence may even be under-estimated because it relies on self-report that tinnitus is modulated by touch or movement (Ward et al., 2015). For example, the prevalence of somatic modulation is higher when the patients are questioned specifically about it rather than spontaneous reports (Sanchez et al., 2002).

For clarity we will use the following definitions: Tinnitus Modulation is the human capability of changing the tinnitus perception (frequency or intensity) by means of performing a certain manouver or movement of the head or neck or jaw or limbs or the eyes. Triggers is the phenomenon that acivates tinnitus modulation, examples: gaze movement, some tactile stimulous, performing a certain manouver or movement of the head or neck or jaw or limbs or the eyes. So the peripheral activity or stimulation are the primary single sources of a precise modulation of the tinnitus sound and it is described as trigger activity and the term modulation is reserved solely for describing the central neural activity that affect changes in tinnitus percept.

In the most comprehensive literature review to date on somatosensory tinnitus, Sanchez and Rocha (2011a,b) spoke of the need to establish evaluation protocols and specific treatments for somatosensory tinnitus that focus on both the auditory pathway and the musculoskeletal system. Yet there has never been a scoping review or systematic review on the topic. In this review, we scope the primary research literature on the pathophysiology, diagnosis, and treatment of somatosensory tinnitus. The aims of the review are to account the breadth and current state of knowledge on somatosensory tinnitus, to consider priority directions for research, and to identify whether any systematic reviews would be informative to the field.

MATERIALS AND METHODS

Literature searches were conducted in November 2016 in Google Scholar, PubMed, and EMBASE databases using the search terms somato* AND tinnitus (see Appendix 1 in Supplementary Material for an example search). Search results were screened to identify original articles and case reports for review. For Google Scholar, results were screened until five consecutive results pages yielded no new potentially relevant results. Additional hand searches of publications were conducted in the same databases using the additional broad search terms etiology, diagnose, treatment. Records were independently reviewed by at least two authors. In cases of disagreement, opinion of a third reviewer was taken as consensus. Inclusion criteria were: somatosensory tinnitus as main or secondary study objective, inclusion of at least one group with patients or case study suffering from somatosensory tinnitus, definition of somatosensory tinnitus, description of somatosensory tinnitus diagnostic approach or treatment. If the focus of the study was somatosensory tinnitus pathophysiology, diagnosis, or management, and at least one of the study groups or case study consisted of somatosensory tinnitus patients, the study was included; otherwise it was excluded. Exclusion criteria were articles written in languages other than English, and records relating solely to objective tinnitus.

Initial screening was based on abstract reading. Where there was uncertainty whether or not a record was relevant the full text record was screened. Records were grouped into three categories: pathophysiology, diagnosis, and treatment. One record could be relevant to more than one category. All records included patients with somatosensory tinnitus (P). Interventions (I) and their effects were recorded. Outcome measures were also identified (O), and comparisons (C) were described either between patients and controls, groups of patients divided by tinnitus type or intervention, as well between groups of patients before and after intervention for somatosensory tinnitus (see **Figure 1**).

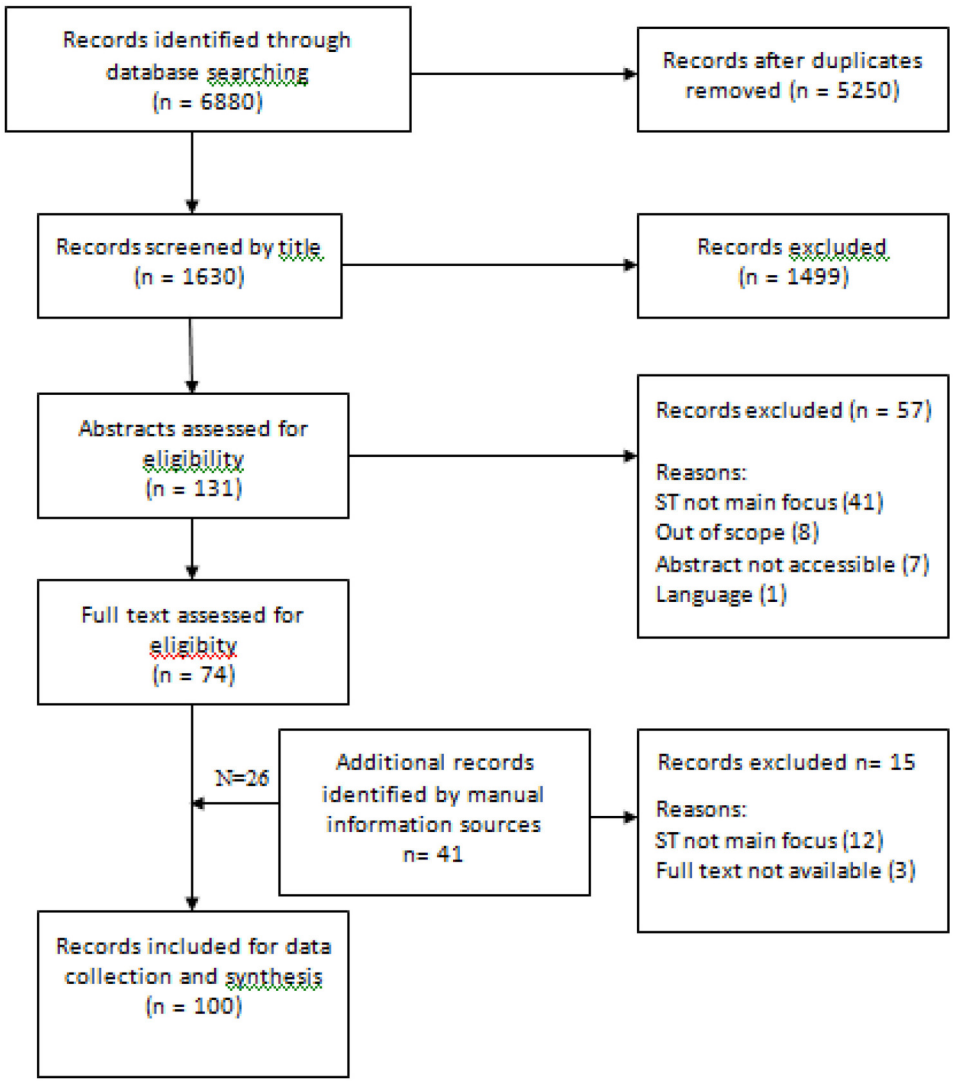

FIGURE 1 | Flow chart of study records.

RESULTS

The initial searches for somato* AND tinnitus yielded 1,630 records of which 100 were suitable for inclusion in the review. Records are subdivided for review according to pathophysiology, diagnosis, and management.

Pathophysiology and Etiology

Records describing studies on the pathophysiology and etiology of somatosensory tinnitus were included and are reviewed here. A table compiling the case controlled studies and cross-sectional studies were summarized in Appendix 2 in Supplementary Material (case reports, reviews and book chapters were excluded).

A number of authors suggest the somatosensory stimuli inducing tinnitus are deeply related to abnormal cross-modal plasticity of somatic-auditory interactions (Cacace, 2003; Levine et al., 2007; Herraiz, 2008; Rocha et al., 2008; Koehler and Shore, 2013) whereby somatic modulations of tinnitus results from abnormal auditory neural interactions—distortion of the normal synaptic activity—within the central nervous system, as Sanchez et al. (2007) describes, *"The information triggered by muscle contractions is carried by the somatosensory system and, upon reaching the cuneiform nucleus, may influence tinnitus through its projection over the auditory pathway due to an overactivitiy in the cochlear nucleus."* In particular, modulation of hyperactivity of neurons in the dorsal cochlear is triggered by the stimulation of specific ipsilateral cranial nerves, i.e., branch of the trigeminal nerve, explaining how ipsilateral tinnitus may be modulated by head and neck's manipulation (see a review, Kaltenbach, 2006). In guinea pigs, it was demonstrated that DCN bimodal plasticity is stimulus timing-dependant and implicated as an underlying mechanism in tinnitus (Shore et al., 2007; Koehler and Shore, 2013).

Levine et al. (2003) found somatic modulation in patients with tinnitus and deafness patients, identifying neural interactions in the central nervous system as the main protagonists in this process. Levine et al. (2008) also suggest that pulsatile tinnitus is modulated by the somatosensory system of the head or upper lateral neck, presenting two mechanisms; (1) cardiac synchronous somatosensory activation of the central auditory pathway, or (2) distortion of the normal synaptic activity between the somatosensory and auditory central nervous system. Simmons et al. (2008), studying patients who could modulate tinnitus with jaw clench found that an alteration in tinnitus loudness related to a variation in neural activity in the auditory cortex, concluding that tinnitus originates in the central auditory pathway. The same effect has been observed in patients who can modulate their tinnitus with eye movements (Lockwood et al., 2001; Sanchez and Akemi, 2008), and in patients whose tinnitus is modulated by intravenous administration of lidocaine (Reyes et al., 2002). Modulation of tinnitus with oral-facial movements suggest that the classical auditory system is not implicated in tinnitus because limbic structures respond to sound stimulation in patients with tinnitus (through hypoactivity localized in the hippocampus), further indicating the central auditory system and not the cochlea as the origin of tinnitus (Lockwood et al., 1998; Cacace, 2003; Schaette and McAlpine, 2011). In his studies, Levine found that patients could better detect changes in their tinnitus when using isometric maneuvers of the extremities, compared to head/neck maneuvers, suggestive of a major role of the central neural pathway as opposed to the auditory periphery (Cacace, 2003). In fact, a higher prevalence of somatoform disorders in individuals with tinnitus may also relate to certain craniocervical pathological features (e.g., herniated discs or temporomandibular joint syndrome; Chole and Parker, 1992; Rubinstein, 1993; Gelb et al., 1997; Levine, 1999b) and dental and jaw diseases (Han et al., 2009). For example, there is a higher than general incidence of tinnitus in patients and normal hearing who have temporomandibular disorder (TMD) (Levine, 1999b), suggesting that it may be associated with other symptoms of TMD (Chole and Parker, 1992; Bernhardt et al., 2011). The temporomandibular joint (TMJ) is thought to be commonly involved in the ability to modulate tinnitus, particularly its loudness (Ralli et al., 2016). Recently the risk of tinnitus was established as 8.37 times higher for patients with TMD (Bürgers et al., 2013), and unilateral tinnitus is even reported to be on the same side as unilateral TMD (Bürgers et al., 2013). These patients are also reportedly able to regulate their tinnitus through certain jaw or neck movements (Wright and Bifano, 1997a; Vielsmeier et al., 2011, 2012; Bürgers et al., 2013). Since tinnitus is normally related to the opposite risk factors (i.e., older males with hearing loss), such findings postulate that TMJ may be the cause and maintenance of tinnitus (Vielsmeier et al., 2011). It is proposed that TMD can cause tinnitus through the disruption of the trigeminal input (Vielsmeier et al., 2012; Ostermann et al., 2016). Another indication supporting the role of TMD in tinnitus is that the two conditions occur simultaneously. Evidence also shows that worsening of tinnitus coincides with aggravation of TMD (Wright and Bifano, 1997b).

Diagnosis

Records describing studies on diagnosis or rate of diagnosis of somatosensory tinnitus were included and are reviewed here. A table compiling the case controlled studies and cross-sectional studies were summarized in Appendixes 3, 4 in Supplementary Material (reviews, thesis, and book chapters were excluded), concerning both epidemiology and diagnosis fields, respectively.

Common attributed risk factors for any subtype of tinnitus are male gender, older in age and hearing problems (i.e., Hazell, 1991; Abel and Levine, 2004; Eggermont and Roberts, 2004; Hoffman and Reed, 2004; Oostendorp et al., 2016), except for TMD-tinnitus patients (Chole and Parker, 1992; Wright and Bifano, 1997b; Vielsmeier et al., 2011; Bürgers et al., 2013). Recent evidence in a British cohort study shows that somatic tinnitus is more common among younger people and it is unrelated to hearing loss or tinnitus severity (Ward et al., 2015). Some of these audiological and demographic traits, may be indeed useful in informing therapy (Won et al., 2013) through the identification of "clinical criteria for useful subtyping of tinnitus patients" (Vielsmeier et al., 2012).

Signs of somatosensory tinnitus include head or neck problems (i.e., temporomandibular joint syndrome, osteophits, arthorosis, spondylosis, myofascial trigger points, etc.), dental or jaw diseases, frequent pain in head, neck, or shoulder girdle, aggravation of events of simultaneous pain and tinnitus, incorrect body postures, and severe bruxism (Sanchez and Rocha, 2011b,c). Such complexity demands a multidisciplinary team (i.e., dentist, physiotherapist) to diagnose.

Somatosensory tinnitus is strongly evidenced when the patient can modulate the loudness or intensity of their tinnitus (Abel and Levine, 2004; Latifpour et al., 2009; Sanchez and Rocha, 2011b,c; Oostendorp et al., 2016). Hence somatic testing may identify patients who could be treated with somatosensory system-related therapies. However, this type of testing receives little attention (Won et al., 2013).

There are various presentations of somatosensory tinnitus to be aware of. Typical cases include gaze-evoked or modulated tinnitus, cutaneous-evoked tinnitus, and tinnitus modulated by movement of corporal elements (i.e., head, fingers, jaw). Gaze-evoked/modulated tinnitus, the modulation of tinnitus by eye movement, provides clues on the potential cortical role in tinnitus (Lockwood et al., 2001). Simmons et al. (2008) found a large sample of patients who were capable of modulating their tinnitus by eye movement, half of whom had developed this ability after undergoing surgery for removal of an acoustic neuroma; these patients were able to change the tinnitus loudness and pitch through eye movement.

Studies of cutaneous-evoked tinnitus (using magnetoencephalographic signals and tactile discrimination tests) have found that cutaneous stimulation of skin on the hand region (specifically palm and fingers) activates the somatosensory system along with the auditory cortical areas in congenitally deaf individuals (Cacace et al., 1999a,b; Cacace, 2003).

In respect to modulation of tinnitus through of head and neck, Levine (1999a) reported that 68% of 70 patients could modulate tinnitus through maneuvers of the head, neck, or

less intensely, maneuvers of limb. Similarly, Sanchez et al. (2002) found both patients with tinnitus (65.3% of 121 persons) and healthy subjects (14% of 100) could modulate or develop, respectively, tinnitus through 16 different maneuvers, and later found 57.9% of a study population could modulate tinnitus using nine different maneuvers (Sanchez et al., 2007). Simmons et al. (2008) found that, in 93 subjects able to modulate tinnitus by jaw clench, 90% could increase the loudness of their tinnitus, and 50% could alter the pitch. In a different assessment, the same authors found that 78% of their sample of 45 subjects could modulate their tinnitus with movement of the head or neck, mainly using the cranial and cervical nerves and using forceful maneuvers. In another study, Won et al. (2013) found that in 57% of tested ears in a population sample of 163 patients, tinnitus (especially unilateral tinnitus) was modulated through neck maneuvers or jaw maneuvers, decreasing and increasing tinnitus loudness respectively. The authors also reported that in their sample bilateral and low-pitch tonal tinnitus was rarely modulated by movement and may even be aggravated by somatic therapy. More distal movement is also observed to modulate tinnitus. Cullington (2001) reported the case of a 78-year-old man with severe hearing loss implanted with a cochlear implant in his right ear was able to modulate his tinnitus by moving his finger. Fascinatingly, this patient reported that the quicker the movement, the more intense was tinnitus loudness; passive or isometric movement did not modulate the tinnitus (Sanchez and Akemi, 2008). See **Table 1** for a summary of somatic maneuvers.

Even when the patient cannot self-modulate tinnitus, it may be altered by other kinds of stimuli, using maneuvers to increase activity of the trigeminal nerve such as passive muscular palpation to find myofascial trigger points (MFT), relaxation, and massage (Simmons et al., 2008; Sanchez and Rocha, 2011b; Shore, 2011; Won et al., 2013).

Treatment

Records describing studies on the treatment of somatosensory tinnitus were included and are reviewed here by treatment category. Case controlled studies and cross-sectional studies were summarized in Appendix 5 in Supplementary Material.

Physiotherapeutic Treatment

Studies have accounted the benefits for tinnitus of treating (temporomandibular disorder) TMD. Wright and Bifano (1997a) studied tinnitus in TMD patients and reported that 56% had been cured and 30% had a significant improvement with cognitive therapy and modulation through maneuvers. However, it has also been found that that severe tinnitus is less likely to improve with TMD therapy (Wright and Bifano, 1997a). Another similar study has shown that younger patients with moderate tinnitus were more likely to experience relief of their tinnitus through TMD therapy (Wright and Bifano, 1997b). Tinnitus severity as a predictor of the effectiveness of TMD therapy has already been proposed by others including Erlandsson et al. (1991) and Bush (1987).

The presence of fluctuating tinnitus is another factor that may associate with TMD treatment effectiveness (e.g., Tullberg and Ernberg, 2006).

One form of TMD treatment is occlusal splint therapy (Attanasio et al., 2015). In their study involving this treatment in patients presenting with chronic subjective tinnitus Attanasio et al. (2015) divided patients into three groups according to whether TMD was absent, present, or the patient was considered predisposed to TMD. Patients were subjected to treatment with

TABLE 1 | Summary of somatic manouvers.

Authors	Body part	Maneuvres (examples)
Cullington, 2001	Finger	Moving up and down the middle finger of left hand**#
Levine, 1999a; Sanchez et al., 2002, 2007; Abel and Levine, 2004; Levine et al., 2007	Extremities	Locking the fingers of the two hands together and pulling as hard as possible, or resisting maximal pressure to. Shoulder abduction. Flexion or abduction of the hip. Resisting or not an applied force.
Lockwood et al., 2001; Sanchez and Akemi, 2008; Simmons et al., 2008	Eye	Moving in the vertical or horizontal axis**
Cacace et al., 1999a,b; Cacace, 2003; Sanchez and Akemi, 2008	Cutaneous	Stimulation of a well-defined region—various regions of the hand and fingers (e. g., palm, dorsal web regions, and fingertips)**&
Pinchoff et al., 1998; Sanchez et al., 2002, 2007; Abel and Levine, 2004; Levine et al., 2007; Simmons et al., 2008; Latifpour et al., 2009; Won et al., 2013	Jaw	Clench the teeth, open and close mouth, protrude jaw, slide jaw. Resisting or not an applied force.
Levine, 1999a; Sanchez et al., 2002, 2007; Abel and Levine, 2004; Simmons et al., 2008; Latifpour et al., 2009; Won et al., 2013	Head and neck	Moving the head back and in front and laterally, resisting or not an applied force (against the head in a neutral position or turned to one of the sides).
		Applying pressure on muscle insertions–esternocleidomastoid, splenius capitis, and posterior auricular.

All the different voluntary muscle contraction manouvers should be sustained during 5–10 s and performed using a moderate degree of force in a silent environment (Levine, 1999a). The idiopathic somotosensorial tinnitus will present more relevant modulation with jaw and head-neck manouvers.

***Very specific to certain cases of patients subjected to brain neurosurgery or cochlear implantation only rarely is it spontaneous.*

#The patient reported that the quicker the movement, the more intense the tinnitus loudness, passive or isometric movement did not modulate the tinnitus.

&Studies of cutaneous-evoked tinnitus, (using magnetoencephalographic signals and tactile discrimination tests) have found that electrical stimulation of the median nerve and hand region or cutaneous stimulation of skin on various regions of the hand including dorsal web regions and fingertips activate the somatosensory system along with the auditory cortical areas in congenitally deaf individuals (Cacace et al., 1999a,b; Cacace, 2003).

a neuromuscular occlusal splint for 6 months (using the splint at night time) and rated for the severity of tinnitus using 10-point visual analog scale and Tinnitus Handicap Inventory (THI; Newman et al., 2004) questionnaire. Post-treatment THI scores were reduced in all groups but was most pronounced in the TMD (experience or predisposed) groups. The authors concluded that, once otologic disorders and neurological diseases are excluded, that clinicians should refer patients for an evaluation of the temporomandibular joint and subsequently to treat patients with TMD or a predisposition to it.

Wright (2000) suggested oro-myofunctional therapy as an effective alternative to occlusal splints therapy. Their study involved patients from the US air force seeking treatment for tinnitus, dizziness, and/or nonotologic otalgia without an identifiable cause and presenting with TMD symptoms in the temple, jaw, or preauricular area. Patients were provided a dental orthotic and TMD self-care instructions. After 3 months of orthotic wear, the percentages of patients reporting at least moderate symptom improvement of their tinnitus, dizziness, otalgia, and/or TMD were 64, 91, 87, and 92%, respectively. Follow-up telephone calls 6 months after completion of TMD therapy revealed that all patients maintained their symptom improvements. These findings imply that TMD was affecting the patients' otologic symptoms.

Stomatognathic Therapy

Usually it includes splints therapy, therapeutic exercises for the lower jaw and occlusal adjustment in combination with counseling.

For a long time, scientists have investigated the effects of dental and stomatognathic therapies in tinnitus (Junemann, 1941; Gelb and Arnold, 1959; Dolowitz et al., 1964; Kelly and Goodfriend, 1964; Gelb et al., 1967; Koskinen et al., 1980; Ioannides and Hoogland, 1983; Cooper et al., 1986; Bush, 1987; Rubinstein and Erlandsson, 1991). According to the findings of Rubinstein (1993), almost one-third of patients report improvement in their tinnitus after mandibula movements and/or pressure on their TMJs. More recently, Bürgers et al. (2013) found that stomatognathic therapy had a positive effect on tinnitus symptoms in 44% of their TMD-tinnitus patients ($n = 25$), up to 3–5 months after the first intervention; while promising it is noted that there was no control group in this study. Using dental functional therapy, the authors found an improvement on acute or subacute tinnitus in 100% of the patients but little improvement in patients with chronic tinnitus. It is important to note that the authors discussed an individual therapeutic strategy with each patient before the start of treatment. The authors suggested long term studies are conducted to assess the outcome and advised caution when interpreting current epidemiological data.

Chiropractic Therapy

Chiropractic therapy is a correction therapeutic treatment of an abnormal movement pattern through the manipulation of the vertebral column and extremities. Only three studies related to chiropractic treatment of tinnitus were identified and all three were case studies. Alcantara et al. (2002) described the chiropractic therapy in a 41-year-old woman with history of ear pain, tinnitus, vertigo, altered hearing, ear infections, and headaches, and who was diagnosed TMD and cervical subluxation. The authors reported a complete relief from the TMD symptoms, including tinnitus, after only 9 treatments (2 months). The treatment involved the application of high-velocity low amplitude adjustments. Kessinger and Boneva (2000) also reported progress in a 75-year-old patient who received upper cervical specific chiropractic care which resulted in improvements in vertigo, tinnitus, and hearing loss. These authors concluded that the success of chiropractic therapy was due to improvement in cervical spine function.

DeVocht et al. (2003) also describes the chiropractic management of a 30-year-old woman with TMJ pain. The patient suffered daily from unremitting jaw pain for 7 years accompanied by headache, tinnitus, decreased hearing, and a feeling of congestion in her right ear. Twenty months of chiropractic treatment resulted in total resolution of all symptoms except fullness of the right cheek.

Muscle Relaxation

Combined with chiropractic care, muscular relaxation (through massage and stretching exercises) is used in clinical practice. For instance, evidence suggests that palpation of masseter, pterygoid, and sternocleidomastoid muscles or myofascial trigger points can modulate tinnitus (Rocha et al., 2008; Teachey et al., 2012). Björne (2007) reported on the effectiveness of stretching exercises targeting the suboccipital muscles, along with rotation movements in the atlanto-occipital joint and relaxing exercises, on a TMD patient population (no control group). Bjorne notes that patients with Ménière's were more likely to present with TMJ and cervical spine disorder's symptoms (including tinnitus), than people who do not have Ménière and using a coordinated therapy of TMJ and cervical spine disorder (relaxation and posture) found improvements in self-reported tinnitus severity that were retained up to 3 year follow up.

Latifpour et al. (2009) evaluated 24 subjects from an original pool of 41 subjects (non-randomized), divided into two groups: treatment and control group. The authors compared self-training of stretching, posture training, and acupuncture, targeting muscle symmetry and balance in the jaw and neck, and later reported an improvement of tinnitus in the treatment group. In this blinded study they observed immediate and long term (3 months) improvements in the treatment group.

Another therapy worth noting here; in a pilot study with 11 patients, Kaute (1998) reported improvement in vestibular disturbances through the method of Arlen's Atlas Therapy, normally applied to whiplash-injured patients, concluding it to be indicated where tinnitus may be caused by neck muscle tension. This study suggest that muscular relaxation may play a significant role in the treatment of tinnitus but high quality explanatory studies (i.e., comparison with a control, blinded, randomized allocation), are needed.

Somatic Modulation Therapy

Somatic modulation therapy (treatment aiming to modulate the intensity of a given symptom, by movement) has rarely been

studied beyond case studies. Sanchez et al. (2007) were the first to investigate the effect of repetitive training maneuvers with head and neck muscle contractions, focusing on its value as a tinnitus retraining therapy. The authors found it to have a significant effect on the modulation patterns but not in the daily perception of tinnitus.

In the case of a 39-year-old woman who developed gaze-evoked tinnitus after surgery to remove a left vestibular Schwannoma, therapy consisted of a repetitive gaze training and tinnitus was resolved after 14 weeks (Sanchez and Akemi, 2008). Interestingly, there was both a "horizontal" and "vertical" gaze effect on tinnitus and the vertical component responded more quickly to treatment suggesting more than one neural network or process was involved in this case.

In another case, a 54-year-old man with severe tinnitus noticed an improvement through tactile stimuli to the ipsilateral postauricular area, head rotation, opening of the mouth, and clenching teeth and mandible lateralization (Sanchez and Akemi, 2008). In another case of tinnitus improvement through tactile stimulation was reported in a single patient by Emmert et al. (2014); the patient reported a decrease in tinnitus intensity in the left ear when a tactile stimulus was applied (block-design using EPI sequence—the patients touched on the right cheek on seven blocks of 25 s, intercalating with 25 vs rest).

Electrical Stimulation

Recent evidence reported a significant improvement in tinnitus using transcutaneous electrical nerve stimulation (Herraiz et al., 2007; Vanneste et al., 2010). Trans-electrical nerve stimulation (TENS) of areas of skin close to the ear increases the activation of the dorsal cochlear nucleus through the somatosensory pathway and may augment the inhibitory role of this nucleus on the CNS and thereby ameliorate tinnitus (Herraiz et al., 2007).

Vanneste et al. (2010) applied transcutaneous nerve stimulation in the upper cervical nerve in 240 patients with the ability to modulate tinnitus and found a significant suppression of tinnitus. Although only 18% of the patients responded to the treatment, 43% declared an improvement and six patients reported a total suppression of tinnitus (Vanneste et al., 2010). Herraiz et al. (2007) showed that trans-electrical nerve stimulation led to improvements in 46% of somatic tinnitus patients (reduced VAS tinnitus severity scores) after 2 weeks of treatment. Intermittent "typewriter"—sound like tinnitus was the most responsiveness. Herraiz et al. (2007) also noted that tinnitus caused by a somatosensory injury had a better response than somatic tinnitus with an otologic disease.

Standardizing the indications and method could increase the efficacy of electrical stimulation in somatic tinnitus according to most authors. These results are promising so further controlled trials are warranted.

Pharmaceutical Treatment

Only one relevant record describing a pharmaceutical treatment was included. In this case study McCormick and Walega (2015) reported the successful treatment of refractory somatic tinnitus with cervical epidural injection of 80 mg triamcinolone acetonide. The patient was 61-year-old male with previous history of bacterial otitis media.

Surgical Treatment

No surgical treatment studies specific to somatosensory tinnitus were identified. One case study worth mentioning however was that of a 65 years old patient with left sided tinnitus and with left sided cervical neck pain who experienced a complete resolution of somatic tinnitus for over 1 year through radiofrequency ablation of the left C2–C3 medial branches of the dorsal ramus ipsilateral to tinnitus symptoms (Gritsenko et al., 2014).

DISCUSSION

Tinnitus is complex in nature and so ideally, and to achieve the best results, diagnosis and treatment should be specific to an individual patients experience. Further research on the physiological processes that lead to somatosensory tinnitus would facilitate the development of a specific protocol and therapy targeting the auditory pathways and musculoskeletal disorders (Sanchez and Rocha, 2011c). Indeed, any holistic view of tinnitus needs to take into consideration the auditory system as a dynamic and active structure, integrating systems of reaction, stimulation, and emotion and tinnitus itself as a symptom with complex causes that indicate hyperactive neural activity (Møller, 2011a) and activation of neural plasticity (Moller and Rollins, 2002; Møller, 2011b; Smith et al., 2013), without the participation of the ear (Møller, 2016).

Evidence points to a high prevalence of somatosensory tinnitus, but that it is under-investigated by clinicians and the processes underlying are still poorly studied. For instance, only very recently have the first steps been made toward understanding the genetic underpinnings of subjective tinnitus (Lopez-Escamez et al., 2016) or the social context and environment which may influence tinnitus, following the new Social-Neurophysiological Model of Tinnitus. This model proposes the integration of the neurophysiological system (Jastreboff, 1990; Jastreboff and Jastreboff, 2000) the relation between psychophysiological and behavioral systems) and the social information system, associated with the emotional experience of tinnitus (Li et al., 2015). These avenues may help develop clinical strategies that adapt to patient's understanding and attitudes toward tinnitus, through social learning. What these will mean for somatosensory tinnitus is an open question.

It is important to note that an early and precise diagnosis, presents the best outcomes for the patient treatment (Herraiz, 2008). Recent research on the treatment of somatosensory tinnitus has focused on bone and muscular disorders, on each structure independently or using multimodal approach including manual therapy and exercise (Michiels et al., 2014, 2016). This demands different practitioners (dentists, neurologists, audiologists, physiatrist etc.) to be involved in treatment. Although such strategies do not target tinnitus directly, such therapies are shown to ameliorate its side effects.

It is not possible to cure tinnitus through dental and TMD therapies. But these same therapies may contribute to a multidisciplinary methodology of tinnitus treatment (Herraiz, 2008; Bürgers et al., 2011). It is a priority to establish how TMD and somatosensory tinnitus are related and what criteria should be used to select tinnitus patients for different TMD therapies.

Further research is needed to attest the efficacy of TMD therapy on tinnitus and to access the placebo effect (Rubinstein, 1993; Tullberg and Ernberg, 2006).

A multidisciplinary approach to managing somatosensory tinnitus may result in different strategies being used by different teams of clinicians if there is poor interdisciplinary communication and the lack of large-scale controlled trials to inform evidence-based clinical guidelines (Møller, 2007). In addition, standardization of core measures hinders the process of any potential meta-analysis on the large datasets, which would aid the development of clinical interventions for tinnitus. However, it will need to be tested whether these standardized outcomes are sensitive to treatment related changes in groups of patients or trail participants who have somatosensory tinnitus.

CONCLUSION

Because somatosensory tinnitus is not judged a disease *per se*, but instead it is considered a symptom, its diagnosis and treatment were related to other disorders. Connection to hearing loss and bone and muscular disorders are evident.

With this scoping review, we intended to give the reader a broad overview of findings to date concerning somatosensory tinnitus, and encourage new systematic and integrative analyses which will hopefully bring the much-needed order to the field of tinnitus research.

We propose several outstanding studies on somatosensory tinnitus:

1. There is some discrepancy over the prevalence of somatosensory tinnitus; a systematic review is needed.
2. The etiology of somatosensory tinnitus needs continued investigation. Particularly, and considering the involvement of neural plasticity, it is necessary to determine the exact processes that initiate the abnormal cross-modal plasticity of somatic-auditory interactions. Moreover, it is important to determine the exact relation between the head/neck maneuvers in the central neural system.
3. There is a lack of objective diagnostic methodology, which may misguide clinical management. Clinical guidelines that consider or are specific to somatosensory tinnitus are needed.
4. There are many and different strategies for managing tinnitus, originating in different clinical fields (audiology, neurology, psychology, etc.), and not all strategies have been trialed in somatosensory tinnitus. Integrating such strategies, and having in mind that each patient is a singular case, may increase the success of clinical management practices for tinnitus.
5. To support further trials and data synthesis in somatosensory tinnitus there needs to be standard research methodologies. Theses should be developed through consensus.
6. A therapeutic intervention combining simultaneously several types of treatment approaches may bring the best results for tinnitus relief, but such combinations may also be individual specific.

AUTHOR CONTRIBUTIONS

HH is the guarantor of the review. DH and DK created the search strategies. DK and CN created the tables in appendix. IP contributed in data extraction and initial manuscript. HH, DH, and RC contributed equally to all other stages of the manuscript development, produced, and approved the manuscript. NT, HC, AL, and JP provided consultative advice and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Abel, M. D., and Levine, R. A. (2004). Muscle contractions and auditory perception in tinnitus patients and nonclinical subjects. *Cranio* 22, 181–191. doi: 10.1179/crn.2004.024

Alcantara, J., Plaugher, G., Klemp, D. D., and Salem, C. (2002). Chiropractic care of a patient with temporomandibular disorder and atlas subluxation. *J. Manipulative Physiol. Ther.* 25, 63–70. doi: 10.1067/mmt.2002.120415

Attanasio, G., Leonardi, A., Arangio, P., Minni, A., Covelli, E., Pucci, R., et al. (2015). Tinnitus in patients with temporo-mandibular joint disorder: proposal for a new treatment protocol. *J. Craniomaxillofac. Surg.* 43, 724–727. doi: 10.1016/j.jcms.2015.02.009

Bernhardt, O., Mundt, T., Welk, A., Köppl, N., Kocher, T., Meyer, G., et al. (2011). Signs and symptoms of temporomandibular disorders and the incidence of tinnitus. *J. Oral Rehabil.* 38, 891–901. doi: 10.1111/j.1365-2842.2011.02224.x

Bjorne, A. (1993). Tinnitus aereum as an effect of increased tension in the lateral pterygoid muscle. *Otolaryngol. Head Neck Surg.* 109:969. doi: 10.1177/019459989310900538

Björne, A. (2007). Assessment of temporomandibular and cervical spine disorders in tinnitus patients. *Prog. Brain Res.* 166, 215–219. doi: 10.1016/S0079-6123(07)66019-1

Bürgers, R., Behr, M., and Gosau, M. (2011). "Treatment strategies of temporomandibular joint and masticatory muscle disorders in patients with tinnitus," in *Textbook of Tinnitus,* eds A. R. Møller, D. DeRidder, B. Langguth, and T. Kleinjung (New York, NY: Springer), 763–767.

Bürgers, R., Kleinjung, T., Behr, M., and Vielsmeier, V. (2013). Is there a link between tinnitus and temporomandibular disorders? *J. Prosthet. Dent.* 111, 222–227. doi: 10.1016/j.prosdent.2013.10.001

Bush, F. M. (1987). Tinnitus and otalgia in temporomandibular disorders. *J. Prosthet. Dent.* 58, 495–498. doi: 10.1016/0022-3913(87)90282-4

Cacace, A. T. (2003). Expanding the biological basis of tinnitus: crossmodal origins and the role of neuroplasticity. *Hear. Res.* 175, 112–132. doi: 10.1016/S0378-5955(02)00717-7

Cacace, A. T., Cousins, J. P., Parnes, S. M., McFarland, D. J., Semenoff, D., Holmes, T., et al. (1999a). Cutaneous-evoked tinnitus. II. Review of neuroanatomical, physiological and functional imaging studies. *Audiol. Neurootol.* 4, 258–268.

Cacace, A. T., Cousins, J. P., Parnes, S. M., McFarland, D. J., Semenoff, D., Holmes, T., et al. (1999b). Cutaneous-evoked tinnitus. I. Phenomenology, psychophysics and functional imaging. *Audiol. Neurootol.* 4, 247–268.

Cacace, A. T., Lovely, T. J., McFarland, D. J., Parnes, S. M., and Winter, D. F. (1994). Anomalous cross-modal plasticity following posterior fossa surgery: some speculations on gaze-evoked tinnitus. *Hear. Res.* 81, 22–32. doi: 10.1016/0378-5955(94)90149-X

Chole, R. A., and Parker, W. S. (1992). Tinnitus and vertigo in patients with temporomandibular disorder. *Arch. Otolaryngol. Head Neck Surg.* 118, 817–821. doi: 10.1001/archotol.1992.01880080039010

Coelho, C. B., Sanchez, T. G., and Tyler, R. S. (2007). Tinnitus in children and associated risk factors. *Prog. Brain Res.* 166, 179–191. doi: 10.1016/S0079-6123(07)66016-6

Coles, R. R. A. (1984). Epidemiology of tinnitus:(1) prevalence. *J. Laryngol. Otol.* 98, 7–15. doi: 10.1017/S1755146300090041

Cooper, B. C., Alleva, M., Cooper, D. L., and Lucente, F. E. (1986). Myofacial pain dysfunction: analysis of 476 patients. *Laryngoscope* 96, 1099–1106. doi: 10.1288/00005537-198610000-00010

Crummer, R. W., and Hassan, G. A. (2004). Diagnostic approach to tinnitus. *Am. Fam. Physician* 69, 120–126.

Cullington, H. (2001). Tinnitus evoked by finger movement: brain plasticity after peripheral deafferentation. *Neurology* 56, 978–979. doi: 10.1212/WNL.56.7.978

DeVocht, J. W., Schaeffer, W., and Lawrence, D. J. (2003). Chiropractic treatment of temporomandibular disorders using the activator adjusting instrument and protocol. *Altern. Ther. Health Med.* 11, 70–73.

Dolowitz, D. A., Ward, J. W., Fingerle, C. O., and Smith, C. C. (1964). The role of muscular incoordination in the pathogenesis of the temporomandibular joint syndrome. *Laryngoscope* 74, 790–801. doi: 10.1288/00005537-196406000-00003

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Emmert, K., Van De Ville, D., Bijlenga, P., Djema, D. A., and Haller, S. (2014). Auditory cortex activation is modulated by somatosensation in a case of tactile tinnitus. *Neuroradiology* 56:511. doi: 10.1007/s00234-014-1360-0

Erlandsson, S. I., Rubinstein, B., Axelsson, A., and Carlsson, S. G. (1991). Psychological dimensions in patients with disabling tinnitus and craniomandibular disorders. *Br. J. Audiol.* 25, 15–24. doi: 10.3109/03005369109077860

Fricton, J. R., Kroening, R., Haley, D., and Siegert, R. (1985). Myofascial pain syndrome of the head and neck: a review of clinical characteristics of 164 patients. *Oral Surg. Oral Med. Oral Pathol.* 60, 615–623. doi: 10.1016/0030-4220(85)90364-0

Gelb, H., and Arnold, G. E. (1959). Syndromes of the head and neck of dental origin. *Plast. Reconstr. Surg.* 26:100. doi: 10.1097/00006534-196007000-00022

Gelb, H., Calderone, J. P., Gross, S. M., and Kantor, M. E. (1967). The role of the dentist and the otolaryngologist in evaluating temporomandibular joint syndromes. *J. Prosthet. Dent.* 18, 497–503. doi: 10.1016/0022-3913(67)90173-4

Gelb, H., Gelb, M. L., and Wagner, M. L. (1997). The relationship of tinnitus to craniocervical mandibular disorders. *Cranio* 15, 136–143. doi: 10.1080/08869634.1997.11746004

Gritsenko, K., Caldwell, W., Shaparin, N., Vydyanathan, A., and Kosharskyy, B. (2014). Resolution of long standing tinnitus following radiofrequency ablation of C2–C3 medial branches–a case report. *Pain Physician* 17:E95–E98.

Han, B. I., Lee, H. W., Kim, T. Y., Lim, J. S., and Shin, K. S. (2009). Tinnitus: characteristics, causes, mechanisms, and treatments. *J. Clin. Neurol.* 5, 11–19. doi: 10.3988/jcn.2009.5.1.11

Hazell, J. (1991). Tinnitus and disability with ageing: adaptation and management. *Acta Otolaryngol.* 111, 202–208. doi: 10.3109/00016489109127279

Herraiz, C. (2008). Assessing the cause of tinnitus for therapeutic options. *Expert Opin. Med. Diagn.* 2, 1183–1196. doi: 10.1517/17530059.2.10.1183

Herraiz, C., Toledano, A., and Diges, I. (2007). Trans-electrical nerve stimulation (TENS) for somatic tinnitus. *Prog. Brain Res.* 166, 389–394. doi: 10.1016/S0079-6123(07)66037-3

Hoffman, H. J., and Reed, G. W. (2004). "*Epidemiology of Tinnitus*", in Tinnitus: Theory and management, ed J. B. Snow (Lewiston, NY: BC Decker Inc.), 16–41.

Ioannides, C. A., and Hoogland, G. A. (1983). The disco-malleolar ligament: a possible cause of subjective hearing loss in patients with temporomandibular joint dysfunction. *J. Maxillofac. Surg.* 11, 227–231. doi: 10.1016/S0301-0503(83)80053-8

Jastreboff, P. J. (1990). Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci. Res.* 8, 221–254. doi: 10.1016/0168-0102(90)90031-9

Jastreboff, P. J., and Hazell, J. W. (1993). A neurophysiological approach to tinnitus: clinical implications. *Br. J. Audiol.* 27, 7–17. doi: 10.3109/03005369309077884

Jastreboff, P. J., and Jastreboff, M. M. (2000). Tinnitus retraining therapy (TRT) as a method for treatment of tinnitus and hyperacusis patients. *J. Am. Acad. Audiol.* 11, 162–177.

Junemann, H. R. (1941). Consequences of shortening the intermaxillary distance. *J. Am. Dent. Assoc.* 28, 1427–1436. doi: 10.14219/jada.archive.1941.0231

Kaltenbach, J. A. (2006). Summary of evidence pointing to a role of the dorsal cochlear nucleus in the etiology of tinnitus. *Acta Otolaryngol.* 126, 20–26. doi: 10.1080/03655230600895309

Kaute, B. B. (1998). The influence of atlas therapy on tinnitus. *Int. Tinnitus* 4, 165–167.

Kelly, H. T., and Goodfriend, D. J. (1964). Vertigo attributable to dental and temporomandibular joint causes. *J. Prosthet. Dent.* 14, 159–173. doi: 10.1016/0022-3913(64)90131-3

Kessinger, R. C., and Boneva, D. V. (2000). Vertigo, tinnitus, and hearing loss in the geriatric patient. *J. Manipulative Physiol. Ther.* 23, 352–362. doi: 10.1016/S0161-4754(00)90211-2

Koehler, S. D., and Shore, S. E. (2013). Stimulus timing-dependent plasticity in dorsal cochlear nucleus is altered in tinnitus. *J. Neurosci.* 33, 19647–19656. doi: 10.1523/JNEUROSCI.2788-13.2013

Koskinen, J., Paavolainen, M., Raivio, M., and Roschier, J. (1980). Otological manifestations in temporomandibular joint dysfunction. *J. Oral Rehabil.* 7, 249–254. doi: 10.1111/j.1365-2842.1980.tb00442.x

Langguth, B., Kreuzer, P. M., Kleinjung, T., and De Ridder, D. (2013). Tinnitus: causes and clinical management. *Lancet Neurol.* 12, 920–930. doi: 10.1016/S1474-4422(13)70160-1

Latifpour, D. H., Grenner, J., and Sjodahl, C. (2009). The effect of a new treatment based on somatosensory stimulation in a group of patients with somatically related tinnitus. *Int. Tinnitus J.* 15, 94.

Levine, R. A. (1999a). "Somatic modulation appears to be a fundamental attribute of tinnitus," in *Proceedings of the Sixth International Tinnitus Seminar* (Cambridge, UK: British Society of Audiology), 193–197.

Levine, R. A. (1999b). Somatic (craniocervical) tinnitus and the dorsal cochlear nucleus hypothesis. *Am. J. Otolaryngol.* 20, 351–362.

Levine, R. A., Abel, M., and Cheng, H. (2003). CNS somatosensory-auditory interactions elicit or modulate tinnitus. *Exp. Brain Res.* 153, 643–648. doi: 10.1007/s00221-003-1747-3

Levine, R. A., Nam, E. C., and Melcher, J. (2008). Somatosensory pulsatile tinnitus syndrome: somatic testing identifies a pulsatile tinnitus subtype that implicates the somatosensory system. *Trends Amplif.* 2, 242–253. doi: 10.1177/1084713808321185

Levine, R. A., Nam, E. C., Oron, Y., and Melcher, J. R. (2007). Evidence for a tinnitus subgroup responsive to somatosensory based treatment modalities. *Prog. Brain Res.* 166, 195–207. doi: 10.1016/S0079-6123(07)66017-8

Li, Z., Gu, R., and Zeng, X. (2015). The social-neurophysiological model of tinnitus: theory and practice. *J. Formos. Med. Assoc.* 114, 201–203. doi: 10.1016/j.jfma.2013.09.003

Lockwood, A. H., Salvi, R. J., Coad, M. L., Towsley, M. L., Wack, D. S., and Murphy, B. W. (1998). The functional neuroanatomy of tinnitus Evidence for limbic system links and neural plasticity. *Neurology* 50, 114–120. doi: 10.1212/WNL.50.1.114

Lockwood, A. H., Wack, D. S., Burkard, R. F., Coad, M. L., Reyes, S. A., Arnold, S. A., et al. (2001). The functional anatomy of gaze-evoked tinnitus and sustained lateral gaze. *Neurology* 56, 472–480. doi: 10.1212/WNL.56.4.472

Lopez-Escamez, J. A., Bibas, T., Cima, R. F., Van de Heyning, P., Knipper, M., Mazurek, B., et al. (2016). Genetics of tinnitus: an emerging area for molecular diagnosis and drug development. *Front. Neurosci.* 10:377. doi: 10.3389/fnins.2016.00377

McCormack, A., Edmondson-Jones, M., Somerset, S., and Hall, D. (2016). A systematic review of the reporting of tinnitus prevalence and severity. *Hear. Res.* 337, 70–79. doi: 10.1016/j.heares.2016.05.009

McCormick, Z. L., and Walega, D. R. (2015). Cervical epidural steroid injection for refractory somatic tinnitus. *Pain Pract.* 15, e28–e33. doi: 10.1111/papr.12255

Michiels, S., De Hertogh, W., Truijen, S., and Van de Heyning, P. (2014). Physical therapy treatment in patients suffering from cervicogenic somatic tinnitus: study protocol for a randomized controlled trial. *Trials* 15, 1. doi: 10.1186/1745-6215-15-297

Michiels, S., Van de Heyning, P., Truijen, S., Hallemans, A., and De Hertogh, W. (2016). Does multi-modal cervical physical therapy improve tinnitus in patients with cervicogenic somatic tinnitus? *Man. Ther.* 26, 125–131. doi: 10.1016/j.math.2016.08.005

Møller, A. R. (2007). Tinnitus and pain. *Prog. Brain Res.* 166, 47–53. doi: 10.1016/S0079-6123(07)66004-X

Møller, A. R. (2011a). "Pathology of the auditory system that can cause tinnitus," in *Textbook of Tinnitus,* eds A. R. Møller, D. DeRidder, B. Langguth, and T. Kleinjung (New York, NY: Springer), 77–93.

Møller, A. R. (2011b). "The role of neural plasticity in tinnitus," in *Textbook of Tinnitus,* eds A. R. Møller, D. DeRidder, B. Langguth, and T. Kleinjung (New York, NY: Springer), 99–102.

Møller, A. R. (2016). Sensorineural tinnitus: its pathology and probable therapies. *Int. J. Otolaryngol.* 2016:2830157. doi: 10.1155/2016/2830157

Moller, A. R., and Rollins, P. R. (2002). The non-classical auditory pathways are involved in hearing in children but not in adults. *Neurosci. Lett.* 319, 41–44. doi: 10.1016/S0304-3940(01)02516-2

Newman, C. W., Sandridge, S. A., and Snow, J. B. (2004). "Tinnitus questionnaires," in *Tinnitus: Theory and Management,* ed J. B. Snow (Hamilton, ON: B.C. Decker), 237–254.

Oostendorp, R. A., Bakker, I., Elvers, H., Mikolajewska, E., Michiels, S., De Hertogh, W., et al. (2016). Cervicogenic somatosensory tinnitus: an indication for manual therapy? Part 1: theoretical concept. *Man. Ther.* 23, 120–123. doi: 10.1016/j.math.2015.11.008

Ostermann, K., Lurquin, P., Horoi, M., Cotton, P., Hervé, V., and Thill, M. P. (2016). Somatic tinnitus prevalence and treatment with tinnitus retraining therapy. *B-ENT* 12, 59–65.

Pinchoff, R. J., Burkard, R. F., Salvi, R. J., Coad, M. L., and Lockwood, A. H. (1998). Modulation of tinnitus by voluntary jaw movements. *Otol. Neurotol.* 19, 785–789.

Ralli, M., Altissimi, G., Turchetta, R., and Cian-frone, G. (2016). Somatic modulation of tinnitus: a review and some open questions. *Otolaryngol. Open J.* 2, 111–114. doi: 10.17140/OTLOJ-2-125

Reyes, S. A., Salvi, R. J., Burkard, R. F., Coad, M. L., Wack, D. S., Galantowicz, P. J., et al. (2002). Brain imaging of the effects of lidocaine on tinnitus. *Hear. Res.* 171, 43–50. doi: 10.1016/S0378-5955(02)00346-5

Rocha, C. A. B., and Sanchez, T. G. (2007). Myofascial trigger points: another way of modulating tinnitus. *Prog. Brain Res.* 166, 209–214. doi: 10.1016/S0079-6123(07)66018-X

Rocha, C. A. B., Sanchez, T. G., and Tesseroli de Siqueira, J. T. (2008). Myofascial trigger point: a possible way of modulating tinnitus. *Audiol. Neurotol.* 13, 153–160. doi: 10.1159/000112423

Rocha, C. A. C. B., Sanchez, T. G., and de Siqueira, J. T. T. (2006). Myofascial trigger points: occurrence and capacity to modulate tinnitus perception. *Arq. Int. Otorrinolaringol.* 10, 210–217.

Rubinstein, B. (1993). Tinnitus and craniomandibular disorders: is there a link? *Swed. Dent. J.* 95, 1–46.

Rubinstein, B., and Erlandsson, S. I. (1991). A stomatognathic analysis of patients with disabling tinnitus and craniomandibular disorders (CMD). *Br. J. Audiol.* 25, 77–83. doi: 10.3109/03005369109079837

Sanchez, T. G., and Akemi, M. (2008). Modulating tinnitus with visual, muscular, and tactile stimulation. *Semin. Hear.* 29, 350–360. doi: 10.1055/s-0028-1095894

Sanchez, T. G., da Silva Lima, A., Brandao, A. L., Lorenzi, M. C., and Bento, R. F. (2007). Somatic modulation of tinnitus: test reliability and results after repetitive muscle contraction training. *Ann. Otol. Rhinol. Laryngol.* 116, 30–35. doi: 10.1177/000348940711600106

Sanchez, T. G., Guerra, G. C. Y., Lorenzi, M. C., Brandão, A. L., and Bento, R. F. (2002). The influence of voluntary muscle contractions upon the onset and modulation of tinnitus. *Audiol. Neurotol.* 7, 370–375. doi: 10.1159/000066155

Sanchez, T. G., and Rocha, C. B. (2011a). "Tinnitus caused and influenced by the somatosensory system," in *Textbook of Tinnitus* (New York, NY: Springer), 363–368.

Sanchez, T. G., and Rocha, C. B. (2011b). "Diagnosis of somatosensory tinnitus," in *Textbook of Tinnitus*, eds A. R. Møller, D., DeRidder, B., Langguth, and T. Kleinjung (New York, NY: Springer), 429–433.

Sanchez, T. G., and Rocha, C. B. (2011c). Diagnosis and management of somatosensory tinnitus: review article. *Clinics* 66, 1089–1094. doi: 10.1590/S1807-59322011000600028

Schaette, R., and McAlpine, D. (2011). Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J. Neurosci.* 31, 13452–13457. doi: 10.1523/JNEUROSCI.2156-11.2011

Shargorodsky, J., Curhan, G. C., and Farwell, W. R. (2010). Prevalence and characteristics of tinnitus among US adults. *Am. J. Med.* 123, 711–718. doi: 10.1016/j.amjmed.2010.02.015

Shore, S. E. (2011). Plasticity of somatosensory inputs to the cochlear nucleus–implications for tinnitus. *Hear. Res.* 281, 38–46. doi: 10.1016/j.heares.2011.05.001

Shore, S., Zhou, J., and Koehler, S. (2007). Neural mechanisms underlying somatic tinnitus. *Prog. Brain Res.* 166, 107–548. doi: 10.1016/S0079-6123(07)66010-5

Simmons, R., Dambra, C., Lobarinas, E., Stocking, C., and Salvi, R. (2008). Head, neck, and eye movements that modulate tinnitus . *Semin. Hear.* 29, 361–370. doi: 10.1055/s-0028-1095895

Smith, G. S., Romanelli-Gobbi, M., Gray-Karagrigoriou, E., and Artz, G. J. (2013). Complementary and integrative treatments: tinnitus. *Otolaryngol. Clin. North Am.* 46, 389–408. doi: 10.1016/j.otc.2013.02.005

Swain, S. K., Nayak, S., Ravan, J. R., and Sahu, M. C. (2016). Tinnitus and its current treatment–Still an enigma in medicine. *J. Formos. Med. Assoc.* 115, 139–144. doi: 10.1016/j.jfma.2015.11.011

Teachey, W. S., Wijtmans, E. H., Cardarelli, F., and Levine, R. A. (2012). Tinnitus of myofascial origin. *Int. Tinnitus J.* 17, 70–73.

Travell, J. (1960). Temporomandibular joint pain referred from muscles of the head and neck. *J. Prosthet. Dent.* 10, 745–763. doi: 10.1016/0022-3913(60)90257-2

Tullberg, M., and Ernberg, M. (2006). Long-term effect on tinnitus by treatment of temporomandibular disorders: a two-year follow-up by questionnaire. *Acta Odontol. Scand.* 64, 89–96. doi: 10.1080/00016350500377842

Vanneste, S., Plazier, M., Van de Heyning, P., and De Ridder, D. (2010). Transcutaneous electrical nerve stimulation (TENS) of upper cervical nerve (C2) for the treatment of somatic tinnitus. *Exp. Brain Res.* 204, 283–287. doi: 10.1007/s00221-010-2304-5

Vielsmeier, V., Kleinjung, T., Strutz, J., Bürgers, R., Kreuzer, P. M., and Langguth, B. (2011). Tinnitus with temporomandibular joint disorders a specific entity of tinnitus patients? *Otolaryngol. Head Neck Surg.* 145, 748–752. doi: 10.1177/0194599811413376

Vielsmeier, V., Strutz, J., Kleinjung, T., Schecklmann, M., Kreuzer, P. M., Landgrebe, M., et al. (2012). Temporomandibular joint disorder complaints in tinnitus: further hints for a putative tinnitus subtype. *PLoS ONE* 7:e38887. doi: 10.1371/journal.pone.0038887

Ward, J., Vella, C., Hoare, D. J., and Hall, D. A. (2015). Subtyping somatic tinnitus: a cross-sectional UK cohort study of demographic, clinical and audiological characteristics. *PLoS ONE* 10:e0126254. doi: 10.1371/journal.pone.0126254

Won, J. Y., Yoo, S., Lee, S. K., Choi, H. K., Yakunina, N., Le, Q., et al. (2013). Prevalence and factors associated with neck and jaw muscle modulation of tinnitus. *Audiol. Neurotol.* 18, 261–273. doi: 10.1159/000351685

Wright, E. F. (2000). Tinnitus, dizziness, and nonotologic otalgia improvement through temporomandibular disorder therapy. *Mil. Med.* 165, 733.

Wright, E. F., and Bifano, S. L. (1997a). Tinnitus improvement through TMD therapy. *J. Am. Dent. Assoc.* 128, 1424–1432.

Wright, E. F., and Bifano, S. L. (1997b). The relationship between Tinnitus and Temporomandibular Disorder (TMD) therapy. *Int. Tinnitus J.* 3, 55–61.

Wyant, G. M. (1979). Chronic pain syndromes and their treatment II. trigger points. *Can. Anaesth. Soc. J.* 26, 216–219. doi: 10.1007/BF03006985

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REVIEW
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The Effect of Physical Therapy Treatment in Patients with Subjective Tinnitus: A Systematic Review

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Background: Tinnitus is a very common symptom that often causes distress and decreases the patient's quality of life. Apart from the well-known causes, tinnitus can in some cases be elicited by dysfunctions of the cervical spine or the temporomandibular joint (TMJ). To date however, it is unclear whether alleviation of these dysfunctions, by physical therapy treatment, also decreases the tinnitus complaints. Such physical therapy could be an interesting treatment option for patients that are now often left without treatment.

Objectives: The aim of this review was to investigate the current evidence regarding physical therapy treatment in patients with tinnitus.

Data sources: The online databases Pubmed, Web of Science, Cochrane, and Embase were searched up to March 2016. Two independent reviewers conducted the data extraction and methodological quality assessment.

Study eligibility criteria: Only randomized controlled trials and quasi-experimental trials were included in the review. Studies had to be written in English, French, Dutch, or German.

Participants and interventions: The included studies investigated the effect of physical therapy treatment modalities on tinnitus severity in patients suffering from subjective tinnitus.

Results: Six studies were included in this review, four investigating cervical spine treatment and two investigating TMJ treatment. These studies show positive effects of cervical spine treatment (manipulations, exercises, triggerpoint treatment) on tinnitus severity. Additionally, decrease in tinnitus severity and intensity was demonstrated after TMJ treatment, following splints, occlusal adjustments as well as jaw exercises.

Limitations: The risk of bias in the included studies was high, mainly due to lack of randomization, lack of blinding of subjects, therapists, and/or investigators. Additionally, risk of bias is present due to incomplete presentation of the data and selective reporting.

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A major issue of the reviewed papers is the heterogeneity of the included study populations, treatments and outcome measures, which inhibit data pooling and meta-analysis.

Conclusions: Despite the methodological issues in the included studies and the consequent low quality evidence, it is noteworthy that all included studies show positive treatment effects. Before recommendations can be made, these results need to be confirmed in larger, high quality studies, using unambiguous inclusion criteria, state-of-the-art treatment, and high quality outcome measures.

Keywords: somatic tinnitus, physical therapy, treatment, cervical spine, temporomandibular joint disorders

INTRODUCTION

Tinnitus or "ringing in the ears" is a conscious perception of an auditory sensation in the absence of a corresponding external stimulus (Baguley et al., 2013). It is a very common symptom [15% of the adult population (Axelsson and Ringdahl, 1989)] that often causes distress and decreases the patient's quality of life. The ability to do intellectual work can be negatively affected and sleeping difficulties are frequently reported (Baguley et al., 2013).

Various types and causes of tinnitus have been described, with two main subtypes of tinnitus described: A subjective and an objective type. Tinnitus is in most cases subjective, meaning that the patient experiences the tinnitus in the absence of any auditory stimulus. In some cases, an internal, measurable, stimulus can cause the tinnitus, for instance turbulences of the blood flow. In these cases the perceived somatosounds can be considered an objective tinnitus (Baguley et al., 2013).

Several risk factors for subjective tinnitus have been described, such as hearing loss (Domenech et al., 1990), ototoxic medication (e.g., salicylates), head injuries (Ceranic et al., 1998), and depression (Trevis et al., 2016). Tinnitus can also occur in association with otological conditions, such as noise exposure or presbyacusis and can co-exist with anxiety or depression (McKenna et al., 1991) and with dysfunctions of the cervical spine (Teachey et al., 2012) or temporomandibular joint (TMJ) (Saldanha et al., 2012).

In these last two cases, tinnitus can be elicited by the somatosensory system of the cervical spine or temporomandibular area. This type of tinnitus is called somatic tinnitus and has been described in 36–43% of a population with subjective tinnitus (Abel and Levine, 2004; Michiels et al., 2015). A physiological explanation is proposed by several animal studies, which have found connections between the somatosensory system of the cervical spine and temporomandibular area on the one hand and the cochlear nuclei (CN) on the other hand (Pfaller and Arvidsson, 1988; Zhan et al., 2006). Cervical and temporomandibular somatosensory information is conveyed to the brain by afferent fibers, the cell bodies of which are located in the dorsal root ganglia or the trigeminal ganglion. Some of these afferent fibers also project to the central auditory system and more specifically to the dorsal CN. This makes the somatosensory system able to influence the auditory system by altering the spontaneous rates (i.e., not driven by auditory stimuli) or the synchrony of firing among neurons in the CN, inferior colliculus or auditory cortex. In this way, the somatosensory system is able to alter the intensity and the character of the tinnitus for instance by forceful muscle contractions of the neck or jaw musculature (Levine, 1999; Shore et al., 2007) or by increased muscle tension in the tensor tympani muscle (Westcott et al., 2013). Langguth et al. (2007) already stated that the investigation of the cervical spine and TMJ should be considered in all subjective tinnitus patients.

In 2011, Sanchez et al. (Sanchez and Rocha, 2011) published a literature overview on the diagnosis and treatment of somatic tinnitus, proposing the now currently used diagnostic criteria for somatic tinnitus. Regarding therapy however, this literature overview was not systematically performed and was based on case studies and case series that do not provide high quality evidence for the effect of physical therapy treatment in tinnitus patients.

Physical therapy could be an interesting treatment option for patients that are now often left without treatment. Therefore, the aim of this review was to investigate the current evidence regarding physical therapy treatment in patients with tinnitus.

METHODS

Search Strategy

A search of the online databases Pubmed, Web of Science, Cochrane, and Embase was performed up until March 2016. The search strategy was based on the PICO-framework and the following search was entered in the different databases: [("Tinnitus"[Mesh]) AND ("Exercise Movement Techniques"[Mesh]) OR ("Musculoskeletal Manipulations"[Mesh]) OR ("Exercise Therapy"[Mesh]) OR ("Myofunctional Therapy"[Mesh])].

Systematic Review Registration Number

A detailed review protocol was composed by the authors and registered at PROSPERO (registration number: CRD42016035834).

Study Selection

For inclusion in the review, studies needed to meet the following inclusion criteria: (1) subjects had to be human, (2) patients had to be adults suffering from subjective tinnitus, (3) the studied intervention was a physical therapy treatment modality, (4) this treatment was compared to no treatment or another treatment, (5) a tinnitus severity measure was one of the outcome

measures, (6) studies had to be written in English, French, Dutch, or German, (7) only randomized controlled trials and quasi-experimental trials were considered for inclusion and (8) articles had to present original research. Articles not meeting all inclusion criteria were excluded.

After the initial search, all retrieved articles were screened for eligibility based on title and abstract. The included articles were then screened again based on the full text.

The inclusion procedure was conducted by the first and third author independently and supervised by the last author. In case of uncertainty about inclusion, a decision was made in a consensus meeting, starting from the three independent opinions.

Qualification of the Investigators

The literature was screened and methodological quality was assessed independently by the first author, PhD. with experience in tinnitus and neck related complaints, and by the second author, MSc. in rehabilitation sciences and physiotherapy. The last author, PhD. with experience in neck related complaints, supervised the process. The third and sixth author, provided overall expertise on tinnitus complaints and the fourth and fifth author provided overall expertise on temporomandibular dysfunction.

Data Items and Collection

All relevant information from each included article was extracted and is presented in **Tables 1, 2**. This table contains the number of patients, used outcome measure for tinnitus severity and the main findings.

Risk of Bias in the Individual Studies

The PEDro scale for randomized controlled trials was used to investigate the methodological quality of the included articles. This scale is recommended by the "Physiotherapy Evidence Database." The PEDro scale was developed to rapidly identify clinical trials that are likely to be internally valid and have sufficient statistical information to make their results interpretable. The scale uses 11 items (Yes, No) to score each article on external validity (item 1), internal validity (item 2–9) and sufficient statistical information to make the results interpretable (item 10–11). A total score is calculated by summing the number of "Yes" answers on item 2–11. Item 1 is not taken into account for the total score.

The methodological quality assessment was performed by two investigators independently. Afterwards, the results were compared and differences were discussed to reach a consensus.

RESULTS

Study Selection

In total, 40 unique articles were retrieved from the 4 databases. After both screening phases, 6 articles were included in our review. In total, 6 studies were excluded due to the described population, 16 because the described intervention was not a physical therapy modality, 10 due to the design of the study (no comparison with "no treatment" or "another treatment" and 2 studies were excluded due to the language. A detailed overview of the selection process can be found in the flowchart in **Figure 1**.

Risk of Bias and Level of Evidence

The results of the risk of bias assessment are presented in **Figures 2**, **3**.

Overall, a high risk of bias was present in the included studies. This risk is mostly due to lack of randomization, lack of blinding of subjects, therapists, and/or investigators. Additionally, risk of bias is present due to incomplete presentation of the data and selective reporting. Therefore, the level of evidence of the included studies is low.

Synthesis of the Results

For each individual study, a summary of the characteristics of the study group, type of intervention and main results is presented in **Tables 1, 2**. **Table 1** presents the studies concerning cervical spine treatment and **Table 2** presents the studies regarding TMD treatment in tinnitus patients.

Cervical Spine Treatment

Four of the included studies investigated the effect of cervical spine treatment on tinnitus complaints. All of these studies had high risk of bias (Latifpour et al., 2009; Amanda et al., 2010; Rocha and Sanchez, 2012; Mielczarek et al., 2013), therefore the quality of the evidence is low.

Based on these studies, there are indications that cervical physical therapy (including stabilizing and mobilizing exercises) improves tinnitus complaints in a population of patients with a combination of tinnitus, sensorineural hearing loss and cervical spine degenerative changes (Mielczarek et al., 2013).

Additionally, there are indications that manipulations of the cervical spine decrease tinnitus severity (measured using the Tinnitus Handicap Inventory THI) in tinnitus patients. The intensity of the tinnitus (measured using visual analog scale VAS) did not decrease after cervical spine manipulations (Amanda et al., 2010).

Another study shows a significantly greater decrease in tinnitus severity (measured using VAS) after a combination of stretching, posture exercises and auricular acupuncture compared to waiting list (Latifpour et al., 2009) in patients with somatically related tinnitus.

Finally, there are indications that a combination of ischemic compression therapy of trigger points, stretching and posture exercises decreases tinnitus severity (measured using THI) in patients with tinnitus and pain complaints in head, neck, or shoulder girdle (Rocha and Sanchez, 2012).

Temporomandibular Joint Treatment

Two included studies investigated the effect of temporomandibular joint treatment on tinnitus complaints. Both studies had high risk of bias (Erlandsson et al., 1991; Tullberg and Ernberg, 2006), causing the quality of the evidence to be low.

TABLE 1 | Summary of studies concerning cervical spine treatment.

Publication	Participants	Intervention and control	Frequency and duration of intervention	Tinnitus severity outcome	Follow-up	Results
Amanda et al., 2010	$N = 40$ Females: 10 Males: 30	Osteopathic manipulations of the cervical spine	Once a week for 2 months	Tinnitus Handicap Inventory (THI)	Post-treatment	**THI**: No difference between treatment groups: TENS: 15.1 points recuction (−27%; $p < 0.001$) Manip: 8.5 points reduction (−16.2%; $p < 0.04$)
	Age: 48.5 (18–65)	vs.		VAS-intensity		
	Design: RCT					**VAS-intensity**: No improvement manip., 1.45 point decrease ($p < 0.006$) in TENS
	Diagnosis: Tinnitus patients otherwise healthy	Transcutaneous electrical nerve stimulation (TENS)				
Latifpour et al., 2009	$N = 24$ Females: 12 Males: 12 Age: 51 (*SD*: 16) Design: Controlled trial Diagnosis: Somatically related tinnitus	Supervised self-stretch of shoulder, neck and jaw muscles (Deltoid, trapezius pars descendens, splenius capitis, levator scapulae and sternocleido-mastoideus, masseter, temporalis and pterygoid), combined with Posture exercises and Auricular accupunture vs. Waiting list	9 sessions of 60 min, 3 per week during 3-week period	VAS-severity	Post-treatment and 3 months follow-up	**VAS-severity**: Significantly greater decrease in treatment group compared to controls after treatment ($p = 0.001$) and after follow-up ($p = 0.006$)
Mielczarek et al., 2013	$N = 80$ Females: 38 Males: 42 Age: 21–74 Design: Controlled trial Diagnosis: Tinnitus and sensorineural hearing loss + cervical spine degenerative changes (radiologically diagnosed)	TENS vs. Cervical physical therapy (stabilizing and mobilizing exercises)	15 TENS applications in a period of 30 days	Author's own questionnaire	Post treatment, 1 and 3 months follow-up	Significant improvement in both groups No significant difference between groups
Rocha and Sanchez, 2012	$N = 71$ Females: / Males: / Age: / Design: RCT Diagnosis: Tinnitus and pain complaints in head, neck or shoulder girdle during the previous 3 months	Ischemic compression therapy of trigger points, stretching and posture exercises vs. Sham deactivation trigger points	10 weekly sessions	Tinnitus Handicap Inventory (THI)	Not mentioned	Improvement in THI in the fifth session ($p < 0.001$)

TABLE 2 | Summary of studies concerning temporomandibular treatment.

Publication	Participants	Intervention and control	Frequency and duration of intervention	Tinnitus severity outcome	Follow-up	Results
Tullberg and Ernberg, 2006	Patients (P): *N* = 73 Controls (C): *N* = 50 Females: 39 (P) / 27 (C) Males: 34 (P) / 23 (C) Age: 48 (*SD*:12) (P) 47 (*SD*:14) (C) Design: Controlled design Diagnosis:Patients suffering from combination of tinnitus and TMD Controls suffering from tinnitus	Splints, occlusal adjustments, jaw exercises and laser therapy vs. Waiting list	1 to 6 sessions	Global perceived effect (GPE) Custom made questionnaire	Post-treatment (GPE) and 2–3 years follow-up (questionnaire)	**GPE**: 73% reported improvement, 27% reported no change **Questionnaire**: Significanty decreased tinnitus severity Significantly more improvement in the patients than in the control group
Erlandsson et al., 1991	*N* = 32 Females: 14 Males: 18 Age: 50 (24–65) Design: RCT with cross-over design Diagnosis: severe tinnitus and self-reported TMD or headaches	Somatognatic treatment (SGT) comprising: occlusal splints, occlusal adjustments and exercise therapy vs. Biofeedback therapy (BFT) comprising biofeedback training, progressive relaxation and counseling	Not specified	VAS-intensity (0–100) NRS-severity (1–9)	Post-treatment, 6 months follow-up	**VAS-intensity:** Significant decrease after SGT or BFT (*n* = 31) No significant changes after SGT or BFT alone (*n* = 13 or 18)

TMJ treatment included splints, occlusal adjustments and jaw exercises in both studies. One study also added laser therapy (Tullberg and Ernberg, 2006).

Based on these studies, there are indications that TMJ treatment decreases tinnitus intensity (measured using VAS) and severity (measured using global perceived effect and a custom made questionnaire). TMJ treatment was more effective than no treatment and equally effective than a combination of biofeedback therapy, progressive relaxation and counseling.

DISCUSSION

The aim of this review was to investigate the current evidence regarding physical therapy treatment in patients with subjective tinnitus.

Physical therapy treatment was either directed to the cervical spine or the temporomandibular area. Overall, positive effects of physical therapy on tinnitus severity were found.

Regarding cervical spine treatment, the effect of exercise therapy on tinnitus severity was proven in two studies (Latifpour et al., 2009; Mielczarek et al., 2013) that treated 40 and 13 patients respectively. A positive effect of manipulations of the cervical spine was found in one randomized controlled trial (RCT) of 20 patients (Amanda et al., 2010) and improvement of tinnitus severity after combination of triggerpoint deactivation and exercise therapy was found in one study of 33 patients (Rocha and Sanchez, 2012).

Regarding TMJ treatment, the effectiveness of splints, occlusal adjustments and jaw exercises was shown in two studies (Erlandsson et al., 1991; Tullberg and Ernberg, 2006) of 104 patients in total.

Unfortunately, none of the data can be pooled, due to heterogeneity of inclusion criteria, outcome measures and applied treatment.

Firstly, an international standard of outcome measurements in clinical trials of tinnitus is lacking. This is mandatory to enable meta-analysis as was also pointed out by Hall et al. (2015). This international standard is being developed, but to date, a clear consensus was not reached yet.

Secondly, the lack of unambiguously composed diagnostic criteria for somatic tinnitus is reflected in the applied inclusion criteria. All researchers define their own inclusion criteria, making it very hard to compare studies and to pool data. Sanchez et al. (Sanchez and Rocha, 2011) suggested a series of diagnostic criteria in a literature review in 2011, but none of the studies used these criteria. Possibly due to the fact that the criteria, seem too broad, since somatic tinnitus is assumed in all patients where tinnitus and neck or TMJ complaints co-occur. Since a recent study (Michiels et al., 2015) showed that neck complaints also occur in patients with other types of tinnitus, modification of the diagnostic criteria for somatic tinnitus is needed. Additionally, when applying cervical spine or TMJ

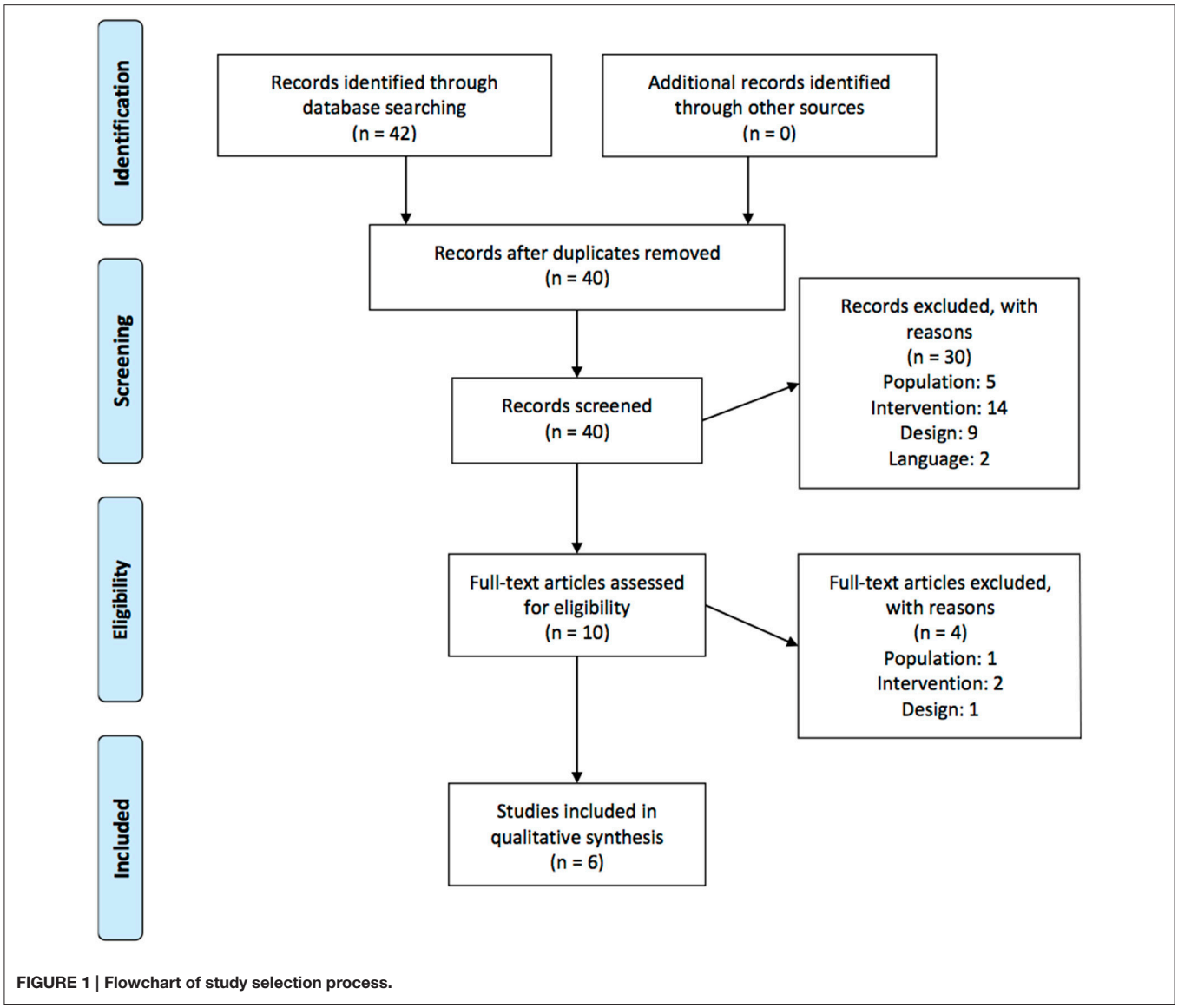

FIGURE 1 | Flowchart of study selection process.

treatment, studies should only include those patients that require this therapy for the treatment of their neck or TMJ complaint. In the study of Amanda et al. (2010) for instance, patients were included in case they had tinnitus and were otherwise healthy. These patients were treated using manipulations of the cervical spine, a treatment modality that is normally performed in case of limited range of motion of the cervical spine. The presence of these limitations in range of motion were however, not a requirement for patients to be included in the study. Therefore, doubts about the usefulness of manipulations in the study population may arise and therapy effects may be underestimated.

Another study (Mielczarek et al., 2013) included patients based on the presence of radiologically confirmed degenerative changes in the cervical spine, though degeneration is not necessarily accompanied with dysfunction and cervical spine complaints.

Thirdly, the applied treatments for cervical spine and TMJ complaints are divergent and do not always match the evidence based practice for cervical spine or TMJ treatment. In patients with somatic tinnitus, tinnitus severity is thought to be altered by cervical spine or TMJ dysfunctions. Therefore, complete alleviation of cervical spine or TMJ complaints using the best available treatment option will be necessary for a maximal decrease in tinnitus severity. Systematic reviews (Kay et al., 2005; Gross et al., 2010, 2015; Miller et al., 2010; Schroeder et al., 2013) have shown that a multimodal physical therapy treatment, combining mobilizations/manipulations and exercises is the best treatment option for cervical spine complaints. For TMJ complaints, treatment options vary (not only in specific choices for exercise or mobilization, but also the care provider), depending on the diagnosis and etiology. Both dentists and physical therapists may play a role in the primary care treatment of these patients (Feine and Lund, 1997; de Souza et al., 2012).

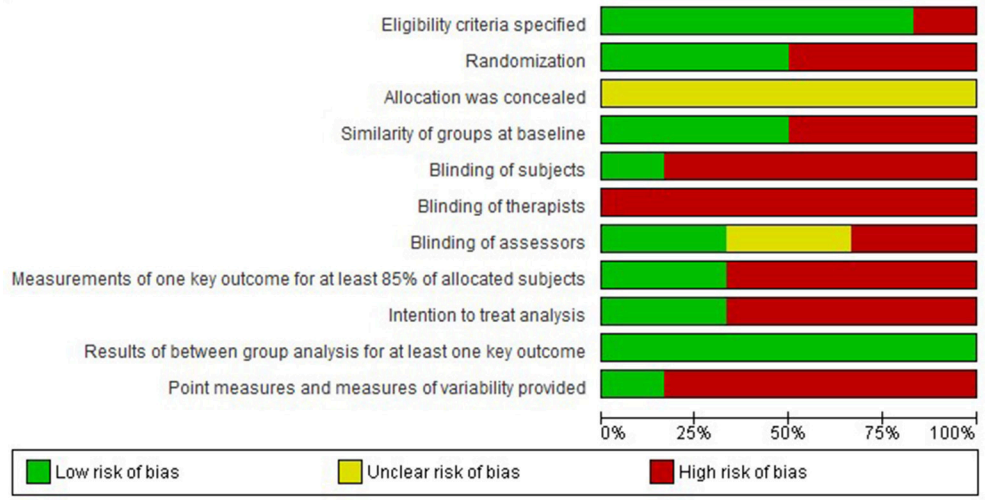

FIGURE 2 | Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all included studies.

Future studies should include these approaches to investigate its effect on tinnitus severity in patients with somatic tinnitus. The use of evidence based cervical spine and TMJ treatment instead of less underpinned therapies is specifically important in patients who already received numerous unsuccessful therapies in the past, as is the case in many patients with tinnitus.

All six studies however, show high risk of bias, limiting the generalizability of the conclusions. The risk was mostly due to lack of randomization, lack of blinding of subjects, therapists and/or assessors, and additionally due to incomplete presentation of the data and selective reporting. Lack of randomization was mostly caused by practical considerations, such as decreasing the waiting period before the start of the treatment. Blinding of subjects and therapists is always an issue in studies investigating physical therapy treatment and is very hard to overcome. Therefore, blinding of the assessor, who performs the follow-up measurements and data processing, is even more important. Although blinding the assessor is perfectly possible in physical therapy studies, only two studies mentioned this type of blinding (Rocha and Sanchez, 2007; Latifpour et al., 2009). Selective reporting and incomplete data presentation was another issue in the included articles. Only two out of six articles (Erlandsson et al., 1991; Mielczarek et al., 2013) presented the results of measurements of at least 85% of the allocated subjects. Additionally, only one study (Latifpour et al., 2009) provided point measures and measures of variability, where most other studies only provided significance figures. These issues of lack of randomization and blinding of assessors and selective reporting should be avoided in future research.

In future studies, researchers should firstly focus on clear patient selection, based on the existing diagnostic criteria and the applied treatment. Secondly, evidence based cervical spine and TMJ treatments should be applied and thirdly, studies should prevent risk of bias by focusing on randomization, blinding of assessors, and complete reporting of data.

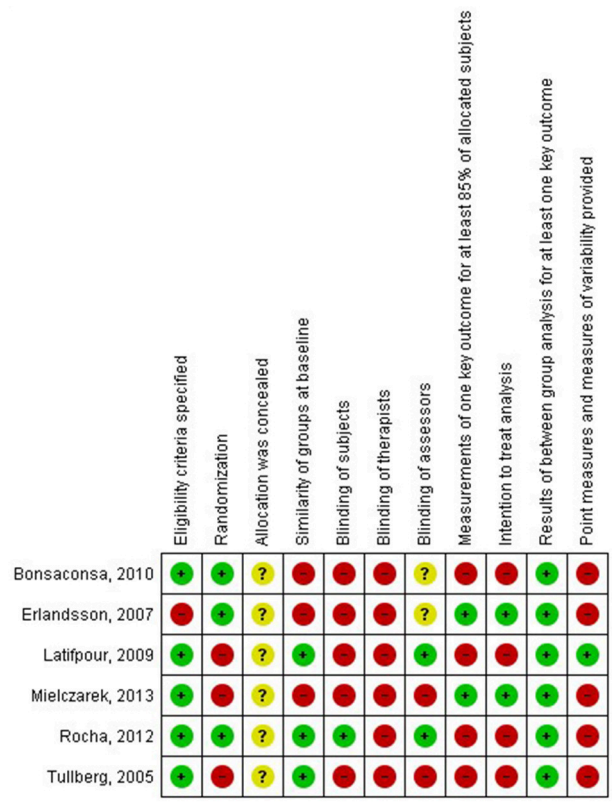

FIGURE 3 | Risk of bias summary: review authors' judgements about each risk of bias item for each included study.

CONCLUSION

Despite the methodological issues in the included studies and the consequent low quality evidence, it is noteworthy that all included studies showed positive treatment effects.

Although the results of the 6 studies are promising, the quality of the studies do not reach a high EBM level, which is necessary to endorse clinical practice and experience with recommendations. Current available effectiveness methodology and assessment has to guide future studies. These studies should focus on larger populations, higher methodological quality and should use unambiguous inclusion criteria, state-of-the-art treatment, and high quality outcome measures.

AUTHOR CONTRIBUTIONS

The literature was screened and methodological quality was assessed independently by SM and SN. WD supervised the process. PV and AG provided overall expertise on tinnitus complaints and MB and CV provided overall expertise on temporomandibular dysfunction. SM drafted the manuscript and all other authors added their comments.

REFERENCES

Abel, M. D., and Levine, R. A. (2004). Muscle contractions and auditory perception in tinnitus patients and nonclinical subjects. *Cranio* 22, 181–191. doi: 10.1179/crn.2004.024

Amanda, B., Manuela, M., Antonia, M., Claudio, M., and Gregorio, B. (2010). Posturography measures and efficacy of different physical treatments in somatic tinnitus. *Int. Tinnitus J.* 16, 44–50.

Axelsson, A., and Ringdahl, A. (1989). Tinnitus–a study of its prevalence and characteristics. *Br. J. Audiol.* 23, 53–62. doi: 10.3109/03005368909077819

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Ceranic, B. J., Prasher, D. K., Raglan, E., and Luxon, L. M. (1998). Tinnitus after head injury: evidence from otoacoustic emissions. *J. Neurol. Neurosurg. Psychiatr.* 65, 523–529. doi: 10.1136/jnnp.65.4.523

de Souza, R. F., Lovato da Silva, C. H., Nasser, M., Fedorowicz, Z., and Al-Muharraqi, M. A. (2012). Interventions for the management of temporomandibular joint osteoarthritis. *Cochrane Database Syst. Rev.* 4:CD007261. doi: 10.1002/14651858.cd007261.pub2

Domènech, J., Cuchí, M. A., and Carulla, M. (1990). High-frequency hearing loss in patients with tinnitus. *Adv. Otorhinolaryngol.* 45, 203–205. doi: 10.1159/000418955

Erlandsson, S. I., Rubinstein, B., and Carlsson, S. G. (1991). Tinnitus: evaluation of biofeedback and stomatognathic treatment. *Br. J. Audiol.* 25, 151–161. doi: 10.3109/03005369109079849

Feine, J. S., and Lund, J. P. (1997). An assessment of the efficacy of physical therapy and physical modalities for the control of chronic musculoskeletal pain. *Pain* 71, 5–23.

Gross, A., Kay, T. M., Paquin, J. P., Blanchette, S., Lalonde, P., Christie, T., et al. (2015). Exercises for mechanical neck disorders. *Cochrane Database Syst. Rev.* 1:CD004250. doi: 10.1002/14651858.cd004250.pub5

Gross, A., Miller, J., D'Sylva, J., Burnie, S. J., Goldsmith, C. H., Graham, N., et al. (2010). Manipulation or mobilisation for neck pain: a cochrane review. *Man. Ther.* 15, 315–333. doi: 10.1016/j.math.2010.04.002

Hall, D. A., Haider, H., Kikidis, D., Mielczarek, M., Mazurek, B., Szczepek, A. J., et al. (2015). Toward a global consensus on outcome measures for clinical trials in tinnitus: report from the first international meeting of the COMiT initiative, november 14, 2014, Amsterdam, The Netherlands. *Trends Hear* 19:2331216515580272. doi: 10.1177/2331216515580272

Kay, T. M., Gross, A., Goldsmith, C., Santaguida, P. L., Hoving, J., Bronfort, G., et al. (2005). Exercises for mechanical neck disorders. *Cochrane Database Syst. Rev.* 3:CD004250. doi: 10.1002/14651858.cd004250.pub3

Langguth, B., Goodey, R., Azevedo, A., Bjorne, A., Cacace, A., Crocetti, A., et al. (2007). Consensus for tinnitus patient assessment and treatment outcome measurement: tinnitus research initiative meeting, Regensburg, July 2006. *Prog. Brain Res.* 166, 525–536. doi: 10.1016/S0079-6123(07)66050-6

Latifpour, D. H., Grenner, J., and Sjödahl, C. (2009). The effect of a new treatment based on somatosensory stimulation in a group of patients with somatically related tinnitus. *Int. Tinnitus J.* 15, 94–99.

Levine, R. A. (1999). "Somatic modulation appears to be a fundamental attribute of tinnitus," in *Proceedings of the Sixth International Tinnitus Seminar*. (London: The Tinnitus Hyperaccusis Center).

McKenna, L., Hallam, R. S., and Hinchcliffe, R. (1991). The prevalence of psychological disturbance in neurotology outpatients. *Clin. Otolaryngol. Allied Sci.* 16, 452–456. doi: 10.1111/j.1365-2273.1991.tb01038.x

Michiels, S., De Hertogh, W., Truijen, S., and Van de Heyning, P. (2015). Cervical spine dysfunctions in patients with chronic subjective tinnitus. *Otol. Neurotol.* 36, 741–745. doi: 10.1097/MAO.0000000000000670

Mielczarek, M., Konopka, W., and Olszewski, J. (2013). The application of direct current electrical stimulation of the ear and cervical spine kinesitherapy in tinnitus treatment. *Auris Nasus Larynx* 40, 61–65. doi: 10.1016/j.anl.2012.05.006

Miller, J., Gross, A., D'Sylva, J., Burnie, S. J., Goldsmith, C. H., Graham, N., et al. (2010). Manual therapy and exercise for neck pain: a systematic review. *Man. Ther.* 15, 334–354. doi: 10.1016/j.math.2010.02.007

Pfaller, K., and Arvidsson, J. (1988). Central distribution of trigeminal and upper cervical primary afferents in the rat studied by anterograde transport of horseradish peroxidase conjugated to wheat germ agglutinin. *J. Comp. Neurol.* 268, 91–108. doi: 10.1002/cne.902680110

Rocha, C. A., and Sanchez, T. G. (2007). Myofascial trigger points: another way of modulating tinnitus. *Prog. Brain Res.* 166, 209–214. doi: 10.1016/S0079-6123(07)66018-X

Rocha, C. B., and Sanchez, T. G. (2012). Efficacy of myofascial trigger point deactivation for tinnitus control. *Braz. J. Otorhinolaryngol.* 78, 21–26.

Saldanha, A. D., Hilgenberg, P. B., Pinto, L. M., and Conti, P. C. (2012). Are temporomandibular disorders and tinnitus associated? *Cranio* 30, 166–171. doi: 10.1179/crn.2012.026

Sanchez, T. G., and Rocha, C. B. (2011). Diagnosis and management of somatosensory tinnitus: review article. *Clinics* 66, 1089–1094. doi: 10.1590/S1807-59322011000600028

Schroeder, J., Kaplan, L., Fischer, D. J., and Skelly, A. C. (2013). The outcomes of manipulation or mobilization therapy compared with physical therapy or exercise for neck pain: a systematic review. *Evid. Based Spine Care J.* 4, 30–41. doi: 10.1055/s-0033-1341605

Shore, S., Zhou, J., and Koehler, S. (2007). Neural mechanisms underlying somatic tinnitus. *Prog. Brain Res.* 166, 107–123. doi: 10.1016/S0079-6123(07)66010-5

Teachey, W. S., Wijtmans, E. H., Cardarelli, F., and Levine, R. A. (2012). Tinnitus of myofascial origin. *Int. Tinnitus J.* 17, 70–73.

Trevis, K. J., McLachlan, N. M., and Wilson, S. J. (2016). Psychological mediators of chronic tinnitus: the critical role of depression. *J. Affect. Disord.* 204, 234–240. doi: 10.1016/j.jad.2016.06.055

Tullberg, M., and Ernberg, M. (2006). Long-term effect on tinnitus by treatment of temporomandibular disorders: a two-year follow-up by questionnaire. *Acta Odontol. Scand.* 64, 89–96. doi: 10.1080/00016350500377842

Westcott, M., Sanchez, T. G., Diges, I., Saba, C., Dineen, R., McNeill, C., et al. (2013). Tonic tensor tympani syndrome in tinnitus and hyperacusis patients: a multi-clinic prevalence study. *Noise Health* 15, 117–128. doi: 10.4103/1463-1741.110295

Zhan, X., Pongstaporn, T., and Ryugo, D. K. (2006). Projections of the second cervical dorsal root ganglion to the cochlear nucleus in rats. *J. Comp. Neurol.* 496, 335–348. doi: 10.1002/cne.20917

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Exploring Tinnitus-Induced Disablement by Persistent Frustration in Aging Individuals: A Grounded Theory Study

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Background: Qualitative research can help to improve the management of patients, meet their expectations and assist physicians in alleviating their suffering. The perception of moment-to-moment variability in tinnitus annoyance is an emerging field of exploration. This study sought to enlighten variability in tinnitus-induced disablement using a qualitative approach.

Methods: Twelve participants (six females, six males, aged 51–79) were recruited via the French Tinnitus Association Journal for participation in recorded semi-structured interviews. Each participant had three interviews lasting 1 h, the sessions being separated one from the other by 2 weeks. Following recommendations of Charmaz (2014), the second and third interviews were aimed at gathering rich data, by enhancing the participants' reflexivity in the circumstances of distress caused by tinnitus. After transcription, the data ($n = 36$ interviews) were analyzed using the approach to Grounded Theory proposed by Strauss and Corbin (1998).

Results: Tinnitus as persistent frustration emerged as being the core category uniting all the other categories of the study. Hence, the core category accounted for the broader scope in participants' experience of chronic tinnitus. It is suggested that tinnitus-induced disablement varied according to the degree of frustration felt by the participants in not being able to achieve their goals. The implications of this were analyzed using the following categories: "Losing body ownership," "Lacking perspectives," and "Persevering through difficulties." Based on these findings, we draw a substantive theory of tinnitus tolerance that promotes an active, disciplined and individualized approach to tinnitus-induced disablement. The model distinguishes pathways from sustained suffering to reduced annoyance (i.e., emerging tolerance). It accounts for difficulties that the participants experienced with a perceived unchanged annoyance over time. Furthermore, this model identifies a set of new attitudes toward oneself and others that tinnitus tolerance would entail.

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Conclusion: The subjective experience of frustration enlightens tinnitus-induced disablement, offering new perspectives for long-term self-management. Modulation of frustration, rather than moderation of tinnitus interference, is suggested as a new approach to the clinical management of tinnitus-related distress.

Keywords: tinnitus, frustration, disablement, long-term suffering, intra-individual variability, grounded theory, qualitative research

INTRODUCTION

Disablement is a concept that refers to the impact of a chronic condition on "people's abilities to act in a common, expected and personally desired way in society" (Verbrugge and Jette, 1994, p. 3). Understanding the disablement process not only accounts for the disabilities that an individual might meet as a consequence of his or her illness; it can in a broader sense also explain the impaired interactions that occur in the individuals' social and physical environment. This process involves a close interest for reciprocal influences between the disabled individuals and others' attitudes toward those restrictions. From a psychosocial perspective, chronic illness was characterized as a threat to self-integrity and "taken-for-granted assumptions about possessing a smoothly functioning body" (Charmaz, 1995, p. 657). It is accompanied by the loss of bodily enjoyment and inevitable limitations in the exercise of one's own desire. Charmaz (1995) further emphasized that chronically suffering patients may undertake divergent relationships toward their illness, whether they refuse the weakening of their life (i.e., they struggle against the condition) or adapt their way of living to accommodate to physical losses. Adaptation to a chronic condition is not a linear process. When body limitations and suffering exceed the threshold of tolerability, frustration may become overwhelming for the disabled individual (Dow et al., 2012).

Over the last decades, numerous studies have documented disabilities reported by tinnitus patients. Heterogeneity of outcomes in the tinnitus literature was recently stressed as a methodological issue that should be considered in a broader, ecologically valid framework (Searchfield, 2014). Recent studies have documented complex changes in patients' perception of their sound environment after tinnitus onset. Enhanced salience of tinnitus in either silent or noisy environments was reported by Pan et al. (2015). The authors point to a diversity of needs among tinnitus patients, reporting that perceived loudness in a noisy place could contribute to worsening of tinnitus in 32% of their population ($n = 258$). Hébert et al. (2013) also demonstrated that a tinnitus patient group ($n = 124$) displayed an increased growth in loudness compared to a control group with only hearing loss ($n = 106$). Matching the level of hearing loss in compared groups, they further suggested that hypersensitivity to noise in tinnitus patients was a phenomenon distinct from loudness recruitment. While tinnitus and hyperacusis do not always overlap clinically, the findings by Hébert et al. (ibid) support the relevance of identifying a sub-group of patients for whom specific treatments may be beneficial (Dauman and Bouscau-Faure, 2005; Schecklmann et al., 2014). Vielsmeier and co-authors reported recently that more than 70% of patients with tinnitus ($n = 351$) experienced difficulties understanding speech (Vielsmeier et al., 2016). While these difficulties were a concern for 40% of the patients when no specific circumstance was considered, 80% acknowledged that speech understanding was a problem in a cocktail party situation, with multiple sources of noise.

Beyond changes in the relationship to the sound environment, studies have documented extra-auditory difficulties among patients with tinnitus. Tinnitus has been considered to be the main sleep deprivation factor among people with hearing impairment (Test et al., 2011). Apart from difficulties falling asleep, tinnitus manifests as the reason for early awakening and mid-sleep awaking, which often lead to the use of hypnotic medication (ibid). Schecklmann et al. (2015) reported that the prevalence of insomnia in a group of chronic tinnitus patients ($n = 182$) reached 76%. Otherwise, lack of sleep can substantially contribute to increase tinnitus-induced disablement. According to Pan et al. (2015) up to 27% of their sample ($n = 258$) reported that lack of sleep was a source of increased annoyance. Tinnitus interference with mental performances has also been documented in the literature (for a review see Mohamad et al., 2016). It was suggested that the level of demand of tasks (Stevens et al., 2007) and executive control deficit (Heeren et al., 2014) contribute to patients' impairment when conflicting information is to be sought during a task. The consequences of tinnitus on social relationships are far less documented, although it was found that relatives' misunderstanding of daily difficulties accompanied by tinnitus could be a source of frustration for patients (Tyler and Baker, 1983; Sullivan et al., 1994).

Aspects of variability in how patients cope with their condition could be found in a treatment trial including 37 patients with severe refractory tinnitus (Zöger et al., 2008). The authors explored the effects of a group psychotherapy approach in comparison with anti-depressive medication. For example, some patients in the group reported in a post-therapy interview that tinnitus varied with their emotional state. Those who felt that they had gained a deeper personal insight into how their suffering from tinnitus varied were also those who were likely to perceive a positive long-term effect as a result of the therapy. These results suggest that patients' narratives could be used in the search for intra-individual variability of tinnitus based on patients' histories. Previous studies of the psychoacoustics of tinnitus have shown to what extent tinnitus may vary (Penner et al., 1981). Burns (1984) confirmed the high intra-individual variability of results in terms of equalization of pitch, intensity, and narrowband noise levels required to continue to mask the tinnitus. Emphasis was placed on perceptual differences between tinnitus and external sounds, the latter having a seemingly much higher stability.

Another example is to be found in a magnetoencephalographic study (MEG) carried out some 15 years ago (Dietrich et al., 2001) with eight subjects with tinnitus who presented a dip in hearing on high frequencies. The study demonstrated broader MEG responses when the sounds used to stimulate the auditory system were situated in the lesion-edge frequency region compared to responses obtained with sounds from pre-lesional frequencies (corresponding to audiometrically healthy zones). One of the subjects was tested four times in 3 months. Quite surprisingly, his MEG responses were not at all stable between the tests. The overrepresentation of lesion-edge frequencies disappeared in a few weeks but reappeared in the next session, before showing up in the last session at a level even higher than in the first one (Figure 4. of Dietrich et al. (2001) article). The authors showed that the observed MEG variations had no link with the perceived seriousness of tinnitus as measured by the Structured Tinnitus Interview (Goebel and Hiller, 2001).

Investigations conducted in a large series of patients also provided information on tinnitus variability. In a sample of 528 tinnitus patients, Stouffer and Tyler (1990) reported the following: (i) only half (52%) considered their tinnitus pitch as stable, and more than a third (36%) perceived fluctuations in pitch during the day; (ii) tinnitus intensity was considered as stable in only 44% of the cases; changes in intensity could occur suddenly (25%) or progressively (31%); (iii) since tinnitus onset annoyance was mainly considered as stable (60%), but 34% of the sample reported more intense tinnitus, and only 7% a lessening of its severity. The study also revealed several factors likely to increase tinnitus, such as staying in a quiet place, being exposed to a lot of noise, and being subjected to stress and lack of sleep. Interestingly, an investigation conducted by Slater et al. (1987) in nearly 1,000 individuals living in Wales mentioned the relief frequently felt by patients when they were engrossed, involved or interested enough in an active occupation. On the other hand, most of the patients had a hard time at night and needed to take sleeping pills or tranquillizers to get to sleep. These findings suggest that individuals may modulate their awareness and annoyance of tinnitus through their behavior (Roberts et al., 2013). However, such an influence may be limited considering the course of tinnitus annoyance in many patients. A previous study from our group (Bouscau-Faure et al., 2003) confirmed the variability factors mentioned in other studies, but these proved elusive to translate into a global handicap score. Data collected from 57 patients at the Clinique des Acouphènes (Tinnitus Clinic, University Hospital) were subjected to a detailed analysis of individual answers to the Iowa questionnaire THQ (Tinnitus Handicap Questionnaire, Kuk et al., 1990). For each item, subjects were invited to answer with a score between 0 and 100, and could note down a free-form comment in another box corresponding to the question. Importantly, to two precise questions: "I am unable to relax because of tinnitus" (item 14) and "I have trouble falling asleep at night because of tinnitus" (item 16), it was not uncommon for subjects to write "It depends on my level of nervousness and tiredness; I hesitate between 50 and 100%". Emerging ecological momentary assessment (EMA) is advocated to overcome these difficulties inherent to one-time static measurements, thereby enabling more accurate measures of moment-to-moment tinnitus variability (Wilson et al., 2015). Preliminary studies suggest that intra-individual variability may be much higher in tinnitus than traditionally thought. A remaining challenge of EMA in tinnitus is that participants are not always able to account for the variability of annoyance that they encounter (Schlee et al., 2016). To date, variability in tinnitus has mainly been investigated by using self-rating questionnaires to score the impact of tinnitus on patients' daily life (e.g., Kuk et al., 1990; Zeman et al., 2012) and to assess treatment outcomes (e.g., Newman et al., 2014). Questionnaires are usually based on personality constructs (e.g., anxiety, depression, acceptance, self-efficacy) that are *applied to* the experience of tinnitus (e.g., Smith and Fagelson, 2011; Weise et al., 2013). However, such measures may not be sufficient for specific features of the experienced condition to emerge. An alternative methodology, like Grounded Theory, uses unstructured data (i.e., individual interviews) and a constant coding procedure to discover constructs that are close to the data. This inductive approach goes beyond the pre-structured instrument level and explores variations in difficulties experienced by participants on an individual level.

In the current study, we propose to contribute to the understanding of the variability of tinnitus-induced disablement from such a qualitative approach, by investigating the inner perspective of a small group of participants who have lived with bothersome tinnitus for some time. Qualitative inquiry and analysis have been promoted by Malterud (2001) with the aim of improving management strategies and better understanding the clinical setting, patients' expectations and physicians' attitudes toward the suffering of their patients. In audiology, qualitative research has previously underlined the contribution of the patients' perspective on the management of hearing impairments (Hallberg and Carlsson, 1993; Laplante-Lêvesque et al., 2012), Méniere's disorder and otosclerosis (Eriksson-Mangold et al., 1996; Erlandsson et al., 1996), hearing aid use and non-use in older people (Lockey et al., 2010), and young people's risk-taking as regards exposure to loud music (Widén and Erlandsson, 2007). To our knowledge, however, no qualitative study has yet been undertaken in tinnitus, especially with regard to the issue of intra-individual variability of tinnitus-induced disablement, and how this relates to tinnitus suffering and to tolerance. From previous studies (Dauman and Erlandsson, 2012; Dauman et al., 2015) we hypothesized that tinnitus patients' narratives offer a substantial contribution to the comprehension of the condition, by taking into account the perspective of individuals in the perception of the circumstances in which their tinnitus varies.

Aims

This study had three aims. First, we wanted to use a more systematic approach (i.e., qualitative research) to investigate tinnitus-induced disablement. Secondly, we aimed at contextualizing the suffering of tinnitus in a social perspective, i.e., taking into account ecological validity by exploring the participants' narratives. The objective was to allow individuals to play an active role in data collection, and acknowledging their agency in how meaning is constructed with regard to their condition. Thirdly, we aimed to enlighten their attitudes toward

health professionals in order to increase knowledge about the difficulties they may encounter in their search for a cure.

MATERIALS AND METHODS

Study Design

This qualitative study was based on Grounded Theory, a well-known research methodology within the field of health and chronic illness (Glaser, 1978; Strauss and Corbin, 1998; Charmaz, 2014). Our approach was especially influenced by Strauss and Corbin's (1998) mode of Grounded Theory data analysis (described below) and by Charmaz's (2014, 1995) approach to open-ended interviews that promotes rich data collection through sensitized listening. The aim was to deepen our understanding of distress and disablement in individuals with chronic tinnitus, through a sequential and intensive interviewing of participants. To achieve this goal, we designed a study consisting of three interview sessions for each participant, each interview lasting 1 h with an interval of 2 weeks between. It was considered that this design would contribute to the building of trust between the participants and the researcher (ND). The second and the third interviews with each participant provided detailed data, with a contribution of the researcher's reflexivity emerging from memo-writing and initial coding between each interview. However, it is acknowledged that this first study using Grounded Theory encountered limitations with respect to theoretical sampling. We address this issue at the end of the article and draw some perspectives for future studies.

Ethical Considerations

The Ethical Research Committee CPP Sud-Ouest Outre Mer III (DC2016/154) approved the study. Each participant was handed an information letter stating the purpose of the study. Prior to participation they were assured that the study would be anonymous and that any personal information would remain confidential. They were told that in order to enable a rigorous analysis of their discourse, the interviews had to be recorded and that the study results would be published in a scientific journal. All of them acknowledged that it was important to contribute to research on chronic tinnitus and gave their written informed consent in accordance with the Declaration of Helsinki (WMA, 2013).

Procedure

The recruitment of participants was conducted with the help of the French Tinnitus Patients Association (France Acouphène, FA). A call for testimonials under the title: "How does one live with tinnitus nowadays?" was published by FA. The national recruitment aimed at exploring a variety of participants' health paths, depending on their residence (i.e., metropolis, medium-sized cities, villages). Individual interviews were conducted via Skype or by phone. By selecting members of the Tinnitus Patients Association to participate in our investigation, we expected to receive data rich enough for a qualitative methodology like Grounded Theory. The participants had had a rather long experience of living with persistent tinnitus and were also used to sharing their experiences with other members of the association. In this way, we believed that their reflections and thoughts would be highly valuable for the investigation and also suit the study methodology.

Participants and Demographics

Table 1 shows the 12 participants by gender, in ascending order of hearing loss. Their median age was 63 years and the majority (9/12) were no longer working for health reasons. At the time of the interviews the median duration of tinnitus was 14 years (range 6–37 years). Seven participants considered that the discomfort due to tinnitus had worsened after the initial onset, while others reported stabilization or waning of symptoms over time. A minority reported more difficulties in falling asleep than before the onset (3/12). However, a majority perceived tinnitus as soon as they awakened with difficulties getting back to sleep (10/12). Finally, only two subjects used hearing aids regularly. The sample was gender-balanced.

Rich Data Gathering

The interviews began with an interview guide (see **Table 2**) based on previous studies on patients' narratives (Dauman and Erlandsson, 2012; Dauman et al., 2015). Five open-ended questions were retained as an outline for the semi-structured interviews, aimed at helping participants to contextualize their experience of tinnitus i.e., to explore circumstances preceding the onset of tinnitus and possible long-term physical and mental health consequences. Information collected included duration, description and laterality of tinnitus, discomfort since onset of symptoms, tinnitus perception (sound/noise), use of hearing aid and the impact of tinnitus on sleep. Other questions focused on the participants' close networks such as family and entourage and strategies undertaken in order to manage suffering and on help received from healthcare providers.

While conducting the interviews, the first author used memos to record initial codes, e.g., participants reactions and experiences (e.g., being overwhelmed, hypersensitivity) and associated ideas and questions about circumstances that arose during the interviews. More abstract memos were used to collect and cluster events of the same type into a broader description of tinnitus in daily living. Significant themes were identified (e.g., sensitivity to noise, tiredness, being misunderstood, suffering), which allowed the second and third interviews to include more focused questions on tinnitus disablement (e.g., What do you do when you have to deal with new worsening of your suffering?; Have you encountered disbelief from others about its seriousness?). Focused questions were asked as a means of enriching descriptions of difficulties when this proved necessary. More abstract memos enabled us (ND & SE) to build the first concepts that clustered and highlighted seemingly different conducts by a suggested underlying process. For instance, comparison between avoiding noises and surrounding oneself with pleasant sounds led to the idea that controlling sounds was an important feature of participants' relationship with their environment. Hence, an initial theme "sensitivity to noise" contributed to an important concept, later labeled "auditory congruence". In turn, abstract memos helped us to formulate more precise questions (e.g., What do you need

TABLE 1 | Study participants by gender and in ascending order of hearing loss, with information on tinnitus perception, overall annoyance, sleep, and wearing of hearing aid.

Sex	Age	Uni HL	Bi HL	AA00 HL	HF HL	Tinnitus lateralization	Tinnitus duration	Type	Predominant description	Overall annoyance	Insomnia before	Insomnia after	Drugs	Tinnitus at awaking	Hearing aid use
F1	75		X	20	55	Left	13 y	Noise	Humming	Reduced	No	No	No*	Yes	No
F2	79		X	25	60	Left	6 y	Noise	Whistling, horn	Enhanced	Yes	Yes	Yes	Yes	No
F3	67		X	30	40	Left	8 y	Noise	Crackling, roaring	Enhanced	No	No	No*	Yes	No
F4	60	X		38,5	42.5	Head	11 y	Noise	Rumbling	Unchanged	No	Yes	No	Yes	No
F5	63	X		73,5	75	Left	13 y	Noise	Crackling	Reduced	No	No	No	No	Yes (unilat and occasional)
F6	56		X	120 46,5	120 47.5	Left	29 y	Noise	Roaring, whistling	Unchanged	No	Yes	No	Yes	Yes (bilat and regular)
M1	56		X	15	42.5	Left	12 y	Noise	Whistling	Enhanced	No	No	No*	Yes	No
M2	51		X	21,5	50	Both	8 y	Noise	Whistling, crackling	Enhanced	No	Yes	Yes	Yes	No
M3	61		X	26,5	67.5	Head	13 y	Sound	Whistling	Enhanced	Yes	Yes	Yes	Yes	No
M4	64		X	31,5	72.5	Left	17 y	Sound	Whistling	Enhanced	No	No	No	Yes	No
M5	57	X		35	112.5 Prog	Right	32 y	Sound	Crackling	Enhanced	No	No	No	No	No
M6	62		X	40	120 Prog	Right	16 y	Sound	Whistling	Unchanged	No	No	No	Yes	Yes (bilat and regular)

*Legend. For each sex group, the ranking of participants based on the amount of hearing loss was determined by averaging the pure-tone hearing thresholds at 500, 1,000, and 2,000 Hz of the poorer ear, according to Merluzzi and Hinchcliffe, 1973. In addition, the average hearing loss over the high frequencies, 4,000 and 8,000 Hz, was calculated in the same ear. By comparing the two hearing threshold averages, it can be seen that hearing loss predominated over the high-frequencies in the majority of participants. *means that participants only used a drug when they needed to be efficient the next day.*

TABLE 2 | List of initial questions used in first interview with participants.

1. Can you tell me how your tinnitus began?
2. What steps have you taken since then?
3. To this day, what are the consequences of tinnitus on your family and social life?
4. What do you do to lessen your suffering?
5. What do you think of the therapeutic approaches provided?

BOX 1 | Excerpt from abstract memo about the concept "Controling auditory environment."

Having tinnitus all day long prone to control one's surrounding environment. Circumstances in which too much noise are to be endured must be avoided (e.g., restaurant, bar, cinema) because of the consecutive worsening of tinnitus. Sudden, unpredictable noises from the outside world seem to bring the worst annoyance (e.g., fire engine, moped). Noise pollution is also a problem when people are talking all together. Decline any invitation may be the only solution, when you don't know if you could participate in meetings. Hard days cannot be foreseen with chronic tinnitus. Then, even sounds from relatives may be too much to endure. Withdrawal can be mandatory for avoiding worsening the situation. Instead of other's sounds, it seems more desirable to surround oneself with sounds that are pleasant and choosen (e.g., music, fontain, radio). These ones may bring comfort, helping to relax a bit from tinnitus presence. They ensure that there can be less discomfortable situations, from time to time. Surrounding oneself with these sounds is even more necessary at the tinnitus onset.

to feel comfortable?). **Box 1** shows an excerpt of abstract memos regarding the need to control one's surrounding auditory environment, leading to focused questions with a more precise content.

Extended Data Analysis

The material collected consisted of 36 in-depth interviews ($n = 12$ participants; 3 interviews per participant). In total, the transcripts from the recordings ran to 678 pages. The detailed material allowed for more systematic scrutinizing of data, which led to the emergence of a central perspective (i.e., a core category) with broad implications for the data material (Glaser, 1978). The core category was judged to be the best fit and to explain variations in the data. Data transcription enabled a constant comparison between codes and concepts, which were easier to cluster and contrast based on the coding procedure. According to the recommendations of Strauss and Corbin (1998), we followed a three-step procedure which was carried out by ND and discussed at each stage with the co-authors (SE, DA, RD) who offered remarks and significant comments. **Table 3** explains the overview of the coding process, including data gathering and extended analysis. The material was first subjected to an open coding with systematic reading of the transcripts, line by line. Our understanding of the participants' daily life situation was significantly improved by this initial stage of analysis. It helped to sustain a balance between previous concepts from data gathering and emergent ones (i.e., involvement, sustainability, independence). DA contributed with reflections regarding the outcomes of open coding and helped in building logical links between individual behaviors. Abstract memos enabled a personal "dialogue" with the data, by the construction of a storyline based on the concepts. This led to performing a second axial coding that combined concepts about similar individual behaviors and circumstances. The rationale of using axial coding was to further increase conceptual density around as few axes as possible. This step entailed searching for reciprocal implications between categories (e.g., articulate "facing disbelief from others" with "developing independence toward misunderstanding"). SE and RD established the appropriateness of the categories and sought the best fit with clinical issues arising from the analysis. Reflexive questions were addressed to foster understanding of axis of analysis with respect to their conditions and consequences (e.g., Why do meaningful activities help in sustaining tolerance? Why is it essential to regulate one's lifestyle?). From conceptual tensions around axes of analysis, a more central perspective was found to account for each axis of analysis and a governing principle between them could be drawn. The last step of selective coding consisted in reconsidering all the results using the perspective that the core category provided. This led SE and ND to search for literature review that would best fit their tentative labeling, and to find more suitable terms to identify the core category and supporting categories. The core category was assumed to offer a high level of explanation of the participants' experience (Glaser and Strauss, 1967; Glaser, 1978) by integrating variations across the participants (Strauss, 1987).

RESULTS

Tinnitus as Persistent Frustration

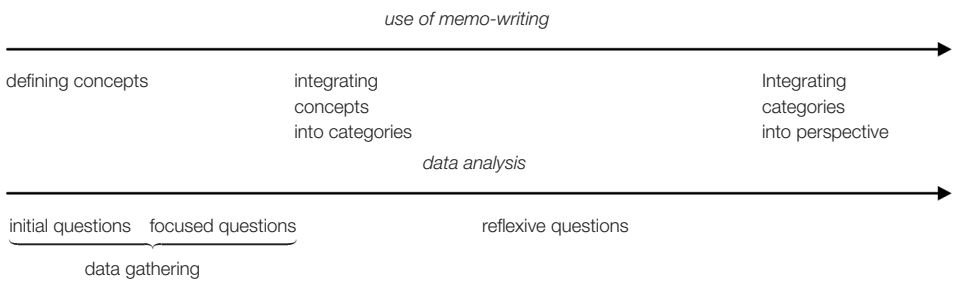
Tinnitus was found to vary in relation to the participants' frustration at being unable to achieve their goals. The term "frustration" is used throughout the results to indicate the subjective experience of being unable to change a situation or to fulfill one's desire. Even though the participants' frustration was mainly related to the auditory field (e.g., having difficulties to communicate in noisy environments), it also concerned other circumstances in which hearing does not play a central role (e.g., when others misunderstand the impairment caused by tinnitus). We hypothesize that variability in tinnitus annoyance reflects participants' inability to act in accordance with their desire in the experiences they have of the condition. In particular, the participants' perceptual awareness of their inability to change the situation seemed to be important to them. The more acute their awareness of the situation was, the greater was the tinnitus annoyance in their perceptual field. Although all sufferers reported variability in their annoyance with tinnitus, their frustration was not easy to grasp, as the following remark illustrates:

> "I cannot compare it with anything else, I don't know, it ruins my life, it prevents me from doing things I'd like to... it bothers me whatever I try to do." Participant F6.

We identified three main implications of tinnitus as a persistent source of frustration: 1. Losing body ownership, 2. Lacking perspectives and 3. Persevering through difficulties. **Table 4** presents an overview of the results.

TABLE 3 | Flowchart of the coding process in relationship to data gathering and data analysis.

Raw data	Open coding	Axial coding		Selective coding
Researcher You were telling me that you never stop working? **Participant M1** In fact, what happens with tinnitus is that since I cannot remain doing nothing, I cannot stay in silence, I must always be busy. I need to do something and when I focus on something very definite, I do not hear my tinnitus, I only hear it when I stop concentrating. If I focus for 10 min on something very definite, then I do not hear it over this period of time. Thus, I am constantly searching for concentration, something to do, and eventually I get tired after a while, because I cannot relax and rest.	being unable to rest doing something forgetting tinnitus while focused getting tired by constant activity	Sustainability Involvement Indifference Enjoyment	**Carrying out reachable activities**	**Persevering through difficulties**
Researcher Was it difficult for you to describe to them the state you were in? **Participant M3** It is somewhat tricky because people are thinking about something else. They presumably feel well, so attempting to provide an explanation implies somehow interrupting their pleasant moment, and they have the right to experience good times. Consequently, the best strategy I have found for the time being, in order to minimize the damage, is to not expose myself and to offer my apologies ... and to say that, I'm going to take a break, in other words, I'm having a rest! Anyhow, embarking on explanations is useless. It's something that nobody else can really be aware of ...	being ignored by others avoid interrupting others finding the lesser evil avoid pointless explanation	Elusiveness Disbelief Withdrawal Independence	**Living with invisible weakness**	



LOSING BODY OWNERSHIP

Tinnitus was found to interfere with the fulfillment of the participants' daily goals. It impinges upon their free will and body enjoyment, interfering with the perception of sounds and making communication difficult in noisy environments. It deprives them of the calming experience of silence. It leads them to undertake activities constantly in order to distract themselves from a phenomenon that above all they want to get rid of. Participants try to counterbalance this unpleasantness by creating an enjoyable sound environment. They also try to regulate their lifestyle in order to limit the variability of their tinnitus and its interference in their lives. However, this task is relentless because of the unyielding symptoms of the condition.

Being Invaded by Inescapable Noise

Tinnitus is perceived as a disagreeable noise. Its intensity varied from one moment to the other, sometimes with extreme awareness of its presence at the heart of the participants' perception. The consciousness of the obstacles they meet when trying to control their sound environment even makes tinnitus worse. It interferes with the search for informative sounds, and with the desire to escape noises that interfered with concentration and rest. In all cases, when they are thwarted in achieving respite, participants report a worsening of their tinnitus that increased and prolonged their frustration. The relationship between variability of tinnitus and experienced frustration varied with the individual's ability to control one's sound environment. The most hearing-impaired participants reported that difficulties in perceiving sounds of low-to-medium

TABLE 4 | An overview of results emerging from the Grounded Theory approach to data.

TINNITUS AS PERSISTENT FRUSTRATION		
1. Losing body ownership	**2. Lacking perspectives**	**3. Persevering through difficulties**
1.1. Being invaded by inescapable noise	2.1. Failing to identify tinnitus	3.1. Living with invisible weakness
1.2. Holding on to a fragile body	2.2. Facing an irreversible condition	3.2. Carrying out achievable activities

intensity (e.g., a fan, fridge or coffee-maker) made their tinnitus even more bothersome.

> "I think that from the moment I focus on a sound that I like to hear clearly and there is a background noise, my brain is disturbed and the tinnitus increases, or at least my perception of tinnitus increases [...] I feel as if the more my ears are prepared to hear, the more likely that my tinnitus does the same and increases." Participant M6

A resolute but unsuccessful effort to listen to sounds could also intensify the presence of tinnitus in the perceptual field. This can happen when someone must pay attention to important information in a professional framework, or needs to focus on a sound of uncertain origin. Searching for sounds that they were unable to grasp could be experienced as a worsening of tinnitus (somewhat similar to the experience in a soundproof chamber, an environment which is known to temporarily enhance the perception of tinnitus).

> "The worst is the room where we took the audiogram. When they told me, behind the window, to: 'Listen to the sound', I felt burning everywhere and then, after a while, a sound appeared besides the tinnitus that I was supposed to hear... the tinnitus was even stronger, maybe because I was expecting this sound I was supposed to hear, I don't know..." Participant F6.

Hearing loss increases the effort made to understand what others are talking about. It is a source of fatigue accumulated during the day, because of the greater attention required to listen and answer appropriately. It forces the hearing-impaired to ask others, whose reply is not always comprehensible, repeat, and this has a negative influence on the conversation. Meeting difficulties in communication situations, the hearing-impaired have to deal with a more intense form of tinnitus that seems to compete with their intention to participate in conversations.

> "At first, it was as if I was wearing a helmet, as if there was something around my head that isolated me completely... and if I wanted to hear when someone talked to me, I had to pull out my ear, take it out of the helmet." Participant F5.

The hypothesized relationship between frustration and tinnitus annoyance is also shown in the participants' thwarted desire to protect themselves from noisy environments. In their experience, the onset of tinnitus heralded a lower tolerance threshold to noise disturbance. Following the onset of tinnitus, sudden noises (e.g., a fire siren) and noises lasting in time (e.g., festivities in the street) are perceived as harmful to the integrity of the body. The reluctance to be exposed to an uncertain sound environment is also dependent on the increased intensity of tinnitus, which can last from one to a few days. The awareness of such a disturbance is perceived intensely during exposure to noise, when being forced to endure passively a constraint that cannot be avoided. In such circumstances sufferers must go on, against their will, to the limits of their resistance to noise disturbance.

> "I know immediately when the evening is going to be too noisy. And when I depend on others to accompany me home, it is really annoying... Because I know that it means that my tinnitus will be back for several days. And that my ears will be buzzing if I expose myself to the noise for too long [...] I then huddle up over my discomfort and irritation and the pleasant moments are gone. I count the minutes go by while thinking: 'When is it going to end?'" Participant F1.

The insistence of tinnitus, from waking up in the morning to difficulties falling asleep, reinforces the sense of its constant presence in one's mind. Some of the participants are preoccupied by this continual appearance of tinnitus with very rare moments when they are able to pay less attention to the sound. Most of them are also confronted with the presence of tinnitus when they wake up at night. Such experiences give the impression that tinnitus is a phenomenon that is omnipresent in their perception.

> "The problem is that you have to make a constant effort. Just to live. You need to make an effort whatever you do, in order to sleep, for everything. It is not natural to make an effort all the time, that's what wears the body out. [...] Tinnitus consumes life, it is so oppressive and intrusive that it steals a part of your life." Participant F4.

This conviction is not contradicted by any perception of a cessation of tinnitus. In anguish, individuals may project their incapacity to put up with tinnitus on their future existence. The absence of the prospect of improvement leaves them with a deep feeling of helplessness.

> "I wrote some notes that I used to put in my pockets. They said 'Yes, I accept to live with it'. Because at first, I couldn't tell myself 'You're going to put up with this all the time, all your life'. It is not possible. [...] And then, once the day was over, even if it had been a bad day, I wrote on the paper 'Yes, well done' or 'To improve'. And when I was in a lot of pain, I put my hand in the pocket and took out the paper to remember what I had to do." Participant M3.

The unyielding nature of tinnitus can push individuals to the limits of their physical endurance. Some cut themselves off from social relations, only seeking to protect themselves from noise pollution which seems to have a negative influence on tinnitus. Above all, they want the agony to cease, so that they can return to the life they used to have. Faced with the absence of silence, some evoke their fear of going mad. For others, the lack of silence is experienced as their biggest frustration. Not being able to perceive silence again is regarded as a fundamental loss in the experience of oneself in the absence of others. If there is no solution to this kind of suffering, tinnitus can at times become overwhelming.

> "I am invaded by noise. When I listen to the television, people speak too loud. Family meals are no longer possible, because of loud sounds when there are too many people around... I no longer celebrate Christmas with my family, I don't feel capable of managing all this, I prefer to isolate myself rather than being invaded by the noise, it's unbearable." Participant M4.

Holding on to a Fragile Body

Confronted with the interference of tinnitus with their hearing experience, participants reported trying to restore the balance between their desires and their sound environment. They adapt the latter to their auditory sensitivity, using recordings (e.g., fountain, natural music) or radio chats to exercise efficiently the desire to choose sounds that bring comfort. Such individualized environments enable them to listen to enjoyable sounds that counterbalance the unpleasantness of tinnitus. Sounds that are personally selected are of special value, also because they are easily accessible.

> "I listen to music for pleasure; I like to listen to music. Well, tinnitus is still in the background, but I enjoy the music, so I don't really care about the tinnitus." Participant F5.

The use of auditory protection in a noisy professional environment fulfills the same purpose with respect to the avoidance of noise disturbance. It enables sufferers to contain the gap experienced between noise intensity that they can tolerate and the noises they are exposed to, being actors in settings they cannot escape.

> "The first thing I think about when I go to work is to put in my earplugs. I couldn't do it without my earplugs. I never forget them. If I do, I get worried, stressed. [...] But I use different types of filters, depending on if I am at work, outside or at home." Participant M2.

Of great importance is to choose how to deal with the sound environment. It is experienced as the assurance of having the choice to select and overlook sounds that are to be perceived.

> "My wife keeps telling me: 'I don't understand why you have that around your neck and why you don't use headphones'. But no, headphones are not convenient for me, because the sounds I hear don't interest me all the time. [...] I don't know how to say it, it is an area of interest for me that I can take on board or drop whenever I want." Participant M6.

Regulation of lifestyle was a second adjustment made by the participants. It implies a stricter management of rest and sleep compared to how routines used to be before the onset of tinnitus. Participants stressed the importance of rest and sleep, as well as the need to sometimes isolate themselves despite the frustrations it might give rise to socially. Lifestyle regulation also includes avoiding any excess of noise, agitation and sometimes stimulants (e.g., alcohol, coffee). In particular, lifestyle regulation enables them to limit the variability of tinnitus-induced disablement and avoid crises resulting from going over their limits.

> "The two main factors are fatigue and noise exposure. If I handle them well, the variability of tinnitus becomes less difficult to handle. But if I fail to handle it, I experience huge variability and the tinnitus can be dreadful." Participant M1.

Trying to cope with the frustration over enduring, permanent tinnitus may lead some individuals to adopt sleeping on their own. This is an attempt to avoid fighting against tinnitus, and instead admitting to its presence in their mind and body.

> "At night, I still prefer to manage my tinnitus in silence and complete darkness. Going to sleep is complicated... So, I cut myself off from external noise, and come to terms with it: 'here you go, you hear it...' I know that at other moments during the day, I won't hear tinnitus for some time. That's what I try to tell myself." Participant M4.

Sufferers often reported limiting their socializing in order to alleviate their frustration being exposed to others' noisy behavior and misunderstandings of what the hearing loss implies. Simultaneously, they escape from potentially demanding situations, which also increase tinnitus annoyance. Even though all sufferers regretted it, these social limitations seem to be efficient in a long-term perspective. Nevertheless, the negative side is that the strategy restricts relationships with close family and friends.

> "Before, we were a family who organized many meals together. But now the social part of my life changed a lot. For some time, I had almost no social contacts; now I'm beginning again, slowly... organizing meals with 6–8 persons. Afterwards I need two, three days to recover, as my ears are more sensitive, and there is systematically a worsening of my tinnitus." Participant M2.

Considering that nothing alters the sustainability of tinnitus leaves many participants with a sense of helplessness. It is described as an inescapable struggle against it.

> "...You end up completely empty at night, knocked out. You think: 'Yet I didn't do much during the day' Yes! You were hiding, fighting against this parasite that is here permanently, 24 h a day, you were fighting to try and do something else. [...] you fight, but after a while, you don't have any energy left to overcome the parasite..." Participant M5.

Even for those who are able to develop some occasional or even more regular tolerance to its presence, invasive thoughts about tinnitus remain a potential risk. Constant effort is needed in order to avoid paying attention to it and at the same time holding on to a controlled lifestyle. If not, tinnitus can "get the upper hand" over the desire not to be dominated by it. Tinnitus is perceived as a handicap to be carried within oneself, whereas the most natural reaction would be to get rid of it once and for all.

LACKING PERSPECTIVES

That something strange is happening in the body becomes an urgent worry after tinnitus onset and is something most participants have experienced. This belief feeds their need to understand what is taking place in their brain and ears, hoping to find a means to act upon it. These expectations are rarely a matter of concern for physicians who view tinnitus as a symptom of brain activity and not as an illness. Perceived as an unreachable goal, the prediction that by time you can get used to it accentuates their sense of incapacity to put up with it. Furthermore, a chat with their physicians regarding the daily experience of tinnitus would allow them to understand more fully how to manage tinnitus-induced disablement. With no perspective of understanding the variability of tinnitus and its influence on daily life, patients are left with their frustration to manage.

Failing to Identify Tinnitus

Tinnitus presence may elicit some serious questioning about the origin of noise in the body which continues for years after tinnitus onset, as shown by a large number of medical consultations. Patients are determined not to miss any solution of how to get rid of it and want to explore every potential source (e.g., ear, brain, teeth, cervical vertebra, etc.). The belief that tinnitus reveals an underlying pathology triggers the search for medical explanations. In a long-term perspective, tinnitus onset may induce preoccupations and thoughts about a degradation of hearing and normal brain activity. Concerns about managing tinnitus without therapeutic help is strengthened by the lack of a satisfactory medical response to the possible etiology. Without a clear understanding of a likely cause and the process of tinnitus, no efficient means of action seem to be at hand.

> "When it happens to you, you ask yourself: 'Why me? Why is this happening to me?... while I always had a quiet life, I paid attention to what I ate, what I did. What on earth have I done to deserve this?... It's human, I think, to ask oneself these questions. And we have no answer." Participant F2.

Medical explanations often alleviate preoccupations about the danger of tinnitus for hearing and brain activity. However, its variability over time also prompts questions like: "what is happening in my brain?" Being able to understand more about tinnitus variability might have helped the participants to better cope with the suffering. This kind of knowledge, however, is seldom addressed in medical consultations. The lack of medical explanation regarding this aspect of tinnitus makes patients observe themselves through the prism of their own physical experience.

> "At the time, I even did Excel tables on my blood pressure, the intensity of my emotions, and tinnitus intensity. I gathered information, as if I was a witness as well as a patient, to somehow be able to understand what was going on!" Participant M3.

Participants highlight the role of internal factors (e.g., tiredness, anxiety) as well as external circumstances (e.g., noises, upsetting events) that might worsen tinnitus-induced disablement. Sometimes, however, tinnitus variability is hard to understand. It may increase when they are engaged in things they have to do, and may compete with their activities, as if tinnitus is "trying" to oppose what they like to do. Some witness a kind of automatic rolling-out of bodily changes, against which they cannot fight.

> "It fluctuates. Today it is bearable, but yesterday it wasn't. Tomorrow it will return more strongly... It is really strange. It changes all the time. One day it is strong, the next it is less, so you never know... But I know the day it will be intense. Today it is not virulent, but tomorrow it will be, as if to make me pay for the day when it was somewhat quieter." Participant F2.

Facing an Irreversible Condition

Participants' belief that they need to be cured from tinnitus does not fit with their physicians' perspective on their health status. Referring to the absence of a visible pathology on MRI scans, physicians try to reassure and clear up the patients' concerns about it. However, the absence of an underlying pathology does not resolve the suffering. It rather means that nothing can be done for what some sufferers experience as agony. Unintentionally, this kind of information may even worsen the patient's view on his or her experience of tinnitus.

> "It is not all about having audiograms, there should be some intervention about our suffering. [...] Doctors don't seem to take us seriously, it makes me angry. Once we tell them we have tinnitus, they raise their arms, as if to say there is nothing they can do..." Participant F2.

The examinations that they received (e.g., routine hearing loss measurement) were quite different from the help and care that they felt they really needed. Their practitioners' view about the condition counteracts their belief that "something must be done" to relieve them from the agony that tinnitus causes. Invariably, participants remember words like: "there is nothing to be done against tinnitus" and they "should better get used" to a noise they will certainly hear "all their life". According to them, practitioners may not realize the impact that such words have on a distressed patient. Indeed, after tinnitus onset most of them did not consider any other solution than relief or suppression of it through treatment.

> "At first, you feel really overwhelmed by it. So you think you will remain this way all your life. First because the ENT specialist I met told me so. He said: 'Your hair cells are destroyed, they will

not grow again. So, you will have tinnitus all your life'. [...] When a physician tells you that, it's crazy..." Participant F5.

Furthermore, participants missed having a conversation with their physicians regarding how they experienced and reacted to the onset of tinnitus. Little or no information on the process of habituation to the symptom was reported. The way physicians explained the habituation process seemed unfathomable to the sufferers, and was even perceived by some as a source of resignation. There were no questions on how they managed the variability of tinnitus that could have helped them to clarify their search for suitable coping behavior. A perceived reluctance from professionals to talk about how tinnitus might fluctuate from one day to another reinforced participants' feeling that they were receiving no medical help. The perceived closed-mindedness of the medical attitude regarding the characteristics of tinnitus might complicate the early stages of their experience.

"At first, they told me several times: 'don't worry Ms., you'll get used to it, it's not serious, it will get better. You are going to live well with it—anyway, you are going to live with it–so you better begin right away.' When I only wanted one thing: to get rid of it. I didn't want to hear this. I couldn't hear it. [...] They were perfectly right, ultimately, but in my opinion, they were wrong to be right at once. What they said disturbed me as much as the tinnitus." Participant F1.

PERSEVERING THROUGH DIFFICULTIES

The experience of being disturbed by tinnitus cannot be shared and often remains unknown to others. Disclosing the hearing impairment is met with disbelief or a lack of regard from interlocutors, which adds to the participants' feeling that they are suffering from an unrecognizable condition. Therefore, they have to find ways on their own to alleviate the hardship related to the impairment. All of them observed variations in the course of their tinnitus-induced disablement. Some of them realized that these variations gave them the opportunity to change their attitude toward it. For example, activities that they are involved in might enable them occasionally to pay less attention to it. These coping experiences are rewarding and full of meaning because they can restore the congruence between what participants want to do and are able to do, enabling them to live momentarily with less suffering.

Living with Invisible Weakness

The invisible presence of tinnitus puts the participants in an awkward position when it comes to social relations. Most of the time, tinnitus is non-existent in contexts of social communication. When informed about it, interlocutors usually appear indifferent or disconcerted. Even close relatives are uncertain about how to react, and hence the subject might create relational tensions. Participants generally decide not to mention tinnitus, even when they are with acquaintances and family members who usually soon forget all about it. To those who do not have their own experience of tinnitus it sounds elusive.

"People who do not have tinnitus don't really manage to grasp the fact that it is permanent. I have friends and family who regularly ask me: 'Do you still hear it, now?' Yet, I told them several times that it is permanent, but I don't know... It is a reaction as permanent as tinnitus is, I think." Participant F5.

Not mentioning tinnitus enables those who suffer to avoid negative comments from people in their entourage. It is aggravating to receive comments about the supposed banality of tinnitus from people who believe it is a lesser problem.

"When I first had the symptoms, people used to tell me: 'you listen to yourself too much, don't listen to yourself, etc. You're getting upset for nothing, I had tinnitus too, etc.' I quickly understood that I couldn't talk about it too much, because I'm not understood. People see me as someone who exaggerates, as a hypochondriac. [...] I only really talk about it when I am very, very tired, and feel crestfallen." Participant M1.

To unwillingly choose social isolation enable the participants to limit their frustration (for oneself and others) stemming from people' misunderstanding of their lesser endurance to loud noise. Participating in social gatherings is bearable if it results from a clear and assumed decision. When the noise gets louder, it becomes mandatory to leave the group and rest for a while. The company of others must not prevail over the pressing need to protect oneself from harmful noises. This requires an independence of mind with regard to others, to communicate a trouble that is not shared, and likely to arouse comments. There is a need sometimes to impose limits on the behavior of young children, and to remind others not to speak all at once. Long-term tinnitus management demands living in accordance with one's individual needs more than ever before, and to stick to these new limits.

"I have one day, two days of activity and usually after two days tinnitus is very loud, because I can't put up with it anymore. [...] Now I know myself, but before I became depressed, because I went too far... Now I try to slow down, to take breaks. I withdraw more often; I don't get in contact with people so often anymore, whereas before I used to wear myself out." Participant F4.

Carrying Out Achievable Activities

All participants notice some kind of reconnection with tinnitus and an increase in its loudness after they have finished an activity they were involved in. Some are surprised to find how easily it comes back into their consciousness, and directly becomes more audible. They would like to know about the mechanisms that make it return as soon as they stop what they are doing.

"Manual work is the most efficient: even in silence. I manage not to hear it then. When I am very busy manually, that is good, I am settled down, my work is perfect and then I don't hear it. But once I stop and look at what I did, it returns. Once I stop concentrating, it returns as fast and goes crescendo, it slowly increases and after 5 min, it's at full pitch." Participant M1.

This feature of tinnitus can drive some participants to maintain a high level of activity. It is also a way to avoid passive suffering from it. Nevertheless, over time, active opposition toward tinnitus proves not to be efficient. An acute awareness of such limitation reduces the perceived relevance of these activities, which may appear insufficient to alleviate the presence of tinnitus. Unlike sustained efforts to overcome it, someone just mentioning tinnitus can immediately recall its presence to a sufferer who was not paying attention to it just before. Likewise, upon the cessation of a musical activity, tinnitus emerges as strong as ever. Also, a worsening of tinnitus follows a sudden noise.

> "Sometimes I hang out with friends, we talk for an hour, and everything's fine. And then, all it takes is that someone tells me: 'It seems your tinnitus is getting better, no?' only that, and I answer: 'Yes, it's somewhat better, it depends on the time', but then, only with that, I feel tinnitus buzzing in my head. Just talking about it puts it back into my brain." Participant M6.

For some participants, the experience of the contrasting variations of tinnitus can shed a new light on the phenomenon. Those who realize that they need to restrict their agenda no longer constantly engage in activities. They understand that their thoughts were absorbed in their hobby the moment before tinnitus suddenly became more intense. For such participants, the brief disappearance of tinnitus is experienced as more valuable than its sudden return into their consciousness.

> "One day, I realized that I didn't hear it for a while. Then, I found it fantastic. It meant that I managed not to have it in my head anymore! [...] But I think it's because I heard it again that I realized I wasn't hearing it before. I don't know in which order it went but... you think about it, you hear it, there is no mystery." Participant F5.

They rediscover the meaning of being positively involved in one's hobbies. This makes them realize that their attempt to escape from tinnitus is more effective when they cease to distract themselves from it. When those who better tolerate it are absorbed in meaningful activities, they are not concerned by its presence or absence; instead they are positively indifferent toward its variability and presence. Listening to music for the pleasure it gives is different from listening to music with the aim at limiting the disablement of tinnitus. The renewed exercise of one's desire detached from tinnitus allows some participants to get a new perspective. It means that they worry less about its virtual presence when sleeping or not paying attention to it. The belief that an absence of impairment can be achieved through the free exercise of one's will testifies to the pointlessness of fighting against its presence. Thus, activities that participants have learnt to engage in become pleasant and gratifying. They are easy to access and are adapted to their mental and physical preferences. Lasting for some time, they might enable them not to be continuously interrupted by monitoring their tinnitus. In particular, such gratifying activities are associated with the perceived interests of others. For example, an interesting conversation has a positive influence on participants' attention (and thereby on the presence of tinnitus) whereas a boring conversation has no effect at all on it.

> "In this self-help association, I found people listening to me in a way the medical profession doesn't. So, my reaction was to return what I received. I thought, if I cannot fight against tinnitus, instead of being passive, I intend to fight for it, within the association. [...] We speak with people who experience the same thing, we help them, and it enables us to put things into perspective." Participant F3.

The fulfillment of pleasant activities convinces some participants that it is possible to escape from tinnitus, without wearing oneself out in a systematic and unsuccessful opposition to it. For example, the pleasure of swimming offers greater comfort than that of permanently listening to the radio in order not to hear it. Spending hours doing craftwork in a workshop relaxes participants more than constantly keeping the mind occupied to forget tinnitus. Writing in the silence of the night is more rewarding than music in the background that is supposed to diminish its presence. The awareness of this possibility offers a wider perspective, greater hope as well as energy for managing tinnitus despite its inevitable variations. Although, it does not guarantee complete control of tinnitus on a day-to day basis, it might lead to greater confidence in the future.

DISCUSSION

The Relevance of Using the Term Frustration in Chronic Tinnitus

It has been recently advocated that an ecological framework should integrate the diversity of tinnitus outcomes into an overall construct of tinnitus experience (Searchfield, 2014). The individual who perceives tinnitus is assumed to be at the core of such an ecological framework. Results of the present study suggest that frustration may enlighten this individual perspective. Frustration as a concept (Berkowitz, 1989) fulfills two important criteria to account for tinnitus annoyance, namely variability and ecological validity. Variability of frustration has been demonstrated in experimental settings (Yu et al., 2014). Asked to complete a rewarded task on a computer, individuals who were thwarted in their attempts experienced greater frustration as their motivations increased. Consistent with the frustration-aggression model (Berkowitz, 1989), subjects' frustration instigated immediate aggressive trends, with higher probability to show open aggression with greater prior expectations. Therefore, frustration as a dynamic construct would be suitable in further investigations of tinnitus variability. The ecological validity of frustration has been documented by studies in chronic pain (Schneider et al., 2012), accounting for a broader scope of patients' difficulties than depression, anxiety or fear (Wade et al., 1990). Frustration in patients with pain can be used as a measure of the perceived lack of understanding from others with regard to the strain that they experience in social contexts (Dow et al., 2012). This testifies to the disruptive consequence of pain on one's existence, impacting life roles and the ability to keep perspectives on

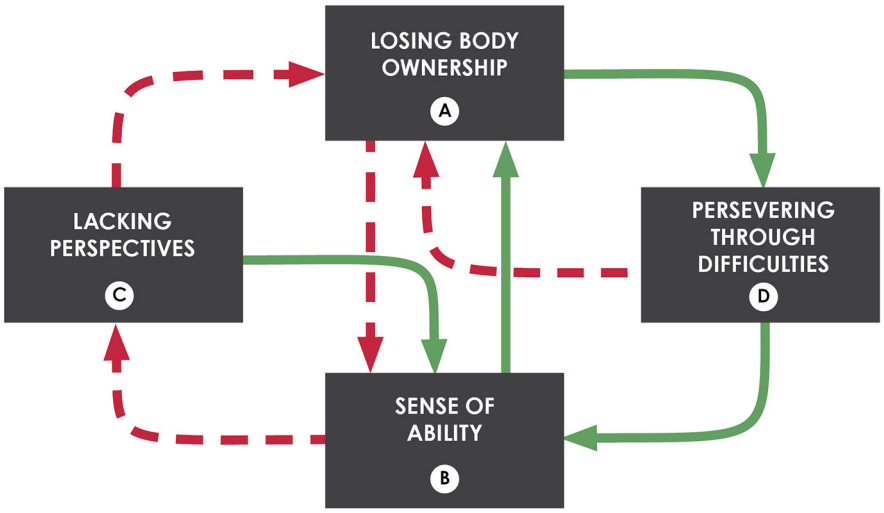
one's future (Harris et al., 2003). Finally, frustration addresses patients' perception of medical diagnosis and management, which has been previously underestimated in the field of pain (Dow et al., 2012). We believe that the frustration hypothesis in chronic tinnitus may also contribute to a broader understanding of tinnitus distress, incorporating patients' impairments with expectancies about tinnitus management.

Modulating Frustration: A Substantive Theory of Tinnitus Tolerance

In the following section, the analysis of the frustration hypothesis in tinnitus-induced disablement is extended with the building of a substantive theory of tinnitus tolerance. A substantive theory consists in exploring relationships between categories that were presented in the results section (see **Table 4**). It accounts for trends in individual experience and changes over time, from one experience to another. It further aims at accounting for which difficulties participants meet and how they try to overcome them (Glaser and Strauss, 1967). Eventually, a substantive theory allows hypotheses to be tested in further research. The following figure (**Figure 1**) establishes predictable relationships between trends in individual conduct in a consistent model that is suitable for implementation in clinical practice. It includes the three previous categories from the results section, "Losing body ownership", "Lacking perspectives" and "Persevering through difficulties". A fourth category, "Sense of ability", is introduced to account for changes in the individual experience of tinnitus, from tinnitus suffering (pathways in hatched red arrows) to tinnitus tolerance (pathways in full green arrows). The figure is based on a continuum of experience that is represented by the horizontal two-way arrow, whose ends lead to suffering and tolerance. A basic assumption of the model is that tolerance to tinnitus entails a sustained and disciplined effort that is challenged by the many circumstances of daily living. In contrast with current models of tinnitus annoyance, this assumption suggests that tolerance cannot be equated with a passive process (e.g., the extinction of a conditioned reflex or over-reaction toward tinnitus stimulus, for a review see Dauman et al., 2013). Therefore, we suggest that the term *tolerance* better describes an active effort from the individual not to suffer from tinnitus than the term habituation. Tinnitus tolerance entails a full reform of oneself with regard to restriction and adaptation of activities, as much as to perceived outcomes that can be achieved through daily efforts.

Losing body ownership sets the scene for the understanding of tinnitus-induced disablement and its relationship to persistent frustration. The model predicts that the more effort the individual has to make to overcome tinnitus interference with daily activities, the weaker he or she will feel facing tinnitus in the long term (pathway AB). Difficulties in resting and being able to relax, along with a strict and rigid control over the sound environment (i.e., a required auditory congruence), increase the burden of accepting tinnitus in the perceptual field. This results in a poor *sense of ability* and a lack of sustainability of effort that is required to tolerate its presence. The model further predicts that an affliction like this does not empower the individual to adapt his or her of lifestyle to the condition, but rather to refuse the mere perspective of dealing with tinnitus in the future. Adaptation seems initially unreachable to somebody who is severely disabled by the condition. The frustration hypothesis enables health professionals to consider that such an experience of tinnitus calls for a radical solution to the condition (i.e., tinnitus suppression or significant alleviation) and will not be satisfied with any other moderate perspective (i.e., improving one's tolerance to the condition over time). A poor sense of ability is also prone to undervaluing emerging experiences of lesser annoyance when involved in achievable activities that seem disproportionate to the overwhelming suffering of each and every moment. The model predicts that discrepancy between individual's expectations and counseling contributes to the *lack of perspectives* for the disabled individual (pathway BC). A large number of consultations represent the desperate search for another perspective than merely having tinnitus for the rest of one's life. In addition to disappointment concerning the perceived attitude of professionals (i.e., a lack of concern for one's disablement), the misunderstanding of one's entourage about the new restrictions stemming from tinnitus add to the frustration and the feeling of not being helped by anyone. The model predicts that frustration is worsened by the lack of such perspectives on tinnitus, which results in a vicious cycle leading back to *losing body ownership*. In the figure, sustained suffering is restricted to this cycle A, B, C, with no external factor that might enable the individual to break out of his or her perceived disablement induced by tinnitus.

If individual expectations toward professional counseling are to remain high, the search for external help can strengthen a poor sense of ability to deal with tinnitus on one's own. This paradox is raised by the frustration hypothesis, all the more in that the individual has to learn to live with tinnitus as a chronic condition. Therefore, the role of health professionals must not be undervalued when restoring a sense of ability in disabled patients. To begin with, confident and trustful professionals can help suffering patients to find a new perspective regarding their condition. Setting up a dialogue concerning the condition may help the patient to adhere to the variability of annoyance, and to identify moments and circumstances during which a lesser presence of tinnitus is experienced (when absorbed in achievable activities). Increased insight regarding the experience of tinnitus may support efforts to reduce the annoyance of tinnitus, thus setting a perspective in which potential improvements could come about following expert advice. Another way to reduce the discrepancy between expectations and counseling is illustrated by participants who suddenly cease to consult health professionals and thus, re-focus their efforts to hold on to their body ownership. The model predicts that highly thwarted expectations prevent any improvement in the sense of ability, while moderation might contribute to lesser tinnitus interference. This entails adapting one's lifestyle to the chronic condition (pathway BA), in contrast to the previous struggle against its presence. Regulating one's socializing and fostering one's endurance are strategies that enable the individual to develop ways to put up with the presence of constant tinnitus. An improved ability to rest and a softened auditory congruence



Legend. Pathways that account for participants' experience of chronic tinnitus, leading to tinnitus suffering (hatched red arrows) or tinnitus tolerance (full green arrows). Enhanced annoyance (i.e. sustained suffering) is mainly restricted to a circular pathway through categories A, B,C. Reduced annoyance (i.e. tinnitus tolerance) is characterized by a circular pathway through categories A, D,B. Unchanged annoyance (i.e. struggle against tinnitus) entails a circular pathway through categories A,B,C,D, with pathways CB and DA respectively prevailing over CA (i.e. chronic suffering) and DB (i.e. strengthened tolerance). The following properties of each category [A,B,C,D] account for trends of individual experience that result in recurrent difficulties and the struggle against tinnitus, or improved tinnitus tolerance and self-adaptation to the chronic condition :

A
Overwhelming : effort to overcome tinnitus interference : *Bearable*
Limited : ability to relax and rest : *Improved*
Required : auditory congruence : *Softened*

B
Desperate : sustainability of effort to reduce annoyance : *Encouraging*
Refused : attempt to adapt lifestyle to chronic condition : *Rewarding*
Unsatisfactory : perception of resulting outcomes : *Good enough*

C
High : discrepancy between expectations and counselling : *Moderate*
Disappointed : expectation about others' understanding : *Tempered*
Poor : ability to handle tinnitus variability : *Effective*

D
Insufficient : ability to carrying out meaningful activities : *Mastered*
Questionable : involvement in communication and social gathering : *Ensured*
Precarious : independence toward others' misunderstanding : *Sustained*

FIGURE 1 | Modulating frustration: a substantive theory of tinnitus tolerance.

(emerging self-confidence toward the sound environment) come by changing one's attitudes and lifestyle, i.e., identified as *persevering through difficulties* (pathway AD).

From this new pathway, a set of outcomes is predicted to emerge in the individual's experience of tinnitus. The most important is assumed to be carrying out meaningful and

pleasant activities which are easily achievable and enable the individual to detach her or himself from the interference of tinnitus. Involvement in communication and social gathering might also bring new insights that diminish the presence of tinnitus. Interesting conversations and captivating interactions can be a distraction that eases the experience of tinnitus. *Perceived outcomes* from these new attitudes toward tinnitus interference are assumed to play a central role in distinguishing reduced annoyance (i.e., improved tolerance, pathway DB) from unchanged annoyance (i.e., merely struggling to limit tinnitus annoyance, pathway DA). In the latter, meaningful and pleasant activities appear to be insufficient and disproportionate to the amount of effort that is required by the unyielding presence of tinnitus. Likewise, communication and interaction offer only restricted periods of relief and are questionable when one contrasts them with the virtual omnipresence of tinnitus. When individuals find that it comes back easily into their consciousness, the effort they have to make in order to cope with it seems somewhat trivial. Moreover, they cannot see that these long-term strategies to escape tinnitus can be successful. Prolonged and unchanged annoyance is dealt with by a struggle to limit its presence through engagement in distracting activities, and is to be distinguished from improved tolerance implying self-induced relief from tinnitus. The model predicts that distracting activities have a limited influence upon tinnitus annoyance over time. It further assumes that the effort to overcome tinnitus interference might again be overwhelming sooner or later for patients who struggle against its presence (regressive pathway DA). Difficulties are predicted to reoccur if perceived outcomes lack in sustainability. It is assumed that difficulties in putting up with tinnitus might be limited by previous experience, as shown in the figure by pathway CB (tempered expectations toward others' help and self-confidence in finding relief) rather than CA (sustained suffering with no perspectives over the condition). Therefore, unchanged and prolonged annoyance is characterized by a circular pathway between the four categories A, B, C, D with pathways CB and DA prevailing over sustained suffering (CA) and improved tolerance (DB).

An improved tolerance is characterized by a distinct attitude toward tinnitus (pathways DB). The model predicts that a *sustained and positive* (i.e., not monitored) involvement in meaningful activities can strengthen the sense of effectiveness in distracting oneself from tinnitus interference. This pathway contrasts with previous attempts to merely cover up its presence in the perceptual field. It is assumed that sustainability of effort should be encouraged by the extension of time resulting in less annoyance from it. This new effectiveness of lifestyle is rewarded by the fresh perspective of being able not to suffer *every* moment of life despite its presence. Finding that they are not continuously interrupted by tinnitus monitoring can make individuals realize that they were *not* thinking about it when they were involved in enjoyable activities. According to the model, retraining oneself with respect to its presence might also change the individual's perceived efforts to integrate the condition into her or his lifestyle. The experience of tolerance can confirm one's ability to limit tinnitus interference (pathway BA) and to enjoy pleasant and uninterrupted activities (pathway DB). The model predicts that a virtuous cycle like this (A, D, B) might improve the individual's ability to be satisfied, and also to allow him to be capable of considering future outcomes as *good enough*. Thus, the model does not equate tinnitus tolerance with an achieved stage of perception, but rather defines it as an improved management of the frustration that it causes.

Current psychological therapies for patients with tinnitus attempt to allow sufferers to cope with the implications of tinnitus frustration. *Lacking perspective* may be the primary concern for Cognitive-Behavioral Therapy which has, for decades, emphasized the need to structure patients' cognitions so that they can appraise their condition. Current hypotheses, which highlight the importance of belief in the tinnitus experience, suggest that advances in CBT will take this search for the ability to cope one step further (see McKenna et al., 2014). Following the trend for approaches raising awareness of mindfulness, more recent psychological approaches to tinnitus address the issue of *losing body ownership*. Mindfulness-Based Stress Reduction Therapy (MBSRT, see e.g., Roland et al., 2015) and Acceptance and Commitment Therapy (ACT, see e.g., Westin et al., 2011) have both led to improvement in tinnitus tolerance. Basically, mindfulness promotes the development of a non-judgemental and non-reactive attitude toward the present-moment self-experience (Zou et al., 2016). It entails self-training exercises in acceptance of the condition on a daily basis (Westin et al., 2011). Recent thinking in psychology considers mindfulness in an even broader perspective than equanimity toward negative experiences (Lindsay and Creswell, 2015). It is suggested that a more flexible appraisal of oneself and others' attitudes translates from (and beyond) self-training, with a sustained influence on daily activities (ibid). This view addresses the issue of *persevering through difficulties*, with emphasis on the daily efforts required by patients with tinnitus. Among other approaches to psychotherapy, the attention that frustration has received in contemporary psychoanalysis (Bion, 2007; Ferro and Civitaresse, 2015) suggests that this approach might also contribute to our understanding of tinnitus tolerance from a psychological perspective.

LIMITATIONS AND STRENGTHS

The present study has two main limitations concerning data analysis and patients selection. Both involve the procedure of theoretical sampling, i.e., purposeful, category-driven choice of questions and participants in accordance with the Grounded Theory methodology (Draucker et al., 2007). First, the mode of recruitment (i.e., through the Internet) and the coding procedure in interviews did not sufficiently meet the requirements of theoretical sampling so this had a broader impact on data gathering. Purposeful sampling will be possible in the future, based on our understanding of a core category in tinnitus-induced disablement. However, we believe that our core category and supporting analysis demonstrate the relevance of the focused questions we used in the interview process. Second, our population cannot be considered as representative of the general tinnitus population. Participants were aging individuals who experienced long-term tinnitus, the shortest duration being 6 years at the time of the interviews. The testimonials gathered do not reflect the experience of a recent onset, nor the experience

of younger participants. Likewise, problems related to work situations might have been underestimated since most of the participants do not work outside their homes. Furthermore, the study participants are members of the French tinnitus association. While the condition is a matter of concern for them, they strive to tolerate it in their daily life. Most of them, if not all, were disappointed as their expectations of finding therapeutic relief in encounters with health care professionals were not met. A different view on tinnitus and its implications for the individual patient might have resulted in a therapeutic approach more in line with what they expected. These limitations suggest the need for further studies using another set of interviews with purposeful selection of participants. Other types of patients (i.e., negative cases) will be sought such as those who do not report any variability of disablement, and others who are not disabled by tinnitus. There is also a need to consider participants' age (young adults), recency of onset and working status. The present study, however, offers an original, substantive theory of tinnitus-induced disablement and progressive tolerance. Predictable relationships are drawn from the present model that can be further tested by focused interviews, psychological investigations (see e.g., Kahn-Greene et al., 2006) or more recent neuroscience investigation of frustration (see e.g., Yu et al., 2014). Identifying a core category in the experience of tinnitus could lead to implementing new strategies to provide help in the various forms of tinnitus, from sustained suffering to reduced disablement through time. It could help in promoting meaningful clinical dialogue between patients and clinicians, which is essential for improving tinnitus tolerance.

CONCLUSION

This study underlines the importance of variability in tinnitus-induced disablement when attempting to understand the condition in a long-term perspective. Furthermore, it highlights frustration as a central issue in chronic tinnitus. To our knowledge, this is the first study that clearly raises frustration to a conceptual level in the understanding of tinnitus-induced disablement, beyond anecdotal observations to date. Based on the results of the qualitative data analysis, we suggest that frustration influences the intra-individual variability of tinnitus annoyance. We further suggest that *modulating frustration* could be a new approach to tinnitus suffering, in contrast with current models of moderating *tinnitus interference* with individual perception. This perspective could have implications for counseling of tinnitus patients, e.g., by highlighting key variables in management such as focusing on the narrative of each individual. It also enlightens the recurrent difficulties in those who previously tolerated tinnitus, but who henceforth have to cope with frustrating events in their life (diseases, mourning, break-ups and so forth). Otherwise, the role of frustration in tinnitus-induced disablement may encapsulate the underlying rationale of person-centred counseling. Acknowledging the importance of tinnitus frustration invites practitioners to take into account the influence of the caregiver's attitude toward the condition. While many may consider that the lack of scientific explanation about the origin of tinnitus is the source of patients' disappointment, the participants in this study complained rather of the lack of dialogue with their practitioner. This means that primary care could start by paying attention to the suffering client and showing interest in his/her experience. We believe that the proposed model raises significant issues that can be incorporated into the clinical dialogue with the patient. The present findings may also have implications for basic research on non-auditory networks involved in tinnitus annoyance. There are many ways to try to ease individual frustration, and some of the current approaches, including counseling and psychotherapy, have a contribution to make. However, to our knowledge, it is still a matter of chance within hearing rehabilitation units whether or not patients with tinnitus receive the special care they need. Finally, this qualitative study will have achieved its goal if it results in future studies based on patients' testimonials, since patients' narratives have much to offer in improving tinnitus management from an individualized perspective.

AUTHOR CONTRIBUTIONS

ND, SE, and RD designed the study. ND led the interviews with participants and collected data. Data analysis was performed by ND, SE, RD, and DA. ND, RD, and SE discussed the results. RD and SE provided critical revisions to the manuscript. All authors approved the final version of the manuscript for submission.

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REFERENCES

Berkowitz, L. (1989). Frustration-aggression hypothesis: examination and reformulation. *Psychol. Bull.* 106, 59–73. doi: 10.1037/0033-2909.106.1.59

Bion, W. R. (2007). *Learning from Experience*. London: Karnac Book.

Bouscau-Faure, F., Keller, P. H., and Dauman, R. (2003). Further validation of the Iowa tinnitus handicap questionnaire. *Acta Otolaryngol.* 123, 227–231. doi: 10.1080/00016480310001051

Burns, E. M. (1984). A comparison of variability among measurements of subjective tinnitus and objective stimuli. *Audiology* 23, 426–440. doi: 10.3109/00206098409081535

Charmaz, K. (1995). The body, identity and self: adapting to impairment. *Sociol. Quart.* 36, 657–680. doi: 10.1111/j.1533-8525.1995.tb00459.x

Charmaz, K. (2014). *Constructing Grounded Theory*. London: SAGE Publication.

Dauman, N., and Erlandsson, S. I. (2012). Learning from tinnitus patients' narratives—A case study in the psychodynamic approach. *Int. J. Qual. Stud. Health Well-Being.* 7:19540. doi: 10.3402/qhw.v7i0.19540

Dauman, N., Erlandsson, S. I., and Carlsson, S. G. (2013). "Habituation theories in current models of chronic tinnitus: evidence and criticism," in *Habituation: Theories, Characteristics and Biological Mechanisms,* ed A. Buskirk (New York, NY: Nova Publisher), 55–90.

Dauman, N., Erlandsson, S. I., Lundin, L., and Dauman, R. (2015). Intra-individual variability in tinnitus patients. Current thoughts and perspectives. *HNO* 63, 302–306. doi: 10.1007/s00106-014-2978-2

Dauman, R., and Bouscau-Faure, F. (2005). Assessment and amelioration of hyperacusis in tinnitus patients. *Acta Otolaryngol.* 125, 503–509. doi: 10.1080/00016480510027565

Dietrich, V., Nieschalk, M., Stoll, W., Rajan, R., and Pantev, C. (2001). Cortical reorganization in patients with high frequency cochlear hearing loss. *Hear. Res.* 158, 95–101. doi: 10.1016/S0378-5955(01)00282-9

Dow, C. M., Roche, P. A., and Ziebland, S. (2012). Talk of frustration in the narratives of people with chronic pain. *Chron. Illn.* 8, 176–191. doi: 10.1177/1742395312443692

Draucker, C. B., Martsolf, D. S., Ross, R., and Rusk, T. B. (2007). Theoretical sampling and category development in grounded theory. *Qual. Health Res.* 17, 1137–1148. doi: 10.1177/1049732307308450

Eriksson-Mangold, M., Erlandsson, S. I., and Jansson, G. (1996). The subjective meaning of illness in severe otosclerosis: a descriptive study in three steps based on focus group interviews and written questionnaire. *Scand. J. Audiol.* 43, 34–44.

Erlandsson, S. I., Eriksson-Mangold, M., and Wiberg, A. (1996). Ménière's disease: trauma, distress and adaptation studied through focus interview analyses. *Scand. J. Audiol. Suppl.* 43, 45–56.

Ferro, A., and Civitaresse, G. (2015). *The Analytic Field and its Transformations*. London: Karnac Book.

Glaser, B. G. (1978). *Theoretical Sensitivity*. Mill Valley, CA: Sociology Press.

Glaser, B. G., and Strauss, A. L. (1967). *The Discovery of Grounded Theory. Strategies for Qualitative Research.* Chicago, IL: Adline Transaction.

Goebel, G., and Hiller, W. (2001). *Das Strukturierte Tinnitus-Interview [Structured Tinnitus Interview STI: Instrument and Manual for Behavioral Medicine Examination of Patients With Chronic Tinnitus]*. Goettingen: Hogrefe & Huber Publishers.

Hallberg, L. R. M., and Carlsson, S. G. (1993). A qualitative study of situations turning a hearing disability into a handicap. *Disab. Hand. Soc.* 8, 71–86. doi: 10.1080/02674649366780051

Harris, S., Morley, S., and Barton, S. B. (2003). Role loss and emotional adjustment in chronic pain. *Pain* 105, 363–370. doi: 10.1016/S0304-3959(03)00251-3

Hébert, S., Fournier, P., and Norena, A. (2013). The auditory sensitivity is increased in tinnitus ears. *J. Neurosci.* 33, 2356–2364. doi: 10.1523/JNEUROSCI.3461-12.2013

Heeren, A., Maurage, P., Perrot, H., De Volder, A., Renier, L., Araneda, R., et al. (2014). Tinnitus specifically alters the top-down executive control subcomponent of attention: evidence from the attention network task. *Beh. Brain Res.* 269, 147–154. doi: 10.1016/j.bbr.2014.04.043

Kahn-Greene, E. T., Lipizzi, E. L., Conrad, A. K., Kamimori, G. H., and Killgore, W. D. S. (2006). Sleep deprivation adversely affects interpersonal responses to frustration. *Pers. Ind. Dif.* 41, 1433–1443. doi: 10.1016/j.paid.2006.06.002

Kuk, F. K., Tyler, R. S., Russell, D., and Jordan, H. (1990). The psychometric properties of a tinnitus handicap questionnaire. *Ear Hear.* 11, 434–445. doi: 10.1097/00003446-199012000-00005

Laplante-Lêvesque, A., Knudsen, L. V., Preminger, J. E., Jones, L., Nielsen, C., Öberg, M., et al. (2012). Hearing help-seeking and rehabilitation: perspectives of adults with hearing impairment. *Int. J. Audiol.* 51, 93–102. doi: 10.3109/14992027.2011.606284

Lindsay, E. K., and Creswell, J. D. (2015). Back to the basics: how attention monitoring and acceptance stimulate positive growth. *Psychol. Inquiry* 26, 343–348. doi: 10.1080/1047840X.2015.1085265

Lockey, K., Jennings, M. B., and Shaw, L. (2010). Exploring hearing aid use in older women through narratives. *Int. J. Audiol.* 49, 542–549. doi: 10.3109/14992021003685817

Malterud, K. (2001). Qualitative research: standards, challenges, and guidelines. *Lancet* 358, 483–488. doi: 10.1016/S0140-6736(01)05627-6

McKenna, L., Handscomb, L., Hoare, D. J., and Hall, D. A. (2014). A scientific cognitive-behavioral model of tinnitus: novel conceptualizations of tinnitus distress. *Front. Neurol.* 5:196. doi: 10.3389/fneur.2014.00196

Merluzzi, F., and Hinchcliffe, R. (1973). Threshold of subjective auditory handicap. *Audiology* 12, 65–69. doi: 10.3109/00206097309089306

Mohamad, N., Hoare, D. J., and Hall, D. A. (2016). The consequences of tinnitus and tinnitus severity on cognition: a review of the behavioural evidence. *Hear. Res.* 332, 199–209. doi: 10.1016/j.heares.2015.10.001

Newman, C. W., Sandridge, S. A., and Jacobson, G. P. (2014). Assessing outcomes of tinnitus intervention. *J. Am. Acad. Audiol.* 25, 76–105. doi: 10.3766/jaaa.25.1.6

Pan, T., Tyler, R. S., Haihong, J., Coelho, C., and Gogel, S. A. (2015). Differences among patients that make their tinnitus worse or better. *Am. J. Audiol.* 24, 469–476. doi: 10.1044/2015_AJA-15-0020

Penner, M. J., Brauth, S., and Hood, L. (1981). The temporal course of the masking of tinnitus as a basis for inferring its origin. *J. Speech Hear. Res.* 24, 257–261. doi: 10.1044/jshr.2402.257

Roberts, L. E., Husain, F. T., and Eggermont, J. J. (2013). Role of attention in the generation and modulation of tinnitus. *Neurosci. Bio. Rev.* 37, 1754–1773. doi: 10.1016/j.neubiorev.2013.07.007

Roland, L. T., Lenze, E. J., Hardin, F. M., Kallogjeri, D., Nicklaus, J., Wineland, A., et al. (2015). Effects of mindfulness-based stress reduction therapy on subjective bother and neural connectivity in chronic tinnitus. *Otolar. Head Neck Surg.* 152, 919–926. doi: 10.1177/0194599815571556

Schecklmann, M., Landgrebe, M., and Langguth, B. (2014). Phenotypic characteristics of hyperacusis in tinnitus. *PLoS ONE* 9:e86944. doi: 10.1371/journal.pone.0086944

Schecklmann, M., Pregler, M., Kreuzer, P. M., Poeppl, T. B., Lehner, A., Crönlein, T., et al. Langguth, B. (2015). Psychophysiological associations between chronic tinnitus and sleep: a cross validation of tinnitus and insomnia questionnaires. *BioMed. Res. Int.* 2015:461090. doi: 10.1155/2015/461090

Schlee, W., Pryss, R. C., Probst, T., Schobel, J., Bachmeier, A., Reichert, M., et al. (2016). Measuring the moment-to-moment variability of tinnitus: the track your tinnitus smart phone app. *Front. Aging Neurosci.* 8:294. doi: 10.3389/fnagi.2016.00294

Schneider, S., Junghaenel, D. U., Keefe, F. J., Schwartz, J. E., Stone, A. A., and Broderick, J. E. (2012). Individual differences in the day-to-day variability of pain, fatigue, and well-being in patients with rheumatic disease: associations with psychological variables. *Pain* 153, 813–822. doi: 10.1016/j.pain.2012.01.001

Searchfield, G. D. (2014). Tinnitus what and where: an ecological framework. *Front. Neurol.* 5:271. doi: 10.3389/fneur.2014.00271

Slater, R., Terry, M., and Davis, B. (1987). *Tinnitus: A Guide for Sufferers and Professionals*. Beckenham: Croon Helm.

Smith, S. L., and Fagelson, M. (2011). Development of the self-efficacy for tinnitus management questionnaire. *J. Am. Acad. Audiol.* 22, 424–440. doi: 10.3766/jaaa.22.7.4

Stevens, C., Walker, G., Boyer, M., and Gallagher, M. (2007). Severe tinnitus and its effect on selective and divided attention. *Int. J. Audiol.* 46, 208–216. doi: 10.1080/14992020601102329

Stouffer, J. L., and Tyler, R. S. (1990). Characterization of tinnitus by tinnitus patients. *J. Speech Hear. Disord.* 55, 439–453. doi: 10.1044/jshd.5503.439

Strauss, A. L. (1987). *Qualitative Analysis for Social Scientists*. Cambridge: Cambridge University Press.

Strauss, A. L., and Corbin, J. (1998). *Basic of Qualitative Research. Techniques and Procedures for Developing Grounded Theory*. Thousand Oaks, CA: SAGE Publication.

Sullivan, M., Katon, W., Russo, J., Dobie, R., and Sakai, C. (1994). Coping and marital support as correlates of tinnitus disability. *Gen. Hosp. Psychia.* 16, 259–266. doi: 10.1016/0163-8343(94)90005-1

Test, T., Canfi, A., Eyal, A., Shoam-Vardi, I., and Sheiner, E. K. (2011). The influence of hearing impairment on sleep quality among workers exposed to harmful noise. *Sleep* 34, 25–30. doi: 10.1093/sleep/34.1.25

Tyler, R. S., and Baker, L. J. (1983). Difficulties experienced by tinnitus sufferers. *J. Speech Hear. Res.* 48, 150–154. doi: 10.1044/jshd.4802.150

Verbrugge, L. M., and Jette, A. M. (1994). The disablement process. *Soc. Sci. Med.* 38, 1–14. doi: 10.1016/0277-9536(94)90294-1

Vielsmeier, V., Kreuzer, P. M., Haubner, F., Steffens, T., Semmler, P. R., Kleinjung, T., et al. (2016). Speech comprehension difficulties in chronic tinnitus and its relation to hyperacusis. *Front. Aging Neurosci.* 8:293. doi: 10.3389/fnagi.2016.00293

Wade, J. B., Price, D. D., Hamer, R. M., Schwartz, S. M., and Hart, R. P. (1990). An emotional component analysis of chronic pain. *Pain* 40, 303–310. doi: 10.1016/0304-3959(90)91127-5

Weise, C., Kleinstäuber, M., Hesser, H., Westin, V. Z., and Andersson, G. (2013). Acceptance of tinnitus: validation of the tinnitus acceptance questionnaire. *Cog. Behav. Therapy*. 42, 100–115. doi: 10.1080/16506073.2013.781670

Westin, V. Z., Schulin, M., Hesser, H., Karlsson, M., Noe, R. Z., Olofsson, U., et al. (2011). Acceptance and commitment therapy vs tinnitus retraining therapy in the treatment of tinnitus: a randomised controlled trial. *Behav. Res. Therapy* 49, 737–747. doi: 10.1016/j.brat.2011.08.001

Widén, S. E., and Erlandsson, S. I. (2007). Risk perception in musical settings – a qualitative study. *Int. J. Qual. Stud. Health Well-Being* 2, 33–44. doi: 10.1080/17482620601121169

Wilson, M. B., Kallogjeri, D., Joplin, C. N., Gorman, M. D., Krings, J. G., Lenze, E. J., et al. (2015). Ecological momentary assessment of tinnitus using smartphone technology: a pilot study. *Otol. Head Neck Surg.* 152, 897–903. doi: 10.1177/0194599815569692

World Medical Association (2013). *Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects*. Available online at: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects

Yu, R., Mobbs, D., Seymour, B., Rowe, J. B., and Calder, A. J. (2014). The neural signature of escalating frustration in humans. *Cortex* 54, 165–178. doi: 10.1016/j.cortex.2014.02.013

Zeman, F., Koller, M., Schecklmann, M., Langguth, B., Landgrebe, M., and the TRI data base study group. (2012). Tinnitus assessment by means of standardized self-report questionnaires: psychometric properties of the Tinnitus Questionnaire (TQ), the Tinnitus Handicap Inventory (THI), and their short versions in an international and multi-lingual sample. *Health Qual. Life Outcomes* 10:128. doi: 10.1186/1477-7525-10-128

Zöger, S., Erlandsson, S. I., Svedlund, J., and Holgers, K. M. (2008). Benefits from group psychotherapy in the treatment of severe refractory tinnitus. *Audiol. Med.* 6, 62–72. doi: 10.1080/16513860801959092

Zou, T., Wu, C., and Fan, X. (2016). The clinical value, principle, and basic practical technique of mindfulness intervention. *Shanghai Arch. Psychiatry* 28, 121–130. doi: 10.11919/j.issn.1002-0829.216060

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Can Animal Models Contribute to Understanding Tinnitus Heterogeneity in Humans?

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The brain activity of humans with tinnitus of various etiologies is typically studied with electro- and magneto-encephalography and functional magnetic resonance imaging-based imaging techniques. Consequently, they measure population responses and mostly from the neocortex. The latter also underlies changes in neural networks that may be attributed to tinnitus. However, factors not strictly related to tinnitus such as hearing loss and hyperacusis, as well as other co-occurring disorders play a prominent role in these changes. Different types of tinnitus can often not be resolved with these brain-imaging techniques. In animal models of putative behavioral signs of tinnitus, neural activity ranging from auditory nerve to auditory cortex, is studied largely by single unit recordings, augmented by local field potentials (LFPs), and the neural correlates of tinnitus are mainly based on spontaneous neural activity, such as spontaneous firing rates and pair-wise spontaneous spike-firing correlations. Neural correlates of hyperacusis rely on measurement of stimulus-evoked activity and are measured as increased driven firing rates and LFP amplitudes. Connectivity studies would rely on correlated neural activity between pairs of neurons or LFP amplitudes, but are only recently explored. In animal models of tinnitus, only two etiologies are extensively studied; tinnitus evoked by salicylate application and by noise exposure. It appears that they have quite different neural biomarkers. The unanswered question then is: does this different etiology also result in different tinnitus?

Keywords: brain imaging, neural responses, neural synchrony, spontaneous activity, burst firing, human, animal

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TINNITUS HETEROGENEITY

One may classify tinnitus types by etiology, phenotype, comorbidity or all these combined, and personal responses to it (Møller, 2011; Kreuzer et al., 2014). Within the etiology one may distinguish noise trauma and ototoxic drugs, whiplash and neck trauma, blast- and other traumatic brain injury, vestibular schwannoma and Ménière's disease, and stress. Phenotype differences such as tinnitus pitch, loudness, and aurality may be important as well, but estimates of pitch and loudness are varying between tests (Hoare et al., 2014). Comorbidities of the neurological type such as migraine or tension-type headaches (Langguth et al., 2015), psychological type, such as depression and distress or finding tinnitus bothersome (Schecklmann et al., 2013; Pattyn et al., 2016), and of the audiological type such as hyperacusis (Schecklmann et al., 2014) seem to be more

important for treatment than etiology. Moreover, these comorbidities together with the amount of hearing loss appear to underlie most of the electro- and magneto-encephalography (EEG/MEG) and brain imaging findings, whereas tinnitus on its own barely affects these (Davies et al., 2014). Here it should be emphasized that in animal experiments one knows the etiology, knows typically exactly what structures, subdivisions and neuron types one is recording from and assumes that optionally resulting stress has no effect. Yet, behavioral test often show that not all animals subjected to a tinnitus-inducing agent will have tinnitus.

What is important from the point of view of animal experiments is how to translate tinnitus types, if they can be solidified, into animal models. Different etiologies that have been studied are noise trauma, ototoxic drugs (i.e., salicylate, quinine, cisplatin), and interaction between somatic stimulation and noise trauma (Eggermont, 2012). In animal research, the only extensive studied etiologies are salicylate application and noise exposure, hence we will compare these two etiologies.

THE NEURAL CORRELATES OF SALICYLATE AND NOISE-EXPOSURE IN ANIMAL MODELS OF TINNITUS

Salicylate

Salicylate induces tinnitus, either following a single high dose (acute) or following repeated administration of low dose (chronic). The result of salicylate application in rodents is predictable and maybe for that reason salicylate has early on been applied in animal experiments (Stypulkowski, 1990; Chen and Jastreboff, 1995; Ochi and Eggermont, 1996). Salicylate interacts with the auditory system in multiple ways in the cochlea and in the central auditory system. In the cochlea, salicylate initially down-regulates the action of prestin in the wall of the outer hair cells (OHCs) and thereby causes a modest hearing loss (Greeson and Raphael, 2009). In addition, salicylate interacts with the arachidonic acid cycle ultimately causing an increase in NMDA receptor activity and increased spontaneous firing rates (SFRs) in a subset of auditory nerve fibers (ANFs; Guitton et al., 2003). Long-duration application reverses its action on prestin and actually enhances its expression (Yu et al., 2008; Yang et al., 2009) and may even lead to ANF degeneration (Deng et al., 2013). Centrally, salicylate down-regulates serotonin and GABA activity, and affects the conductivity of some K^+ channels (Wang et al., 2008). Cochlear perfusion with salicylate does not produce the central effects of systemically applied salicylate. This makes searching for neural substrates of tinnitus difficult at the least. Salicylate also increases the gain of the more central parts of the auditory system for sound, reflected in increased startle responses and potentially inducing hyperacusis (Sun et al., 2009). So it is not clear what enhanced gap-startle responses after salicylate application imply: tinnitus or hyperacusis (Salloum et al., 2016). This also may depend on the presence or absence of modulation by auditory cortical activity of the gap-startle reflex. As far as SFRs are concerned, high levels of salicylate result in variable changes in ANF, dorsal cochlear nucleus (DCN), inferior colliculus (IC) including central nucleus (ICC) and external cortex (ICX), and auditory cortex (ACx) particularly in primary (A1) and second auditory cortical area (A2), depending on the species, the dose, and type of neuron. An overview is presented in **Table 1**.

Noise Trauma

The findings for traumatic noise exposure are summarized in **Table 2**, using the same format as for salicylate. The primary targets of noise trauma (and ototoxic drugs) are the cochlear hair cells. The most vulnerable are the OHCs in the first row followed by the inner hair cells (IHCs). If the noise is not excessively loud and of short duration, the minimal structural damage that correlates with hearing loss is related to changes in the hair cell stereocilia, which contain the transduction channels. If the result of noise exposure is just a temporary threshold shift (TTS), the only consequence may be loss of IHC ribbon synapses followed by permanent loss of the Type I spiral ganglion cells that innervate the IHC (Kujawa and Liberman, 2009). Consequently, central nerve degeneration may ensue. Noise trauma rarely caused increases in SFR of ANFs but more generally a reduction. The result of reduced auditory nerve output is typically an imbalance between neural excitation and inhibition in the central auditory system (Potashner et al.,

TABLE 1 | Changes after salicylate application.

Structure	Cell density	SFR	2-DG	Glu	Gly/GABA	5-HT
OHC IHC	≈[19]					
ANF	⇓ (chronic)[18]	≈[4] ⇑[5] ⇓[13*]				
DCN		⇓ (FF)[1] ≈ (CW)[1]	⇓[15]		⇓[9]	
ICC		⇓[11] ⇑[13*]	⇓[15] ⇑[16*]		⇓[8,10,20]	⇑[17]
ICX		⇑[14]	⇑[15]			
MGB		⇑[13*]		⇓[7*]	⇓[7*]	
A1		≈[2] ⇓[3*,6]	⇑[15]			⇑[17]
A2		⇑[12,13*]	⇑[15]			

[1]*Superfusion in slice FF, fusiform cells; CW, cartwheel cells (Wei et al., 2010);* [2]*cat (Ochi and Eggermont, 1996);* [3*]*rat (Yang et al., 2007);* [4]*Stypulkowski (1990) (≤200 mg/kg, acute);* [5]*Kumagai (1992) (≥400 mg/kg; chronic);* [6]*cat (Zhang et al., 2011),* [7*]*Su et al. (2012) slice,* [8]*Butt et al. (2016),* [9]*Zugaib et al. (2015),* [10]*Zou and Shang (2012),* [11]*Ma et al. (2006),* [12]*Eggermont and Kenmochi (1998),* [13*]*Chen et al. (2015),* [14]*Chen and Jastreboff (1995),* [15]*Wallhäusser-Franke et al. (1996),* [16*]*Paul et al. (2009),* [17]*Caperton and Thompson (2011),* [18]*Deng et al. (2013),* [19]*Feng et al. (2010),* [20]*Bauer et al. (2000),* * *indicates behavioral tinnitus.*

TABLE 2 | Changes after chronic NIHL.

Structure	Cell density	SFR	2-DG	Glu	Gly/GABA	5-HT
OHC IHC	⇓[14]					
ANF	⇓[18]	⇓[8]				
VCN		⇑[9]				
DCN	⇓[18]	⇓[1] ⇑[2, 12*]	⇑[3*]	⇑[16]	⇓[15]	
ICC		⇑[6, 7, 10*]		⇑[16]	⇓→⇑[16]	⇑[17]
ICX				⇑[16]		
MGB		⇑[4*]			⇓[5*]	
A1		⇑[11,13*]				⇑[17]
A2						

[1]*Fusiform cells (in vivo, Ma and Young, 2006),* [2]*fusiform cells (slice, Finlayson and Kaltenbach, 2009), cartwheel cells (slice Chang et al., 2002),* [3]**Middleton et al. (2011) using flavoprotein imaging,* [4]**Kalappa et al. (2014),* [5]**Llano et al. (2012) using flavoprotein,* [6]*Ma et al. (2006),* [7]*Manzoor et al. (2012, 2013),* [8]*Liberman and Kiang (1978),* [9]*Vogler et al. (2011),* [10]**Coomber et al. (2014),* [11]*Noreña and Eggermont (2006),* [12]**Brozoski et al. (2002),* [13]**Basura et al. (2015),* [14]*Liberman and Beil (1979),* [15]*Potashner et al. (1997),* [16]*Suneja et al. (1998a; 1998b),* [17]*Caperton and Thompson (2011),* [18]*Morest et al. (1998),* * *indicates behavioral tinnitus.*

1997; Llano et al., 2012; Schreiner and Polley, 2014). This causes strong hyperactivity in the DCN (Kaltenbach et al., 2000), and can result in tonotopic map reorganization, likely only in thalamic and cortical areas, accompanied by increased SFR and increased spike-firing synchrony (Noreña and Eggermont, 2003). This trio of changes is considered to comprise potential neural substrates of tinnitus. The balance between the excitatory and inhibitory transmitter efficacy in the central nervous system (CNS) is only temporarily changed in the first few weeks to months after the trauma (Suneja et al., 1998a,b). It is believed that during that period restoration of the excitatory–inhibitory balance can prevent tonotopic map reorganization as well as increases in SFR and neural synchrony, and thus likely also tinnitus (Noreña and Eggermont, 2005). Lesion studies suggest that the DCN may function as a source of increased SFR without ascending cochlear input and descending input from the CNS (Zacharek et al., 2002; Brozoski et al., 2012). However, these studies also suggest that behavioral tinnitus persists in animals for which the DCN output is isolated from central auditory structures. In contrast, the increased SFR in IC is dependent on output of the cochlea (Robertson et al., 2013), at least for the first 8–12 weeks after the trauma (Mulders and Robertson, 2013). This suggests that the induced increased central gain amplifies the remaining SFR from the auditory periphery. If the SFR from the periphery was not amplified the total result would not be an increased SFR in the IC. Species dependence and recovery times may play a role in these discrepancies.

Heterogeneity in the Salicylate and Noise Exposure Induced Markers for Tinnitus

Comparing the findings in salicylate and chronic noise trauma (**Figure 1**) indicates strong differences in SFR and 2-DG, but more correspondence for neurotransmitter action. This is surprising unless we abandon the hypothesis that hyperactivity reflected in increased SFR and 2-DG is a biomarker for tinnitus. In TTS-induced tinnitus, Wu et al. (2016) showed that increased SFRs, burst firing, and spike-firing synchrony in the fusiform cells of the DCN correlated with behavioral evidence for tinnitus. In recordings from cat A1 following salicylate application, Ochi and Eggermont (1996) could not demonstrate an overall change in SFR, however, units that initially had SFRs < 1 sp/s showed a significant increase and units with SFRs > 1 sp/s showed a significant decrease after acute salicylate application. However, Eggermont and Kenmochi (1998) did find a significant increase in SFR in A2 following salicylate application. In neither case could a change in spike-firing synchrony be demonstrated. Noreña and Eggermont (2003) have also shown that immediately after noise exposure, the SFR in A1 was not increased, whereas after more than 2 h it was. In contrast, the spike-firing synchrony was significantly increased immediately after exposure and continued to increase in parallel with the increase in SFR. In the IC, the delay to increased SFR was about 12 h (Mulders and Robertson, 2013), and in the DCN at least 2 days (Kaltenbach et al., 2000). This suggests that the locus of spike recording can result in quite different conclusions if one uses the SFR as a metric.

Structure	Cell density	SFR	2-DG	Glu	Gly/GABA	5-HT
Hair cells	≈ ⇓					
ANFs	⇓ ⇓	≈ ⇑ ⇓ ⇓				
DCN		⇓ ⇑	⇓ ⇑	⇑ ⇑	⇓	
ICC	⇓	⇓ ⇑	⇓ ⇑	⇑	⇓ ⇓→⇑	⇑ ⇑
ICX		⇑	⇑	⇑		
MGB		⇑ ⇑		⇓	⇓ ⇓	
A1	≈	≈ ⇓ ⇑	⇑	⇑→⇓		⇑ ⇑
A2	≈	⇑	⇑			

≈ no effect; ⇓ significant decrease; ⇑ significant increase; ⇓→⇑ change from decrease to increase; ⇑→⇓ change from increase to decrease; (blue salicylate, red NIHL).

FIGURE 1 | Comparing the effects of salicylate and noise-induced hearing loss.

TABLE 3 | Burst-firing and Tinnitus.

Structure	Agent	PTS	TTS	SFR	Bursting	Synchrony
ANF	Noise	•		$\Downarrow\approx$[1]	$\Uparrow$[1]	
DCN	Noise	•		$\Uparrow$[2]	$\Uparrow$[2]	
	Noise	•		$\Uparrow$[4*]	$\Uparrow$[3]	$\Uparrow$[4*]
	Noise		•		$\Uparrow$[4*]	
ICC	Noise		•	$\Uparrow$[5*]	$\Uparrow$[5*]	$\Uparrow$[5*]
	Noise	•		$\Uparrow$[6*]	$\Uparrow$[6*]	
ICC	Noise	•		$\approx$[7]	$\approx$[7]	
	Salicylate		•	$\Downarrow$[7]	$\approx$[7]	
ICX	Salicylate		•	$\Uparrow$[8]	$\Uparrow$[8]	
MGBv	Noise		•	$\Uparrow$[9*]	$\Uparrow$[9*]	
A1	Noise		•	$\Uparrow$[10]	$\Uparrow\approx$[10]	$\Uparrow$[10]
A1	Noise	•		$\Uparrow$[11]	$\approx$[11]	$\Uparrow$[11]

[1]*Liberman and Kiang (1978)*, [2]*Finlayson and Kaltenbach (2009)*, [3]*Pilati et al. (2012)*, [4]**Wu et al. (2016)*, [5]**Bauer et al. (2008)*, [6]**Coomber et al. (2014)*, [7]*Ma et al. (2006)*, [8]*Chen and Jastreboff (1995)*, [9]**Kalappa et al. (2014)*, [10]*Noreña and Eggermont (2003)*, [11]*Noreña and Eggermont (2006)*, * *indicates behavioral tinnitus,* • *indicates PTS or TTS present.*

It is instructive to look at changes in SFR, burst firing, and spike-firing synchrony associated with tinnitus (**Table 3**). Burst firing has been implicated with plastic changes in many neural systems (Eggermont, 2015), and has been evaluated in DCN (Wu et al., 2016), ICC (Bauer et al., 2008; Coomber et al., 2014), and medial geniculate body (MGB; Kalappa et al., 2014) in animals with behaviorally demonstrated putative signs of tinnitus. Increased burst firing correlates strongly with increased SFR in all central areas including ACx. Increased neural spike-firing synchrony, increased bursting and increased SFR correlate in DCN. In recordings from A1 increased spike-firing synchrony is found in the absence of bursting and initially unchanged SFR, but corresponds, after a few hours delay, to increased SFR. This strengthens the idea that increased SFR, at least in subcortical structures, is a biomarker for tinnitus. In salicylate, there is only evidence for bursting and increased SFR in the ICX (Chen and Jastreboff, 1995), but not in the ICC (Ma et al., 2006). In ANFs, bursting only occurs in neurons with very low SFR after noise trauma. This survey suggests that changes in bursting in subcortical structures are not independent of changes in SFR or in spike-firing synchrony. Burst firing and spike-firing synchrony in primary ACx appear to be independent, at least under ketamine anesthesia.

DO ANIMAL MODELS OF TINNITUS RELATE TO TINNITUS FINDINGS IN HUMANS?

The effects of tinnitus were until recently (Chen et al., 2014, 2015) studied very differently in animal models compared to humans. First of all detecting tinnitus is straightforward in humans—one just has to ask, whereas in animals it has to be inferred from behavioral tests. This is not straightforward (Eggermont, 2013; Lobarinas et al., 2013; Salloum et al., 2014, 2016), but let's assume that it can be done unambiguously. Secondly, putative electrophysiological correlates of tinnitus in animal models are increased SFRs, increased pair-wise spike-firing synchrony, and changes in the tonotopic maps in the auditory system (Eggermont and Roberts, 2004; Eggermont, 2012). In human studies one finds reduced or increased power of certain brain rhythms, interpreted as increased neural synchrony (Weisz et al., 2011; Weisz and Obleser, 2014), and changes in connectivity between brain areas based on EEG or functional magnetic resonance imaging (fMRI; Vanneste et al., 2011; Husain and Schmidt, 2014). Here, it is important to distinguish spike-firing synchrony and neural synchrony. I used spike firing synchrony as correlated firing times between two simultaneously recorded neurons. I use neural synchrony as in phase responding of population responses, typical EEG/MEG or slow BOLD fluctuations, at two brain sites.

Humans potentially may show changes in tonotopic maps but these will be more likely related to hearing loss than to tinnitus (Langers et al., 2012). More indirect correlates of tinnitus can be deduced from stimulus-evoked activity (Gu et al., 2010; Roberts et al., 2010, 2013) but are more sensitive to co-occurring hyperacusis.

TINNITUS NETWORKS

Putative Networks in Humans

Tinnitus may be related to changes in the resting-state neural networks of the brain. In a recent meta analysis of reported neural network changes in tinnitus patients, Husain and Schmidt (2014) found changes in the default network, in the connectivity between ACx and the limbic system that mediates stress, in the connection of the auditory system with the limbic system and attention network, and also in connections between visual cortex and the ACx, and between visual cortex and the attention network (Roberts et al., 2013). In contrast, Davies et al. (2014) did not find "significant differences in auditory network connectivity between groups after correcting for multiple statistical comparisons in the analysis. This contradicts previous findings reporting reduced auditory network connectivity; albeit at a less stringent statistical [significance] threshold."

Non-auditory areas have been identified as involved in people with tinnitus, using non-invasive functional and structural imaging. Resting state connectivity between brain areas is, by definition, based on spontaneous fluctuations in brain activity that can be reliably organized into coherent networks. The term "resting state" differentiates this type of activity from that obtained as a result of some task or stimulus (Husain and Schmidt, 2014). The finding of several resting state networks allows studying the neural mechanisms of tinnitus or auditory processing in general. See **Figure 2** for a representative set of these networks, the human network connectivities are indicated in red. It should be emphasized that the first insights into the role of inherent long-range cortical coupling in tinnitus were provided by resting-state studies probed by MEG (e.g., Weisz et al., 2007) and EEG (Vanneste et al., 2011).

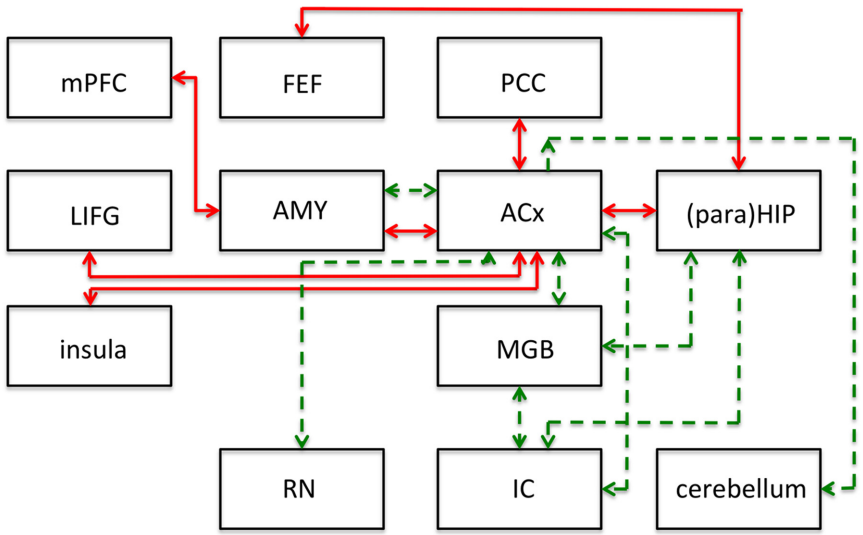

FIGURE 2 | Summary of main results of resting-state functional connectivity studies in tinnitus in humans (red lines) and following salicylate application in rats (green lines). This figure shows modifications to the connections of the networks and does not represent the networks in their entirety. ACx, auditory cortex; AMY, amygdala; FEF, frontal eye fields; IC, inferior colliculus; LIFG, left inferior frontal gyrus; MGB, medial geniculate body; mPFC, medial prefrontal cortex; PCC, posterior cingulate cortex; (para)HIP, parahippocampus and hippocampus. Based on human data from Husain and Schmidt (2014), and animal data from Chen et al. (2015).

A Salicylate-Activated Tinnitus Network

To identify putative neural substrates for tinnitus and hyperacusis in an animal model, Chen et al. (2015) applied salicylate to rats and used behavioral, electrophysiological, and fMRI (7T animal MRI scanner) techniques to identify a putative tinnitus–hyperacusis network. They found that salicylate application depressed the neural output of the cochlea, as measured by the compound action potential. In contrast, strongly amplified sound-evoked local field potentials (LFPs) were obtained in the amygdala (AMY), MGB, and ACx. These findings relate in principle to central gain changes and potentially to hyperacusis. Resting-state fMRI, which may be more relevant to understand tinnitus, showed a hyperactive auditory network composed of IC, MGB, and ACx. This network was also connected to parts of the cerebellum, AMY, and reticular formation (RN; **Figure 2**; dashed green lines).

The connectivity analysis was done by seeding various voxels in the regions-of-interest. This basically shows one-way connectivity from the seed region to other areas, by combining the findings from various seed regions a putative network can be built up. When the IC was seeded, they found that activity changes in the IC correlated significantly with that in voxels of the MGB, interpreted as an increase in functional connectivity (FC; **Figure 2**). Similarly, when changes in MGB voxels showed increased FC with voxels in the ACx. With the seed in the ACx, increased FC was seen in the same two lower auditory centers, the MGB and IC, which suggests a recurrent feedback loop in this auditory subnetwork (IC, MGB, and ACx in **Figure 2**). FC further revealed enhanced coupling between the ACx and the cerebellum, the reticular nuclei, and the AMY, and between the IC, MGB, and hippocampus. These subdivisions all show large salicylate-induced increases in the amplitude of low-frequency fluctuations, as well as increased FC with the ACx.

Comparing the animal (green dashed lines in **Figure 2**) and human networks (red full lines) does not tell us too much; the only correspondence is in the connection between ACx and AMY, and the involvement of the hippocampus and the area surrounding it, the parahippocampus. The animal model emphasizes the strengthening of the connections of the auditory structures and the relevance of subcortical structures such as the reticular activating system and parts of the cerebellum. The human network in particular adds the involvement of the attention network (frontal eye fields, left inferior frontal gyrus, insula).

In this comparison, one should note that the human network covers tinnitus in humans regardless of its etiology, whereas the animal network is limited to the putative effects of salicylate: tinnitus as well as hyperacusis.

Tinnitus without Hearing Loss

If one believes that increased SFR in the auditory nervous system, and particularly in ACx, is a neural correlate of tinnitus (Eggermont and Roberts, 2004; Roberts et al., 2010; Basura et al., 2015), then a few additional noise-exposure effects demand attention. After a single TTS-causing exposure—which constitutes the bulk of current animal experiments involving gap-startle indications of tinnitus—one often finds increased SFRs and the gap-startle reflex indicates (Salloum et al., 2014, 2016) the presence of tinnitus. Even more intriguing is that after long-term exposure ($\geq$6 weeks) to 4–20 kHz sound (noise or multi-tone) with levels $\leq$80 dB SPL one finds in ACx that the exposure frequency range causes strong suppression of driven

and spontaneous firing rates, whereas the edge regions (extending about one octave above and two octaves below the band-pass exposure range) show increased gain for sound stimuli and also increased SFR and increased neural synchrony (Noreña et al., 2006; Pienkowski and Eggermont, 2009; Munguia et al., 2013).

It is instructive to look at several cases with relatively low-level noise exposures in some more detail. Brozoski et al. (2002) behaviorally trained and tested chinchillas before and after unilateral exposure to a unilateral 80 dB SPL 4 kHz tone for 30–60 min. This elevated the ABR thresholds by 20–30 dB. In comparison to a non-exposed control group, they found that putative fusiform cells of exposed animals showed significantly elevated spontaneous activity. Compared with cells of unexposed animals, the exposed group displayed enhanced discrimination of 1 kHz tones and putative fusiform cells of exposed animals showed a greater stimulus-evoked response to tones at 1 kHz and at characteristic-frequency. This fits with the enhanced sound responses two octaves below our long-term 4–20 kHz exposure (Noreña et al., 2006). These are potential correlates of hyperacusis.

Noreña et al. (2006) continuously exposed four adult cats in their free-running room so that there was no time relationship with the feeding and cleaning period of about 0.5 h/day. More than 4 months exposure of these normal hearing adult cats with a 4–20 kHz band of multi-frequency tone pips—termed an enhanced acoustic environment (EAE)—continuously presented at 80 dB SPL, did not result in changes in ABR thresholds. However, there was a strong reduction in the driven firing rates to frequencies between 4 and 20 kHz, and an increase for frequencies below or above that range. The mean SFRs for CFs in the exposure frequency range was not significantly changed compared to controls, but the SFRs were significantly increased for units with CFs below and above the exposure frequencies. The similarity between the increases for the SFR and driven firing rate suggests an underlying synaptic gain change as the main cause. Neural synchrony was vastly increased as well, particularly when involving units with CFs above and below the exposure frequency range. Tonotopic maps were reorganized with CFs > 20 kHz taking over the normal 4–20 kHz CF range (Noreña et al., 2006).

We followed this up with several studies where the 4–20 kHz sound was presented at 68 dB SPL, and only for about 6 weeks. In our first study (Pienkowski and Eggermont, 2009), we reported basically the same pattern as in the Noreña et al.'s (2006) study. ABR thresholds were completely normal and so were DPOAEs. Tonotopic maps were reorganized, a process that surprisingly started during the 3-month recovery period in quiet (Pienkowski and Eggermont, 2009). Munguia et al. (2013) reported that for the 4–20 kHz multi-tone EAE, the SFR for MUs with CFs in the EAE range was significantly smaller than for those with CFs outside the EAE frequency region. In addition, the SFR for MUs with CFs outside the EAE frequency range (non-EAE) was significantly larger than for controls in the same frequency range. The increases in SFR were most often observed on the high-frequency side of the EAE. For instance, for the 4–20 kHz EAE, the mean ratios of the SFRs in exposed to control cats were 0.91 (below EAE range), 0.39 (within EAE range), and 1.47 (above EAE range).

An overview of some of these findings, augmented with results from Basura et al. (2015) and Wu et al. (2016) that are likely TTS causing, is presented in **Table 4**. Again, assuming that increased SFRs in ACx suggest the presence of tinnitus, one has to come to the conclusion that tinnitus cannot only occur in humans with clinical normal thresholds (≤25 dB HL) but also with absolute normal thresholds (Gu et al., 2010; Melcher et al., 2013). It should be noted that tonotopic map changes are not a requisite for tinnitus in humans with clinically normal audiograms (Langers et al., 2012), whereas the equivalent in noise-exposed animal suggests that tonotopic map changes do not occur for hearing losses <25 dB, whereas increased SFR may still be present (Seki and Eggermont, 2002, 2003).

MAKING ANIMAL MODELS AND HUMAN TINNITUS RESEARCH MORE COMPATIBLE

It is obvious that making the research approach between animal models and humans more comparable would require that animal recordings of neural activity include spontaneous LFPs, study the

TABLE 4 | Effects of non-traumatic noise exposure.

Structure	Exposure level (SPL)	SFR	Tonotopic map	Synchrony	GABA	Tinnitus
ANF	96 dB; 5 days	≈[1]				
VCN	80, 103 dB; 2 h				⇑[2]	
DCN	80, 103 dB; 2 h				⇑[2]	
	80 dB, 30–60 min	⇑[3]				Yes
	97 dB; 2 h	⇑[4]		⇑[4]		Yes
IC	120 dB; 4 h	⇑[5]				
MGB						
A1	80 dB; ≥4 months	⇑[6]	Changed[6]	⇑[6]		
	68 dB; ∼6 weeks	⇑[7]	Changed[8]	⇑[8]		Yes
	97 dB; 2 h	⇑[9]				

[1]*Salvi et al. (1983)*, [2]*Idrizbegovic et al. (1998)*, [3]*Brozoski et al. (2002)*, [4]*Wu et al. (2016)*, [5]*Wang et al. (2013)*, [6]*Noreña et al. (2006)*, [7]*Munguia et al. (2013)*; [8]*Pienkowski and Eggermont (2009)*, [9]*Basura et al. (2015)*.

power in the various EEG frequency bands (delta, theta, alpha, beta, and gamma), and use simultaneous recordings in several auditory and non-auditory areas to assess changes in connectivity (Weisz and Obleser, 2014). This will require recording from awake animals.

Recently, Salvi and colleagues have made a start on this by recording LFPs and carrying out resting state and connectivity (fMRI) recordings in anesthetized rats (Chen et al., 2014, 2015). Human research using neural spiking activity can only be done in pre-surgical conditions such as for relief of epilepsy, but so far only depth-recorded LFPs are have been obtained (Sedley et al., 2015).

Thus, the large differences in what is recorded in animal models with those obtained in humans makes a direct approach to the heterogeneity of tinnitus difficult. The most human-compatible animal model currently is that from Chen et al. (2015), albeit that it is based on salicylate-induced tinnitus, and that provides for only a minute fraction of the etiology of tinnitus in humans.

CONCLUSION

In humans with tinnitus, several biomarkers for tinnitus have been proposed based on spontaneous brain rhythms, both decreased and increased power in several frequency bands, and largely increased neural network connectivity between auditory and attention as well as limbic networks. In animal models, tinnitus biomarkers—increased SFR, burst-firing, and neural synchrony—are the same for acute noise trauma, chronic effects with permanent threshold shifts after recovery from trauma, but also for long-term non-traumatic exposure without hearing loss as measured by ABR, and normal DPOAEs. All the noise-exposure animal models reviewed here show signs of increased central gain (hyperacusis?) and increased SFR (tinnitus?). Salicylate application in animals, chronic as well as acute, despite causing a mild hearing loss, has different electrophysiological characteristics compared to chronic noise, both in periphery and in the cortex. Salicylate animals showed behavioral signs of hyperacusis as well as tinnitus, whereas the electrophysiological signs reflected increased central gain but no change in SFRs. Both noise exposure and salicylate application may cause tinnitus and hyperacusis-like effects, but differ in their effects on SFR. This is an illustration of heterogeneity in electrophysiological correlates of tinnitus for these two etiologies.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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REFERENCES

Basura, G. J., Koehler, S. D., and Shore, S. E. (2015). Bimodal stimulus timing-dependent plasticity in primary auditory cortex is altered after noise exposure with and without tinnitus. *J. Neurophysiol.* 114, 3064–3075. doi: 10.1152/jn.00319.2015

Bauer, C. A., Brozoski, T. J., Holder, T. M., and Caspary, D. M. (2000). Effects of chronic salicylate on GABAergic activity in rat inferior colliculus. *Hear. Res.* 147, 175–182. doi: 10.1016/S0378-5955(00)00130-1

Bauer, C. A., Turner, J. G., Caspary, D. M., Myers, K. S., and Brozoski, T. J. (2008). Tinnitus and inferior colliculus activity in chinchillas related to three distinct patterns of cochlear trauma. *J. Neurosci. Res.* 86, 2564–2578. doi: 10.1002/jnr.21699

Brozoski, T. J., Bauer, C. A., and Caspary, D. M. (2002). Elevated fusiform cell activity in the dorsal cochlear nucleus of chinchillas with psychophysical evidence of tinnitus. *J. Neurosci.* 22, 2383–2390.

Brozoski, T. J., Wisner, K. W., Sybert, L. T., and Bauer, C. A. (2012). Bilateral dorsal cochlear nucleus lesions prevent acoustic-trauma induced tinnitus in an animal model. *J. Assoc. Res. Otolaryngol.* 13, 55–66. doi: 10.1007/s10162-011-0290-3

Butt, S., Ashraf, F., Porter, L. A., and Zhang, H. (2016). Sodium salicylate reduces the level of GABAB receptors in the rat's inferior colliculus. *Neuroscience* 316, 41–52. doi: 10.1016/j.neuroscience.2015.12.021

Caperton, K. K., and Thompson, A. M. (2011). Activation of serotonergic neurons during salicylate-induced tinnitus. *Otol. Neurotol.* 32, 301–307. doi: 10.1097/MAO.0b013e3182009d46

Chang, H., Chen, K., Kaltenbach, J. A., Zhang, J., and Godfrey, D. A. (2002). Effects of acoustic trauma on dorsal cochlear nucleus neuron activity in slices. *Hear. Res.* 164, 59–68. doi: 10.1016/S0378-5955(01)00410-5

Chen, G. D., and Jastreboff, P. J. (1995). Salicylate-induced abnormal activity in the inferior colliculus of rats. *Hear. Res.* 82, 158–178. doi: 10.1016/0378-5955(94)00174-O

Chen, G. D., Radziwon, K. E., Kasanian, N., Manohar, S., and Salvi, R. (2014). Salicylate-induced auditory perceptual disorders and plastic changes in nonclassical auditory centers in rats. *Neural Plast.* 2014:658741. doi: 10.1155/2014/658741

Chen, Y.-C., Li, X., Liu, L., Wang, J., Lu, C.-Q., Yang, M., et al. (2015). Tinnitus and hyperacusis involve hyperactivity and enhanced connectivity in auditory-limbic-arousal-cerebellar network. *Elife* 4:e06576. doi: 10.7554/eLife.06576

Coomber, B., Berger, J. I., Kowalkowski, V. L., Shackleton, T. M., Palmer, A. R., and Wallace, M. N. (2014). Neural changes accompanying tinnitus following unilateral acoustic trauma in the guinea pig. *Eur. J. Neurosci.* 40, 2427–2441. doi: 10.1111/ejn.12580

Davies, J., Gander, P. E., Andrews, M., and Hall, D. A. (2014). Auditory network connectivity in tinnitus patients: a resting-state fMRI study. *Int. J. Audiol.* 53, 192–198. doi: 10.3109/14992027.2013.846482

Deng, L., Ding, D., Su, J., Manohar, S., and Salvi, R. (2013). Salicylate selectively kills cochlear spiral ganglion neurons by paradoxically up-regulating superoxide. *Neurotox. Res.* 24, 307–319. doi: 10.1007/s12640-013-9384-5

Eggermont, J. J. (2012). *The Neuroscience of Tinnitus*. Oxford: Oxford University Press.

Eggermont, J. J. (2013). Hearing loss, hyperacusis, and tinnitus: what is modeled in animal research? *Hear. Res.* 295, 140–149. doi: 10.1016/j.heares.2012.01.005

Eggermont, J. J. (2015). Animal models of spontaneous activity in the healthy and impaired auditory system. *Front. Neural Circuits* 9:19. doi: 10.3389/fncir.2015.00019

Eggermont, J. J., and Kenmochi, M. (1998). Salicylate and quinine selectively increase spontaneous firing rates in secondary auditory cortex. *Hear. Res.* 117, 149–160. doi: 10.1016/S0378-5955(98)00008-2

Eggermont, J. J., and Roberts, L. E. (2004). The Neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Feng, H., Yin, S.-H., Tang, A.-Z., Cai, H.-W., Chen, P., Tan, S.-H., et al. (2010). Caspase-3 activation in the guinea pig cochlea exposed to salicylate. *Neurosci. Lett.* 479, 34–39. doi: 10.1016/j.neulet.2010.05.023

Finlayson, P. G., and Kaltenbach, J. A. (2009). Alterations in the spontaneous discharge patterns of single units in the dorsal cochlear nucleus following intense sound exposure. *Hear. Res.* 256, 104–117. doi: 10.1016/j.heares.2009.07.006

Greeson, J. N., and Raphael, R. M. (2009). Amphipath-induced nanoscale changes in outer hair cell plasma membrane curvature. *Biophys. J.* 96, 510–520. doi: 10.1016/j.bpj.2008.09.016

Gu, J. W., Halpin, C. F., Nam, E. C., Levine, R. A., and Melcher, J. R. (2010). Tinnitus, diminished sound-level tolerance, and elevated auditory activity in humans with clinically normal hearing sensitivity. *J. Neurophysiol.* 104, 3361–3370. doi: 10.1152/jn.00226.2010

Guitton, M. J., Caston, J., Ruel, J., Johnson, R. M., Pujol, R., and Puel, J. L. (2003). Salicylate induces tinnitus through activation of cochlear NMDA receptors. *J. Neurosci.* 23, 3944–3952.

Hoare, D. J., Edmondson-Jones, M., Gander, P. E., and Hall, D. A. (2014). Agreement and reliability of tinnitus loudness matching and pitch likeness rating. *PLoS ONE* 9:e114553. doi: 10.1371/journal.pone.0114553

Husain, F. T., and Schmidt, S. A. (2014). Using resting state functional connectivity to unravel networks of tinnitus. *Hear. Res.* 307, 153–162. doi: 10.1016/j.heares.2013.07.010

Idrizbegovic, E., Bogdanovic, N., and Canlon, B. (1998). Modulating calbindin and parvalbumin immunoreactivity in the cochlear nucleus by moderate noise exposure in mice. A quantitative study on the dorsal and posteroventral cochlear nucleus. *Brain Res.* 800, 86–96. doi: 10.1016/S0006-8993(98)00504-6

Kalappa, B. I., Brozoski, T. J., Turner, J. G., and Caspari, D. M. (2014). Single unit hyperactivity and bursting in the auditory thalamus of awake rats directly correlates with behavioural evidence of tinnitus. *J. Physiol.* 592, 5065–5078. doi: 10.1113/jphysiol.2014.278572

Kaltenbach, J. A., Zhang, J., and Afman, C. E. (2000). Plasticity of spontaneous neural activity in the dorsal cochlear nucleus after intense sound exposure. *Hear. Res.* 147, 282–292. doi: 10.1016/S0378-5955(00)00138-6

Kreuzer, P. M., Landgrebe, M., Vielsmeier, V., Kleinjung, T., De Ridder, D., and Langguth, B. (2014). Trauma-associated tinnitus. *J. Head Trauma Rehabil.* 29, 432–442. doi: 10.1097/HTR.0b013e31829d3129

Kujawa, S. G., and Liberman, M. C. (2009). Adding insult to injury: cochlear nerve degeneration after 'temporary' noise-induced hearing loss. *J. Neurosci.* 29, 14077–14085. doi: 10.1523/JNEUROSCI.2845-09.2009

Kumagai, M. (1992). Effect of intravenous injection of aspirin on the cochlea. *Hokkaido Igaku Zasshi* 67, 216–233.

Langers, D. M., de Kleine, E., and van Dijk, P. (2012). Tinnitus does not require macroscopic tonotopic map reorganization. *Front. Syst. Neurosci.* 6:2. doi: 10.3389/fnsys.2012.00002

Langguth, B., Hund, V., Busch, V., Jürgens, T. P., Lainez, J.-M., Landgrebe, M., et al. (2015). Tinnitus and headache. *Biomed Res. Int.* 2015:797416. doi: 10.1155/2015/797416

Liberman, M. C., and Beil, D. G. (1979). Hair cell condition and auditory nerve response in normal and noise-damaged cochleas. *Acta Otolaryngol.* 88, 161–176. doi: 10.3109/00016487909137156

Liberman, M. C., and Kiang, N. Y. (1978). Acoustic trauma in cats. Cochlear pathology and auditory-nerve activity. *Acta Otolaryngol. Suppl.* 358, 1–63.

Llano, D. A., Turner, J., and Caspary, D. M. (2012). Diminished cortical inhibition in an aging mouse model of chronic tinnitus. *J. Neurosci.* 32, 16141–16148. doi: 10.1523/JNEUROSCI.2499-12.2012

Lobarinas, E., Hayes, S. H., and Allman, B. L. (2013). The gap-startle paradigm for tinnitus screening in animal models: limitations and optimization. *Hear. Res.* 295, 150–160. doi: 10.1016/j.heares.2012.06.001

Ma, W. L., Hidaka, H., and May, B. J. (2006). Spontaneous activity in the inferior colliculus of CBA/J mice after manipulations that induce tinnitus. *Hear. Res.* 212, 9–21. doi: 10.1016/j.heares.2005.10.003

Ma, W. L., and Young, E. D. (2006). Dorsal cochlear nucleus response properties following acoustic trauma: response maps and spontaneous activity. *Hear. Res.* 21, 176–188. doi: 10.1016/j.heares.2006.03.011

Manzoor, N. F., Gao, Y., Licari, F., and Kaltenbach, J. A. (2013). Comparison and contrast of noise-induced hyperactivity in the dorsal cochlear nucleus and inferior colliculus. *Hear. Res.* 295, 114–123. doi: 10.1016/j.heares.2012.04.003

Manzoor, N. F., Licari, F., Klapchar, M., Elkin, R. L., Gao, Y., Chen, G., et al. (2012). Noise-induced hyperactivity in the inferior colliculus: its relationship with hyperactivity in the dorsal cochlear nucleus. *J. Neurophysiol.* 108, 976–988. doi: 10.1152/jn.00833.2011

Melcher, J. R., Knudson, I. M., and Levine, R. A. (2013). Subcallosal brain structure: correlation with hearing threshold at supra-clinical frequencies (>8 kHz), but not with tinnitus. *Hear. Res.* 295, 79–86. doi: 10.1016/j.heares.2012.03.013

Middleton, J. W., Kiritani, T., Pedersen, C., Turner, J. G., Shepherd, G. M., and Tzounopoulos, T. (2011). Mice with behavioral evidence of tinnitus exhibit dorsal cochlear nucleus hyperactivity because of decreased GABAergic inhibition. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7601–7606. doi: 10.1073/pnas.1100223108

Møller, A. R. (2011). "Different forms of tinnitus," in *Textbook of Tinnitus*, eds A. R. Møller, B. Langguth, D. De Ridder, and T. Kleinjung (New York, NY: Springer), 9–12.

Morest, D. K., Kim, J., Potashner, S. J., and Bohne, B. A. (1998). Long-term degeneration in the cochlear nerve and cochlear nucleus of the adult chinchilla following acoustic overstimulation. *Microsc. Res. Tech.* 41, 205–216. doi: 10.1002/(SICI)1097-0029(19980501)41:3<205::AID-JEMT4>3.0.CO;2-S

Mulders, W. H., and Robertson, D. (2013). Development of hyperactivity after acoustic trauma in the guinea pig inferior colliculus. *Hear. Res.* 298, 104–108. doi: 10.1016/j.heares.2012.12.008

Munguia, R., Pienkowski, M., and Eggermont, J. J. (2013). Spontaneous firing rate changes in cat primary auditory cortex following long-term exposure to non traumatic noise. Tinnitus without hearing loss? *Neurosci. Lett.* 546, 46–50. doi: 10.1016/j.neulet.2013.04.048

Noreña, A. J., and Eggermont, J. J. (2003). Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. *Hear. Res.* 183, 137–153. doi: 10.1016/S0378-5955(03)00225-9

Noreña, A. J., and Eggermont, J. J. (2005). Enriched acoustic environment after noise trauma reduces hearing loss and prevents cortical map reorganization. *J. Neurosci.* 25, 699–705. doi: 10.1523/JNEUROSCI.2226-04.2005

Noreña, A. J., and Eggermont, J. J. (2006). Enriched acoustic environment after noise trauma abolishes neural signs of tinnitus. *Neuroreport* 17, 559–563. doi: 10.1097/00001756-200604240-00001

Noreña, A. J., Gourévitch, B., Aizawa, N., and Eggermont, J. J. (2006). Enriched acoustic environment disrupts frequency representation in cat auditory cortex. *Nat. Neurosci.* 9, 932–939. doi: 10.1038/nn1720

Ochi, K., and Eggermont, J. J. (1996). Effects of salicylate on neural activity in cat primary auditory cortex. *Hear. Res.* 95, 63–76. doi: 10.1016/0378-5955(96)00019-6

Pattyn, T., Van Den Eede, F., Vanneste, S., Cassiers, L., Veltman, D. J., Van De Heyning, P., et al. (2016). Tinnitus and anxiety disorders: a review. *Hear. Res.* 333, 255–265. doi: 10.1016/j.heares.2015.08.014

Paul, A. K., Lobarinas, E., Simmons, R., Wack, D., Luisi, J. C., and Spernyak, J. (2009). Metabolic imaging of rat brain during pharmacologically-induced tinnitus. *Neuroimage* 44, 312–318. doi: 10.1016/j.neuroimage.2008.09.024

Pienkowski, M., and Eggermont, J. J. (2009). Recovery from reorganization induced in adult cat primary auditory cortex by a band-limited spectrally enhanced acoustic environment. *Hear. Res.* 257, 24–40. doi: 10.1016/j.heares.2009.07.011

Pilati, N., Large, C., Forsythe, I. D., and Hamann, M. (2012). Acoustic over-exposure triggers burst firing in dorsal cochlear nucleus fusiform cells. *Hear. Res.* 283, 98–106. doi: 10.1016/j.heares.2011.10.008

Potashner, S. J., Suneja, S. K., and Benson, C. G. (1997). Regulation of D-aspartate release and uptake in adult brain stem auditory nuclei after unilateral middle ear ossicle removal and cochlear ablation. *Exp. Neurol.* 148, 222–235. doi: 10.1006/exnr.1997.6641

Roberts, L. E., Eggermont, J. J., Caspary, D. M., Shore, S. E., Melcher, J. R., and Kaltenbach, J. A. (2010). Ringing ears: the neuroscience of tinnitus. *J. Neurosci.* 30, 14972–14979. doi: 10.1523/JNEUROSCI.4028-10.2010

Roberts, L. E., Husain, F. T., and Eggermont, J. J. (2013). Role of attention in the generation and modulation of Tinnitus. *Neurosci. Biobehav. Rev.* 37, 1754–1773. doi: 10.1016/j.neubiorev.2013.07.007

Robertson, D., Bester, C., Vogler, D., and Mulders, W. H. (2013). Spontaneous hyperactivity in the auditory midbrain: relationship to afferent input. *Hear. Res.* 295, 124–129. doi: 10.1016/j.heares.2012.02.002

Salloum, R. H., Sandridge, S., Patton, D. J., Stillitano, G., Dawson, G., Niforatos, J., et al. (2016). Untangling the effects of tinnitus and hypersensitivity to sound (hyperacusis) in the gap detection test. *Hear. Res.* 331, 92–100. doi: 10.1016/j.heares.2015.10.005

Salloum, R. H., Yurosko, C., Santiago, L., Sandridge, S. A., and Kaltenbach, J. A. (2014). Induction of enhanced acoustic startle response by noise exposure: dependence on exposure conditions and testing parameters and possible relevance to hyperacusis. *PLoS ONE* 9:e111747. doi: 10.1371/journal.pone.0111747

Salvi, R. J., Henderson, D., Hamernik, R., and Ahroon, W. A. (1983). Neural correlates of sensorineural hearing loss. *Ear Hear.* 4, 115–129.

Schecklmann, M., Landgrebe, M., Langguth, B., and the Tri Database Study Group (2014). Phenotypic characteristics of hyperacusis in tinnitus. *PLoS ONE* 9:e86944. doi: 10.1371/journal.pone.0086944

Schecklmann, M., Lehner, A., Poeppl, T. B., Kreuzer, P. M., Rupprecht, R., Rackl, J., et al. (2013). Auditory cortex is implicated in tinnitus distress: a voxel-based morphometric study. *Brain Struct. Funct.* 218, 1061–1070. doi: 10.1007/s00429-013-0520-z

Schreiner, C. E., and Polley, D. B. (2014). Auditory map plasticity: diversity in causes and consequences. *Curr. Opin. Neurobiol.* 24, 143–156. doi: 10.1016/j.conb.2013.11.009

Sedley, W., Gander, P. E., Kumar, S., Oya, H., Kovach, C. K., Nourski, K. V., et al. (2015). Intracranial mapping of a cortical tinnitus system using residual inhibition. *Curr. Biol.* 25, 1208–1214. doi: 10.1016/j.cub.2015.02.075

Seki, S., and Eggermont, J. J. (2002). Changes in cat primary auditory cortex after minor-to-moderate pure-tone induced hearing loss. *Hear. Res.* 173, 172–186. doi: 10.1016/S0378-5955(02)00518-X

Seki, S., and Eggermont, J. J. (2003). Changes in spontaneous firing rate and neural synchrony in cat primary auditory cortex after localized tone-induced hearing loss. *Hear. Res.* 180, 28–38. doi: 10.1016/S0378-5955(03)00074-1

Stypulkowski, P. H. (1990). Mechanisms of salicylate ototoxicity. *Hear. Res.* 46, 113–146. doi: 10.1016/0378-5955(90)90144-E

Su, Y.-Y., Luo, B., Jin, Y., Wu, S.-H., Lobarinas, E., Salvi, R. J., et al. (2012). Altered neuronal intrinsic properties and reduced synaptic transmission of the rat's medial geniculate body in salicylate-induced tinnitus. *PLoS ONE* 7:e46969. doi: 10.1371/journal.pone.0046969

Sun, W., Lu, J., Stolzberg, D., Gray, L., Deng, A., Lobarinas, E., et al. (2009). Salicylate increases the gain of the central auditory system. *Neuroscience* 159, 325–334. doi: 10.1016/j.neuroscience.2008.12.024

Suneja, S. K., Benson, C. G., and Potashner, S. J. (1998a). Glycine receptors in adult guinea pig brain stem auditory nuclei: regulation after unilateral cochlear ablation. *Exp. Neurol.* 154, 473–488. doi: 10.1006/exnr.1998.6946

Suneja, S. K., Potashner, S. J., and Benson, C. G. (1998b). Plastic changes in glycine and GABA release and uptake in adult brain stem auditory nuclei after unilateral middle ear ossicle removal and cochlear ablation. *Exp. Neurol.* 151, 273–288. doi: 10.1006/exnr.1998.6812

Vanneste, S., van de Heyning, P., and De Ridder, D. (2011). The neural network of phantom sound changes over time: a comparison between recent onset and chronic tinnitus patients. *Eur. J. Neurosci.* 34, 718–731. doi: 10.1111/j.1460-9568.2011.07793.x

Vogler, D. P., Robertson, D., and Mulders, W. H. A. M. (2011). Hyperactivity in the ventral cochlear nucleus after cochlear trauma. *J. Neurosci.* 31, 6639–6645. doi: 10.1523/JNEUROSCI.6538-10.2011

Wallhäusser-Franke, E., Braun, S., and Langner, G. (1996). Salicylate alters 2-DG uptake in the auditory system: a model for tinnitus? *Neuroreport* 7, 1585–1588. doi: 10.1097/00001756-199607080-00010

Wang, F., Zuo, L., Hong, B., Han, D., Range, E. M., Zhao, L., et al. (2013). Tonotopic reorganization and spontaneous firing in inferior colliculus during both short and long recovery periods after noise overexposure. *J. Biomed. Sci.* 20:91. doi: 10.1186/1423-0127-20-91

Wang, H. T., Luo, B., Zhou, K. Q., and Chen, L. (2008). Sodium salicylate suppresses serotonin-induced enhancement of GABAergic spontaneous inhibitory postsynaptic currents in rat inferior colliculus in vitro. *Hear. Res.* 236, 42–51. doi: 10.1016/j.heares.2007.11.015

Wei, L., Ding, D., Sun, W., Xu-Friedman, M. A., and Salvi, R. (2010). Effects of sodium salicylate on spontaneous and evoked spike rate in the dorsal cochlear nucleus. *Hear. Res.* 267, 54–60. doi: 10.1016/j.heares.2010.03.088

Weisz, N., Hartmann, T., Müller, N., Lorenz, I., and Obleser, J. (2011). Alpha rhythms in audition: cognitive and clinical perspectives. *Front. Psychol.* 2:73. doi: 10.3389/fpsyg.2011.00073

Weisz, N., Müller, S., Schlee, W., Dohrmann, K., Hartmann, T., and Elbert, T. (2007). The neural code of auditory phantom perception. *J. Neurosci.* 27, 1479–1484. doi: 10.1523/JNEUROSCI.3711-06.2007

Weisz, N., and Obleser, J. (2014). Synchronisation signatures in the listening brain: a perspective from non-invasive neuroelectrophysiology. *Hear. Res.* 307, 16–28. doi: 10.1016/j.heares.2013.07.009

Wu, C., Martel, D. T., and Shore, S. E. (2016). Increased synchrony and bursting of dorsal cochlear nucleus fusiform cells correlate with tinnitus. *J. Neurosci.* 36, 2068–2073. doi: 10.1523/JNEUROSCI.3960-15.2016

Yang, G., Lobarinas, E., Zhang, L., Turner, J., Stolzberg, D., Salvi, R., et al. (2007). Salicylate induced tinnitus: behavioral measures and neural activity in auditory cortex of awake rats. *Hear. Res.* 226, 244–253. doi: 10.1016/j.heares.2006.06.013

Yang, K., Huang, Z. W., Liu, Z. Q., Xiao, B. K., and Peng, J. H. (2009). Long-term administration of salicylate enhances prestin expression in rat cochlea. *Int. J. Audiol.* 48, 18–23. doi: 10.1080/14992020802327998

Yu, N., Zhu, M. L., Johnson, B., Liu, Y. P., Jones, R. O., and Zhao, H. B. (2008). Prestin upregulation in chronic salicylate (aspirin) administration: an implication of functional dependence of prestin expression. *Cell. Mol. Life Sci.* 65, 2407–2418. doi: 10.1007/s00018-008-8195-y

Zacharek, M. A., Kaltenbach, J. A., Mathog, T. A., and Zhang, J. (2002). Effects of cochlear ablation on noise induced hyperactivity in the hamster dorsal cochlear nucleus: implications for the origin of noise induced tinnitus. *Hear. Res.* 172, 137–143. doi: 10.1016/S0378-5955(02)00575-0

Zhang, X., Yang, P., Cao, Y., Qin, L., and Sato, Y. (2011). Salicylate induced neural changes in the primary auditory cortex of awake cats. *Neuroscience* 172, 232–245. doi: 10.1016/j.neuroscience.2010.10.073

Zou, Q.-Z., and Shang, X.-L. (2012). Effect of salicylate on the large GABAergic neurons in the inferior colliculus of rats. *Acta Neurol. Belg.* 112, 367–374. doi: 10.1007/s13760-012-0090-5

Zugaib, J., Ceballos, C. C., and Leão, R. M. (2015). High doses of salicylate reduces glycinergic inhibition in the dorsal cochlear nucleus of the rat. *Hear. Res.* 332, 188–198. doi: 10.1016/j.heares.2015.10.008

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Non-Monotonic Relation between Noise Exposure Severity and Neuronal Hyperactivity in the Auditory Midbrain

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The occurrence of tinnitus can be linked to hearing loss in the majority of cases, but there is nevertheless a large degree of unexplained heterogeneity in the relation between hearing loss and tinnitus. Part of the problem might be that hearing loss is usually quantified in terms of increased hearing thresholds, which only provides limited information about the underlying cochlear damage. Moreover, noise exposure that does not cause hearing threshold loss can still lead to "hidden hearing loss" (HHL), i.e., functional deafferentation of auditory nerve fibers (ANFs) through loss of synaptic ribbons in inner hair cells. While it is known that increased hearing thresholds can trigger increases in spontaneous neural activity in the central auditory system, i.e., a putative neural correlate of tinnitus, the central effects of HHL have not yet been investigated. Here, we exposed mice to octave-band noise at 100 and 105 dB SPL to generate HHL and permanent increases of hearing thresholds, respectively. Deafferentation of ANFs was confirmed through measurement of auditory brainstem responses and cochlear immunohistochemistry. Acute extracellular recordings from the auditory midbrain (inferior colliculus) demonstrated increases in spontaneous neuronal activity (a putative neural correlate of tinnitus) in both groups. Surprisingly, the increase in spontaneous activity was most pronounced in the mice with HHL, suggesting that the relation between hearing loss and neuronal hyperactivity might be more complex than currently understood. Our computational model indicated that these differences in neuronal hyperactivity could arise from different degrees of deafferentation of low-threshold ANFs in the two exposure groups. Our results demonstrate that HHL is sufficient to induce changes in central auditory processing, and they also indicate a non-monotonic relationship between cochlear damage and neuronal hyperactivity, suggesting an explanation for why tinnitus might occur without obvious hearing loss and conversely why hearing loss does not always lead to tinnitus.

Keywords: tinnitus, hearing loss, mouse model, computational model, noise exposure, neuronal hyperactivity, cochlear damage, hidden hearing loss

INTRODUCTION

Epidemiological data suggest a close relation between hearing loss and tinnitus. For example, most tinnitus patients also have a certain degree of hearing loss (1, 2), tinnitus prevalence rises with hearing loss (3), 75–90% of patients with otosclerosis experience tinnitus (4, 5), as do 80% of patients with idiopathic sudden sensorineural hearing loss (6). However, upon closer inspection, the relation

between hearing loss and tinnitus appears quite heterogeneous. For example, even though there is a general trend for the tinnitus pitch to coincide with frequencies affected by hearing loss (7), clear correlations between the shape of the hearing threshold curve in the audiogram and tinnitus characteristics have only been observed for homogeneous study groups like noise-exposed workers (8), but not for general tinnitus patient samples (9, 10). Moreover, a significant fraction of individuals with tinnitus show no obvious signs of hearing loss (11, 12), and conversely many hearing-impaired listeners do not experience tinnitus (13).

Clinically, hearing loss is quantified through pure-tone audiometry, which measures thresholds for detecting tones in quiet. However, hearing thresholds alone only convey a limited picture of actual cochlear damage. The degree of outer hair cell loss, for example, only shows a moderate correlation to hearing threshold shifts (14), and cochlear dead regions, i.e., cochlear regions with severe loss of inner hair cells (IHCs), cannot be reliably detected through audiometry alone (15). Moreover, hearing thresholds are poor predictors of listening performance (16), and some hearing problems might not be detected by audiometry at all.

Thus, hearing thresholds do not provide a complete picture of hearing function. Indeed, recent evidence even suggests a considerable degree of "hidden hearing loss" (HHL) might be present despite a normal audiogram. Investigations in mice have shown that noise exposures producing only a temporary elevation of hearing thresholds can lead to loss of synaptic ribbons at synapses between IHCs and auditory nerve fibers (ANFs), permanently reducing the amplitude of wave I of the auditory brainstem response (ABR) (17). ANFs with high response thresholds appear particularly vulnerable to this kind of deafferentation, whereas fibers with low thresholds seem to be more resilient (18). Moreover, HHL might also develop through age-related processes in humans (19) as well as mice (20). Significant reductions of ABR wave I have also been reported for tinnitus subjects with clinically normal audiograms (21, 22), suggesting that HHL might not only cause a degradation of the auditory nerve response but also affect auditory processing beyond the ear.

Peripheral hearing loss can lead to pronounced changes in the central auditory system, with reduced inhibitory neurotransmission (23–27), increased excitatory neurotransmission or neuronal excitability (23, 25, 27, 28), and changes reported in gene expression (29). Moreover, spontaneous neuronal activity in the auditory system is altered in a non-intuitive fashion after hearing loss. While ANFs generally show reduced (30) or unchanged (31) spontaneous activity following induction of various kinds of cochlear damage, spontaneous firing rates in the central auditory system are reported to increase. Cochlear damage through exposure to noise or ototoxic drugs generates elevated spontaneous firing rates in the dorsal (32–34) and ventral (35) cochlear nuclei, the inferior colliculus (36–38), and the auditory cortex (39, 40). This neuronal hyperactivity has been linked to behavioral markers of tinnitus (34, 41–43). Moreover, the level of hyperactivity was proportional to the degree of hearing loss or cochlear damage (33, 36, 44).

Here, we investigate how noise-induced HHL affects spontaneous and evoked neuronal activity in the auditory midbrain of mice. In contrast to most previous studies employing unilateral noise exposure, we employed bilateral noise exposure – more typical of the noise exposure generally encountered by human listeners. After exposing two groups of mice to different levels of noise (100 dB SPL to induce HHL, 105 dB SPL to induce permanent changes in hearing thresholds), we compared the effects of "hidden" and "obvious" hearing loss on IC responses. Although spontaneous firing rates increased in both exposure groups, the increase was greatest in the animals exposed to the lower (100 dB SPL) sound level, which showed ANF deafferentation without permanent shifts in hearing thresholds. Using a computational model, we demonstrate that this non-monotonic relationship between the degree of cochlear damage and the degree of spontaneous neuronal hyperactivity is explicable in terms of different degrees of deafferentation of high- and low-threshold ANFs, suggesting that specific ANF deafferentation patterns could differ significantly in their effect on auditory function. Our results thus indicate that differences in the specific patterns of cochlear damage, which cannot be reliably determinated from hearing threshold measurements alone, might account for some of the heterogeneity observed in the relation between hearing loss and tinnitus.

MATERIALS AND METHODS

Subjects

Subjects were 23 male CBA/Ca mice. Mice were 7–19 weeks old at the time of noise exposure. Control animals were age-matched littermates. ABRs were recorded 1–14 days prior to, 1 day after, and 4 weeks after noise exposure. IC recordings took place 4 weeks after noise exposure. At the end of the final experiment, mice were overdosed with an intra-peritoneal (i.p.) injection of sodium pentobarbital. All experiments were performed in accordance with the United Kingdom Animal (Scientific Procedures) Act of 1986.

Noise Exposure

Mice were anesthetized with ketamine and medetomidine (i.p.) and positioned in a custom-made sound-proof booth on a heated pad directly underneath the center of a speaker (Stage Line PA Horn Tweeter MHD-220N/RD) positioned 45 cm above. The speaker was calibrated prior to each use to ensure that the frequency response was flat (±2 dB) over the 8–16 kHz range. Noise exposure was performed with an octave-band noise (8–16 kHz) at 100 or 105 dB SPL for 2 h. Noise stimuli were generated using an RX6 processor (Tucker-Davis Technologies, TDT), attenuated as required (TDT PA5) and amplified (TDT SA2). During the noise exposure, pedal reflex and breathing rate were checked every 30 min. Control animals were not exposed to any additional sound beyond the normal background noise in the animal unit.

Auditory Brainstem Response Recording and Analysis

For ABR recordings, animals were anesthetized with an i.p. injection of ketamine and medetomidine. ABR recordings were obtained using subdermal needle electrodes (Rochester Medical), one inserted at the vertex, and one each behind the

ipsilateral and contralateral pinnae. Electrode signals were low-pass filtered (7.5 kHz cut-off frequency, 12 dB per octave) and recorded at 24 kHz sampling rate (TDT RA4LI, RA4PA and RX5). For analysis, ABR data were filtered using a bandpass filter (100–3000 Hz, 5th-order Butterworth filter). Stimuli were either tone pips (5 ms total duration with 1.5 ms rise/fall time; frequencies 6, 8, 11, 16, 24, 32, and 48 kHz; intensities 0–80 dB SPL in 5 dB steps) or clicks (50 μs duration, 0–80 dB SPL in 5 dB steps), both delivered at a rate of 20/s. Stimuli were generated using a TDT RX6 processor, attenuated as needed (TDT PA5), amplified (TDT SA2), and presented in free-field condition with the speaker (TDT FF1) positioned at a 45° to the animal's axis at a distance of approximately 15 cm. The ear contralateral to the speaker was blocked using a foam earplug. Before the start of each experiment, the transfer function of the speaker was measured with a microphone (4939, Brüel and Kjaer) placed at the location of the animal's ear with the animal in place. This function was used to calibrate individual tones so that the overall output of the speaker was flat across frequency to ±3 dB. ABR thresholds were determined visually by estimating the lowest sound level at which deflections in the ABR waveform were judged to be greater than the background variability in the waveforms. Measurements of wave amplitudes were performed using custom Matlab software: a time window containing the wave of interest was selected by the user, and the software then detected maxima and minima of the ABR traces within that window. ABR wave amplitudes were measured from the peak to the following trough.

Extracellular Recordings in Inferior Colliculus

Animals were anesthetized with an i.p. injection of ketamine and medetomidine, followed by administration of dexamethasone and atropine sulfate. Lactated Ringers solution was given every 2 h to maintain hydration. The animal's temperature was maintained at 37.5°C using a homeothermic blanket connected to a rectal thermistor. Breathing rate was monitored throughout the surgery, and then at 45-min intervals throughout the recording. Once the pedal reflex had been abolished, the mouse was placed in a nose clamp to stabilize the head while leaving the ears free. To access the IC, a large craniotomy (≈4.5–6.5 mm posterior to Bregma, 0.5–3.5 mm lateral to midline) was performed on the right-hand side, revealing the full surface of the right IC. Extracellular, multi-unit recordings were made using single-shank silicon multi-electrodes with 16 recording sites (1 × 16 linear array with 100 μm spacing; NeuroNexus). This arrangement of electrode sites enabled sampling across the full extent of the central nucleus of the IC. The probe was advanced manually until the tip just touched the collicular surface. Using a remote hydraulic microdrive (Neurocraft, FHC Inc.), the electrode array was then advanced rapidly by 2000 μm, to minimize the duration of tissue compression during the initial penetration, and then retracted by 500 μm. Electrode signals were recorded at 24 kHz sampling rate and bandpass-filtered between 300 and 9000 Hz (TDT RX5).

To record frequency-response areas (FRAs), tone pips were played in sequential order from 4 to 70 kHz in one-eighth octave steps, with sound level ranging from 0 to 80 dB SPL in 5 dB steps. Tone pip duration was 100 ms, with a rise/fall time of 5 ms, and the inter-stimulus-interval was 400 ms. The speaker was positioned at a 45° to the animal's axis and at a distance of approximately 15 cm from the animal's left ear. The right ear was plugged with a sound-attenuating plug. The equipment for sound generation and delivery was the same as for the ABR measurements. The FRA measurement was repeated three times.

To obtain a measure of the spontaneous firing rates, we recorded 100 epochs of 500 ms duration without sound presentation. The speaker was unplugged during the recording of spontaneous activity.

Analysis of IC Data

To obtain multiunit activity, the electrode signals were first stripped of the local field potential by applying an additional high-pass filter with cut-off at 600 Hz, and action potentials were then classified using a latent variable spike-sorting algorithm (45) to separate multi-unit clusters from background noise. Characteristic frequencies (CFs) and thresholds of the multiunits were determined visually from the FRAs. Multi-unit recordings were considered to originate from the central nucleus of the IC (ICc) when there was a clear progression of CFs along the length of the linear electrode array. Recordings from electrodes that deviated from this CF progression were excluded from further analysis. Rate-level functions at CF were then derived from the FRA data. Spontaneous firing rates were determined by calculating the average firing rate over the 100 repetitions of the silent epoch of 500 ms duration.

Cochlear Immunohistochemistry for Synaptic Ribbon Counts

After the end of an IC recording, the cochleae were harvested and fixed in 4% paraformaldehyde. For immunohistochemistry, the organ of Corti was left in the temporal bone in position with no decalcification in order to identify the precise location of the cells in each cochlea. The tissue was accessed by removing the overlying apical bony covering and the tectorial membrane removed using micropipette aspiration or fine forceps. The procedure therefore differs from more conventional histological procedures and is a novel approach. To identify the synaptic structures after tissue permeabilization, we employed mouse anti-CtBP2 (#612044, BD Transduction Laboratories), used at 1:200, or simultaneously with rabbit anti-GluR2/3 (#AB1506, Millipore Bioscience), used at 1:50, incubated at 4°C overnight with a standard lysine block. The ribbon protein CtBP2 was then labeled by secondary antibodies conjugated to ATTO425 (TEFLabs); the postsynaptic terminals were labeled using biotinylated goat anti-rabbit IgG (#BA-1000, Vector) and subsequent incubation with either fluorescein or Alexa 488 conjugated to streptavidin (#SA-5001 Vector). The temporal bone was mounted for viewing hair cells from scala media and images were acquired as z-stacks with step sizes of 0.2–0.5 μm with a 63 × 1.0 NA objective. For such *in situ* identifications, multiphoton imaging was used in an upright LSM 510 confocal microscope (Zeiss). Ribbons were identified from z-projections and only those with both labels positively identified and were manually counted. Ribbons per cell were typically

determined as averages from the groups of 8–12 IHCs from the 10–30 kHz region of the cochleae. No account was taken individual variations in the cochlear frequency place map between animals. The differences between the resulting data and reported figures from decalcified and dissected tissue [e.g., Ref. (17)] may reflect differences in the sampling processes.

Computational Model

We have set up a computational model of the early auditory system, as described below, to study the effects of different patterns of ANF deafferentation on spontaneous activity in the central auditory system. The model is based on previous models of the effects of loss of inner and outer hair cells and damage to hair cell stereocilia (46, 47) as well as selective deafferentation of high-threshold ANFs (21).

Auditory Nerve Response Stage of the Model

Auditory nerve responses are modeled with a rate-level function that represents the average response of a small population of ANFs, comprising low-, medium-, and high-threshold fibers, with similar CFs (46). The effects of different types of cochlear damage on AN responses are then captured by adjusting the shape of the ANF population rate–intensity function. Selective loss of high-threshold ANFs is captured by scaling down responses to high sound intensities and reducing the maximum firing rate of the ANF population. The resulting rate–intensity function $f^*(I)$ is then

$$f^*(I) = f(I) - d_h\left[f(I) - f_h\right]_+$$

where $[]_+$ denotes positive rectification, $0 \le d_h < 1$ represents the fraction of missing high-threshold fibers, and $f_h = 100$ spikes/s is the firing rate where deafferentation starts to influence the AN population response. In addition to selective deafferentation of high-threshold ANFs, we also consider cases where all fiber types get deafferented:

$$f^*(I) = \left(f(I) - d_h\left[f(I) - f_h\right]_+\right)d_a$$

The parameter d_a scales the whole AN population rate-level function, and thus (for $d_h = 1$) models equal deafferentation of all fiber types when it is set to a value between 0 and 1. When both d_h and d_a are set to values < 1, deafferentation with a bias toward high-threshold fibers is created. Examples of the resulting AN population rate-level functions are shown in **Figure 4A**. To model hearing threshold increase, we increase the response threshold without changing spontaneous and maximum firing rate, based on the model for outer hair cell loss presented in Ref. (46, 47). Examples of rate–intensity functions with threshold increase and ANF deafferentation can be seen in **Figure 4B**.

Central Auditory System Stage of the Model

The central auditory system stage comprises a neuronal circuit where projection neurons (PNs) receive inhibition from two different types of inhibitory interneurons, one of them providing wide- and the other narrow-band inhibition. This circuit architecture is based on the circuitry of the dorsal cochlear nucleus (DCN) [reviewed in Ref. (48)], as this circuit is probably the best-characterized circuit in the auditory brainstem. However, by adjusting the strength of inhibition (governed by the two parameters g_w and g_n), a variety of different response types can be generated [see Ref. (47), for more details]. In this study, we used $g_w = 0.5$ and $g_n = 0.6$, which yields model PNs with monotonous rate-level functions, as typically observed in the inferior colliculus. Moreover, for the application of the model to data from the IC, we have now also included a response threshold for the PN as an additional parameter to account for the fact that spontaneous firing rates in the IC are generally quite low, and especially lower than in the cochlear nucleus (36, 48, 49). The firing rate response r of the PN to excitatory input at rate f (provided here through the activity of ANFs) and input from the inhibitory interneurons at rates w and n is then given by

$$r = R(f,w,n) = r_{\max}\tanh\left(\left[f - g_w w - g_n n - \mathrm{th}\right]_+ / r_{\max}\right)$$

where $r_{\max} = 300$ spikes/s is the maximum firing rate of the PN, and $th = 40$ spikes/s is the response threshold.

In the model, we assume that the mean firing rate of the PNs is stabilized at a certain target level (i.e., the mean activity obtained for input from an undamaged cochlea) through homeostatic plasticity, which scales the strength of excitatory and inhibitory synapses onto the PN in opposite directions. When the mean activity of the PN is permanently below the target, homeostatic plasticity increases excitation and decreases inhibition to bring activity back up to the target level. Prolonged increases in activity lead to changes in the opposite direction. Synaptic scaling is implemented through the homeostasis factor h (limited to values between 1/3 and 3 to account for physiological constraints on synaptic strength):

$$r = R(f,w,n,h) = r_{\max}\tanh\left(\left[hf - \frac{g_w}{h}w - \frac{g_n}{h}n - \mathrm{th}\right]_+ / r_{\max}\right)$$

The exact value of h required to reach the desired target mean activity for a certain pattern of cochlear damage is determined numerically.

All data analysis as well as implementation and evaluation of the model were done using MATLAB (The MathWorks Inc., Natick, MA, USA). To test for significant differences, t tests were used unless otherwise stated.

RESULTS

We investigated the effects of two different levels of noise exposure – 100 dB SPL and 105 dB SPL – on young adult male CBA/Ca mice, using ABR measurements, cochlear immunohistochemistry, and multiunit recordings from the IC. Noise exposure was performed with an octave-band noise (8–16 kHz) at 100 dB SPL ($n = 6$ animals) or 105 dB SPL ($n = 10$ animals) for 2 h, while the animals were anesthetized. The control group consisted of eight age-matched mice.

The Effect of Noise Exposure on ABR Responses

Auditory brainstem responses were measured before, 1 day after, and 4 weeks following noise exposure. At 1 day postexposure, both exposure groups exhibited a shift in response thresholds for tone pips of around 20–40 dB at frequencies above 11 (105-dB-SPL exposure, **Figure 1C**) or 16 kHz (100-dB-SPL exposure, **Figure 1A**). Four weeks after noise exposure, ABR thresholds had recovered in the 100-dB-SPL exposure group, such that they were no longer significantly different from the pre-exposure values at all test frequencies (all p-values > 0.1). In the 105-dB-SPL group, thresholds remained significantly elevated by around 20–25 dB at 11 and 16 kHz ($p < 0.01$ at both frequencies), but recovered, and were no longer significantly

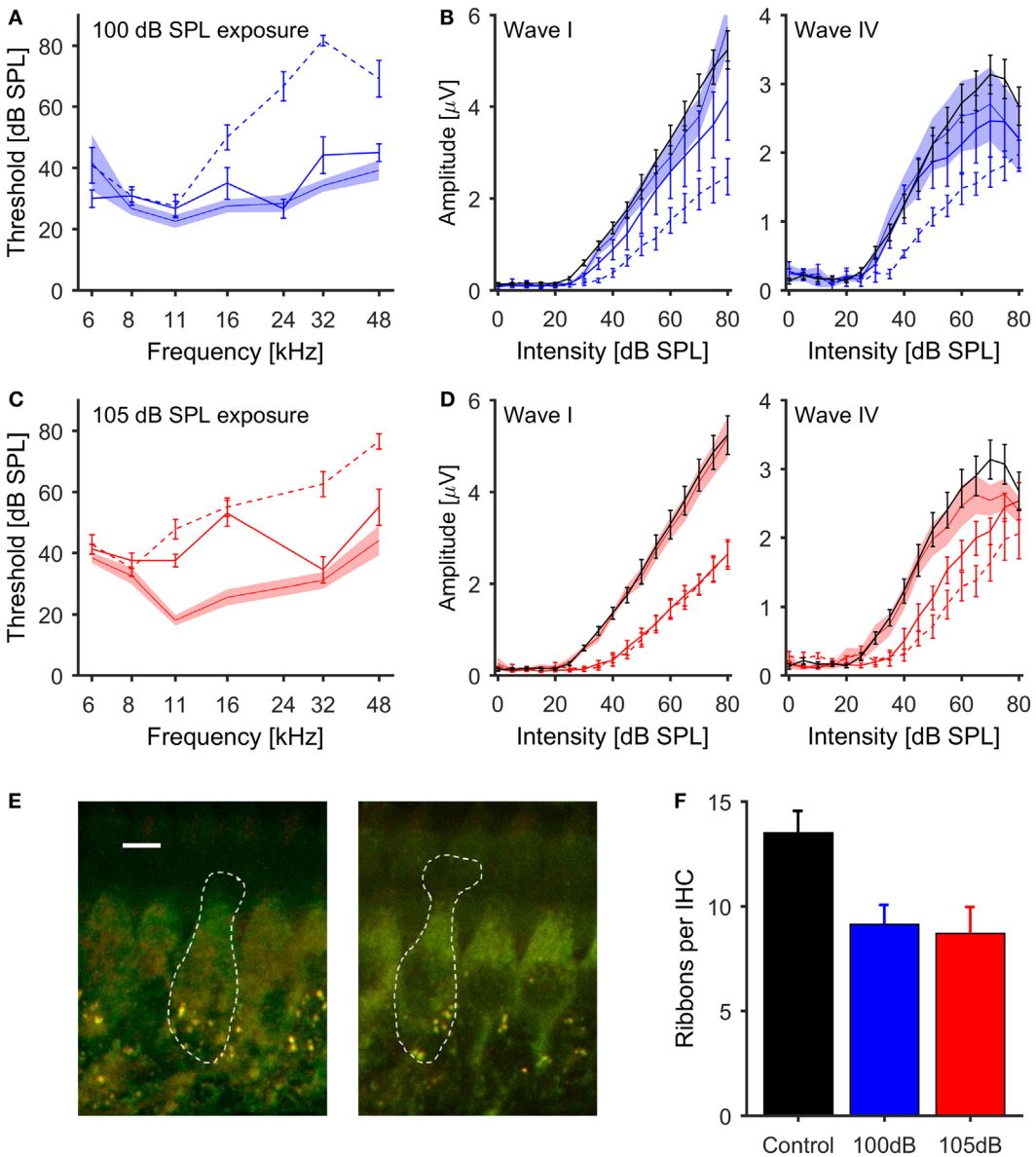

FIGURE 1 | **Effects of noise exposure on the auditory periphery of mice (A,C)**. ABR thresholds for tone-pip stimulation. Pre-exposure thresholds ±1 SEM are indicated by the shaded areas, thresholds 1 day postexposure by dashed lines, and 4 weeks postexposure by solid lines [**(A)** – 100 dB SPL exposure, blue; **(C)** – 105 dB SPL exposure, red]. **(B,D)** Growth function of the amplitudes of ABR waves I and IV (50 μs clicks) for control (black lines) and noise-exposed mice [**(B)** – 100 dB SPL exposure in blue; **(D)** – 105 dB SPL exposure in red]. Pre-exposure data ± 1 SEM are indicated by the shaded areas, 1 day postexposure by dashed and 4 weeks postexposure by solid lines. Both levels of noise exposure caused a significant reduction of ABR wave I amplitude. **(E)** Examples of inner hair cells from a control mouse (left, scale bar = 5μm) and a mouse exposed to 105 dB SPL noise (right), with synaptic ribbons labeled green (anti-CtBP2) and postsynaptic terminals red (anti-GluR2/3). **(F)** Mean number of synaptic ribbons per IHC; black, control ($n = 6$ cochleas); blue, 100 dB SPL exposure ($n = 3$ cochleas); red, 105 dB SPL ($n = 4$ cochleas). There was a significant difference in the ribbon count per IHC between control and noise exposed ears for both noise conditions (ANOVA, $p = 0.03$).

different from pre-exposure levels, at 32 and 48 kHz ($p > 0.1$ at both frequencies, **Figure 1C**).

The effects of noise exposure on the ABR were further investigated by analyzing the amplitudes of ABR waves I and IV in response to broadband clicks. Four weeks after noise exposure, both exposure groups displayed significantly lower amplitudes of click-evoked ABR wave I compared to control mice ($p < 0.01$, repeated measures ANOVA). The strongest reduction in ABR amplitude, in conjunction with an increase of the response threshold, was observed in the group exposed to 105-dB-SPL noise (**Figure 1D**). These changes in the magnitude of ABR wave I are indicative of deafferentation of ANFs (17, 18, 50).

In contrast to the effects of noise exposure on wave I of the ABR, amplitudes of wave IV in the 100-dB-SPL exposure group were virtually identical to those in control animals for click intensities up to 45 dB SPL, and then showed a slightly shallower growth at higher intensities (**Figure 1B**, right panel). In contrast, in the 105-dB-SPL group, the average amplitudes of ABR wave IV were reduced at low and medium sound intensities, but reached those of control animals at high sound intensities, even though the threshold was shifted by about 15 dB, similar to the elevation in wave I thresholds for this group. Moreover, the slope of the amplitude growth function above threshold was very similar to that of the control group (**Figure 1D**, right panel).

The Effect of Noise Exposure on Inner Hair Cell Synapses

To confirm that the observed reduction of ABR wave I amplitude was related to deafferentation of AN fibers, cochleae were extracted from representative animals from the control ($n = 6$), the 100-dB-SPL ($n = 3$) and the 105-dB-SPL ($n = 4$) groups, and loss of synaptic ribbons was quantified using immunohistochemistry (see Materials and Methods). Representative examples from a control mouse and a mouse exposed at 105 dB SPL are shown in **Figure 1E**. Ribbon counting was carried out in up to three regions of the cochlea for each animal, and the counts were averaged across these regions. Compared to control animals, both the 100- and 105-dB-SPL group showed a reduced number of synaptic ribbons per IHC (**Figure 1F**). There was a significant effect of noise exposure on the ribbon count ($p = 0.03$, ANOVA).

Elevated Spontaneous Neural Activity Following Noise Exposure

In addition to measuring wave IV of the ABR, we also assessed the effects of noise exposure on central auditory function by making multi-unit recordings in the central nucleus of the right IC (ICc) of (anesthetized) noise-exposed and control mice using 16-channel single-shank electrode arrays (see Materials and Methods). A total of 66 multi-unit recordings were obtained from control animals, 54 recordings from animals exposed at 100 dB SPL, and 111 recordings from animals exposed at 105 dB SPL. In control animals, the largest fraction of multi-unit recordings had CFs in the range of 16–24 kHz, commensurate with the most sensitive region of the mouse audiogram. However, in both noise exposure groups, CFs in the range of 8–12 kHz, i.e., near the low-frequency end of the octave-band noise used for the acoustic over-exposure, were most frequently encountered (**Figure 2A**). Similar results

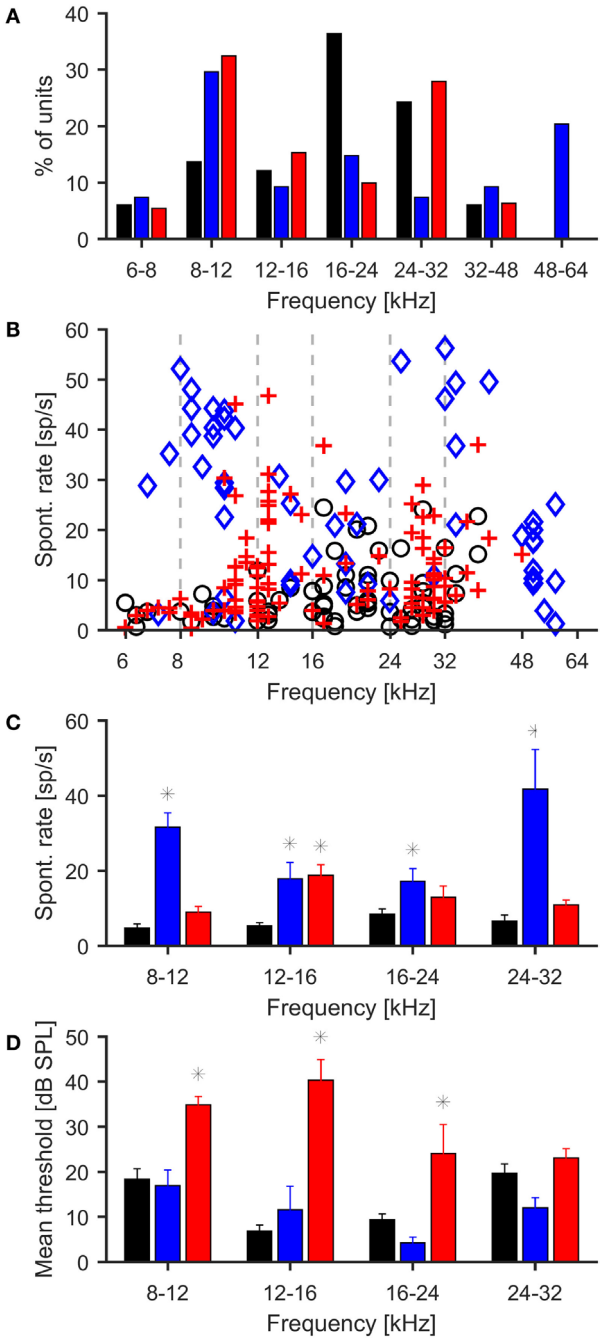

FIGURE 2 | Effects of noise exposure on neuronal responses in the inferior colliculus. **(A)** Distribution of characteristic frequencies of multi-units for control (black bars) and mice exposed to 100 (blue bars) and 105 dB SPL noise (red bars). In control mice, most units are found in the range from 16 to 24 kHz, whereas in both groups of exposed mice, CFs in the range from 8 to 12 kHz are most frequently encountered. **(B)** Spontaneous firing rates of individual IC multi-units, black = control, blue = 100-dB-SPL exposure, red = 105-dB-SPL exposure. **(C)** Spontaneous firing rates of multi-unit recordings grouped by characteristic frequencies. Asterisks denote significant differences from control ($p < 0.01$, t test). Only frequency ranges with at least five recordings from the control mice have been included in this analysis. **(D)** Average response thresholds of IC recordings, conventions as in plots above. Thresholds were significantly increased in the 105 dB exposure group for 8–12, 12–16, and 16–24 kHz.

were obtained for best frequencies, i.e., the tone frequencies evoking maximal firing rates (not shown).

In control animals, CF-tone evoked thresholds of ICc recordings ranged from 0 to 45 dB SPL, with a mean of 15.7 ± 1.3 dB SPL. The range of thresholds recorded from the ICc of noise-exposed mice was similar (0–60 dB SPL for 100-dB-SPL exposure and 0–65 dB SPL for 105-dB-SPL exposure); however, the mean threshold of 31.6 ± 1.4 dB SPL in the 105 dB SPL group was significantly higher than in the control group ($p < 0.01$, **Figure 2D**), corresponding to the threshold shift that was also observed in the ABR recordings. The mean threshold for recordings from the 100 dB SPL exposure group was 17.4 ± 1.7 dB SPL, which did not differ significantly from control.

Spontaneous firing rates were generally low in the control animals at 7.0 ± 1.3 spikes/s. In both exposure groups, the average spontaneous firing rates were significantly increased compared to control animals ($p < 0.01$ in both cases). The average spontaneous firing rate was 26.3 ± 4.2 spikes/s in the 100-dB-SPL exposure group and 11.7 ± 3.1 spikes/s in the 105-dB-SPL exposure group. The difference in spontaneous firing rate between the two noise exposure groups was also significant ($p < 0.01$). To test for significant differences in spontaneous firing rates in different frequency regions, recordings were grouped according to their CFs (8–12 kHz, 12–16 kHz, 16–24 kHz, 24–32 kHz). For CFs <8 kHz and >32 kHz, there were too few recordings in the control data to allow for meaningful statistical analysis. In the control group, average spontaneous rates were around 6–8 spikes/s across frequencies. Spontaneous firing rates in the 100-dB-SPL group were significantly increased in recordings with CFs from 8 to 12 kHz, 12 to 16 kHz, and 16 to 24 kHz ($p < 0.01$ in all cases). In the 105-dB-SPL group, a significant increase in spontaneous firing rates was seen in the 12–16 kHz range ($p < 0.01$). Spontaneous firing rates were also higher than in control mice in the 8–12, 16–24, and 24–32 kHz frequency ranges, but the increases did not achieve significance.

To further characterize the effects of noise exposure on the response properties of ICc recordings, we also analyzed rate-vs.-intensity functions for responses to CF tones. For this purpose, the multi-unit recordings were grouped according to their CFs, using the same frequency ranges as for the analysis of spontaneous firing rates. The average rate-vs.-intensity functions for the control group showed a monotonic response growth with increasing stimulus intensity, reaching discharge rates of 150–200 spikes/s at 80 dB SPL (**Figure 3**). In both exposure groups, on the other hand, the slopes of the rate-vs.-intensity functions were shallower on average, and the maximum responses were reduced compared to those in control animals (**Figure 3**). However, recordings from the 100-dB-SPL group showed elevated spike rates at sound intensities close to threshold, consistent with greater spontaneous activity.

Accounting for the Non-Monotonic Effect of Noise Exposure on Spontaneous Neural Activity

Perhaps the most surprising outcome of the ICc recording experiments was that noise exposure at 100 dB SPL resulted in a greater increase in spontaneous firing rates than did the 105-dB-SPL exposure. This was despite the fact that 100 dB SPL exposure generated less hearing loss (**Figure 1A**) and had less effect on the magnitude of ABR wave I (**Figure 1B**). Our expectation had been that animals in the 105-dB-SPL exposure group would show the greatest increase in spontaneous firing rates, since earlier studies had reported a proportionality between the degree of cochlear damage or threshold increase and the magnitude of spontaneous neuronal hyperactivity [e.g., Ref. (33, 36)], which we had also observed in our earlier modeling results (21, 46, 47, 51).

However, our most recent modeling study has suggested that whereas deafferentation of high-threshold ANFs (which show little or no spontaneous activity) leads to neuronal hyperactivity in the central auditory system, deafferentation of low-threshold fibers (with high spontaneous activity) could even reduce spontaneous firing rates (52). Moreover, while high-threshold ANFs appear to be most susceptible to noise-induced deafferentation (18), reductions in the amplitude of ABR wave I might arise when only low-threshold ANFs are deafferented; recent data suggest that the contribution of high-threshold fibers to the amplitude of ABR wave I is negligible (50). Since mice from the 105-dB-SPL group showed a greater reduction in ABR wave I amplitude with a shallower slope of the amplitude growth function than the mice

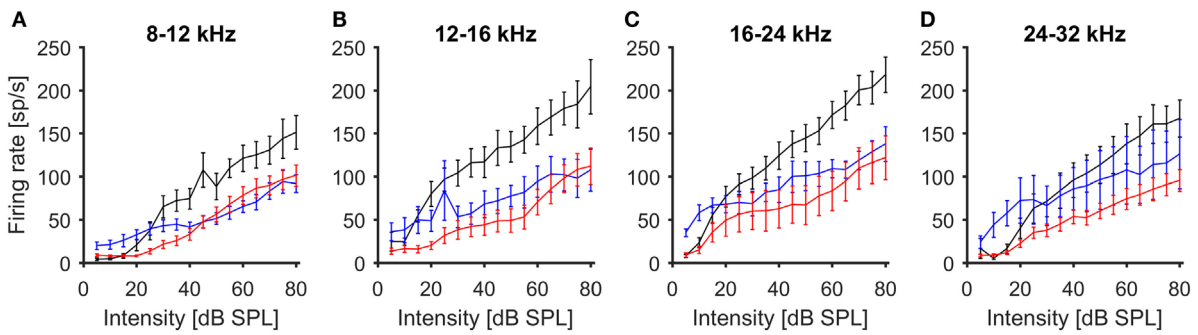

FIGURE 3 | Average rate-level functions of IC units in response to stimulation with 100 ms tone pips (mean ± 1 SEM). Multi-unit recordings have been grouped by CF [**(A)** 8–12 kHz, **(B)** 12–16 kHz, **(C)** 16–24 kHz, **(D)** 24–32 kHz]. Black – control, blue 100-dB-SPL exposure, red 105-dB-SPL exposure.

exposed to noise at 100 dB SPL (**Figure 1B**), we hypothesized that the 105-dB-SPL-exposed mice might have a higher degree of deafferentation of low-threshold fibers than the 100-dB-SPL mice. We therefore employed the model to explore whether this hypothesis might account for the observed non-monotonic relationship between cochlear damage and IC hyperactivity.

In the model, we first created different patterns of ANF deafferentation, ranging from selective deafferentation of high-threshold ANFs to a pattern where all fiber types are affected to a similar degree (see Materials and Methods and **Figure 4A** for an example), and combined these patterns of deafferentation with different degrees of hearing loss (i.e., increased thresholds; see Materials and Methods and **Figure 4B**). By this means, we explored a three-dimensional parameter space of damage parameters spanning (i) the degree of deafferentation, (ii) the pattern of deafferentation (i.e., low, medium and high spontaneous ANFs), and (iii) the degree of hearing loss (threshold increase). The model is based on the assumption that in attempting to stabilize neuronal activity following hearing loss, homeostatic plasticity elevates neuronal response gain in the central auditory system, thereby generating increased spontaneous firing rates through over-amplification of spontaneous activity (21, 46, 47, 53, 54). Hyperactivity thus depends first on how much the gain is increased by homeostatic plasticity and, second, on how much spontaneous activity in the AN is reduced through cochlear damage. If the increase in response gain is greater than the reduction of the spontaneous excitatory input from the ANFs, spontaneous hyperactivity develops (46, 47).

Figure 4C shows model results for four different degrees of overall ANF deafferentation from 10 to 40% of the total number of ANFs. For the case where deafferentation affects only high-threshold ANFs ("Ht only"), and thus does not reduce the amount of spontaneous excitatory input provided to the central auditory system by the ANFs, the greater the number of deafferented fibers the higher the spontaneous rate in the central auditory system following homeostasis. However, when low-threshold (and high spontaneous) fibers are also deafferented (i.e., moving from the bottom to the top, "Ht only" to "Equal" in each plot in **Figure 4C**), the resulting spontaneous firing rates are always lower than when only high-threshold fibers are deafferented. In the "balanced case" where deafferentation affects all fiber types equally (i.e., at the top of each plot in **Figure 4C**), there is even the suggestion of *hypo*-activity with spontaneous firing rates lower than before cochlear damage (the normal, healthy spontaneous rate is indicated by the dashed line in all plots). Finally, in the model, additional increases of peripheral thresholds always increase spontaneous firing rates in the central auditory system. Therefore, the model demonstrates that the same degree of elevation of spontaneous firing rates can be generated by different types of cochlear damage, and that trajectories exist through the "cochlear damage space" where more damage can even lead to a reduction in spontaneous firing rates in the central auditory nervous system, despite the presence of homeostatic plasticity mechanisms.

Comparison with Human Data

The ABR results from the 100-dB exposed mice with HHL resembled our findings from an earlier study in which we compared

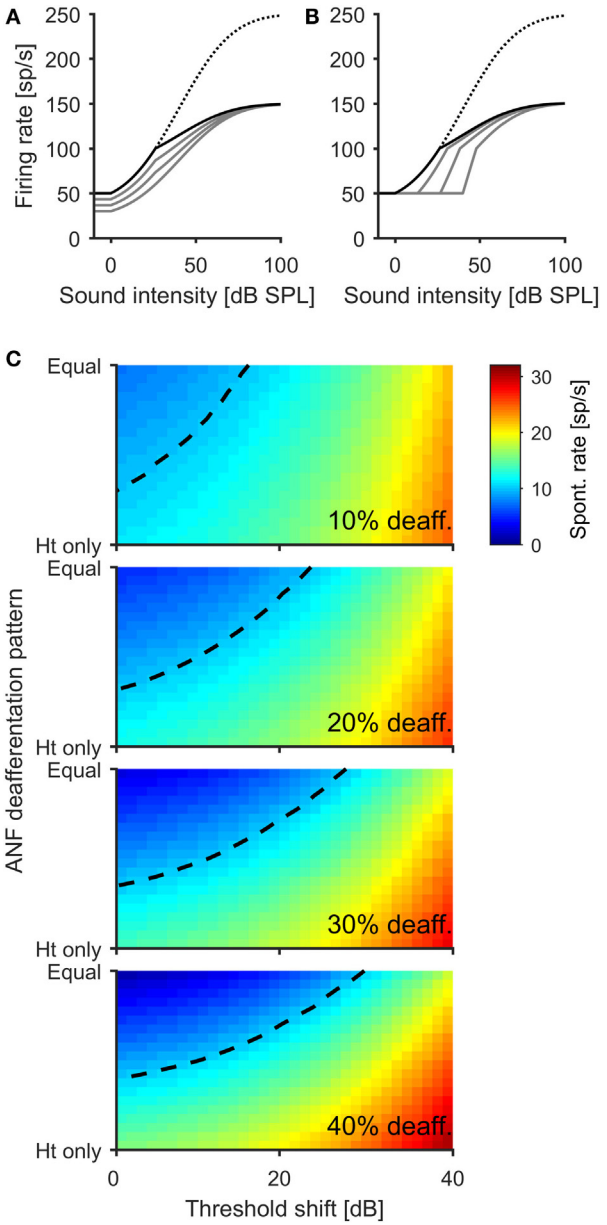

FIGURE 4 | A computational model offers an explanation for the non-linear relation between the degree of cochlear damage and spontaneous firing rates in the IC. **(A)** Illustration of how different ANF deafferentation patterns is modeled for an overall deafferentation 40% of ANFs. The dotted line indicates normal AN population rate–intensity function with no deafferentation, and the solid black line depicts the AN population rate–intensity function for a scenario in which only high-threshold fibers become deafferented. The gray lines show the transition with increasing deafferentation of low-threshold fibers, until all fibers are affected to the same degree (lowest gray line). **(B)** Modeling the effect of additional hearing threshold increase. The black line depicts the AN population response function for the scenario with 40% AN fiber deafferentation affecting only high-threshold fibers. The gray lines show the effects of 13.3, 26.7, and 40 dB threshold increase through damage to outer hair cells. **(C)** Color coded depiction of the spontaneous firing rate of a principal neuron in the central auditory system after activity stabilization through homeostatic plasticity in dependence upon ANF deafferentation pattern and threshold increase.

(Continued)

FIGURE 4 | Continued
Four different degrees of overall ANF deafferentation were modeled, from 10 (top panel) to 40% (bottom panel). The dashed line indicates the normal, healthy level of spontaneous activity. While both deafferentation of high-threshold fibers and threshold increase lead to an increase of spontaneous activity of the model neuron, deafferentation of low-threshold fibers can produce decreases of spontaneous rates below the normal level (e.g., dark blue regions in upper left corner of bottom panel).

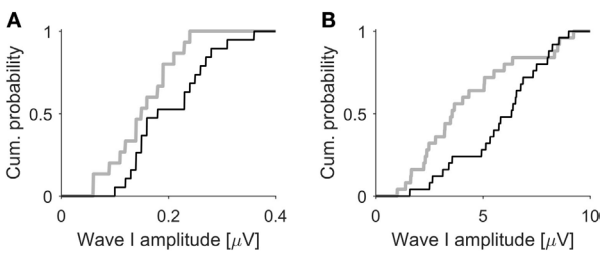

FIGURE 5 | ABR wave I amplitudes in humans and mice. **(A)** Cumulative distributions of ABR wave I amplitudes (50 μs clicks, 100 dB SPL) from tinnitus (gray line, $n = 15$) and control participants (black line, $n = 18$), both with normal hearing thresholds. Data from Schaette and McAlpine (21). **(B)** Cumulative distributions of ABR wave I of mice (50 μs clicks, 80 dB SPL, 25 ears from 13 mice) before (black line) and 4 weeks after exposure to octave-band noise at 100 dB SPL (gray line) causing hidden hearing loss. Mice show a similar degree of separation of the ABR amplitude distribution before and after noise-induced hidden hearing loss as do human subjects with and without tinnitus.

human tinnitus subjects with clinically normal hearing thresholds to age-, gender-, and hearing-matched control subjects, showing that the tinnitus group had significantly smaller amplitudes of ABR wave I (21). A direct comparison between humans and mice is hampered by the fact that ABR wave I amplitudes in mice are more than a magnitude larger than in humans, due to differences in head size and data acquisition methods. We therefore performed a qualitative comparison of the cumulative distributions of ABR wave I amplitudes. **Figure 5A** shows the cumulative distribution of the amplitudes of ABR wave I elicited by 50 μs clicks at 100 dB SPL for human tinnitus (gray line, $n = 15$ ears) and control participants (black line, $n = 18$ ears). Despite the significant difference in average amplitude, there is a considerable overlap in the distributions. **Figure 5B** shows cumulative distributions of ABR wave I amplitudes (50 μs clicks at 80 dB SPL) for mice before (black line) and 4 weeks after (gray line, 25 ears from 13 mice in both cases) exposure to octave-band noise at 100 dB SPL (note that this data set includes additional mice that did not undergo IC recordings). On a qualitative level, there is a great degree of similarity between mice before and after noise-induced HHL and normal-hearing humans with and without tinnitus, suggesting that mice with noise-induced HHL approximate the ABR-phenotype of human tinnitus subjects with normal hearing thresholds and could therefore, if tinnitus is verified through behavioral tests, potentially serve as an animal model for the condition.

DISCUSSION

We examined peripheral and central auditory function in mice exposed to different levels of noise; 100 dB SPL to elicit temporary shifts in hearing thresholds but long-term deafferentation of IHC synapses, and 105 dB SPL to elicit both permanent threshold shifts and deafferentation. A striking finding is the non-monotonic relationship between the severity of cochlear damage and the degree to which spontaneous firing rates in the ICc were elevated, with less damage leading to more hyperactivity (**Figures 2B,C**). From previous physiological studies (33, 36), it might have been expected that this relationship would be reversed with higher spontaneous rates following exposure to the more intense sound. Nevertheless, a relatively simple computational model suggests that this seemingly paradoxical outcome might be explained by different degrees of deafferentation of high-spontaneous-rate ANFs (**Figure 4C**). In particular, the model can account for the data in the frequency range from 12 to 24 kHz, where IC recordings from both exposure groups show similar spontaneous firing rates (**Figure 2C**) despite significant differences in thresholds following noise exposure (**Figures 1A** and **2D**). The model therefore provides a cogent explanation as to why the permanent hearing threshold shift in mice exposed to the higher intensity (105 dB SPL) noise did not lead to an additional increase in spontaneous firing rates: in these animals, a higher proportion of low-threshold ANFs with high spontaneous rates might have been deafferented. While the exact "trajectory" through the "deafferentation and damage plane" generated by different noise exposures remains to be determined, the modeling results provide a simple framework for understanding the complex relationship between cochlear damage and central auditory hyperactivity.

Our noise exposure paradigm for HHL was modeled on that of Kujawa and Liberman (17), who exposed awake mice to octave-band noise (8–16 kHz) at 100 dB SPL. They reported only temporary shifts in ABR thresholds, but permanently reduced ABR wave I amplitudes at higher sound intensities, as well as a reduced number of synaptic ribbons of IHCs, indicative of deafferentation. Our other noise exposure at 105 dB SPL generated additional, permanent shifts in hearing thresholds and a slightly higher degree of loss of synaptic ribbons in the cochlea, qualitatively similar to the findings of Rüttiger et al., who used noise exposure durations of 1 and 1.5 h at 120 dB SPL, and observed more hearing threshold increase and loss of synaptic ribbons after the longer exposure. In both of our exposure groups, the centrally generated ABR wave IV showed a certain degree of recovery of the amplitudes, a finding also consistent with results of recent studies in mice (55) and rats (56).

We observed significant increases in ICc spontaneous firing rates in both exposure groups. Data from the animals exposed to 100-dB-SPL noise suggest that HHL can lead to the development of increased spontaneous firing rates, an outcome we previously predicted from a computational model designed to account for the effects of deafferentation of high-threshold ANFs (21). In mice with HHL, a prominent peak in the profile of spontaneous firing rates was seen in the CF range of 8–12 kHz,

toward the lower end of the octave-band noise used for noise exposure (another, slightly higher peak was found in the CF range of 24–32 kHz, but only four units were recorded there). A similar relationship between noise exposure frequency range, temporary threshold shift, and neuronal hyperactivity has recently been reported for the DCN (57). In this frequency range, noise exposure might only cause a limited degree of ANF deafferentation (17, 58), accentuating the non-linear nature of the relation between cochlear damage and central hyperactivity, which might be influenced by a combination of factors, e.g., central compensation for peripheral damage (46, 54) in conjunction with a loss of lateral inhibition (59–62).

In the animals exposed to noise at 105 dB SPL, we observed the strongest increase in spontaneous firing rates at around 12–16 kHz, where hearing thresholds were permanently increased, matching recent reports from guinea pigs (36) and hamsters (63). It should be noted that previous studies usually only employed a single noise exposure level, either a high-level noise exposure causing permanent hearing loss (36, 41, 42, 49, 63), or a less severe exposure inducing only temporary shifts in hearing thresholds (26, 40, 57, 64), which could explain why the non-monotonic relation between the extent of cochlear damage and neuronal hyperactivity has not been reported before. Also, results indicating a monotonic relation between threshold increase and hyperactivity [e.g., Ref. (36, 44)] have been obtained analyzing different degrees of threshold elevation along the tonotopic axis for a single noise exposure level, and we have observed a similar pattern in the 105 dB SPL group.

An important difference between the two exposure group in our study could be the degree of damage to outer hair cells. In the 105 dB SPL group, which suffered a permanent threshold increase due to the noise exposure, permanent damage to or even loss of outer hair cells might have occurred (65). In the 100 dB SPL exposure group, the absence of a permanent threshold shift suggests that OHCs might have remained largely intact (17, 65). However, to quantify the contribution of OHC damage to our findings, measurements of otoacoustic emissions and detailed hair cell counts would be required. A quantification of hair cell loss would also be an interesting aspect for future studies, as there is also a non-linear relation between cisplatin-induced hair cell loss and central auditory hyperactivity (33): DCN hyperactivity was proportional to the degree of outer hair cell loss for low doses of cisplatin that did not cause IHC loss, whereas additional IHC loss decreased hyperactivity. We have replicated this effect in an earlier modeling study (46) using a simpler version of our current model. These findings indicate that there might be at least one additional point where the relation between noise-induced cochlear damage and hyperactivity is non-monotonic: Noise exposures causing slightly more damage than our 105 dB SPL exposure (e.g., 110 or 115 dB SPL for 2 h) may cause a stronger elevation of spontaneous firing rates, whereas for noise exposures severe enough to cause death of IHCs, another drop in spontaneous firing rates might occur. Such effects could further contribute to the heterogeneity observed in the relation between hearing loss and tinnitus. However, a detailed mapping of the effects of severe noise exposures was beyond the scope of our current study.

Several recent studies (40, 57, 66) and our own results indicate that noise exposure leading to TTS is sufficient to trigger the development of neuronal hyperactivity, but a recent study using has reported that spontaneous firing rates in the guinea pig IC remained normal after bilateral exposure to a loud tone that caused TTS (67). It remains to be determined which factors are responsible for this discrepancy (or heterogeneity). For example, there might be a certain "damage threshold" for the development of hyperactivity after acoustic trauma, i.e., a certain minimum degree of TTS or ANF deafferentation might be required to trigger the development of neuronal hyperactivity. A future study could thus investigate a range of noise exposure severities including even lower intensities than employed in our study to test this hypothesis. Moreover, certain types of sound exposures at relatively low levels that are unlikely to cause cochlear damage could still have an effect on spontaneous neuronal activity in the central auditory system. For example, passive long-term exposure to an "enhanced acoustic environment" consisting of random tone pips at 70–80 dB SPL can have a pronounced effect on spontaneous firing rates in the auditory cortex, with decreases in units with CFs in the frequency range of the tone pips, and increases in units with CFs above and below the exposure frequency range (68, 69). These "side-band" increases have been attributed to plastic changes in gain and lateral inhibition (62). The shape of the profile of spontaneous firing rates that we observed in the mice exposed to noise at 100 dB SPL bears a certain resemblance to the pattern observed for low-level noise, albeit with the crucial difference that we found increases in spontaneous firing also in the frequency range of the noise exposure stimulus (**Figures 2B,C**). A possible explanation might be an interaction between changes in lateral inhibition and homeostatic compensation for decreased input from the auditory nerve. This scenario could be investigated in more detail in future studies. Another interesting finding of the low-level noise exposure studies was changes in the tonotopic organization of the auditory cortex (68, 70) and the auditory midbrain (71) through exposure to tone pip environments or broadband noise for weeks at levels of 70–80 dB SPL. Such reorganization processes could also explain why we encountered relatively more units with CFs in the range of 8–12 kHz in the noise-exposed groups compared to the control group (**Figure 2A**), but it remains to be determined if a relatively short duration, as the 2 h used in our study, is sufficient to trigger this process in order to determine whether reorganization is driven by the exposure itself, or a consequence of cochlear damage.

Our data do not enable us to determine whether the increase in spontaneous activity in the ICc is generated locally, or inherited from another processing stage in the auditory pathway. It has recently been demonstrated that ablation of the contralateral DCN abolishes noise-induced hyperactivity in the IC (63), suggesting that neuronal hyperactivity in the IC could be generated through propagation of elevated spontaneous activity from the DCN. A potential mechanism might be amplification of spontaneous excitatory input from the auditory nerve, as silencing the auditory nerve 4 weeks after noise trauma through cooling or application of TTX abolishes hyperactivity in the IC (36). Beyond 4 weeks, however, spontaneous neuronal hyperactivity appears

to become a persistent feature in the central auditory system, as silencing spontaneous auditory nerve activity no longer reduces central hyperactivity (37). In the DCN, neuronal hyperactivity has been linked to a reduction of neural inhibition (26), and altered auditory-somatosensory integration might also play a role (72, 73). In the IC of normal-hearing animals, blocking inhibition through iontophoretic application of the GABA antagonist bicuculline leads to a substantial increase in spontaneous activity (74), suggesting a potential mechanism for the generation of hyperactivity within the IC.

An open question is whether the mice in our study were experiencing tinnitus – we did not perform behavioral tests. Several recent studies have reported behavioral evidence of tinnitus in mice (26, 66, 75), rats (40), gerbils (76), and guinea pigs (34, 57) following noise exposure leading to temporary shifts in hearing thresholds. Tinnitus-like behavior was reported for the majority, but not all of the animals (26, 34, 40). Similarly, several previous studies have demonstrated that noise exposure leading to permanently elevated hearing thresholds can also generate behavioral signs of tinnitus (41–43, 56, 77). Therefore, some proportion of the mice in our study with "hidden" as well as "normal" hearing loss might be expected to have had tinnitus. Behavioral verification of tinnitus would also help to clarify whether mice with HHL might be a suitable model for studying tinnitus with normal hearing, as suggested by the qualitative similarity between the ABR phenotype of our mice with HHL and human tinnitus subjects with normal hearing (**Figure 5**). Moreover, it has recently also been suggested that mice might show signs of hyperacusis rather than tinnitus after exposure to octave-band noise at 100 dB SPL (55), emphasizing the importance of behavioral testing for future studies to disentangle the effects of different auditory pathologies.

Could the increased spontaneous firing rates we observed in the IC of noise-exposed mice represent a neural correlate of tinnitus? The relationship between exposure frequency range and the location of increases in spontaneous activity on the tonotopic axis of the IC in our study is similar to the relation between exposure frequency range and the putative tinnitus pitch in gerbils (76). Moreover, in both the cochlear nucleus (34) and the inferior colliculus (43), a close link between spontaneous neuronal hyperactivity and behavioral evidence for tinnitus has been reported. Interestingly, it has recently been demonstrated that acoustic trauma that induces only temporary hearing threshold shifts might actually be more likely to induce tinnitus than a noise exposure causing permanent threshold elevation (78), an observation that is closely matched by our finding that the "HHL" noise exposure evoked a greater elevation of spontaneous firing rates in the IC than the more severe noise exposure. However, neuronal hyperactivity may not always be tinnitus-specific; in a recent study in guinea pigs, increases in spontaneous firing rates were observed in all noise-exposed animals, regardless of whether they showed behavioral signs of tinnitus or not (79).

One important difference between our study and most other studies on animal models of tinnitus is that we employed bilateral, rather than unilateral, noise exposure. Although unilateral noise exposure has the advantage of preserving hearing in one ear, which can then serve as a within-subject control (and ensures normal hearing abilities for behavioral testing in at least one ear), noise exposure bilaterally is more representative of the case in human listeners, where usually both ears are exposed to noise (with rifle shooting being possibly the most prominent exception). It remains to be determined whether bilateral noise exposure is more or less likely to lead to tinnitus in animals. We have recently demonstrated that unilateral earplugging can lead to the perception of phantom sounds in normal-hearing human volunteers (51), which is qualitatively similar to the reports of tinnitus during bilateral auditory deprivation in an anechoic chamber (80, 81). Further, the degree of elevation of spontaneous activity in the inferior colliculus we observed following bilateral noise exposure appears similar to the effects reported for unilateral noise exposure (36, 38, 79, 82).

An important insight from our electrophysiological and modeling results is that they offer an indication of why hearing loss might not always lead to tinnitus. If the relationship between cochlear damage and the strength of a putative neuronal correlate for tinnitus is indeed non-monotonic, then some specific patterns or configurations of cochlear damage might be more likely to lead to tinnitus than others. This finding could offer an interesting new approach toward understanding the pathophysiology of tinnitus and tinnitus heterogeneity, and for evaluating potential tinnitus triggers.

ETHICS

The human data presented in this manuscript is from a previous study (21), which had been approved by the University College London Research Ethics Committee.

AUTHOR CONTRIBUTIONS

LH designed research, performed experiments, analysed data, and wrote the manuscript. WB performed experiments and wrote the manuscript. LA performed experiments. H-CO performed experiments and analysed data. JA supervised experiments, analysed data, and wrote the manuscript. DM designed research, supervised experiments, and wrote the manuscript. JL designed research, supervised experiments, and wrote the manuscript. RS designed research, analysed data, produced all figures, and wrote the manuscript.

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REFERENCES

1. Axelsson A, Ringdahl A. Tinnitus – a study of its prevalence and characteristics. *Br J Audiol* (1989) 23:53–62. doi:10.3109/03005368909077819
2. Nicolas-Puel C, Faulconbridge RL, Guitton M, Puel JL, Mondain M, Uziel A. Characteristics of tinnitus and etiology of associated hearing loss: a study of 123 patients. *Int Tinnitus J* (2002) 8:37–44.
3. Chung DY, Gannon RP, Mason K. Factors affecting the prevalence of tinnitus. *Audiology* (1984) 23:441–52. doi:10.3109/00206098409070084
4. Ayache D, Earally F, Elbaz P. Characteristics and postoperative course of tinnitus in otosclerosis. *Otol Neurotol* (2003) 24:48–51. doi:10.1097/00129492-200301000-00011
5. Sobrinho PG, Oliveira CA, Venosa AR. Long-term follow-up of tinnitus in patients with otosclerosis after stapes surgery. *Int Tinnitus J* (2004) 10:197–201.
6. Nosrati-Zarenoe R, Arlinger S, Hultcrantz E. Idiopathic sudden sensorineural hearing loss: results drawn from the Swedish national database. *Acta Otolaryngol* (2007) 127:1168–75. doi:10.1080/00016480701242477
7. Henry JA, Meikle M, Gilbert A. Audiometric correlates of tinnitus pitch: Insights from the Tinnitus Data Registry. In: Hazell J, editor. *VI. International Tinnitus Seminar*. London: The Tinnitus and Hyperacusis Centre (1999). p. 51–7.
8. König O, Schaette R, Kempter R, Gross M. Course of hearing loss and occurrence of tinnitus. *Hear Res* (2006) 221:59–64. doi:10.1016/j.heares.2006.07.007
9. Pan T, Tyler RS, Ji H, Coelho C, Gehringer AK, Gogel SA. The relationship between tinnitus pitch and the audiogram. *Int J Audiol* (2009) 48:277–94. doi:10.1080/14992020802581974
10. Sereda M, Hall DA, Bosnyak DJ, Edmondson-Jones M, Roberts LE, Adjamian P, et al. Re-examining the relationship between audiometric profile and tinnitus pitch. *Int J Audiol* (2011) 50:303–12. doi:10.3109/14992027.2010.551221
11. Barnea G, Attias J, Gold S, Shahar A. Tinnitus with normal hearing sensitivity: extended high-frequency audiometry and auditory-nerve brain-stem-evoked responses. *Audiology* (1990) 29:36–45. doi:10.3109/00206099009081644
12. Sanchez TG, Medeiros IR, Levy CP, Ramalho Jda R, Bento RF. Tinnitus in normally hearing patients: clinical aspects and repercussions. *Braz J Otorhinolaryngol* (2005) 71:427–31. doi:10.1016/S1808-8694(15)31194-0
13. Lockwood AH, Salvi RJ, Burkard RF. Tinnitus. *N Engl J Med* (2002) 347:904–10. doi:10.1056/NEJMra013395
14. Davis B, Qiu W, Hamernik RP. The use of distortion product otoacoustic emissions in the estimation of hearing and sensory cell loss in noise-damaged cochleas. *Hear Res* (2004) 187:12–24. doi:10.1016/S0378-5955(03)00339-3
15. Moore BC, Huss M, Vickers DA, Glasberg BR, Alcantara JI. A test for the diagnosis of dead regions in the cochlea. *Br J Audiol* (2000) 34:205–24. doi:10.3109/03005364000000131
16. Summers V, Makashay MJ, Theodoroff SM, Leek MR. Suprathreshold auditory processing and speech perception in noise: hearing-impaired and normal-hearing listeners. *J Am Acad Audiol* (2013) 24:274–92. doi:10.3766/jaaa.24.4.4
17. Kujawa SG, Liberman MC. Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. *J Neurosci* (2009) 29:14077–85. doi:10.1523/JNEUROSCI.2845-09.2009
18. Furman AC, Kujawa SG, Liberman MC. Noise-induced cochlear neuropathy is selective for fibers with low spontaneous rates. *J Neurophysiol* (2013) 110:577–86. doi:10.1152/jn.00164.2013
19. Makary CA, Shin J, Kujawa SG, Liberman MC, Merchant SN. Age-related primary cochlear neuronal degeneration in human temporal bones. *J Assoc Res Otolaryngol* (2011) 12:711–7. doi:10.1007/s10162-011-0283-2
20. Sergeyenko Y, Lall K, Liberman MC, Kujawa SG. Age-related cochlear synaptopathy: an early-onset contributor to auditory functional decline. *J Neurosci* (2013) 33:13686–94. doi:10.1523/JNEUROSCI.1783-13.2013
21. Schaette R, McAlpine D. Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J Neurosci* (2011) 31:13452–7. doi:10.1523/JNEUROSCI.2156-11.2011
22. Gu JW, Herrmann BS, Levine RA, Melcher JR. Brainstem auditory evoked potentials suggest a role for the ventral cochlear nucleus in tinnitus. *J Assoc Res Otolaryngol* (2012) 13(6):819–33. doi:10.1007/s10162-012-0344-1
23. Vale C, Sanes DH. The effect of bilateral deafness on excitatory and inhibitory synaptic strength in the inferior colliculus. *Eur J Neurosci* (2002) 16:2394–404. doi:10.1046/j.1460-9568.2002.02302.x
24. Caspary DM, Schatteman TA, Hughes LF. Age-related changes in the inhibitory response properties of dorsal cochlear nucleus output neurons: role of inhibitory inputs. *J Neurosci* (2005) 25:10952–9. doi:10.1523/JNEUROSCI.2451-05.2005
25. Whiting B, Moiseff A, Rubio ME. Cochlear nucleus neurons redistribute synaptic AMPA and glycine receptors in response to monaural conductive hearing loss. *Neuroscience* (2009) 163:1264–76. doi:10.1016/j.neuroscience.2009.07.049
26. Middleton JW, Kiritani T, Pedersen C, Turner JG, Shepherd GM, Tzounopoulos T. Mice with behavioral evidence of tinnitus exhibit dorsal cochlear nucleus hyperactivity because of decreased GABAergic inhibition. *Proc Natl Acad Sci U S A* (2011) 108:7601–6. doi:10.1073/pnas.1100223108
27. Wang H, Yin G, Rogers K, Miralles C, De Blas AL, Rubio ME. Monaural conductive hearing loss alters the expression of the GluA3 AMPA and glycine receptor alpha1 subunits in bushy and fusiform cells of the cochlear nucleus. *Neuroscience* (2011) 199:438–51. doi:10.1016/j.neuroscience.2011.10.021
28. Kotak VC, Fujisawa S, Lee FA, Karthikeyan O, Aoki C, Sanes DH. Hearing loss raises excitability in the auditory cortex. *J Neurosci* (2005) 25:3908–18. doi:10.1523/JNEUROSCI.5169-04.2005
29. Dong S, Mulders WH, Rodger J, Woo S, Robertson D. Acoustic trauma evokes hyperactivity and changes in gene expression in guinea-pig auditory brainstem. *Eur J Neurosci* (2010) 31:1616–28. doi:10.1111/j.1460-9568.2010.07183.x
30. Liberman MC, Dodds LW. Single-neuron labeling and chronic cochlear pathology. II. Stereocilia damage and alterations of spontaneous discharge rates. *Hear Res* (1984) 16:43–53. doi:10.1016/0378-5955(84)90024-8
31. Dallos P, Harris D. Properties of auditory nerve responses in absence of outer hair cells. *J Neurophysiol* (1978) 41:365–83.
32. Kaltenbach JA, Godfrey DA, Neumann JB, McCaslin DL, Afman CE, Zhang J. Changes in spontaneous neural activity in the dorsal cochlear nucleus following exposure to intense sound: relation to threshold shift. *Hear Res* (1998) 124:78–84. doi:10.1016/S0378-5955(98)00119-1
33. Kaltenbach JA, Rachel JD, Mathog TA, Zhang J, Falzarano PR, Lewandowski M. Cisplatin-induced hyperactivity in the dorsal cochlear nucleus and its relation to outer hair cell loss: relevance to tinnitus. *J Neurophysiol* (2002) 88:699–714.
34. Koehler SD, Shore SE. Stimulus timing-dependent plasticity in dorsal cochlear nucleus is altered in tinnitus. *J Neurosci* (2013) 33:19647–56. doi:10.1523/JNEUROSCI.2788-13.2013
35. Vogler DP, Robertson D, Mulders WH. Hyperactivity in the ventral cochlear nucleus after cochlear trauma. *J Neurosci* (2011) 31:6639–45. doi:10.1523/JNEUROSCI.6538-10.2011
36. Mulders WH, Robertson D. Hyperactivity in the auditory midbrain after acoustic trauma: dependence on cochlear activity. *Neuroscience* (2009) 164:733–46. doi:10.1016/j.neuroscience.2009.08.036
37. Mulders WH, Robertson D. Progressive centralization of midbrain hyperactivity after acoustic trauma. *Neuroscience* (2011) 192:753–60. doi:10.1016/j.neuroscience.2011.06.046
38. Vogler DP, Robertson D, Mulders WH. Hyperactivity following unilateral hearing loss in characterized cells in the inferior colliculus. *Neuroscience* (2014) 265:28–36. doi:10.1016/j.neuroscience.2014.01.017
39. Norena AJ, Eggermont JJ. Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. *Hear Res* (2003) 183:137–53. doi:10.1016/S0378-5955(03)00225-9
40. Engineer ND, Riley JR, Seale JD, Vrana WA, Shetake JA, Sudanagunta SP, et al. Reversing pathological neural activity using targeted plasticity. *Nature* (2011) 470:101–4. doi:10.1038/nature09656
41. Kaltenbach JA, Zacharek MA, Zhang J, Frederick S. Activity in the dorsal cochlear nucleus of hamsters previously tested for tinnitus following intense tone exposure. *Neurosci Lett* (2004) 355:121–5. doi:10.1016/j.neulet.2003.10.038
42. Ahlf S, Tziridis K, Korn S, Strohmeyer I, Schulze H. Predisposition for and prevention of subjective tinnitus development. *PLoS One* (2012) 7:e44519. doi:10.1371/journal.pone.0044519
43. Mulders WH, Barry KM, Robertson D. Effects of furosemide on cochlear neural activity, central hyperactivity and behavioural tinnitus after cochlear

trauma in Guinea pig. *PLoS One* (2014) 9:e97948. doi:10.1371/journal.pone.0097948
44. Mulders WH, Ding D, Salvi R, Robertson D. Relationship between auditory thresholds, central spontaneous activity, and hair cell loss after acoustic trauma. *J Comp Neurol* (2011) 519:2637–47. doi:10.1002/cne.22644
45. Sahani M. *Latent Variable Models for Neural Data Analysis*. Pasadena, CA: California Institute of Technology (1999).
46. Schaette R, Kempter R. Development of tinnitus-related neuronal hyperactivity through homeostatic plasticity after hearing loss: a computational model. *Eur J Neurosci* (2006) 23:3124–38. doi:10.1111/j.1460-9568.2006.04774.x
47. Schaette R, Kempter R. Development of hyperactivity after hearing loss in a computational model of the dorsal cochlear nucleus depends on neuron response type. *Hear Res* (2008) 240:57–72. doi:10.1016/j.heares.2008.02.006
48. Young ED, Davis KA. Circuitry and function of the dorsal cochlear nucleus. In: Oertel D, Fay RR, Popper AN, editors. *Integrative Functions in the Mammalian Auditory Pathway*. New York: Springer (2002). p. 121–57.
49. Manzoor NF, Gao Y, Licari F, Kaltenbach JA. Comparison and contrast of noise-induced hyperactivity in the dorsal cochlear nucleus and inferior colliculus. *Hear Res* (2013) 295:114–23. doi:10.1016/j.heares.2012.04.003
50. Bourien J, Tang Y, Batrel C, Huet A, Lenoir M, Ladrech S, et al. Contribution of auditory nerve fibers to compound action potential of the auditory nerve. *J Neurophysiol* (2014) 112:1025–39. doi:10.1152/jn.00738.2013
51. Schaette R, Turtle C, Munro KJ. Reversible induction of phantom auditory sensations through simulated unilateral hearing loss. *PLoS One* (2012) 7(6):e35238. doi:10.1371/journal.pone.0035238
52. Schaette R. Computational modeling of tinnitus development. *J Acoust Soc Am* (2013) 133:3560–3560. doi:10.1121/1.4806483
53. Schaette R, Kempter R. Predicting tinnitus pitch from patients' audiograms with a computational model for the development of neuronal hyperactivity. *J Neurophysiol* (2009) 101:3042–52. doi:10.1152/jn.91256.2008
54. Norena AJ. An integrative model of tinnitus based on a central gain controlling neural sensitivity. *Neurosci Biobehav Rev* (2011) 35:1089–109. doi:10.1016/j.neubiorev.2010.11.003
55. Hickox AE, Liberman MC. Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus? *J Neurophysiol* (2014) 111:552–64. doi:10.1152/jn.00184.2013
56. Rüttiger L, Singer W, Panford-Walsh R, Matsumoto M, Lee SC, Zuccotti A, et al. The reduced cochlear output and the failure to adapt the central auditory response causes tinnitus in noise exposed rats. *PLoS One* (2013) 8:e57247. doi:10.1371/journal.pone.0057247
57. Dehmel S, Pradhan S, Koehler S, Bledsoe S, Shore S. Noise overexposure alters long-term somatosensory-auditory processing in the dorsal cochlear nucleus – possible basis for tinnitus-related hyperactivity? *J Neurosci* (2012) 32:1660–71. doi:10.1523/JNEUROSCI.4608-11.2012
58. Liberman LD, Suzuki J, Liberman MC. Dynamics of cochlear synaptopathy after acoustic overexposure. *J Assoc Res Otolaryngol* (2015) 16:205–19. doi:10.1007/s10162-015-0510-3
59. Gerken GM. Central tinnitus and lateral inhibition: an auditory brainstem model. *Hear Res* (1996) 97:75–83. doi:10.1016/S0378-5955(96)80009-8
60. Kral A, Majernik V. On lateral inhibition in the auditory system. *Gen Physiol Biophys* (1996) 15:109–27.
61. Parra LC, Pearlmutter BA. Illusory percepts from auditory adaptation. *J Acoust Soc Am* (2007) 121:1632–41. doi:10.1121/1.2431346
62. Pienkowski M, Eggermont JJ. Reversible long-term changes in auditory processing in mature auditory cortex in the absence of hearing loss induced by passive, moderate-level sound exposure. *Ear Hear* (2012) 33:305–14. doi:10.1097/AUD.0b013e318241e880
63. Manzoor N, Licari FG, Klapchar M, Elkin R, Gao Y, Kaltenbach JA. Noise-induced hyperactivity in the inferior colliculus: its relationship with hyperactivity in the dorsal cochlear nucleus. *J Neurophysiol* (2012) 108:976–88. doi:10.1152/jn.00833.2011
64. Brozoski TJ, Bauer CA, Caspary DM. Elevated fusiform cell activity in the dorsal cochlear nucleus of chinchillas with psychophysical evidence of tinnitus. *J Neurosci* (2002) 22:2383–90.
65. Chen GD, Fechter LD. The relationship between noise-induced hearing loss and hair cell loss in rats. *Hear Res* (2003) 177:81–90. doi:10.1016/S0378-5955(02)00802-X
66. Longenecker RJ, Galazyuk AV. Development of tinnitus in CBA/CaJ mice following sound exposure. *J Assoc Res Otolaryngol* (2011) 12:647–58. doi:10.1007/s10162-011-0276-1
67. Heeringa AN, van Dijk P. The dissimilar time course of temporary threshold shifts and reduction of inhibition in the inferior colliculus following intense sound exposure. *Hear Res* (2014) 312:38–47. doi:10.1016/j.heares.2014.03.004
68. Norena AJ, Gourevitch B, Aizawa N, Eggermont JJ. Spectrally enhanced acoustic environment disrupts frequency representation in cat auditory cortex. *Nat Neurosci* (2006) 9:932–9. doi:10.1038/nn0906-1193a
69. Munguia R, Pienkowski M, Eggermont JJ. Spontaneous firing rate changes in cat primary auditory cortex following long-term exposure to non-traumatic noise: tinnitus without hearing loss? *Neurosci Lett* (2013) 546:46–50. doi:10.1016/j.neulet.2013.04.048
70. Pienkowski M, Eggermont JJ. Long-term, partially-reversible reorganization of frequency tuning in mature cat primary auditory cortex can be induced by passive exposure to moderate-level sounds. *Hear Res* (2009) 257:24–40. doi:10.1016/j.heares.2009.07.011
71. Lau C, Pienkowski M, Zhang JW, McPherson B, Wu EX. Chronic exposure to broadband noise at moderate sound pressure levels spatially shifts tone-evoked responses in the rat auditory midbrain. *Neuroimage* (2015) 122:44–51. doi:10.1016/j.neuroimage.2015.07.065
72. Zeng C, Nannapaneni N, Zhou J, Hughes LF, Shore S. Cochlear damage changes the distribution of vesicular glutamate transporters associated with auditory and nonauditory inputs to the cochlear nucleus. *J Neurosci* (2009) 29:4210–7. doi:10.1523/JNEUROSCI.0208-09.2009
73. Zeng C, Yang Z, Shreve L, Bledsoe S, Shore S. Somatosensory projections to cochlear nucleus are upregulated after unilateral deafness. *J Neurosci* (2012) 32:15791–801. doi:10.1523/JNEUROSCI.2598-12.2012
74. McAlpine D, Palmer AR. Blocking GABAergic inhibition increases sensitivity to sound motion cues in the inferior colliculus. *J Neurosci* (2002) 22:1443–53.
75. Turner J, Larsen D, Hughes L, Moechars D, Shore S. Time course of tinnitus development following noise exposure in mice. *J Neurosci Res* (2012) 90:1480–8. doi:10.1002/jnr.22827
76. Nowotny M, Remus M, Kossl M, Gaese BH. Characterization of the perceived sound of trauma-induced tinnitus in gerbils. *J Acoust Soc Am* (2011) 130:2827–34. doi:10.1121/1.3646902
77. Heffner HE, Heffner RS. Behavioural tests for tinnitus in animals. In: Eggermont JJ, Zeng FG, editors. *Tinnitus (Springer Handbook of Auditory Research)*. New York, Heidelberg, Dordrecht, London: Springer (2012). p. 21–58.
78. Kiefer L, Schauen A, Abendroth S, Gaese BH, Nowotny M. Variation in acoustic overstimulation changes tinnitus characteristics. *Neuroscience* (2015) 310:176–87. doi:10.1016/j.neuroscience.2015.09.023
79. Coomber B, Berger JI, Kowalkowski VL, Shackleton TM, Palmer AR, Wallace MN. Neural changes accompanying tinnitus following unilateral acoustic trauma in the guinea pig. *Eur J Neurosci* (2014) 40:2427–41. doi:10.1111/ejn.12580
80. Heller MF, Bergman M. Tinnitus aurium in normally hearing persons. *Ann Otol Rhinol Laryngol* (1953) 62:73–83. doi:10.1177/000348945306200107
81. Del Bo L, Forti S, Ambrosetti U, Costanzo S, Mauro D, Ugazio G, et al. Tinnitus aurium in persons with normal hearing: 55 years later. *Otolaryngol Head Neck Surg* (2008) 139:391–4. doi:10.1016/j.otohns.2008.06.019
82. Mulders WH, Robertson D. Development of hyperactivity after acoustic trauma in the guinea pig inferior colliculus. *Hear Res* (2013) 298:104–8. doi:10.1016/j.heares.2012.12.008

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Reactive Neurogenesis and Down-Regulation of the Potassium-Chloride Cotransporter KCC2 in the Cochlear Nuclei after Cochlear Deafferentation

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While many studies have been devoted to investigating the homeostatic plasticity triggered by cochlear hearing loss, the cellular and molecular mechanisms involved in these central changes remain elusive. In the present study, we investigated the possibility of reactive neurogenesis after unilateral cochlear nerve section in the cochlear nucleus (CN) of cats. We found a strong cell proliferation in all the CN sub-divisions ipsilateral to the lesion. Most of the newly generated cells survive up to 1 month after cochlear deafferentation in all cochlear nuclei (except the dorsal CN) and give rise to a variety of cell types, i.e., microglial cells, astrocytes, and neurons. Interestingly, many of the newborn neurons had an inhibitory (GABAergic) phenotype. This result is intriguing since sensory deafferentation is usually accompanied by enhanced excitation, consistent with a reduction in central inhibition. The membrane potential effect of GABA depends, however, on the intra-cellular chloride concentration, which is maintained at low levels in adults by the potassium chloride co-transporter KCC2. The KCC2 density on the plasma membrane of neurons was then assessed after cochlear deafferentation in the cochlear nuclei ipsilateral and contralateral to the lesion. Cochlear deafferentation is accompanied by a strong down-regulation of KCC2 ipsilateral to the lesion at 3 and 30 days post-lesion. This study suggests that reactive neurogenesis and down-regulation of KCC2 is part of the vast repertoire involved in homeostatic plasticity triggered by hearing loss. These central changes may also play a role in the generation of tinnitus and hyperacusis.

Keywords: neurogenesis, KCC2 transporter, hearing loss, sensorineural, tinnitus and hyperacousis, homeostatic plasticity

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INTRODUCTION

It has been suggested that homeostatic regulation applies to the averaged neural activity as many neural processes, such as long-term potentiation and depression, can produce unstable activity, i.e., runaway activity or no activity at all, respectively (Davis and Goodman, 1998; Turrigiano and Nelson, 1998; Turrigiano et al., 1998). Changes compatible with homeostatic regulation of neural activity have been reported throughout the central nervous system, including the sensory

systems (Turrigiano, 1999; Turrigiano and Nelson, 2004; Watt and Desai, 2010), and after various manipulations or lesions. In the auditory modality, an enriched acoustic environment provided for a few weeks has been shown to reduce cortical excitability within the frequency region stimulated by the acoustic environment (Noreña et al., 2006; Pienkowski et al., 2011, 2013). On the other hand, moderate to profound hearing loss is accompanied by an increase of spontaneous and stimulus-induced activity at many levels of the central auditory system, from the cochlear nucleus (CN) up to the auditory cortex (Kaltenbach et al., 2000; Noreña et al., 2003; Sumner et al., 2005; Mulders and Robertson, 2009; Kalappa et al., 2014).

The repertoire of cellular and molecular mechanisms involved in homeostatic plasticity is considerable (Burrone and Murthy, 2003; Turrigiano and Nelson, 2004; Davis, 2006; Turrigiano, 2008; Watt and Desai, 2010). Among many other mechanisms, it has been shown that the number of post-synaptic receptors and voltage-gated channels at the level of the axon initial segment are precisely regulated (Turrigiano, 1999; Grubb and Burrone, 2010; Kuba et al., 2010). It is also well known that the balance between excitation and inhibition is finely adjusted (Rutherford et al., 1998; Kilman et al., 2002). Moreover, reactive neurogenesis has been demonstrated in the vestibular nuclei after a unilateral vestibular nerve section. Interestingly, many of the newborn neurons following the deafferentation were of GABAergic phenotype (Tighilet et al., 2007; Dutheil et al., 2013). At first sight, this result is surprising as sensory deafferentation is usually followed by neural hyperexcitability (Noreña et al., 2003; Sumner et al., 2005; Mulders and Robertson, 2009; Kalappa et al., 2014), consistent with a reduction of inhibitory neurotransmission (Suneja et al., 1998; Milbrandt et al., 2000; Argence et al., 2006). Importantly, however, the polarity of GABA action on membrane potential is regulated in adults by KCC2 co-transporters which maintain intra-cellular concentration of chloride ion at low levels (Payne et al., 2003; De Koninck, 2007). As a consequence, a down-regulation of KCC2 after various manipulations have been shown to make the polarity of GABA depolarizing (Coull et al., 2003; Boulenguez et al., 2010).

By definition, homeostatic plasticity in the nervous system is considered to be adaptive. In some cases, however, and for reasons that are still unclear, homeostatic plasticity may be accompanied by collateral effects that can lead to neurological diseases (Schaette and Kempter, 2006; Wondolowski and Dickman, 2013; Kotas and Medzhitov, 2015). In particular, a down-regulation of KCC2 may play a key role in the pathogenesis of epilepsy, neuropathic pain, and spasticity (Cohen et al., 2002; Coull et al., 2003; De Koninck, 2007; Doyon et al., 2013; Kaila et al., 2014). In this regard, investigating how the central nervous system maintains a stable averaged activity around a set point level is a fundamental question in neuroscience and also a pre-requisite to understand some pathological conditions of the central nervous system.

The aim of the present study was to further explore the cellular and molecular mechanisms of homeostatic plastic after unilateral cochlear nerve section in the first relay of the central auditory system, the CN. Tinnitus, an auditory perception not induced by an external sound, and hyperacusis, a hypersensitivity to sound, have been suggested to be a by-product of homeostatic plasticity triggered by hearing loss (Schaette and Kempter, 2006; Noreña, 2011; Noreña and Farley, 2012). In this context, a secondary goal of the present study was to gain further insight into the mechanisms of tinnitus and hyperacusis. Neural proliferation and the phenotype of the newborn neurons were studied in the different sub-divisions of the CN ipsilateral and contralateral to the lesion. Moreover, in order to investigate whether the sensory deafferentation reverses the hyperpolarizing effect of GABA on the membrane potential, the density of the co-transporter KCC2 on the neuronal plasma membrane was also assessed.

MATERIALS AND METHODS

Ethics Statement

All experiments were carried out in strict accordance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (NIH Publication n° 80–23) revised 1996 for the UK Animals (Scientific Procedures) Act 1986 and associated guidelines, or the Policy on Ethics approved by the Society for Neuroscience in November 1989, and amended in November 1993. Male European cats used in the experiments were supplied by ISOQUIMEN (Barcelona, SPAIN) and were housed in our animal housing facility (Fédération 3C, Centre Saint-Charles, Aix-Marseille University) under the veterinary and National Ethical Committee supervision (French Agriculture Ministry Authorization: B13-055-25). Every attempt was made to minimize both the number and the suffering of animals used in this experiment. We selected only the most important post-UCN time delay in light of the findings of our previous studies and in order to limit the number of cats used. Animals were housed in a large confined space with normal diurnal light variations and free access to water and food.

Surgery

Adult male cats weighing between 4 and 5 kg were anesthetized with ketamine (20 mg/kg, i.m.; Rhône-Poulenc, Mérieux, France), received an analgesic (Tolfedine, 0.5 ml, i.m.; Vetoquinol, Lure, France) and were kept at physiological body temperature using a blanket. The cochlear nerve was sectioned on the left-side at the post-ganglion level after mastoidectomy, partial destruction of the bony labyrinth, and surgical exposure of the internal auditory canal. Animals were maintained under antibiotics for 7 days and analgesics for 3 days.

Implantation and Use of Osmotic Minipumps for Drug Infusion in the Fourth Ventricle

For the implantation and use of osmotic minipumps containing cytosine-ß-D arabinofuranoside (AraC, S-phase-specific antimitotic drug, Sigma–Aldrich, Saint-Quentin Fallavier, France), Muscimol ($GABA_A$ receptor agonist, Sigma–Aldrich, Saint-Quentin Fallavier, France) or 0.9% sodium chloride (NaCl), a stainless steel cannula was implanted under anesthesia into the fourth ventricle of the brain and connected

to a subcutaneous minipump (Alzet, Alza Corporation, Palo Alto, CA, USA; flow rate 2.5 μl/h for 30 days). A midline incision was made through the skin and musculature in the back of the neck, and a cannula connected to plastic tubing was inserted between the dorsal wall of the brainstem and the ventral face of the cerebellum and then cemented with dental cement to the skull. The air in the system was removed by filling up with saline, muscimol or AraC, after which the tubing was connected to an osmotic minipump, and the skin was incised. Muscimol was diluted to the required concentration using artificial cerebrospinal fluid (124 mM NaCl, 5 mM KCl, 1.2 mM KH2PO4, and 1.3 mM MgSO4) and tested for pH and adjusted to a pH 7.0, if necessary. As detailed in the study of Gliddon et al. (2005), we chose concentrations that provided an effect on vestibular neurogenesis without adverse side effects on animals (Dutheil et al., 2013). AraC was diluted in a NaCl solution at 0.13 mM, this concentration was demonstrated to inhibit totally the mitotic activity in the deafferented vestibular nuclei without adverse side effects on animals (Dutheil et al., 2009). Cats were infused continuously into the cerebrospinal fluid of the fourth ventricle during 3 or 30 days to study the consequences of the drugs on cell proliferation, survival, and differentiation.

Study Design

A total of 24 cats were used to determine the time course of cell proliferation in the cochlear nuclei (first experimental protocol: **Figure 1**). Among these cats, 20 were subjected to a unilateral cochlear neurectomy (UCN). They were injected intraperitoneally (i.p.) with 5-bromo-2′deoxyuridine (BrdU: 200 mg/kg) 3 h before they were killed and perfused. BrdU is a thymidine analog that can be incorporated into DNA during the S phase of the cell cycle. Five post-UCN survival periods were used: 1, 3, 7, 15, and 30 days. Each of these survival groups was composed of four cats. The survival periods were selected based on our previous investigations, which had showed a high number of BrdU-immunoreactive nuclei in the deafferented vestibular nuclei with a peak at 3 days after unilateral vestibular neurectomy and a decrease at 30 days (Tighilet et al., 2007). A control group was made up of four sham-operated cats. This group was subjected to the same anesthetic procedure and surgical approach on the cochlear nerve but without sectioning the nerve. They were injected with the same amount and route of administration of BrdU 3 days after sham surgery and killed 3 h later.

To study the survival and the differentiation of the proliferating cells and to determine the effects of NaCl, muscimol or AraC infusion after UCN on the different steps of reactive neurogenesis (proliferation, survival, and differentiation) at the cellular level (second experimental protocol, **Figure 1**), we studied six groups of male adult cats: (i) UCN-NaCl groups, animals underwent UCN with continuous NaCl infusion, then received an, i.p., BrdU injection (200 mg/kg, i.p.) and were killed at either D3 when cell proliferation reached a peak (group 1, $n = 4$) or D30 to study the survival and the differentiation of the proliferating cells (group 2, $n = 4$); (ii) UCN-Muscimol groups, animals underwent UCN with continuous Muscimol infusion, then received an, i.p., BrdU injection and were killed at D3 (group 3, $n = 4$) or D30 (group 4, $n = 4$); (iii) UCN-AraC groups, animals underwent UCN with continuous AraC infusion, received an, i.p., BrdU injection and were killed at D3 (group 5, $n = 4$) or D30 (group 6, $n = 4$). The two post-deafferentation survival periods (D3 and D30) were selected on the basis of our anterior data (Tighilet et al., 2007).

Cellular Investigations

Tissue Preparation

BrdU (10 mg/ml, Sigma, Saint Quentin Fallavier, France) was dissolved in a solution of sodium chloride (NaCl) 0.9% heated to 56°C and injected into animals (200 mg/kg). It has been shown in adult rat dentate gyrus that a single dose of BrdU 100, 50, or 25 mg/kg (body weight, i.p.) labeled 60, 45, and 8% of S-phase cells, respectively (Cameron and McKay, 2001). At 300 mg/kg, BrdU labeled most S-phase cells and had no physiological side effects. So, in line with the conclusions of Taupin (2007), we considered 200 mg/kg as a saturating concentration of BrdU for studying adult neurogenesis. BrdU doses were not likely to generate side effects, but were sufficient to mark the cells in S-phase synthesizing DNA. Before BrdU administration, the cats of each group were deeply anesthetized with ketamine dihydrochloride (20 mg/kg, i.m., Merial, Lyon, France) and killed by 0.9% NaCl (1L per animal) then paraformaldehyde 4% (2L per animal) transcardiac perfusion either 3 h or 27 days later according to their experimental group. After removal from the skull, brains were cut into several blocks containing the cochlear nuclei. The blocks were rapidly frozen with dry ice and stored at −80°C. Coronal sections (40-μm-thick) were cut in a cryostat (Leica, Rueil-Malmaison, France) for immunochemistry.

Immunochemistry

Immunochemical labeling was performed according to previously validated protocols (Tighilet et al., 2007). Ki67 marker was used in addition to BrdU to confirm that BrdU had been incorporated into mitotic cells and did not correspond to dying cells or a DNA repair mechanism (Dutheil et al., 2009). For BrdU immunostaining, free-floating sections were first rinsed in 0.1 M PBS and incubated with 2N HCl and 0.5% Triton-X100 in PBS (30 min, 37°C) for DNA hydrolysis. Then sections were rinsed in 0.1 M sodium tetraborate buffer, pH 8.5 before overnight incubation with the primary antibody at 4°C, followed by incubation with the secondary antibody for 1.5 h at room temperature, and visualized using horseradish peroxidase avidin-D (Vector). GFAP and GAD67 immunoreactivity assays were also performed (Tighilet et al., 2007). After several rinses, sections were mounted on gelatin-coated slides, dehydrated, and cover-slipped in Depex mounting medium for peroxidase staining. The differentiation of the newly generated cells was analyzed in the group of cats injected with BrdU 3 days after UCN and killed after 1 month. We used double immunofluorescent stained sections incubated with BrdU and one of four antibodies: NeuN, a post-mitotic neuronal nuclei marker expressed in most neurons; the glial fibrillary acidic protein (GFAP), a specific type of intermediate filament protein used as astrocyte marker; IBA1, a ionized calcium binding adapter molecule 1, specific to microglia and macrophages but not cross-reactive with neurons and astrocytes; and GAD 67, the enzyme that

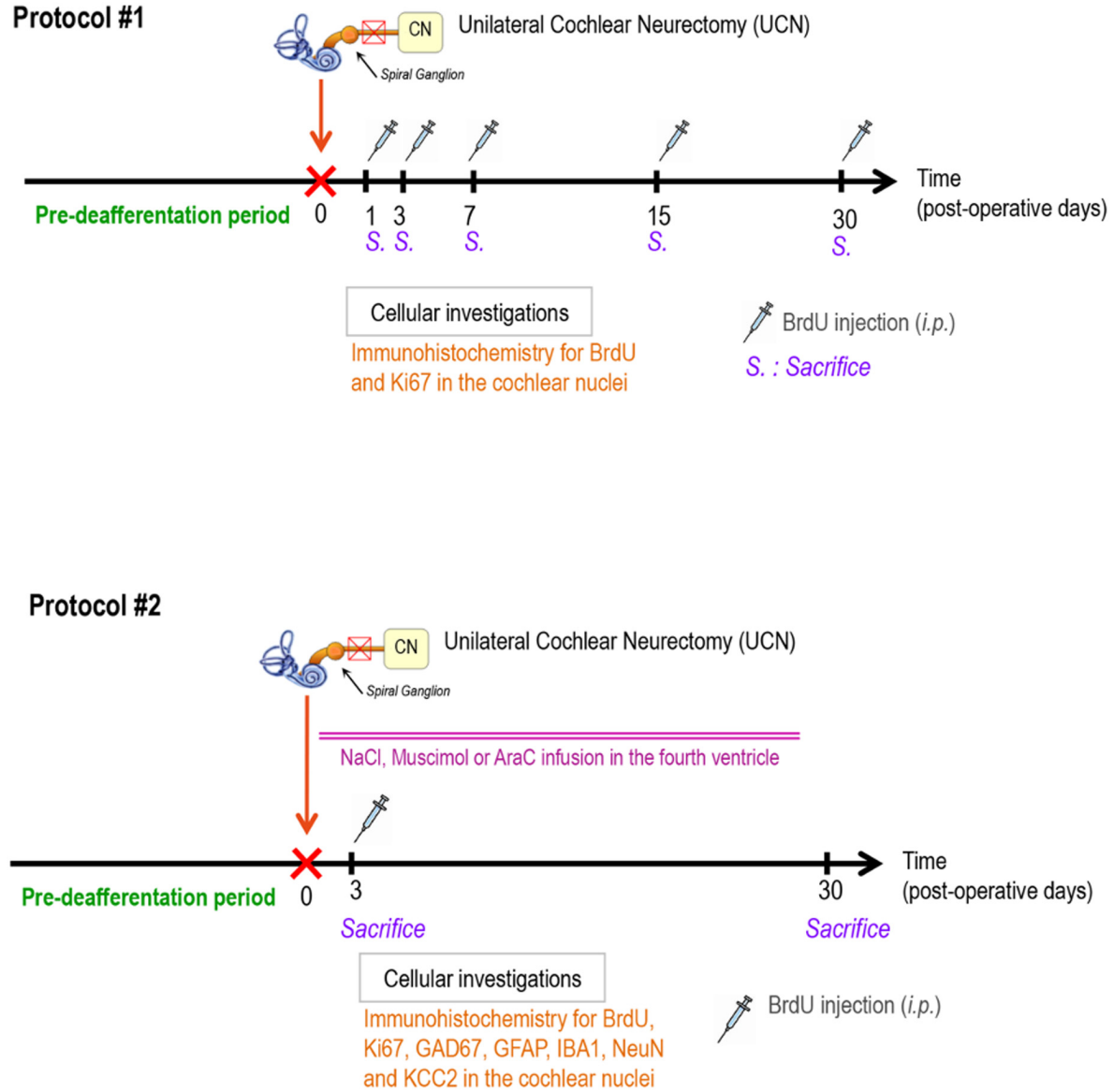

FIGURE 1 | Study designs. The first protocol (#1) was elaborated using BrdU and Ki67 as cell proliferation markers to study the time-dependent effect of a UCN on reactive cell proliferation in cochlear nuclei. Protocol number 2 (#2) was designed using a continuous infusion of NaCl, AraC, or Muscimol in the fourth ventricle to examine alterations in newborn cell survival (BrdU marker), astrogenesis (GFAP marker), GABA cells (GAD67 marker), neuronal cells (NeuN marker), and microglial cells (IBA1 marker) in the cochlear nuclei. BrdU, 5-bromo-2′deoxyuridine; i.p., intraperitoneal injection; GAD67, glutamic acid decarboxylase; GFAP, glial fibrillary acidic protein; CN, cochlear nuclei; $n = 4$ animals per group.

catalyzes the decarboxylation of glutamate to GABA and expressed in GABAergic neurons. Each antibody was processed sequentially, the differentiation marker detection first and then the BrdU labeling. For fluorescent labeling, sections were incubated with a secondary antibody cover-slipped in Mowiol. Differentiation of the newly generated cells was analyzed with double-labeling analysis performed using confocal imaging with a Zeiss LM 710 NLO laser scanning microscope equipped with a 63x/1.32 NA oil immersion lens. The fields of view were then examined by confocal microscopy, and 1-μm-step Z series were obtained.

Immunochemical labeling for KCC2 was performed according to previously validated protocols (Boulenguez et al., 2010; Bos et al., 2013). We incubated sections overnight at 22°C in a mixture of affinity-purified rabbit KCC2-specific polyclonal antibody (1:200; Millipore). We then revealed the labeling with a mixture of donkey Cy3-conjugated rabbit-specific antibody (1:500, Jackson Immunoresearch), and mounted coverslips with a gelatinous aqueous medium. We analyzed the patterns of immunolabeling by means of a laser scanning confocal microscope (Zeiss LSM 710 META) at high magnification (Plan Apochromat 63x 1.4 (N.A) oil immersion objective).

Negative Control and Antibody Specificity

We performed control experiments consisting in omitting successively one of the primary antibodies resulting in a complete absence of cross-reactivity.

Cell Counts and Statistical Analysis

Cell counts were performed according to a previously validated protocol (Dutheil et al., 2009, 2011). Great care was taken not to count blood cells as $BrdU^+$ cells. The cochlear nuclei were identified according to Berman's stereotaxic atlas (Berman, 1968). $BrdU^+$, $Ki67^+$, $GFAP^+$, and $GAD67^+$ were quantified for each subdivision of the CN, namely dorsal, posteroventral, anteroventral, and cochlear granular cell layer (DCN, PVCN, AVCN, and CGL, respectively). $BrdU^+$ cells, $Ki67^+$cells, $GFAP^+$ cells, and $GAD67^+$ neurons were analyzed in each CN on both sides (left/right in sham-operated cats and ipsilateral/contralateral to the lesion in UCN-lesioned cats). While it is usually straightforward to distinguish large- and middle-sized neurons from glial cells, the distinction between small neurons and large glial cells can be challenging. Thus, the following criteria were used as characteristic to distinguish small GAD 67^+ neurons from $GFAP^+$ glial cells at D30: a centrally located nucleolus, a distinctive nucleus, visible cytoplasm, presence of dendritic processes, and larger cell body size. Hence, glial cells were identified by sparse cytoplasm and smaller cell body size (Christensen et al., 2007). The cell count was done with a Nikon microscope (Eclipse 80 i) equipped with a motorized X-Y-Z sensitive stage and a video camera connected to a computerized image analysis system (Mercator; Explora Nova, La Rochelle, France). The total number of immunolabeled cells was estimated using the optical fractionator method (West et al., 1991). $BrdU^+$, $Ki67^+$, $GFAP^+$, $NeuN^+$, $IBA1^+$, and $GAD67^+$ were counted and sampled according to the so-called fractionator principles, that is, a combination of the optical dissector, a three-dimensional probe used for counting, and fractionator sampling, a scheme involving the probing of a known fraction of the tissue (West, 1993). This cell counting method has been described and validated in previous publications (Dutheil et al., 2009, 2011). Accordingly, the statistical analyses were evaluated by ANOVA to test the effects of the group (UCN-NaCl, -Musimol or -AraC), the CN sub-division (DCN, PVCN, AVCN, and CGL), and the post-operative time on Ki 67^+, $BrdU^+$, $GFAP^+$, $KCC2^+$, and $GAD67^+$ cells and to determine whether there were any interactions between these variables. ANOVA was followed by *post hoc* analysis with the Scheffé test. Differences between sub-divisions of CN were considered statistically significant when $p < 0.05$ (StatView II, SAS software Inc., Cary, NC, USA).

Quantification of KCC2 Immunohistological Labeling

Double fluorescent labelings were captured using frame-channel mode to avoid any cross talk between the channels. Each optical section resulted from two scanning averages. Excitation of the fluorochromes was performed with an argon ion laser set at 488 nm, and a helium/neon laser set at 575 nm. At high magnification, we only scanned the ventral CN (PVCN and AVCN regions), which exhibited reactive neurogenesis. We digitized stacks of 1 μm-thick optical sections.

A custom program written in Matlab® (The Mathworks, Inc.) was developed to analyze fluorescence at the plasma membrane of neurons. The background or non-specific immunofluorescence, was assessed by calculating the average fluorescence in a visually selected area devoid of neurons or any other stained structures. From this region, we then derived a threshold equal to the average immunofluorescence plus three times the standard deviation. All data were then subtracted from this threshold value and only positive values were conserved for further analysis. A region of interest was then drawn around the neuronal plasma membrane of each cell body. The program calculated the average fluorescence within the region of interest over data that were 20% above the maximum values. This thresholding insured that all pixels taken for calculating the average was part of the plasma membrane and that the same criterion was used for all slices in all conditions (otherwise, the averaged fluorescence can depend on how large the region of interest was manually drawn around the plasma membrane).

Also, to avoid any biases at the stage of extracting the averaged fluorescence level in each condition, the experimenter in charge of the analysis (M.I.S.) ignored the conditions corresponding to the slices. We used the non-parametric Mann–Whitney test to compare control and lesioned animals, and the lesioned and intact side at both 3 and 30 days post-lesion in lesioned animals. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Time Course of BrdU and Ki67 Immunoreactivity in Cochlear Nuclei

In sham-operated cats, no BrdU-Ir nuclei were detected in the CN. The regions of interest and the lesion-induced changes in BrdU-Ir within the CN are illustrated in **Figures 2A,B**. In the UCN group of cats, BrdU-Ir cells were exclusively restricted to the deafferented CN. The onset of cell proliferation began 1 day after the cochlear nerve section, peaked at 3 days and then decreased to reach control values at 30 days post-UCN. The quantitative analysis showed a significantly increased number of BrdU-Ir nuclei by the first day and peaked at 3 days after UCN for the four main CN sub-divisions (+1382% in the CGL, +2140.93% in the AVCN, +2252.07% in the PVCN, and +2667.03% in the DCN compared to controls, $P < 0.0001$). Repeated measures analysis of variance of the data are given in **Table 1**. They indicate that the number of BrdU immunoreactive cells differed significantly with regards to group ($P < 0.0001$), side ($P < 0.0001$), and CN ($P < 0.0001$). The interaction between these three factors (group, side, and CN) was also highly significant ($P < 0.0001$) (**Table 1**). Treatment with Muscimol significantly enhanced the number of BrdU-Ir cells at day 3 post-lesion in all subdivisions of cochlear nuclei compared with the UCN-NaCl group (+125% in the CGL, +125% in the AVCN, +117% in the PVCN, and +112% in the DCN compared to UCN-NaCl group, $P < 0.0001$).

The time course of Ki67 immunoreactivity was similar to that observed for BrdU immunoreactivity (**Figure 3** and **Table 2**).

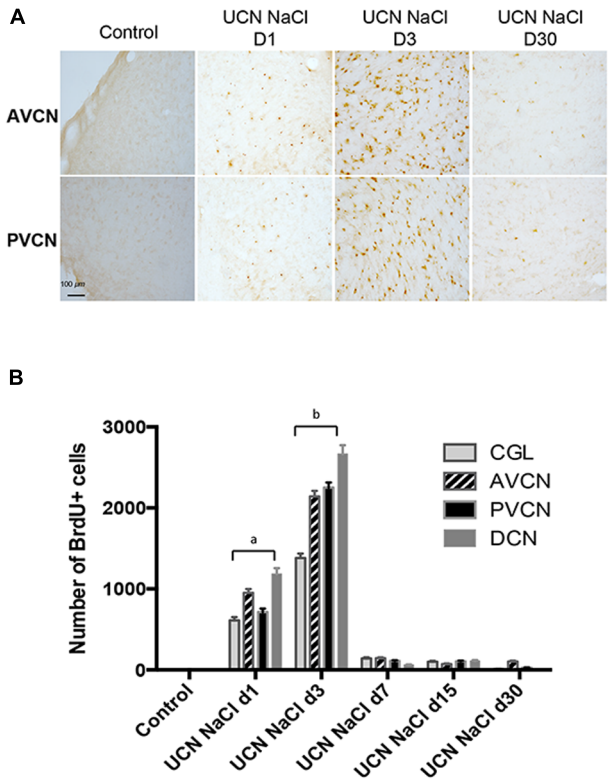

FIGURE 2 | Reactive cell proliferation occurs after unilateral cochlear neurectomy (UCN) in a time-sensitive manner. (A) Photomicrographs showing 5-bromo-2′-deoxyuridine (BrdU) immunostainings in the deafferented cat AVCN and PVCN submitted to UCN and analyzed at post-lesion days 1, 3, and 30 (D1, D3, and D30, respectively). Animals received a BrdU injection (i.p., 200 mg/kg body weight) 3 h before sacrifice. Scale bar: 100 μm for lower magnification. **(B)** Quantitative evaluation. Histograms comparing the mean values (±Standard Error of the Mean, SEM) of the number of BrdU-immunopositive cells in the deafferented CN at D1, D3, and D30 after UCN. Different letters indicate significant differences between groups of animals and assessed by ANOVA followed by the Scheffé test, $p < 0.0001$, see **Table 1**. a: significantly different from control, UCN d3, UCN d7, UCN d15, and UCN d30 groups. b: significantly different from control, UCN d1, UCN d7, UCN d15, and UCN d30 groups. Only values recorded on the lesioned side are illustrated. $n = 4$ animals per group.

TABLE 1 | Statistical analysis of the effects of unilateral cochlear neurectomy (UCN) on the time course of cell proliferation in the cochlear nuclei complex of adult cats.

Source of variation	df	*F*	*P*
Number of BrdU positive cells			
Group	5	7702.02	0.0001*
Side	1	19064.15	0.0001*
Group × side	5	7702.02	0.0001*
Cochlear nucleus	3	248.74	0.0001*
Group × Cochlear nucleus	15	146.74	0.0001*
Side × Cochlear nucleus	3	248.74	0.0001*
Group × side × Cochlear nucleus	15	146.74	0.0001*

*Repeated measures analysis of variance of the changes in the BrdU-immunoreactive cells after UCN. The main fixed effects providing the source of variation among animals were group (that is, sham versus unilateral- cochlear -lesioned cats as a function of the survival postoperative period) and side (intact versus deafferented), and cochlear nucleus (AVCN: anteroventral cochlear nucleus; PVCN: posteroventral cochlear nucleus; CDN: dorsal cochlear nucleus and CGL: cochlear granular cell layer). Degree of freedom (d.f.), Scheffe test (F), and probability level (p) are reported. *Indicates significant variations between variables and/or their interactions.*

Ki67 marker was used in addition to BrdU to confirm that BrdU had been incorporated into mitotic cells and did not correspond to dying cells or a DNA repair mechanism (Dutheil et al., 2009). In sham-operated cats, no Ki67 nuclei were detected in the CN. A strong and significant number of Ki67 immunoreactive cells were observed 3 days after cochlear nerve section in the whole CN (+10616% in the CGL, +2006% in the AVCN, +2310% in the PVCN, and +2536% in the DCN compared to controls, $P < 0.0001$). In contrast, 3 days after UCN, AraC blocked the cell proliferation in the whole CN. The number of ki67-Ir nuclei in the CN was close to zero in cats infused with the antimitotic drug immediately after UCN (UCN-AraC D3 group), thus confirming the efficacy of AraC in blocking cell proliferation. The same result was previously described in deafferented vestibular nuclei after unilateral vestibular neurectomy and AraC infusion in the adult cat (Dutheil et al., 2009). Repeated measures analysis of variance of the data are given in **Table 2**. They indicate that the number of Ki67 immunoreactive cells differed significantly with regards to group ($P < 0.0001$), side ($P < 0.0001$), and CN ($P < 0.0001$). The interaction between these three factors (group, side, and CN) was also highly significant ($P < 0.0001$). There were no Ki67-immunoreactive cells in any of the subdivisions of the CN contralateral to the lesion.

Survival of BrdU Immunoreactive Cells

To study the survival of the newly generated cells stained with BrdU at 3 days (peak of cell proliferation), the sub-groups of animals were killed 27 days after BrdU injection. We found that ∼60% of $BrdU^+$ cells survived in the UCN-NaCL group. These data indicate a loss of BrdU-Ir nuclei in the CN of cats injected at 3 days and killed 30 days after UCN compared to the group of cats injected at the same post-lesion delay and killed 3 h later. This reduction suggests that some of the cells that incorporated BrdU died. **Figure 4** shows the mean numbers of BrdU-Ir nuclei that survived in the subdivisions of CN in the different groups of cats. In the UCN-NaCl group, survival ratio was highest in the AVCN (87.3%) than in the other CN subdivisions (48.3 and 12.2% in the PVCN and the CGL, respectively) (**Table 3**). The survival ratio was very low (<2%) in the DCN (UCN-NaCl group) and in all CN subdivisions in animals treated with muscimol (UCN-muscimol group) and AraC (UCN-AraC group). Repeated measures analysis of variance of the data are given in **Table 3**. They indicate that the number of surviving BrdU immunoreactive cells differed significantly with regard to group ($P < 0.0001$), side ($P < 0.0001$), and CN ($P < 0.0001$). The interaction between these three factors (group, side, and CN) was also highly significant ($P < 0.0001$).

Phenotype of Newly Generated Cells

Cell differentiation was investigated by double immuno-histochemical labeling using BrdU combined with the four

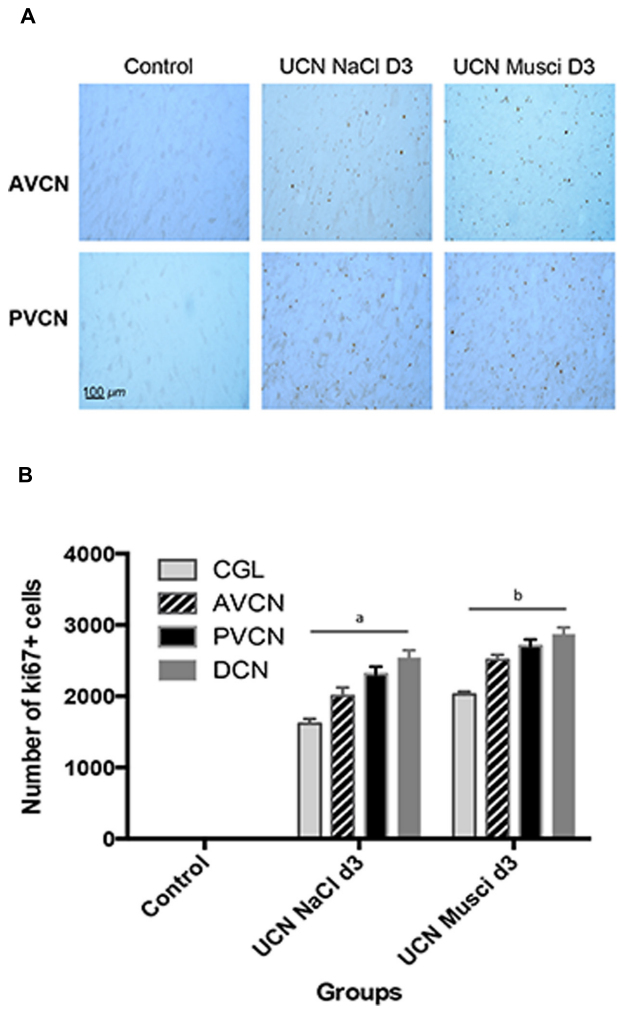

FIGURE 3 | Ki67 immunostaining was used to confirm BrdU cell proliferation. (A) Photomicrographs showing Ki67 immunostainings in the deafferented AVCN and PVCN of control cats and cats infused with NaCl or Muscimol immediately after UCN and at sacrifice (D3). Scale bar: 100 µm. **(B)** Quantitative evaluation. Histograms comparing the mean values (±SEM) of Ki67-immunopositive cells in the deafferented CN 3 days after UCN in control cats or cats submitted to NaCl or Muscimol infusion using ANOVA followed by the Scheffé test ($p < 0.0001$, see **Table 2**). Data from both sides of control animals were pooled for direct comparison with the subgroups of lesioned cats. Different letters indicate significant differences between groups of animals in each CN: a: significantly different from control and UCN Muscimol groups; b: significantly different from control and UCN NaCl groups. Only values recorded on the lesioned side are illustrated. $n = 4$ animals per group.

TABLE 2 | Statistical analysis of the effects of UCN on the cell proliferation in the cochlear nuclei complex of adult cats.

Source of variation	df	*F*	*P*
Number of Ki positive cells			
Group	2	5293.69	0.0001*
Cochlear nucleus	3	137.64	0.0001*
Group × Cochlear nucleus	6	35.39	0.0001*
Side	1	20692.91	0.0001*
Group × side	2	5293.69	0.0001*
Cochlear nucleus × side	3	137.64	0.0001*
Group × side × Cochlear nucleus	6	35.39	0.0001*

*Repeated measures analysis of variance of the changes in the Ki67-immunoreactive cells after UCN. The main fixed effects providing the source of variation among animals were group (that is, control cats or unilateral cochlear neurectomized cats infused with NaCl or Muscimol durind 3 days), side (intact versus deafferented), and cochlear nucleus (AVCN: anteroventral cochlear nucleus; PVCN: posteroventral cochlear nucleus; CDN: dorsal cochlear nucleus and CGL: cochlear granular cell layer). Degree of freedom (d.f.), Scheffe test (F), and probability level (p) are reported. *Indicates significant variations between variables and/or their interactions.*

specific cell type markers: GFAP, NeuN, IBA1, and GAD67 (see Materials and Methods). The double labeling has been quantified only at 30 days in the UCN-NaCl group since co-labeled cells were not observed at the day 3 and no BrdU immunoreactive cells survived in the UCN-AraC and UCN-muscimol groups. The photomicrographs in **Figure 5A** show the colocalization of $BrdU^+$ with $GFAP^+$, $IBA1^+$ $NeuN^+$, and GAD 67^+ cells observed in the deafferented AVCN at 30 days post-lesion in the UCN-NaCl group. The fate of the newly generated cells varied depending on the CN sub-division (**Figure 5B**). The results are expressed in percent defined as the ratio between the mean number of immunopositive-elements colocalizing a cell type marker (GFAP, IBA1, NeuN, or GAD 67) and BrdU relative to the total mean number of $BrdU^+$ nuclei counted in the areas of quantification. For the glial (GFAP and IBA1) and neuronal marker (NeuN), the newly generated cells differentiated approximately in similar proportion in the AVCN (20, 25, and 20%) and the PVCN (22, 23, and 20%). In the CGL, by contrast, GFAP and IBA1 labeling were higher (40 and 45%) than NeuN labeling (5%). The mean number of newly generated $GAD67^+$ neurons varied depending on the CN subdivision (**Figure 5B**): 16, 13, and 0% in the AVCN, PVCN, and CGL, respectively. A non-negligible percentage of cells with an undetermined phenotype was observed in all the CN subdivisions (19% in the CVA, 22% in the CVP, and 10% in the CGL).

KCC2

Simple visual inspection of examples shown in **Figures 6** and **7** suggests that KCC2 expression is dramatically downregulated in the ventral CN ipsilateral to the lesion at 3 and 30 days post-lesion. The regions of interest delineated by the rectangles in **Figure 6** are shown in **Figure 7** at a larger magnification, allowing for a better view of the cell bodies and membrane fluorescence. Contours (gray: ipsilateral to the lesion, and blue: contralateral to the lesion) drawn around the cell body membrane define the region of interest from which fluorescence level is derived. At this magnification, it is again very clear that KCC2 density at the plasma membrane is dramatically downregulated in the cochlear nuclei ipsilateral to the lesion. The distribution of membrane fluorescence is shown in the right column of **Figure 7**.

The averaged KCC2 fluorescence level for each condition (control animals: $n = 110$ cells; lesioned animals, ipsilateral to the lesion, day 3 $n = 101$ cells; lesioned animals, contralateral to the lesion, day 3 $n = 75$ cells; lesioned animals, ipsilateral to the lesion, day 30 $n = 97$ cells; lesioned animals, contralateral

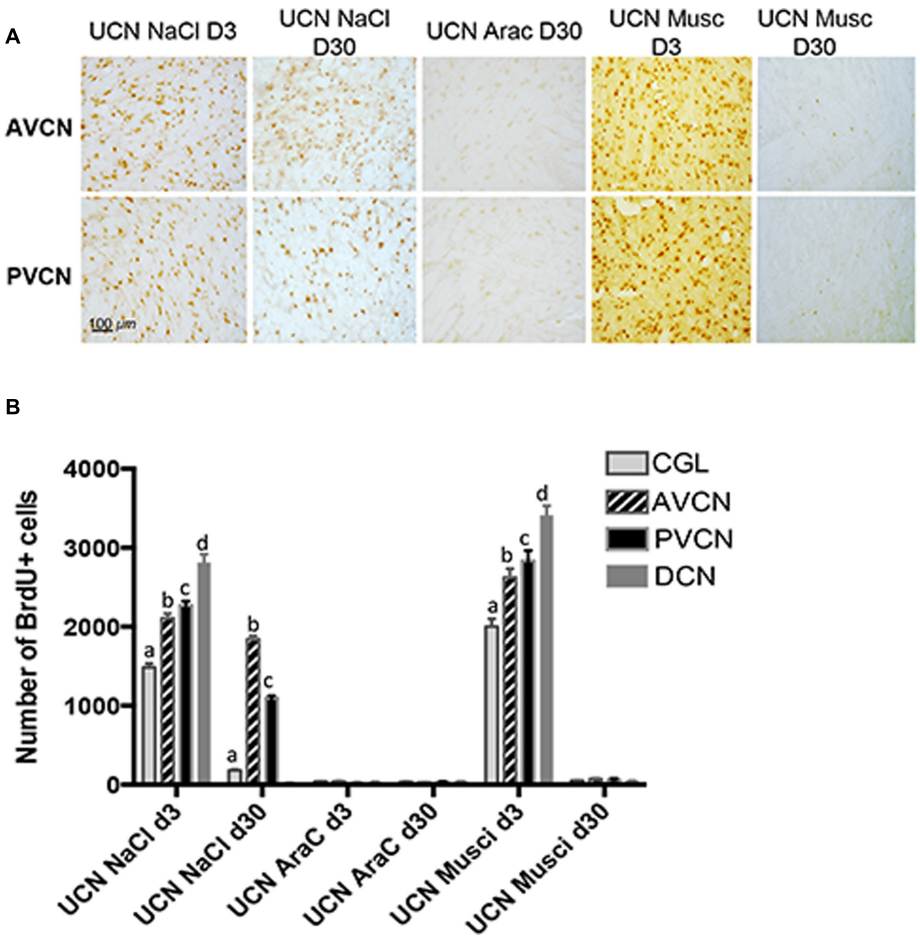

FIGURE 4 | Newborn cells generated 3 days after UCN survived up to 1 month depending on the treatment administrated. (A) Illustrations of BrdU immunoreactivity in both the deafferented AVCN and PVCN at different time points (post-lesion D3 or D30) in UCN animals treated with NaCl-, AraC-, or Muscimol-intra-cerebroventricular infusion. Note that in AraC treated animals, no BrdU+ cells were observed at D3 (data not shown) or D30. Scale bar: 100 µm and $n = 4$ animals per group. **(B)** Histograms showing the effects of drug infusion (NaCl, AraC, or Muscimol) on the number of BrdU-immunopositive cells observed in the deafferented cochlear nuclei. Only values recorded on the lesioned side are illustrated. Different letters indicate significant differences between all other groups of animals. Analyzes were assessed by ANOVA followed by the Scheffé test for all VN and groups ($p < 0.0001$, see **Table 3**).

to the lesion, day 30 $n = 85$ cells) is shown in **Figure 8**. In lesioned animals, the Mann–Whitney test revealed that KCC2-related fluorescence in the ventral CN ipsilateral to the lesion is significantly lower than fluorescence in ventral CN contralateral to the lesion at days 3 and 30 post-lesion ($p < 0.05$). The level of KCC2-related fluorescence was also significantly reduced in the CN ipsilateral to the lesion when compared to the CN in control animals ($p < 0.05$).

DISCUSSION

Cochlear Lesion-Induced Mitotic Activity in the Deafferented Cochlear Nuclei

The present study shows an intense BrdU immunolabeling after unilateral section of the cochlear nerve in the adult cat that is restricted exclusively to the cochlear nuclei ipsilateral to the lesion. The presence of Ki67-Ir cells in the CN after UCN strongly suggests that BrdU immunolabeling is related to mitotic activity instead of DNA repair or apoptotic events. Cell proliferation was observed as early as 1 day post-lesion, with a peak at 3 days, a reduction at 7 days, and a total lack of BrdU-Ir cells at 30 days. At 1 month post-lesion, many of the BrdU-Ir cells survived and differentiated giving rise to microglia, astrocytes and neurons in all CN subdivisions (except DCN). A substantial part of the newborn neurons acquired a GABAergic phenotype, especially in the AVCN.

Our study corroborates an earlier study that reported cell proliferation, survival, and differentiation of the newborn cells in the CN of rats after bilateral cochlear lesions (Zheng et al., 2011). The two studies differ, however, on several aspects. The peak of cell proliferation is delayed in our study (day 3 post-lesion) compared to that of Zheng's study (day 2 post-lesion). Moreover, the number of newborn cells observed at the peak of cell proliferation was much higher

TABLE 3 | Statistical analysis of the survival of newly generated cells by comparing cats receiving BrdU injection 3 days after UCN and perfused 3 h after (protocol 1) and those receiving BrdU at the same time but perfused 27 days after (protocol 2).

Source of variation	df	*F*	*P*
Number of BrdU surviving cells			
Group	2	6360.35	0.0001*
Cochlear nucleus	3	369.20	0.0001*
Group × Cochlear nucleus	6	205.55	0.0001*
Postoperative time	1	13207.51	0.0001*
Group × Postoperative time	2	4296.23	0.0001*
Cochlear nucleus × Postoperative time	3	435.34	0.0001*
Group × Cochlear nucleus × Postoperative time	6	214.46	0.0001*

*Repeated measures analysis of variance of the changes in the BrdU-immunoreactive cells after UCN. The main fixed effects providing the source of variation among animals were group (unilateral cochlear neurectomized cats infused with NaCl, Muscimol, or AraC), the survival postoperative (period 3 days versus 30 days), side (intact versus deafferented) and cochlear nucleus (AVCN: anteroventral cochlear nucleus; PVCN: posteroventral cochlear nucleus; CDN: dorsal cochlear nucleus and CGL: cochlear granular cell layer). Degree of freedom (d.f.), Scheffe test (F), and probability level (p) are reported. *Indicates the significant variations between variables and/or their interactions.*

in our study compared to that reported in Zheng's study. Concerning the survival ratio, the two studies cannot be compared as, unlike in Zheng's study, our study analyzed the different sub-divisions of CN separately. The type of cochlear lesion can in part account for the discrepancies between these two studies. Indeed, the cochleas are bilaterally destroyed in Zheng's study, which largely spares the cochlear nerve. In our study, on the other hand, the cochlear nerve is sectioned unilaterally. Nerve section is known to produce dramatic effects on the cellular microenvironment in the innervated tissue, including Wallerian degeneration and strong inflammatory processes (Liberge et al., 2010; Gaudet et al., 2011). This particular biochemical environment triggered by cochlear nerve section may have promoted reactive cell proliferation in the CN.

Cell proliferation, survival, and differentiation have also been observed in the vestibular nuclei after unilateral vestibular nerve section, but not after unilateral intratympanic injection of tetrodotoxin or labyrinthectomy (Tighilet et al., 2007; Dutheil et al., 2009, 2011). Labyrinthectomy in the vestibular system can be considered equivalent to cochlear destruction in the auditory system, i.e., the sensory organs are destroyed but the vestibular nerve is left intact. These results (cell proliferation and differentiation after cochlear destruction but not after labyrinthectomy) may indicate a proneness of the auditory system to reactive neurogenesis over the vestibular system. A putatively stronger inflammatory reaction in the central auditory system to a peripheral lesion may account for the differences observed between the two sensory systems (Baizer et al., 2015). Recently, cell proliferation has been reported in the cochlear nuclei after unilateral noise trauma (Zheng et al., 2015). This result is consistent with the idea that even moderate cochlear lesions

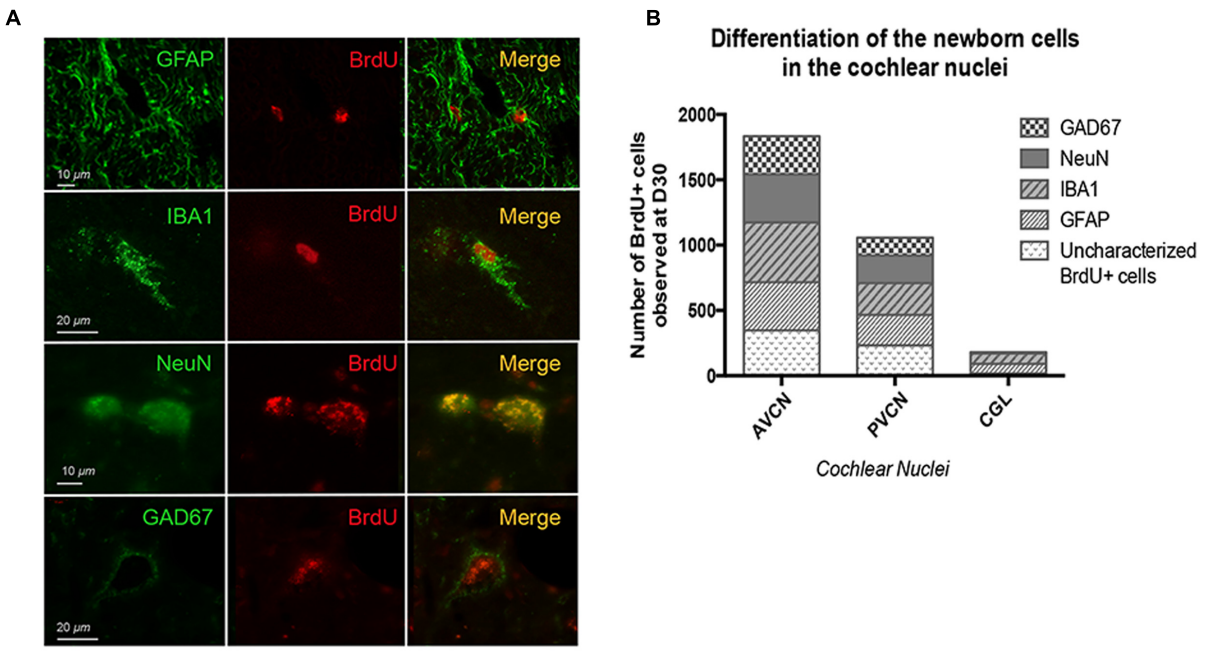

FIGURE 5 | Thirty days after UCN, glial fibrillary acidic protein (GFAP) and glutamic acid decarboxylase (GAD67) immunoreactivity are differentially expressed in the cochlear nuclei of cats submitted to a cochlear neurectomy. (A) Double immunostaining confocal analysis of differentiated newly generated cells processed on consecutive serial sections. The BrdU+ nuclei are in red and the other markers of differentiation: GFAP+, IBA1+, NeuN+, and GAD 67+ in green. Illustrations were made in the deafferented AVCN nucleus of cats 30 days after cochlear neurectomy. **(B)** Histograms illustrating the percentage of GFAP, IBA1, NeuN, and GAD67 positive cells among the BrdU positive cells observed in the different sub-divisions of CN.

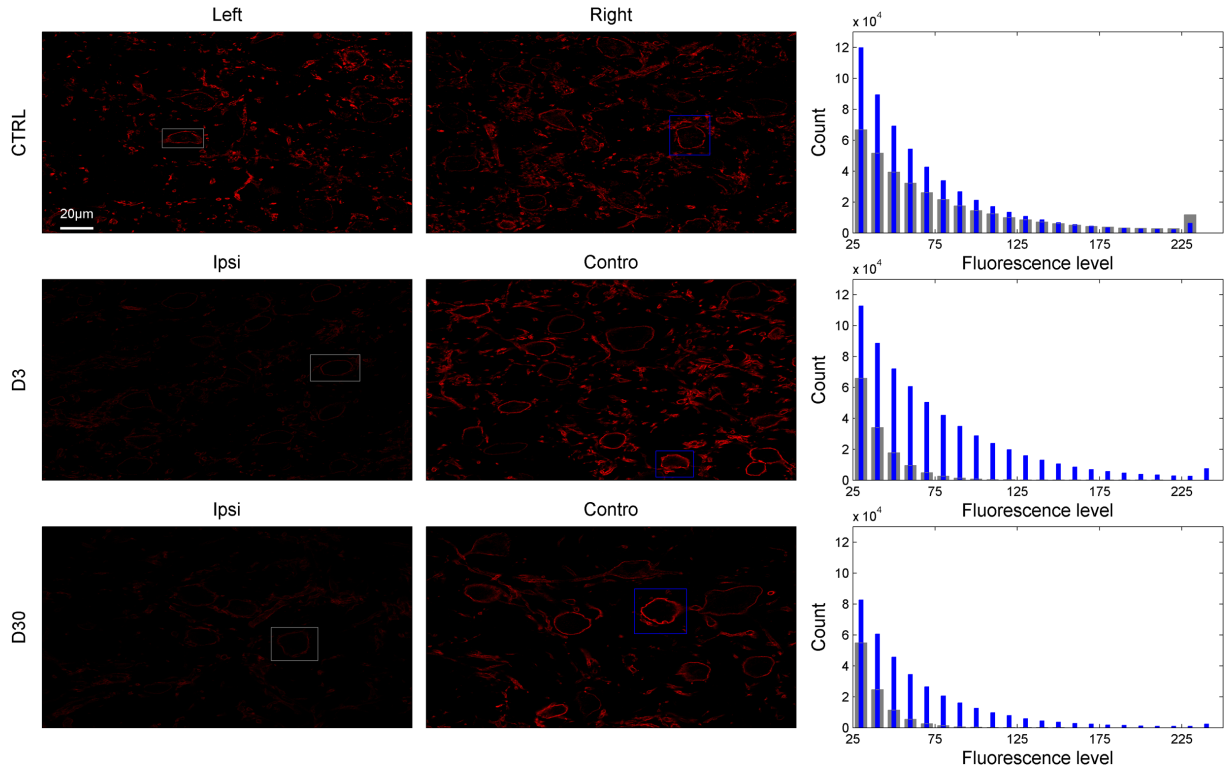

FIGURE 6 | Examples of entire slices showing KCC2-related fluorescence in control and lesioned animals (D3 and D30 post-lesion) in the left and right VCN (or VCN ipsilateral and contralateral side to the lesion; first and second column). The corresponding distribution of fluorescence from the slices is shown in the third column (gray bars: left or ipsilateral to the lesion, blue bars: right or contralateral to the lesion). The region of interests delineated by gray and blue rectangles in the first and second columns are showed in **Figure 7** at higher magnification.

may trigger central modifications that are favorable to cell proliferation.

Compared to the neurogenesis occurring spontaneously, the high percentage of cell proliferation and survival ratio reported in this and other studies can be surprising. However, the two types of cell proliferation (spontaneous and reactive) are very different. Indeed, the adult mammalian brain is considered mostly as non-neurogenic, except in the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus. It is only in pathological conditions or after severe injury that anti-neurogenic influences can be removed and that cell proliferation can occur in usually non-neurogenic regions. It is likely that the two types of cell proliferation are governed by different mechanisms. *In fine*, this could explain the high level of cell proliferation and survival observed in the deafferented cochlear nuclei after unilateral cochlear nerve section.

Regarding the origin of the reactive proliferating cells in the cochlear nuclei several hypotheses can be proposed. Either they result from a local origin, i.e., the intraparenchymental neural precursors in the cochlear nuclei (Manohar et al., 2012) or they migrate from other brain structures. In the present study, we observed cell proliferation as early as 1 day post-lesion, with a peak at 3 days. The precociousness of the cell proliferation suggests a local origin of the precursors.

While many of the newborn cells survive in the ventral CN 30 days after the cochlear nerve section, there were no surviving cells in the DCN. Moreover, the treatment with the $GABA_A$ agonist muscimol (starting with UCN and continued until sacrifice) does not prevent cell proliferation but inhibits completely survival. These results raise questions concerning the mechanisms that facilitate/prevent neuronal survival after cell proliferation. It has been proposed that the survival of newborn neurons is activity-dependent, requiring normal activation of NMDA receptors and the sub-sequent influx of calcium into the cells (Tashiro et al., 2006). However, the NMDA receptor activity should not be abnormally strong as too much intracellular calcium can cause cell death (Hardingham and Bading, 2003). The origin of intra-cellular calcium, whether it comes from intra- or extra-synaptic NMDA receptor activity, may also be important for the fate of neural cells (Hardingham and Bading, 2003). The absence of survival in the dorsal CN after cochlear nerve section may result from the relatively preserved (and maybe too large) neural activity in this nucleus. This is possibly due to non-auditory inputs (Shore and Zhou, 2006), even after cochlear destruction (Koerber et al., 1966). In the same vein, muscimol could have increased neural activity (see below the functional implications of KCC2 down-regulation) thereby leading to an excessive increase of intracellular calcium concentration and death of newborn neurons (Furukawa et al., 2000).

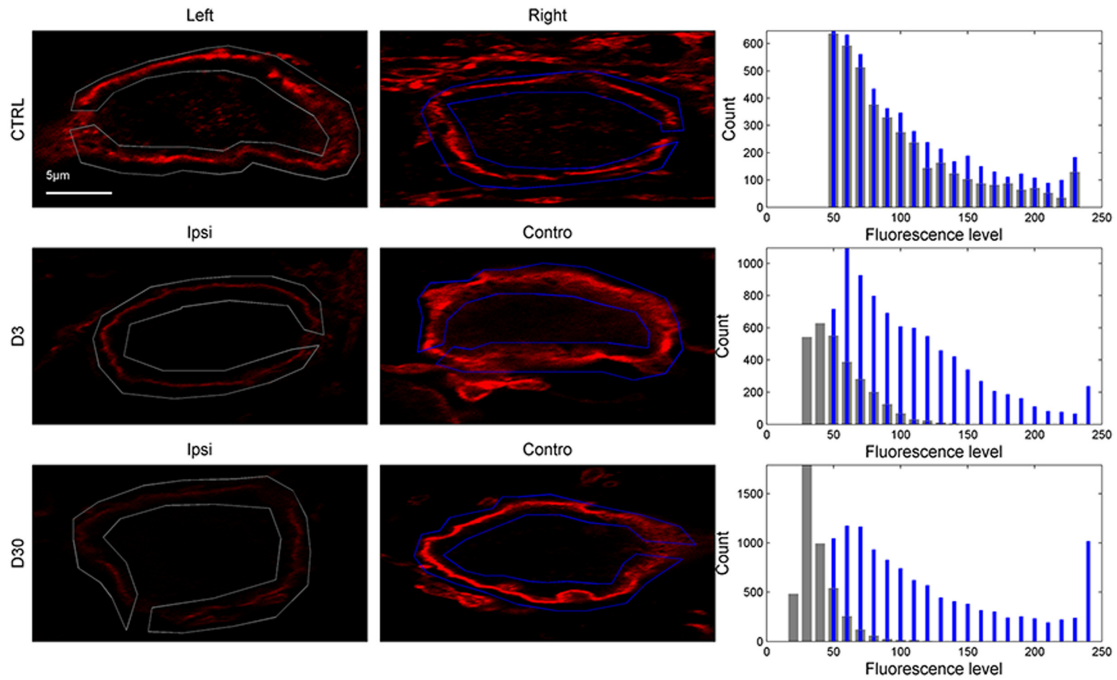

FIGURE 7 | KCC2-related fluorescence of cells bodies in control and lesioned animals from the region of interests delineated in Figure 6 (first and second column). The distribution of fluorescence levels from the region of interest drawn around the plasma membrane is shown in the third column (gray bars: left or ipsilateral to the lesion, blue bars: right or contralateral to the lesion).

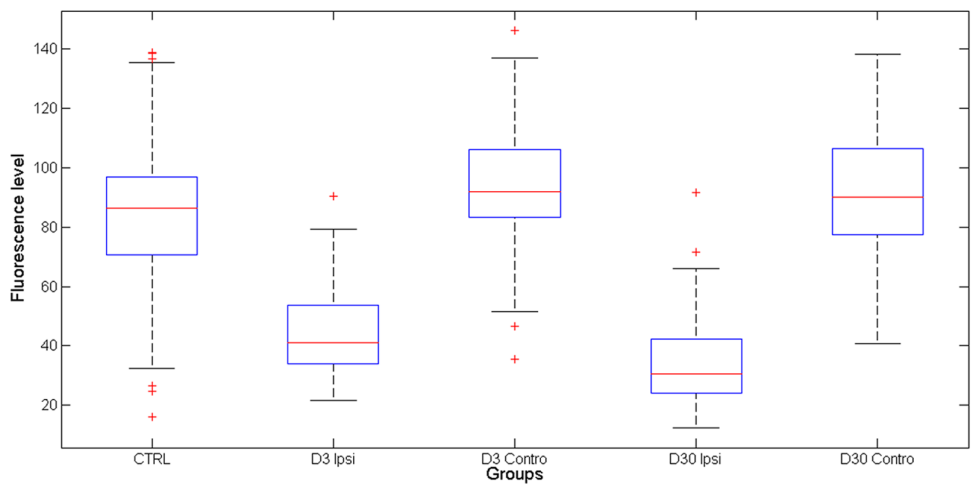

FIGURE 8 | Averaged KCC2-related fluorescence in control and lesioned (in CN ipsilateral and contralateral to the lesion) animals. KCC2 is strongly downregulated on the VCN ipsilateral to the lesion.

Deafferentation Induced a Down-Regulation of KCC2 Co-Transporters in the Ventral CN

For the first time in the auditory modality, the present study shows that KCC2 co-transporters are dramatically downregulated (by 50% on average) in the cochlear nuclei after unilateral deafferentation. This reduction in KCC2 density was observed on the membrane of the cell body as early as 3 days post-lesion and did not recover by 30 days post-lesion. Our results are in strong agreement with other studies showing a down-regulation of KCC2 after stroke or other neural injuries at different locations (spinal chord, sciatic, and vagal nerve) (Nabekura et al., 2002; Coull et al., 2003; Boulenguez et al., 2010; Jaenisch et al., 2010). In auditory modality, only one study investigated the putative changes of KCC2 in auditory centers after hearing loss.

It reported that KCC2 gene expression was unchanged after a bilateral cochlear ablation (Vale et al., 2003). Taken together, these results suggest that hearing loss can downregulate KCC2 in the plasma membrane of central neurons without changing the global expression level of these proteins.

Much effort has been devoted to understanding the regulatory mechanisms of KCC2 activity including: their transcriptional levels, their density in the neuronal plasma membrane and the fine-tuning of their intrinsic transport properties. It is known that KCC2 activity is regulated, at least in part, by phosphorylation/dephosphorylation mechanisms (Kahle and Delpire, 2016). The WNK-SPAK/OSR1 kinase complex has been suggested to play an important regulatory role on cation–chloride cotransporters. Indeed, WNKs stimulate the kinases SPAK and OSR1, which directly phosphorylate and inhibit KCC2, while stimulating sodium-driven cation–chloride cotransporters (NKCC, which enhances intra-cellular chloride level) (Alessi et al., 2014). On the contrary, the dephosphorylation of KCC2 at Thr906/Th1007 stimulates KCC2 activity by preventing the phosphorylation of WNK1-SPAK at these sites (Rinehart et al., 2009). Moreover, the phosphorylation of KCC2 at another site (Ser940) by protein kinase C has been shown to raise the membrane expression and intrinsic transport rate of KCC2 (Lee et al., 2007; Chamma et al., 2013).

The reduction of the sensory inputs and/or the modifications of the cellular micro-environment produced by unilateral nerve section have somehow to be sensed by neurons triggering a molecular cascade leading to dramatic reduction of KCC2 levels. The brain-derived neurotrophic factor (BDNF) has been suggested as playing a role in the activity-dependent regulation of KCC2 through neuronal TrKB receptors (Rivera et al., 2004). Indeed, the BDNF released by activated microglia has been shown to downregulate KCC2 (Coull et al., 2005; Ferrini and De Koninck, 2013). Microglia, activated by various molecules such as cytokine (Tsuda et al., 2009), up-regulates the expression of the purinergic receptor P2X4R, which is required for synthesizing and releasing BDNF (Ferrini and De Koninck, 2013). The expression of BDNF is also enhanced during chronic stress (Lippmann et al., 2007). In our unilateral vestibulocochlear neurectomy model, we demonstrated a long-lasting activation of the hypothalamo–pituitary–adrenal axis and an up-regulation of BDNF and its TrKB receptor in the deafferented vestibular nuclei (Tighilet et al., 2009). Similar results have been observed in the CN (data not shown). After unilateral vestibular neurectomy, we showed recently that intracerebroventricular infusion of BDNF increases dramatically cell proliferation and survival in the vestibular nuclei, whereas the antagonist of TrKB receptor K252a produced the reverse. Interestingly, K252a has been shown to upregulate the KCC2 co-transporters (Dutheil et al., 2016). Further studies are needed to investigate whether BDNF has the same effects in the cochlear nuclei after cochlear nerve section.

Functional Implications of Cell Proliferation and KCC2 Down-Regulation

Previous work in our laboratory described the occurrence of reactive neurogenesis after unilateral vestibular nerve section (Tighilet et al., 2007; Dutheil et al., 2009, 2011). Interestingly, this reactive neurogenesis played a critical role in restoring the vestibular function (Dutheil et al., 2009, 2011). This result suggests that newborn neurons can be integrated in a functional neural network and play an adaptive role in the vestibular recovery after peripheral damage. Newborn neurons could have contributed to restore a normal neuronal activity on the lesioned side (Zennou-Azogui et al., 1993; Ris et al., 1995). In the auditory modality, many studies showed that cochlear lesions are followed by neural hyperactivity in the auditory centers (Kaltenbach et al., 2000; Noreña and Eggermont, 2003; Noreña et al., 2003; Cai et al., 2009; Mulders and Robertson, 2009; Kalappa et al., 2014). For example, neural activity estimated from 2-deoxyglucose was reduced 2 h post cochlear nerve section but was restored (and even potentially enhanced) a few weeks after the lesion (Sasaki et al., 1980). In conclusion, this study and others suggest that reactive neurogenesis may be part of the homeostatic mechanism repertoire rapidly triggered after sensory deafferentation to restore a normal averaged activity in sensory centers.

In the general context of the many resources and energy deployed by the auditory system to restore neural activity after deafferentation (Noreña, 2011), the differentiation of a substantial amount of newborn neurons into the GABAergic phenotype (i.e., inhibitory) can appear contradictory. However, this contradiction is only apparent. Indeed, the action of GABA on the membrane potential depends on the intra-cellular concentration of chloride ion which is maintained at low levels in adults by KCC2 co-transporters. By showing that KCC2 co-transporters are strongly downregulated after cochlear deafferentation, the present study suggests that the polarity of GABA on membrane potential became depolarizing (Coull et al., 2003; Boulenguez et al., 2010). *In fine*, this result implies that GABAergic neurons may contribute to restore normal neural activity after sensory deafferentation.

Finally, while the down-regulation of KCC2 in adults after nerve injury, ischemia, inflammation, or deafferentation can be adaptive in most cases, it may sometimes come at a price, i.e., it can be accompanied by abnormal neural activity resulting in various diseases, namely autism, neuropathic pain, spasticity, and epilepsy (Coull et al., 2003; Boulenguez et al., 2010; Jaenisch et al., 2010; Toda et al., 2014; Sivakumaran et al., 2015). In the auditory modality, tinnitus and hyperacusis have been suggested to be a by-product of homeostatic mechanisms triggered by hearing loss (Schaette and Kempter, 2006; Noreña, 2011). Tinnitus is largely prevalent in the general population and even more so in subjects with profound hearing loss, including subjects who underwent cochlear nerve section surgery (Quaranta et al., 2004; Baguley et al., 2006; Baguley and Atlas, 2007; McCormack et al., 2014). Regarding the possible contribution of KCC2 down-regulation in some neurological diseases, including neuropathic pain [which is often compared to tinnitus – (Møller, 2007)], and the dramatic decrease of KCC2 after cochlear nerve section (this study), it is tempting to propose that down-regulation of KCC2 after hearing loss may play a role in the generation of tinnitus and hyperacusis.

Clinical Relevance

The present study shows that KCC2 is downregulated after hearing loss suggesting that GABA can become excitatory. This result is very important as it implies that GABA agonists are not necessarily adapted to treat neurological disorders associated to neural hyperactivity, including tinnitus and hyperacusis. Moreover, the putative down-regulation of KCC2 after hearing loss may account for the mixed effects of GABA agonist on tinnitus (Johnson et al., 1993; Jalali et al., 2009; Aazh et al., 2011). In this context, KCC2 may represent a much more promising pharmacotherapeutic target for treating tinnitus and hyperacusis. This approach is being developed for treating neurological diseases such as chronic pain (Doyon et al., 2013; Gagnon et al., 2013; Kahle and Delpire, 2016).

Also, it is likely that the inflammatory processes in the cochlear nuclei triggered by cochlear nerve section contribute to the neural changes observed in this study. One can speculate that reducing central inflammation, and/or limiting cell proliferation and neural differentiation triggered by sensory deafferentation, may prevent the emergence of abnormal activity, and tinnitus and hyperacusis. A recent study from our group reported that an antagonist of BDNF can reduce cell proliferation and survival and up-regulate KC22 co-transporters (Dutheil et al., 2016). This suggests that antagonizing the BDNF signaling pathway after cochlear lesion may counteract the mechanisms resulting in central hyperactivity and potentially those involved in the generation of tinnitus and hyperacusis.

CONCLUSION

The present study demonstrates cell proliferation and differentiation in the cochlear nuclei after cochlear nerve section. It also shows a dramatic down-regulation of KCC2 in the CN after sensory deafferentation, suggesting that GABA became excitatory. These two mechanisms add to the vast repertoire of neural mechanisms triggered by hearing loss thought to be homeostatic regarding neural activity. However, these mechanisms may come at a price: they may be involved in the generation of tinnitus and hyperacusis.

AUTHOR CONTRIBUTIONS

BT designed, made experiments, analyzed data, and wrote paper. AN designed, analyzed data, and wrote paper. SD made experiments and analyzed data. MS analyzed data and wrote paper.

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REFERENCES

Aazh, H., El Refaie, A., and Humphriss, R. (2011). Gabapentin for tinnitus: a systematic review. *Am. J. Audiol.* 20, 151–158. doi: 10.1044/1059-0889(2011/10-0041)

Alessi, D. R., Zhang, J., Khanna, A., Hochdörfer, T., Shang, Y., and Kahle, K. T. (2014). The WNK-SPAK/OSR1 pathway: master regulator of cation-chloride cotransporters. *Sci. Signal.* 7:re3. doi: 10.1126/scisignal.2005365

Argence, M., Saez, I., Sassu, R., Vassias, I., Vidal, P. P., and de Waele, C. (2006). Modulation of inhibitory and excitatory synaptic transmission in rat inferior colliculus after unilateral cochleectomy: an in situ and immunofluorescence study. *Neuroscience* 141, 1193–1207. doi: 10.1016/j.neuroscience.2006.04.058

Baguley, D. M., and Atlas, M. D. (2007). Cochlear implants and tinnitus. *Prog. Brain Res.* 166, 347–355. doi: 10.1016/S0079-6123(07)66033-6

Baguley, D. M., Phillips, J., Humphriss, R. L., Jones, S., Axon, P. R., and Moffat, D. A. (2006). The prevalence and onset of gaze modulation of tinnitus and increased sensitivity to noise after translabyrinthine vestibular schwannoma excision. *Otol. Neurotol.* 27, 220–224. doi: 10.1097/01.mao.0000172412.87778.28

Baizer, J. S., Wong, K. M., Manohar, S., Hayes, S. H., Ding, D., Dingman, R., et al. (2015). Effects of acoustic trauma on the auditory system of the rat: the role of microglia. *Neuroscience* 303, 299–311. doi: 10.1016/j.neuroscience.2015.07.004

Berman, A. L. (1968). *The Brain Stem of the Cat: A Cytoarchitectonic Atlas with Stereotaxic Coordinates*. Madison, WI: University of Wisconsin Press.

Bos, R., Sadlaoud, K., Boulenguez, P., Buttigieg, D., Liabeuf, S., Brocard, C., et al. (2013). Activation of 5-HT2A receptors upregulates the function of the neuronal K-Cl cotransporter KCC2. *Proc. Natl. Acad. Sci. U.S.A.* 110, 348–353. doi: 10.1073/pnas.1213680110

Boulenguez, P., Liabeuf, S., Bos, R., Bras, H., Jean-Xavier, C., Brocard, C., et al. (2010). Down-regulation of the potassium-chloride cotransporter KCC2 contributes to spasticity after spinal cord injury. *Nat. Med.* 16, 302–307. doi: 10.1038/nm.2107

Burrone, J., and Murthy, V. N. (2003). Synaptic gain control and homeostasis. *Curr. Opin. Neurobiol* 13, 560–567. doi: 10.1016/j.conb.2003.09.007

Cai, S., Ma, W.-L. D., and Young, E. D. (2009). Encoding intensity in ventral cochlear nucleus following acoustic trauma: implications for loudness recruitment. *J. Assoc. Res. Otolaryngol.* 10, 5–22. doi: 10.1007/s10162-008-0142-y

Cameron, H. A., and McKay, R. D. (2001). Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *J. Comp. Neurol.* 435, 406–417. doi: 10.1002/cne.1040

Chamma, I., Heubl, M., Chevy, Q., Renner, M., Moutkine, I., Eugène, E., et al. (2013). Activity-dependent regulation of the K/Cl transporter KCC2 membrane diffusion, clustering, and function in hippocampal neurons. *J. Neurosci.* 33, 15488–15503. doi: 10.1523/JNEUROSCI.5889-12.2013

Christensen, J. R., Larsen, K. B., Lisanby, S. H., Scalia, J., Arango, V., Dwork, A. J., et al. (2007). Neocortical and hippocampal neuron and glial cell numbers in the rhesus monkey. *Anat. Rec. (Hoboken)* 290, 330–340. doi: 10.1002/ar.20504

Cohen, I., Navarro, V., Clemenceau, S., Baulac, M., and Miles, R. (2002). On the origin of interictal activity in human temporal lobe epilepsy in vitro. *Science* 298, 1418–1421. doi: 10.1126/science.1076510

Coull, J. A. M., Beggs, S., Boudreau, D., Boivin, D., Tsuda, M., Inoue, K., et al. (2005). BDNF from microglia causes the shift in neuronal anion gradient underlying neuropathic pain. *Nature* 438, 1017–1021. doi: 10.1038/nature04223

Coull, J. A. M., Boudreau, D., Bachand, K., Prescott, S. A., Nault, F., Sík, A., et al. (2003). Trans-synaptic shift in anion gradient in spinal lamina I neurons as a mechanism of neuropathic pain. *Nature* 424, 938–942. doi: 10.1038/nature01868

Davis, G. W. (2006). Homeostatic control of neural activity: from phenomenology to molecular design. *Annu. Rev. Neurosci.* 29, 307–323. doi: 10.1146/annurev.neuro.28.061604.135751

Davis, G. W., and Goodman, C. S. (1998). Synapse-specific control of synaptic efficacy at the terminals of a single neuron. *Nature* 392, 82–86. doi: 10.1038/32176

De Koninck, Y. (2007). Altered chloride homeostasis in neurological disorders: a new target. *Curr. Opin. Pharmacol.* 7, 93–99. doi: 10.1016/j.coph.2006.11.005

Doyon, N., Ferrini, F., Gagnon, M., and De Koninck, Y. (2013). Treating pathological pain: is KCC2 the key to the gate? *Expert Rev. Neurother.* 13, 469–471. doi: 10.1586/ern.13.40

Dutheil, S., Brezun, J.-M., Léonard, J., Lacour, M., and Tighilet, B. (2009). Neurogenesis and astrogenesis contribution to recovery of vestibular functions in the adult cat following unilateral vestibular neurectomy: cellular and behavioral evidence. *Neuroscience* 164, 1444–1456. doi: 10.1016/j.neuroscience.2009.09.048

Dutheil, S., Escoffier, G., Gharbi, A., Watabe, I., and Tighilet, B. (2013). GABA(A) receptor agonist and antagonist alter vestibular compensation and different steps of reactive neurogenesis in deafferented vestibular nuclei of adult cats. *J. Neurosci.* 33, 15555–15566. doi: 10.1523/JNEUROSCI.5691-12.2013

Dutheil, S., Lacour, M., and Tighilet, B. (2011). Neurogenic potential of the vestibular nuclei and behavioural recovery time course in the adult cat are governed by the nature of the vestibular damage. *PLoS ONE* 6:e22262. doi: 10.1371/journal.pone.0022262

Dutheil, S., Watabe, I., Sadlaoud, K., Tonetto, A., and Tighilet, B. (2016). BDNF signaling promotes vestibular compensation by increasing neurogenesis and remodeling the expression of potassium-chloride cotransporter KCC2 and GABAA receptor in the vestibular nuclei. *J. Neurosci.* 36, 6199–6212. doi: 10.1523/JNEUROSCI.0945-16.2016

Ferrini, F., and De Koninck, Y. (2013). Microglia control neuronal network excitability via BDNF signalling. *Neural Plast.* 2013, 429815. doi: 10.1155/2013/429815

Furukawa, Y., Okada, M., Akaike, N., Hayashi, T., and Nabekura, J. (2000). Reduction of voltage-dependent magnesium block of N-methyl-D-aspartate receptor-mediated current by in vivo axonal injury. *Neuroscience* 96, 385–392. doi: 10.1016/S0306-4522(99)00553-9

Gagnon, M., Bergeron, M. J., Lavertu, G., Castonguay, A., Tripathy, S., Bonin, R. P., et al. (2013). Chloride extrusion enhancers as novel therapeutics for neurological diseases. *Nat. Med.* 19, 1524–1528. doi: 10.1038/nm.3356

Gaudet, A. D., Popovich, P. G., and Ramer, M. S. (2011). Wallerian degeneration: gaining perspective on inflammatory events after peripheral nerve injury. *J. Neuroinflammation* 8:110. doi: 10.1186/1742-2094-8-110

Gliddon, C. M., Darlington, C. L., and Smith, P. F. (2005). Effects of chronic infusion of a GABAA receptor agonist or antagonist into the vestibular nuclear complex on vestibular compensation in the guinea pig. *J. Pharmacol. Exp. Ther.* 313, 1126–1135. doi: 10.1124/jpet.104.082172

Grubb, M. S., and Burrone, J. (2010). Activity-dependent relocation of the axon initial segment fine-tunes neuronal excitability. *Nature* 465, 1070–1074. doi: 10.1038/nature09160

Hardingham, G. E., and Bading, H. (2003). The Yin and Yang of NMDA receptor signalling. *Trends Neurosci.* 26, 81–89. doi: 10.1016/S0166-2236(02)00040-1

Jaenisch, N., Witte, O. W., and Frahm, C. (2010). Downregulation of potassium chloride cotransporter KCC2 after transient focal cerebral ischemia. *Stroke* 41, e151–e159. doi: 10.1161/STROKEAHA.109.570424

Jalali, M. M., Kousha, A., Naghavi, S. E., Soleimani, R., and Banan, R. (2009). The effects of alprazolam on tinnitus: a cross-over randomized clinical trial. *Med. Sci. Monit.* 15, I55–I60.

Johnson, R. M., Brummett, R., and Schleuning, A. (1993). Use of alprazolam for relief of tinnitus. A double-blind study. *Arch. Otolaryngol. Head Neck Surg.* 119, 842–845. doi: 10.1001/archotol.1993.01880200042006

Kahle, K. T., and Delpire, E. (2016). Kinase-KCC2 coupling: Cl- rheostasis, disease susceptibility, therapeutic target. *J. Neurophysiol.* 115, 8–18. doi: 10.1152/jn.00865.2015

Kaila, K., Price, T. J., Payne, J. A., Puskarjov, M., and Voipio, J. (2014). Cation-chloride cotransporters in neuronal development, plasticity and disease. *Nat. Rev. Neurosci.* 15, 637–654. doi: 10.1038/nrn3819

Kalappa, B. I., Brozoski, T. J., Turner, J. G., and Caspary, D. M. (2014). Single-unit hyperactivity and bursting in the auditory thalamus of awake rats directly correlates with behavioral evidence of tinnitus. *J. Physiol.* 592, 5065–5078. doi: 10.1113/jphysiol.2014.278572

Kaltenbach, J. A., Zhang, J., and Afman, C. E. (2000). Plasticity of spontaneous neural activity in the dorsal cochlear nucleus after intense sound exposure. *Hear. Res.* 147, 282–292. doi: 10.1016/S0378-5955(00)00138-6

Kilman, V., van Rossum, M. C. W., and Turrigiano, G. G. (2002). Activity deprivation reduces miniature IPSC amplitude by decreasing the number of postsynaptic GABA(A) receptors clustered at neocortical synapses. *J. Neurosci.* 22, 1328–1337.

Koerber, K. C., Pfeiffer, R. R., Warr, W. B., and Kiang, N. Y. (1966). Spontaneous spike discharges from single units in the cochlear nucleus after destruction of the cochlea. *Exp. Neurol.* 16, 119–130. doi: 10.1016/0014-4886(66)90091-4

Kotas, M. E., and Medzhitov, R. (2015). Homeostasis, inflammation, and disease susceptibility. *Cell* 160, 816–827. doi: 10.1016/j.cell.2015.02.010

Kuba, H., Oichi, Y., and Ohmori, H. (2010). Presynaptic activity regulates Na(+) channel distribution at the axon initial segment. *Nature* 465, 1075–1078. doi: 10.1038/nature09087

Lee, H. H. C., Walker, J. A., Williams, J. R., Goodier, R. J., Payne, J. A., and Moss, S. J. (2007). Direct protein kinase C-dependent phosphorylation regulates the cell surface stability and activity of the potassium chloride cotransporter KCC2. *J. Biol. Chem.* 282, 29777–29784. doi: 10.1074/jbc.M705053200

Liberge, M., Manrique, C., Bernard-Demanze, L., and Lacour, M. (2010). Changes in TNFα, NFκB and MnSOD protein in the vestibular nuclei after unilateral vestibular deafferentation. *J. Neuroinflammation* 7, 91. doi: 10.1186/1742-2094-7-91

Lippmann, M., Bress, A., Nemeroff, C. B., Plotsky, P. M., and Monteggia, L. M. (2007). Long-term behavioural and molecular alterations associated with maternal separation in rats. *Eur. J. Neurosci.* 25, 3091–3098. doi: 10.1111/j.1460-9568.2007.05522.x

Manohar, S., Paolone, N. A., Bleichfeld, M., Hayes, S. H., Salvi, R. J., and Baizer, J. S. (2012). Expression of doublecortin, a neuronal migration protein, in unipolar brush cells of the vestibulocerebellum and dorsal cochlear nucleus of the adult rat. *Neuroscience* 202, 169–183. doi: 10.1016/j.neuroscience.2011.12.013

McCormack, A., Edmondson-Jones, M., Fortnum, H., Dawes, P., Middleton, H., Munro, K. J., et al. (2014). The prevalence of tinnitus and the relationship with neuroticism in a middle-aged UK population. *J. Psychosom. Res.* 76, 56–60. doi: 10.1016/j.jpsychores.2013.08.018

Milbrandt, J. C., Holder, T. M., Wilson, M. C., Salvi, R. J., and Caspary, D. M. (2000). GAD levels and muscimol binding in rat inferior colliculus following acoustic trauma. *Hear. Res.* 147, 251–260. doi: 10.1016/S0378-5955(00)00135-0

Møller, A. R. (2007). Tinnitus and pain. *Prog. Brain Res.* 166, 47–53. doi: 10.1016/S0079-6123(07)66004-X

Mulders, W. H. A. M., and Robertson, D. (2009). Hyperactivity in the auditory midbrain after acoustic trauma: dependence on cochlear activity. *Neuroscience* 164, 733–746. doi: 10.1016/j.neuroscience.2009.08.036

Nabekura, J., Ueno, T., Okabe, A., Furuta, A., Iwaki, T., Shimizu-Okabe, C., et al. (2002). Reduction of KCC2 expression and GABAA receptor-mediated excitation after in vivo axonal injury. *J. Neurosci.* 22, 4412–4417.

Noreña, A. J. (2011). An integrative model of tinnitus based on a central gain controlling neural sensitivity. *Neurosci. Biobehav. Rev.* 35, 1089–1109. doi: 10.1016/j.neubiorev.2010.11.003

Noreña, A. J., and Eggermont, J. J. (2003). Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. *Hear. Res.* 183, 137–153. doi: 10.1016/S0378-5955(03)00225-9

Noreña, A. J., and Farley, B. J. (2012). Tinnitus-related neural activity: theories of generation, propagation, and centralization. *Hear. Res.* 295, 161–171. doi: 10.1016/j.heares.2012.09.010

Noreña, A. J., Gourévitch, B., Aizawa, N., and Eggermont, J. J. (2006). Spectrally enhanced acoustic environment disrupts frequency representation in cat auditory cortex. *Nat. Neurosci.* 9, 932–939. doi: 10.1038/nn1720

Noreña, A. J., Tomita, M., and Eggermont, J. J. (2003). Neural changes in cat auditory cortex after a transient pure-tone trauma. *J. Neurophysiol.* 90, 2387–2401. doi: 10.1152/jn.00139.2003

Payne, J. A., Rivera, C., Voipio, J., and Kaila, K. (2003). Cation-chloride co-transporters in neuronal communication, development and trauma. *Trends Neurosci.* 26, 199–206. doi: 10.1016/S0166-2236(03)00068-7

Pienkowski, M., Munguia, R., and Eggermont, J. J. (2011). Passive exposure of adult cats to bandlimited tone pip ensembles or noise leads to long-term response suppression in auditory cortex. *Hear. Res.* 277, 117–126. doi: 10.1016/j.heares.2011.02.002

Pienkowski, M., Munguia, R., and Eggermont, J. J. (2013). Effects of passive, moderate-level sound exposure on the mature auditory cortex: spectral edges, spectrotemporal density, and real-world noise. *Hear. Res.* 296, 121–130. doi: 10.1016/j.heares.2012.11.006

Quaranta, N., Wagstaff, S., and Baguley, D. M. (2004). Tinnitus and cochlear implantation. *Int. J. Audiol.* 43, 245–251. doi: 10.1080/14992020400050033

Rinehart, J., Maksimova, Y. D., Tanis, J. E., Stone, K. L., Hodson, C. A., Zhang, J., et al. (2009). Sites of regulated phosphorylation that control K-Cl cotransporter activity. *Cell* 138, 525–536. doi: 10.1016/j.cell.2009.05.031

Ris, L., de Waele, C., Serafin, M., Vidal, P. P., and Godaux, E. (1995). Neuronal activity in the ipsilateral vestibular nucleus following unilateral labyrinthectomy in the alert guinea pig. *J. Neurophysiol.* 74, 2087–2099.

Rivera, C., Voipio, J., Thomas-Crusells, J., Li, H., Emri, Z., Sipilä, S., et al. (2004). Mechanism of activity-dependent downregulation of the neuron-specific K-Cl cotransporter KCC2. *J. Neurosci.* 24, 4683–4691. doi: 10.1523/JNEUROSCI.5265-03.2004

Rutherford, L. C., Nelson, S. B., and Turrigiano, G. G. (1998). BDNF has opposite effects on the quantal amplitude of pyramidal neuron and interneuron excitatory synapses. *Neuron* 21, 521–530. doi: 10.1016/S0896-6273(00)80563-2

Sasaki, C. T., Kauer, J. S., and Babitz, L. (1980). Differential [14C]2-deoxyglucose uptake after deafferentation of the mammalian auditory pathway–a model for examining tinnitus. *Brain Res.* 194, 511–516. doi: 10.1016/0006-8993(80)91233-0

Schaette, R., and Kempter, R. (2006). Development of tinnitus-related neuronal hyperactivity through homeostatic plasticity after hearing loss: a computational model. *Eur. J. Neurosci.* 23, 3124–3138. doi: 10.1111/j.1460-9568.2006.04774.x

Shore, S. E., and Zhou, J. (2006). Somatosensory influence on the cochlear nucleus and beyond. *Hear. Res.* 21, 90–99. doi: 10.1016/j.heares.2006.01.006

Sivakumaran, S., Cardarelli, R. A., Maguire, J., Kelley, M. R., Silayeva, L., Morrow, D. H., et al. (2015). Selective inhibition of KCC2 leads to hyperexcitability and epileptiform discharges in hippocampal slices and in vivo. *J. Neurosci.* 35, 8291–8296. doi: 10.1523/JNEUROSCI.5205-14.2015

Sumner, C. J., Tucci, D. L., and Shore, S. E. (2005). Responses of ventral cochlear nucleus neurons to contralateral sound after conductive hearing loss. *J. Neurophysiol.* 94, 4234–4243. doi: 10.1152/jn.00401.2005

Suneja, S. K., Potashner, S. J., and Benson, C. G. (1998). Plastic changes in glycine and GABA release and uptake in adult brain stem auditory nuclei after unilateral middle ear ossicle removal and cochlear ablation. *Exp. Neurol.* 151, 273–288. doi: 10.1006/exnr.1998.6812

Tashiro, A., Sandler, V. M., Toni, N., Zhao, C., and Gage, F. H. (2006). NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. *Nature* 442, 929–933. doi: 10.1038/nature05028

Taupin, P. (2007). Protocols for studying adult neurogenesis: insights and recent developments. *Regen. Med.* 2, 51–62. doi: 10.2217/17460751.2.1.51

Tighilet, B., Brezun, J. M., Sylvie, G. D. D., Gaubert, C., and Lacour, M. (2007). New neurons in the vestibular nuclei complex after unilateral vestibular neurectomy in the adult cat. *Eur. J. Neurosci.* 25, 47–58. doi: 10.1111/j.1460-9568.2006.05267.x

Tighilet, B., Manrique, C., and Lacour, M. (2009). Stress axis plasticity during vestibular compensation in the adult cat. *Neuroscience* 160, 716–730. doi: 10.1016/j.neuroscience.2009.02.070

Toda, T., Ishida, K., Kiyama, H., Yamashita, T., and Lee, S. (2014). Down-regulation of KCC2 expression and phosphorylation in motoneurons, and increases the number of in primary afferent projections to motoneurons in mice with post-stroke spasticity. *PLoS ONE* 9:e114328. doi: 10.1371/journal.pone.0114328

Tsuda, M., Masuda, T., Kitano, J., Shimoyama, H., Tozaki-Saitoh, H., and Inoue, K. (2009). IFN-gamma receptor signaling mediates spinal microglia activation driving neuropathic pain. *Proc. Natl. Acad. Sci. U.S.A.* 106, 8032–8037. doi: 10.1073/pnas.0810420106

Turrigiano, G. G. (1999). Homeostatic plasticity in neuronal networks: the more things change, the more they stay the same. *Trends Neurosci.* 22, 221–227. doi: 10.1016/S0166-2236(98)01341-1

Turrigiano, G. G. (2008). The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell* 135, 422–435. doi: 10.1016/j.cell.2008.10.008

Turrigiano, G. G., Leslie, K. R., Desai, N. S., Rutherford, L. C., and Nelson, S. B. (1998). Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* 391, 892–896. doi: 10.1038/36103

Turrigiano, G. G., and Nelson, S. B. (1998). Thinking globally, acting locally: AMPA receptor turnover and synaptic strength. *Neuron* 21, 933–935. doi: 10.1016/S0896-6273(00)80607-8

Turrigiano, G. G., and Nelson, S. B. (2004). Homeostatic plasticity in the developing nervous system. *Nat. Rev. Neurosci.* 5, 97–107. doi: 10.1038/nrn1327

Vale, C., Schoorlemmer, J., and Sanes, D. H. (2003). Deafness disrupts chloride transporter function and inhibitory synaptic transmission. *J. Neurosci.* 23, 7516–7524.

Watt, A. J., and Desai, N. S. (2010). Homeostatic plasticity and STDP: keeping a neuron's cool in a fluctuating world. *Front. Synaptic Neurosci.* 2:5. doi: 10.3389/fnsyn.2010.00005

West, M. J. (1993). New stereological methods for counting neurons. *Neurobiol. Aging* 14, 275–285. doi: 10.1016/0197-4580(93)90112-O

West, M. J., Slomianka, L., and Gundersen, H. J. (1991). Unbiased stereological estimation of the total number of neurons in the subdivisions of the rat hippocampus using the optical fractionator. *Anat. Rec.* 231, 482–497. doi: 10.1002/ar.1092310411

Wondolowski, J., and Dickman, D. (2013). Emerging links between homeostatic synaptic plasticity and neurological disease. *Front. Cell Neurosci.* 7:223. doi: 10.3389/fncel.2013.00223

Zennou-Azogui, Y., Borel, L., Lacour, M., Ez-Zaher, L., and Ouaknine, M. (1993). Recovery of head postural control following unilateral vestibular neurectomy in the cat. Neck muscle activity and neuronal correlates in Deiters' nuclei. *Acta Otolaryngol. Suppl.* 509, 1–19.

Zheng, Y., Begum, S., Zhang, C., Fleming, K., Masumura, C., Zhang, M., et al. (2011). Increased BrdU incorporation reflecting DNA repair, neuronal de-differentiation or possible neurogenesis in the adult cochlear nucleus following bilateral cochlear lesions in the rat. *Exp. Brain Res.* 210, 477–487. doi: 10.1007/s00221-010-2491-0

Zheng, Y., Smithies, H., Aitken, P., Gliddon, C., Stiles, L., Darlington, C. L., et al. (2015). Cell proliferation in the cochlear nucleus following acoustic trauma in rat. *Neuroscience* 303, 524–534. doi: 10.1016/j.neuroscience.2015.07.033

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Brain Metabolic Changes in Rats following Acoustic Trauma

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Acoustic trauma is the most common cause of hearing loss and tinnitus in humans. However, the impact of acoustic trauma on system biology is not fully understood. It has been increasingly recognized that tinnitus caused by acoustic trauma is unlikely to be generated by a single pathological source, but rather a complex network of changes involving not only the auditory system but also systems related to memory, emotion and stress. One obvious and significant gap in tinnitus research is a lack of biomarkers that reflect the consequences of this interactive "tinnitus-causing" network. In this study, we made the first attempt to analyse brain metabolic changes in rats following acoustic trauma using metabolomics, as a pilot study prior to directly linking metabolic changes to tinnitus. Metabolites in 12 different brain regions collected from either sham or acoustic trauma animals were profiled using a gas chromatography mass spectrometry (GC/MS)-based metabolomics platform. After deconvolution of mass spectra and identification of the molecules, the metabolomic data were processed using multivariate statistical analysis. Principal component analysis showed that metabolic patterns varied among different brain regions; however, brain regions with similar functions had a similar metabolite composition. Acoustic trauma did not change the metabolite clusters in these regions. When analyzed within each brain region using the orthogonal projection to latent structures discriminant analysis sub-model, 17 molecules showed distinct separation between control and acoustic trauma groups in the auditory cortex, inferior colliculus, superior colliculus, vestibular nucleus complex (VNC), and cerebellum. Further metabolic pathway impact analysis and the enrichment overview with network analysis suggested the primary involvement of amino acid metabolism, including the alanine, aspartate and glutamate metabolic pathways, the arginine and proline metabolic pathways and the purine metabolic pathway. Our results provide the first metabolomics evidence that acoustic trauma can induce changes in multiple metabolic pathways. This pilot study also suggests that the metabolomic approach has the potential to identify acoustic trauma-specific metabolic shifts in future studies where metabolic changes are correlated with the animal's tinnitus status.

Keywords: metabolomics, acoustic trauma, tinnitus, brain, rats

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INTRODUCTION

Acoustic trauma is the most common cause of hearing loss and tinnitus in humans (Cooper, 1994). In fact, noise exposure is the most frequently reported cause of occupational disorders in the world (Nelson et al., 2005; Dobie, 2008) and the fourth leading cause of medical referral for combatants returning from deployment (Schulz, 2004). Therefore, the consequences of exposure to acoustic trauma will impose a significant negative economic burden on healthcare systems worldwide. Using tinnitus as an example, this phantom sound is severe enough in 1% of adults to affect their day-to-day normal life (Vio and Holme, 2005). In severe cases, it can be extremely disturbing, and even lead to suicide (Shargorodsky et al., 2010). In the USA, tinnitus affects 25% of the population at some stage in their life, with 8% of people experiencing persistent or chronic tinnitus (Shargorodsky et al., 2010), and this is similar to the prevalence for the rest of the world (Henry et al., 2005; McCormack et al., 2014; Wu et al., 2015). While the prevalence of tinnitus normally increases with age, noise exposure is believed to be the most common cause of tinnitus in humans (Cooper, 1994).The prevalence of tinnitus is increased by several-fold among military populations, especially those who have served in battle zones (Shah et al., 2014). A recent study analyzed the incidence of hearing loss and tinnitus in a group of people who work in a noisy environment and found that approximately 40% of them had tinnitus (Hong et al., 2016). It is also alarming that an increasing number of adolescents and young adults are experiencing tinnitus due to risky music-listening behaviors (Vogel et al., 2014).

Tinnitus treatment options are very limited and none of the currently available approaches, such as hearing aids, sound masking, drug treatments, hyperbaric oxygen therapy, acupuncture or neuromodulation, show benefit following meta-analytic scrutiny (Hesser et al., 2011). There is no FDA-approved drug marketed for tinnitus, but a wide variety of drugs have been prescribed off-label with no added benefits (Langguth and Elgoyhen, 2012). One of the reasons accounting for the lack of effective treatment for tinnitus is its heterogeneity. First, there is large etiologic heterogeneity in tinnitus. Tinnitus can be caused by many factors including noise exposure, head and neck injury, drug toxicity, ear infection, Meniere's disease, aging and even affective disorders, such as depression (see Baguley et al., 2013, for a review), which suggests that the underlying mechanisms of tinnitus can be very different for different causes. Second, heterogeneity also exists in an individual's reaction to tinnitus. For example, tinnitus severity or tinnitus-related distress is not always proportional to the loudness of the sound of the tinnitus, but can be related to certain personality traits such as anxiety, depression, sleeping difficulties and life satisfaction (Langenbach et al., 2005; Langguth et al., 2007). Therefore, it is not surprising that the outcomes of currently available tinnitus therapies are highly variable (Hoare et al., 2011).

Traditionally, the gold standard, modern drug discovery approach seeks to design more selective drugs with ideally one specific target in order to reduce adverse side effects. Despite excessive efforts and investment routinely made in discovering individual molecular targets over the last two decades, the rate of new drug candidates being translated to clinical use is decreasing (Kola and Landis, 2004). Hopkins (2008) argued that the "one gene, one drug, one disease" drug design philosophy might be the fundamental problem. This is because biological functions, or dysfunctions in disease status, are more likely to be a consequence of complex biochemical regulation processes driven by interactive networks within the genome (Chen et al., 2008), transcriptome (Iancu et al., 2014), proteome (Ebhardt et al., 2015), and metabolome (Shah et al., 2015). Targeting such dynamic network biology by identifying disease-causing networks rather than disease-causing genes, is likely to be a more effective approach for drug discovery (Roth et al., 2004; Hopkins, 2007, 2008; Kell and Goodacre, 2014).

Since genomics, epigenetics, transcriptomics, and proteomics all converge at the level of metabolomics and changes in metabolite concentrations are more substantial and defining than signals in other "omics," metabolomics is considered to be the integration of all "omics." Therefore, it is more reliable, sensitive and powerful in reflecting changes in biological functions due to either disease or drug action. By systematically analyzing low molecular weight metabolites in biological samples, metabolomics has been used to discover new biomarkers for diagnosis, monitoring and understanding the mechanisms of diseases and drugs in many areas, such as cancer (Olivares et al., 2015), diabetes (Li et al., 2013), psychotic disorders (Sethi and Brietzke, 2016), depression (Huang and Lin, 2015), cardiovascular diseases (Rankin et al., 2014), Alzheimer's disease (Graham et al., 2015), and epilepsy (Loeb, 2011).

In comparison, a significant gap in tinnitus research and drug discovery is readily apparent. On the one hand, numerous studies focus on one type of receptor, ion channel, neurotransmitter or neuron in one area of the brain or one particular pathway through molecular, electrophysiological and pharmacological studies seeking to elucidate the underlying neural mechanisms to cure tinnitus (Milbrandt et al., 2000; Liu et al., 2003; Eggermont and Roberts, 2004; Brozoski et al., 2007, 2012; Kaltenbach and Godfrey, 2008; Dong et al., 2010; Middleton et al., 2011; Zhang et al., 2011; Richardson et al., 2012; Zheng et al., 2012; Bauer et al., 2013; Kalappa et al., 2014). In contrast, advanced neuroimaging technology reveals tinnitus-associated changes in neuronal activity and connectivity involving multiple neural networks, both in human patients (Leaver et al., 2011; Vanneste et al., 2011; Kraus and Canlon, 2012; Maudoux et al., 2012; Song et al., 2012; Vanneste and De Ridder, 2012; Boyen et al., 2014; Husain and Schmidt, 2014) and an animal model (Chen et al., 2014). It has been increasingly recognized by the international tinnitus research community that tinnitus is unlikely to be generated by a single pathological source, but rather by complex network changes involving not only the auditory system but also systems related to memory, emotion and stress (see Roberts et al., 2010; Henry et al., 2014; Simonetti and Oiticica, 2015; Leaver et al., 2016, for reviews). One obvious and significant gap is a lack of biomarkers that reflect the consequences of this interactive "tinnitus-causing" network. So far, only one study has attempted to identify protein targets for tinnitus by analyzing the side effect targets for 275 drugs that cause tinnitus (Elgoyhen et al., 2014). Although this is the first study using a network approach to

identify targets related to tinnitus, the significance is limited by the fact that tinnitus as a side effect may not necessarily be generated through the drug's pharmacological targets that were analyzed in the study. Therefore, a more direct approach by analyzing metabolite profiles in relation to tinnitus may provide insights into the phenotype, underlying pathophysiology as well as potential therapeutic targets of acoustic trauma and tinnitus. In the present study, we made the first attempt in analyzing metabolite profiles in different brain areas of rats at 6 months after exposure to acoustic trauma. This was a pilot study in which tissue from animals that had been subjected to acoustic trauma, but no longer exhibited tinnitus-related behavior, was used to investigate the potential application of metabolomics to the study of the effects of acoustic trauma on the brain, with a view to its potential relevance for tinnitus in the longer term.

METHODS

Animals and Tissue Sample Collection

The University of Otago Committee on Ethics in the Care and Use of Laboratory Animals approved all experimental procedures.

All the tissue samples were collected from our previous study which investigated the effects of L-baclofen on acoustic trauma-induced tinnitus (see Zheng et al., 2012, for details). Briefly, 16 male Wistar rats were divided into sham and acoustic trauma groups ($n = 8$ per group) and tested for the behavioral signs of tinnitus before receiving vehicle and 3 doses of L-baclofen (1, 3, and 5 mg/kg). Following the last L-baclofen treatment (5 mg/kg), a 2-week washout period was allowed before the final tinnitus behavioral testing (Zheng et al., 2012). At the end of the final tinnitus testing, the animals were sacrificed and the brains were rapidly removed, placed on ice and dissected into 12 different brain regions. These included the cochlear nucleus (CN), vestibular nucleus complex (VNC), inferior colliculus (IC), superior colliculus (SC), auditory cortex (AC), subregions of the hippocampus [CA1, CA2/3 and dentate gyrus (DG)], frontal cortex (FC), perirhinal cortex (PC), entorhinal cortex (ERC), and cerebellum (CB). Dissections were made according to the Paxinos and Watson brain atlas (Paxinos and Watson, 2008). Tissues were snap frozen on dry ice and kept at −80°C until the time of the assay.

Acoustic Trauma

Unilateral acoustic trauma was delivered using the procedure described in Zheng et al. (2012). Animals were anesthetized with ketamine HCl (75 mg/kg, s.c.) and medetomidine hydrochloride (0.3 mg/kg, s.c.) and then placed inside a sound attenuation chamber for a 1 h exposure to a 16 kHz 110 dB SPL pure tone delivered to one of the ears (Zheng et al., 2012). The pure tone was generated by an NI 4461 Dynamic Signal Acquisition and Generation system (National Instruments New Zealand Ltd) and was delivered to one of the ears through a closed field magnetic speaker with a tapered tip (Tucker-Davis Technologies), attached to a 3-mm cone-shaped speculum that was fitted tightly into the external auditory canal. The sound pressures were calibrated before noise exposure by connecting the speaker to a ¼-inch prepolarized free-field microphone (Type 40BE, GRAS Sound, and Vibration) via the speculum used to fit into the external auditory canal. The unexposed ear was blocked with cone-shaped foam and taped against the foam surface. The sham animals were kept under anesthesia for the same duration as the noise trauma animals, but without noise exposure.

Auditory Brainstem-Evoked Potentials

The auditory function of both ears of the exposed and sham animals before and immediately after the acoustic trauma or sham treatment was measured using auditory brainstem-evoked response (ABR) thresholds, as described in Zheng et al. (2012). Briefly, the animals were anesthetized as described in the Section Acoustic Trauma and subdermal needle electrodes were placed at the vertex and over the bullae with a reference electrode at the occiput. ABR thresholds were tested for tone bursts (2 ms rise/decay, 1 ms plateau) presented at a rate of 50/s, in a decreasing intensity series, beginning with levels that elicited distinct evoked potentials. Hearing threshold was indicated by the lowest intensity that produced visually distinct potentials. Unilateral acoustic trauma produced an immediate elevation of the ABR threshold in the exposed ear across all of the frequencies tested (Zheng et al., 2012). However, the auditory function of the ears of sham exposed animals and the unexposed ears of the acoustic trauma exposed animals, were not affected. Although we did not test the animal's ABR thresholds at 6 months post-exposure prior to sacrificing the animals in this study, we reported in a separate study that ABR thresholds in the exposed ear returned to the same level as that in sham animals at 6 months after exposure (Zheng et al., 2015). This suggests that hearing loss caused by the specific acoustic trauma we used is not permanent and is most likely to subside by 6 months post-exposure.

Tinnitus Assessment

In the original study of Zheng et al. (2012), from which the tissue used in this study was taken, the presence of tinnitus was tested after the acoustic trauma using a conditioned lick suppression paradigm, as described in detail in Zheng et al. (2012). Briefly, tinnitus assessment was conducted in an operant conditioning test chamber in which drinking activity was measured by a lickometer with a photobeam. A speaker generated broadband noise (BBN; white noise ranging from 3 to 20 kHz) or pure tones of different frequencies and intensities via a sound generator. The chamber floor delivered an electric shock produced by a constant current shock source. The conditioned lick suppression paradigm consisted of 15 min of testing daily and the animals went through an acclimation phase, a Pavlovian conditioned suppression training phase, and a frequency discrimination phase. During the acclimation phase, the BBN was played throughout the 15 min session except at 10 random intervals, at which point 15 s acoustic stimuli were inserted. Two of the 10 presentations were always speaker off periods (i.e., silence) and the remaining 8 were one of BBN, 10 kHz tones or 20 kHz tones at one of 4 different intensity levels in a random order with each stimulus repeated twice within each session. The type of stimulus varied randomly between sessions, but remained constant within a session. Following acclimation, each animal

received conditioned suppression training in which a 3 s foot shock (0.35 mA) was presented at the end of each speaker off period. The foot shock acted as an unconditioned stimulus (UCS) and the speaker off period acted as a conditioned stimulus (CS). Over a few sessions, the rats reacted to the speaker off by stopping licking (i.e., the conditioned suppression). Once the lick suppression was established, the rats were subjected to the frequency discrimination test, during which the acoustic stimuli were presented in the same way as in the acclimation and the suppression training. If a rat did not have tinnitus, the presentation of the stimuli had no effect on its drinking activity. However, if a rat had tinnitus, the tinnitus served as the conditioned stimulus (CS) during the training sessions, therefore, a stimulus with sensory features resembling tinnitus during the testing session would produce greater suppression. Tinnitus was assessed in these rats at 2 weeks after the noise exposure, during the L-baclofen or vehicle treatment as well as at the end of the experiment (Zheng et al., 2012).

Tissue Preparation

Frozen brain tissue samples were ground to an homogeneous powder using a liquid nitrogen-chilled mortar and pestle and rapidly transferred to a 1.5 mL microcentrifuge tube. Nine hundred μl of methanol containing the internal standard, [$^{13}C_2$]-myristic acid (12.5 μg/ml), was added to each tube. The mixture was vigorously vortexed for 3 min and centrifuged at 20,000 g for 10 min at 4°C.

An aliquot of 100 μl supernatant was transferred to a gas chromatography (GC) vial and evaporated to dryness using a SPD2010-230 SpeedVac Concentrator (Thermo Savant, Holbrook, USA). The dried residue was then dissolved in 30 μl of methoxyamine in pyridine (10 mg/ml) and vigorously vortexed for 2 min. The methoximation reaction was carried out for 16 h at room temperature, followed by trimethylsilylation for 1 h by adding 30 μl of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) with 1% trimethylchlorosilane (TMCS) as the catalyst. Finally, the solution was vortex-mixed again for 30 s after adding the external standard, methyl myristate in heptane (30 μg/ml), to each GC vial for GC/mass spectrometry (MS) analysis.

GC/MS

Chromatographic separation of the analytes was achieved with a Shimadzu GCMSQP2010 (Shimadzu Corp., Tokyo, Japan) equipped with a RTx-5MS column (30 mm × 0.25 mm i.d. fused-silica capillary column chemically bonded with a 0.25 μm cross bond, 5% diphenyl/95% dimethyl polysiloxane, Restek Corporation, PA, USA). Helium was used as the carrier gas and the temperature was initially set at 80°C for 3 min, which was then increased to 300°C at 20°C/min. Once the temperature was at 300°C, it was maintained for another 3 min. The eluate was introduced through the transfer line into the mass spectrometer, where the molecules were ionized with a current beam of 70 eV. The masses were scanned over m/z 50–700 with a detector voltage of −1050 V. To minimize systematic variations, all samples were analyzed in a randomized order, and the quantitative data were normalized to the internal standard. The metabolites were identified by automatically comparing the MS spectra, in-source fragments and ion features of each peak in the experimental samples with those of reference standards or those available in libraries, such as mainlib and publib in the National Institute of Standards and Technology (NIST) library 2.0 (2012); Wiley 9 (Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, Germany); the in-house mass spectra library database established by Umeå Plant Science Center (Umeå University, Sweden) and our own Laboratory at China Pharmaceutical University.

Data Analysis

Mean differences between the sham and acoustic trauma groups were compared using two-sample independent Student's *t*-tests. However, since the metabolomics data have multiple dependent variables, it required multivariate statistical analysis that takes account of changes in systems of variables and would be sensitive to changes that may occur at the system level between sham and acoustic trauma animals. One approach to analyzing changes in systems of variables is principal component analysis (PCA), in which large numbers of variables (i.e., "high dimensional data") are reduced to components or eigenvalues that represent combinations of variables that account for most of the variation in the data (Manly, 2005). In PCA, these components also have weightings or "eigenvectors" that indicate the weight of each variable within a component. The different components are intended to be independent of one another ("orthogonal") so that they capture unique aspects of the variation in the data. PCA, unlike Factor Analysis, does not assume a formal statistical model and therefore assumptions such as multivariate normality are unnecessary. PCA was performed on the correlation matrix (i.e., the data were z transformed) (Manly, 2005). Loading plots were used to determine the clustering of variables and whether their relationship within components changed as a result of acoustic trauma. Another way of analyzing multivariate changes is linear discriminant analysis (LDA), which attempts to identify a linear equation that combines the metabolomics variables, with different weightings, in order to predict whether animals belong to the sham or acoustic trauma groups. Partial least squares discriminant analysis (PLS-DA, equivalent to "projection to latent structures DA") is a method that uses partial least squares regression in the discriminant analysis, which can be used to elucidate the separation between groups of variables through rotating the principal components obtained in PCA, and is particularly useful when there are more variables than observations and when there is correlation or "multicollinearity" amongst the variables (Tang et al., 2014). In orthogonal projection to latent structures DA (OPLS-DA), an extension of PLS-DA, the data from the continuous variables in the discriminant analysis function are separated into those that are predictive of the dependent variable (e.g., sham or acoustic trauma) and those that are not, resulting in enhanced diagnostics. We performed OPLS-DA and then tested the efficacy of the discriminant functions in predicting whether the animals were exposed to acoustic trauma, using cross-validation (Manly, 2005). The goodness of fit for a model was evaluated using three quantitative parameters: i.e., R^2X, the explained variation in X, R^2Y, the explained variation in Y, and Q^2Y, the predicted variation in Y based on the model using cross-validation

(Sedghipour and Homayoun Sadeghi-Bazargani, 2012). The range of these parameters is between 0 and 1; the closer they approach 1, the better they can be predicted or explained. All analyses were carried out using R (R Core Team, 2013). In formal statistical tests, $P \leq 0.05$ was considered significant. Variable importance was used to summarize the importance of the X-variables, both for the X- and Y-models and was measured by the variable influence on projection (VIP). The VIP value is a parameter indicating the importance of a variable that contributes to the model. In a model, one can compare the VIP of one variable to the others. Variables with a large VIP, larger than 1, are the most relevant for explaining Y. The S-plot is an easy way to visualize an OPLS discriminant analysis model of two classes, since it provides visualization of the OPLS/OPLS-DA predictive component loading to facilitate model interpretation (Wiklund et al., 2008). The S-plot is used to visualize both the covariance and the correlation structure between the X-variables and the predictive score t[1]. Thus, the S-plot is a scatter plot of the p[1] vs. p(corr)[1] vectors of the predictive component. The axes in the S-plot from the predictive component are p1 and p(corr)1, representing the magnitude (modeled covariation) and reliability (modeled correlation), respectively. Hence, in the S-plot both magnitude (intensity) and reliability are visualized. In spectroscopic data, the magnitude of the peak is very important as peaks with low magnitude are close to the noise level and thus have a higher risk for spurious correlation. Ideally, biomarkers would have both high reliability and magnitude. This plot often takes the shape of the letter "S." X-variables situated far out on the wings of the S shape represent higher model influence with higher reliability and are of relevance in the search for biomarkers that are up- or down-regulated. In addition, metabolic pathway analysis was performed by inputting discriminant molecules into Metaboanalyst (available online at http://www.metaboanalyst.ca) and network analysis was processed by Metscape 3.1-based on Cytoscape 3.3.0. "Relative abundance" was calculated by summing the integral areas of metabolites with high variable importance scores, normalizing them, and comparing the sham vs. acoustic trauma groups using a two-sample, independent Student's *t*-test (Gray et al., 2015).

RESULTS

GC/MS analysis of the brain tissue extracts revealed a large number of peaks (**Figure 1**). Deconvolution of the chromatograms produced a total of 107 distinct peaks and 88 were authentically identified by comparing the mass spectrum of the peak with that available in the libraries and that of the reference compounds. These included amino acids, small organic acids, carbohydrates, fatty acids, lipids, and amines. To acquire the quantitative data, a feature mass (m/z) was chosen for each peak, and the peak area was obtained for each deconvoluted peak/molecule.

A data matrix of molecules in samples from the sham and acoustic trauma animals for the 12 brain areas was initially analyzed using PCA and PLS-DA. PLS-DA score plots, in which the scores for PC2 (i.e., t[2]) were plotted against those for PC1 (t[1]), were used to show the distribution of the analyzed samples containing the information from all the metabolites in different brain areas (**Figure 2**). According to the PLS-DA algorithm, each dot represents the summarized information from all the 88 molecules measured in a single sample for a particular brain region. Therefore, the distance between the dots indicates the similarity of the metabolic composition between the samples, i.e., the closer they cluster together the more similar they are. An overview of the PLS-DA score plot of the sham animals revealed that the composition of metabolites varied between different brain regions; however, brain regions with similar functions seemed to have a similar metabolite composition (**Figure 2A**). For example, metabolites from the temporal lobe area, such as the CA1, CA2/3, DG, ERC, and PC, were located close to each other on the PLS-DA score plots but were further apart from those from midbrain and hindbrain areas. Animals that received acoustic trauma showed similar metabolite clustering to the sham animals (**Figure 2B**). This was confirmed by an OPLS-DA analysis, which generated a model comparing the total metabolites in all the brain areas between sham and acoustic trauma animals (PC1: $R^2X = 0.21$, $R^2Y = 0.0286$, $Q^2Y = -0.0245$; PC2: $R^2X = 0.112$, $R^2Y = 0.0354$, $Q^2Y = -0.038$). OPLS-DA visualized the clustering samples within a group and the separation of two groups of

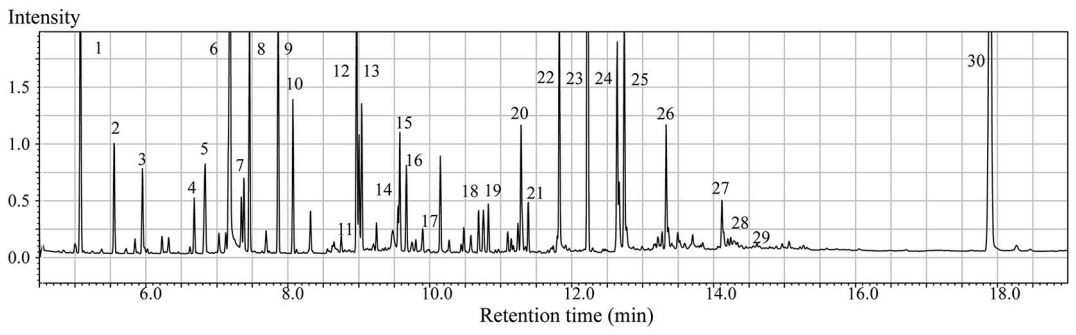

FIGURE 1 | Typical GC/MS chromatograms of extracts from brain tissue of a sham animal. The compounds were identified as: 1. Lactic Acid 2. Alanine 3. Oxalic acid 4. Valine 5. Urea 6. Phosphoric acid 7. Proline 8. Glycine 9. Serine 10. Threonine 11. Malic acid 12. Aspartic acid 13.γ-Aminobutanoic acid 14.Creatinine 15. Glutamic acid 16. Phenylalanine 17. N-Acetylaspartic acid 18. Hypoxanthine 19. Citric acid 20. Lysine 21. Tyrosine 22. Palmitic acid 23. Myo-Inositol 24. Oleic acid 25. Stearic acid 26. Arachidonic acid 27. Docosahexaenoic acid 28. Inosine 29. Glycerol monostearate 30. Cholesterol

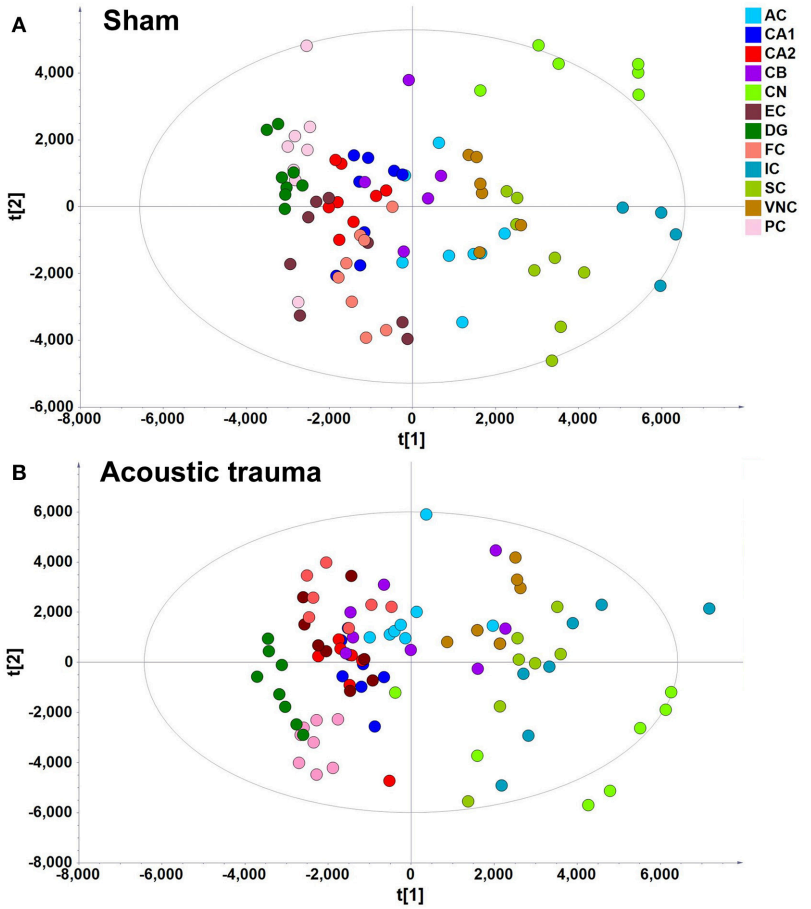

FIGURE 2 | PLS-DA score plot of different brain regions from sham (A) and acoustic trauma **(B)** groups. **(A)** sham (PC1: $R^2X = 0.344$, $R^2Y = 0.0842$, Q2 = 0.0726; PC2: $R^2X = 0.249$, $R^2Y = 0.0525$, Q2 = 0.0373; All 6 PCs: R^2X (cum) = 0.84, R^2Y (cum) = 0.381, Q2 (cum) = 0.286.); **(B)** acoustic trauma (PC1: $R^2X = 0.336$, $R^2Y = 0.0759$, Q2 = 0.0683; PC2: $R^2X = 0.307$, $R^2Y = 0.0513$, Q2 = 0.0407; All 6 PCs: R^2X (cum) = 0.875, R^2Y (cum) = 0. 384, Q2 (cum) = 0.325. AC, auditory cortex; CB, cerebellum; IC, inferior colliculus; CN, cochlear nucleus; VCN, vestibular nucleus complex; SC, superior colliculus; CA1 and CA2 of the hippocampus; DG, dentate gyrus; FC, frontal cortex; PC, perirhinal cortex; EC, entorhinal cortex.

samples. The closer clustering of samples indicates the more similar composition of the detected variables, while the more distant scattering of samples indicates the larger variation in the composition of the detected variables. These results suggested that, taking all brain regions together, the acoustic trauma and sham animals could not be discriminated from one another.

However, when the metabolite profiles in individual areas were analyzed using the OPLS-DA model, acoustic trauma (purple dots in left panel, **Figure 3**) significantly shifted the metabolite profile away from the sham animals (red dots in right panel, **Figure 3**) in the AC (Predictive component: $R^2X = 0.194$, $R^2Y = 0.76$, $Q^2Y = 0.45$; Orthogonal component: $R^2X = 0.446$; All components: R^2X(cum) = 0.64), IC (Predictive component: $R^2X = 0.293$, $R^2Y = 0.978$, $Q^2Y = 0.702$; Orthogonal component 1: $R^2X = 0.417$; All components: R^2X (cum) = 0.905), SC (Predictive component: $R^2X = 0.238$, $R^2Y = 0.791$, $Q^2Y = 0.691$; Orthogonal component: $R^2X = 0.562$; All components: R^2X (cum) = 0.8), VNC (Predictive component: $R^2X = 0.403$, $R^2Y = 0.779$, $Q^2Y = 0.445$; Orthogonal component: $R^2X = 0.389$; All components: R^2X (cum) = 0.792) and CB (Predictive component: $R^2X = 0.152$, $R^2Y = 0.973$, $Q^2Y = 0.68$; Orthogonal component 1: $R^2X = 0.364$; All components: R^2X (cum) = 0.927) (**Figure 3**, left panel). In addition, the discriminant molecules or potential markers for the two groups could be visualized in an S-plot (**Figure 3**, right panel).The X- and Y-axes are p[1] and p(corr)[1] vectors of the predictive component, which describes the magnitude and reliability of each variable in X, respectively. X-variables situated far out on the tips of the wings of the S shape represent high model influence with high reliability and are of relevance in the search for biomarkers. Further analysis of the relative concentrations using Student's *t*-tests revealed a total of 17 differentially changed molecules associated with acoustic trauma (**Figure 4**). These included urea, amino acids, fatty acids, sugar acids, nucleosides and organic acids and the changes were region-specific. For example, the level of GABA was significantly increased only in the AC, while an increase in glutamic acid was observed in both the CB and VNC (**Figure 4**).

Based on the discriminant molecules identified, a metabolic pathway analysis was conducted by entering the discriminant molecules into the available online Metaboanalyst programme.

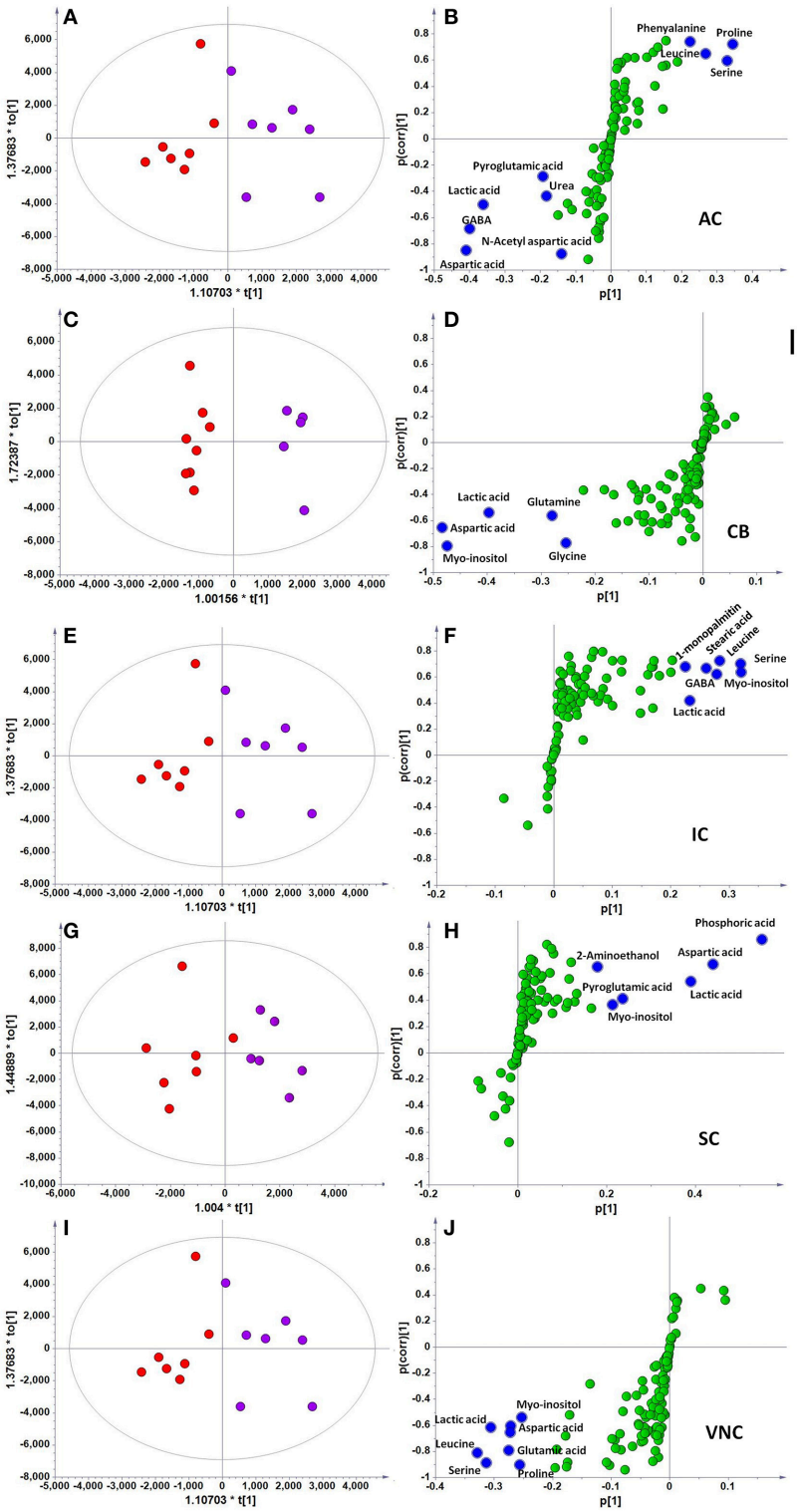

FIGURE 3 | OPLS-DA and S-plot analysis comparing the OPLSDA scores between sham and acoustic trauma animals in different brain regions. Left panel, OPLSDA scores plots, red dots: Sham, purple dots: Acoustic trauma; Right panel, S-plots. **(A,B)** AC (Predictive component: $R^2X = 0.194$, $R^2Y = 0.76$, Q2 = 0.45; Orthogonal component 1: $R^2X = 0.446$; All components: R^2X (cum) = 0.64); **(C,D)** CB (Predictive component: $R^2X = 0.152$, $R^2Y = 0.973$, Q2 = 0.68; Orthogonal component 1: $R^2X = 0.364$; All components: R^2X (cum) = 0.927); **(E,F)** IC (Predictive component: $R^2X = 0.293$, $R^2Y = 0.978$, Q2 = 0.702; Orthogonal component 1: $R^2X = 0.417$; All components: R^2X (cum) = 0.905); **(G,H)** SC (Predictive component: $R^2X = 0.238$, $R^2Y = 0.791$, Q2 = 0.691; Orthogonal component: $R^2X = 0.562$; All components: R^2X (cum) = 0.8); **(I,J)** VNC(Predictive component: $R^2X = 0.403$, $R^2Y = 0.779$, Q2 = 0.445; Orthogonal component: R^2X = 0.389; All components: R^2X (cum) = 0.792). AC, auditory cortex; CB, cerebellum; IC, inferior colliculus; CN, cochlear nucleus; VCN, vestibular nucleus complex.

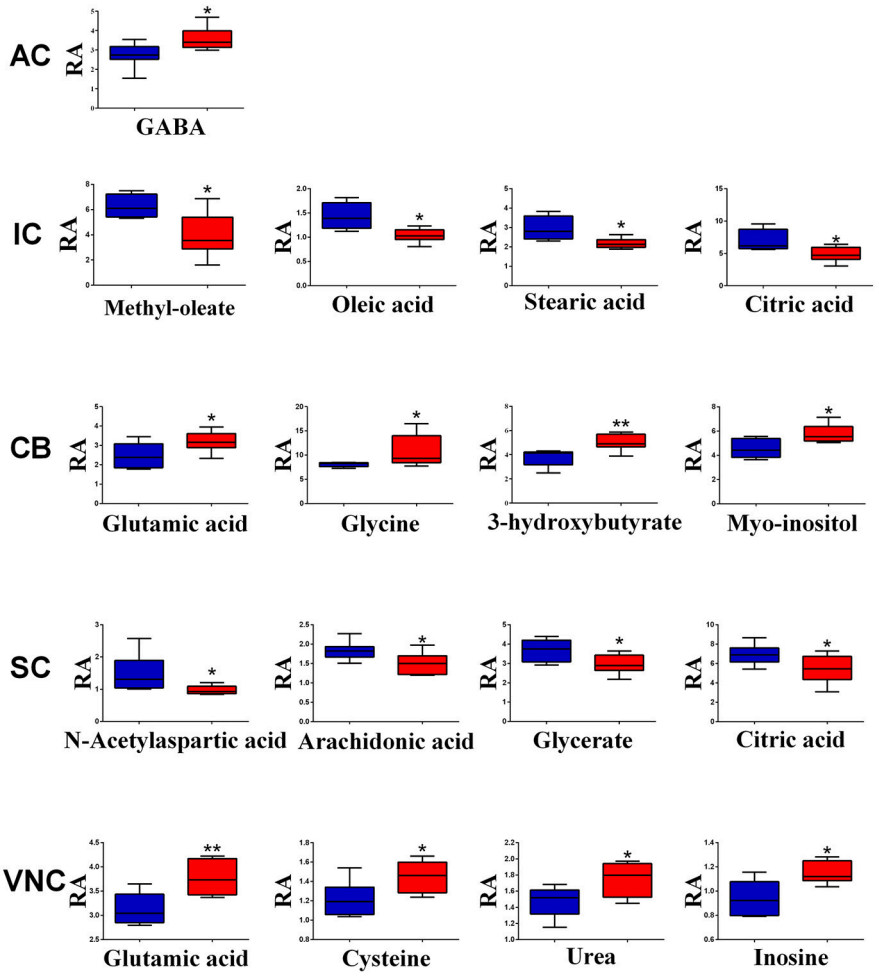

FIGURE 4 | Relative abundance (RA) of the discriminant metabolites in different brain regions of sham (blue) and acoustic trauma (red) groups. *$p < 0.05$; **$p < 0.01$. AC, auditory cortex; CB, cerebellum; IC, inferior colliculus; CN, cochlear nucleus; VCN, vestibular nucleus complex.

The pathway impact analysis suggested that acoustic trauma produced a significant impact on a number of metabolic pathways. A set of 4 pathways was selected. These included glutathione metabolism, alanine aspartate and glutamate metabolism, arginine and proline metabolism and glycine, serine and threonine metabolism (**Figure 5A**) The enrichment overview of the network ranked the acoustic trauma-perturbed metabolite sets by their respective fold enrichment, which provides information on the significant and coordinated changes in metabolites. As shown in **Figure 5B**, the identified metabolites were involved in multiple pathways and the *p*-values indicated whether a particular metabolite set was represented more than expected by chance. The most enriched pathway was the glutathione metabolism pathway.

Further analysis on the correlation was conducted based on the 88 identified metabolites from both sham and acoustic trauma animals to further study the association and interaction patterns between metabolites. Nodes represent the identified metabolites and edges indicate significant correlation between nodes. As can be seen in **Figure 6**, some nodes had more connections with others, while other nodes had fewer links. There were also nodes that were clustered together with a short distance between them. Based on the degree (k) of each node, the top 10 highly connected metabolites were considered as hubs of the network and these metabolites are involved in purine metabolism (green circle), glutamate metabolism (blue circle) and arginine metabolism (red circle) (**Figure 6**).

DISCUSSION

We reported here that acoustic trauma in rats caused a significant shift in metabolite profiles in a number of brain areas. These included both auditory and non-auditory areas. In addition, acoustic trauma imposed a significant impact on a number of metabolic pathways including those involved in purine, glutamate and arginine metabolism.

To our knowledge, this is the first study that has used metabolomics to profile brain metabolite changes in relation to acoustic trauma. Over the last decade, metabolomics has

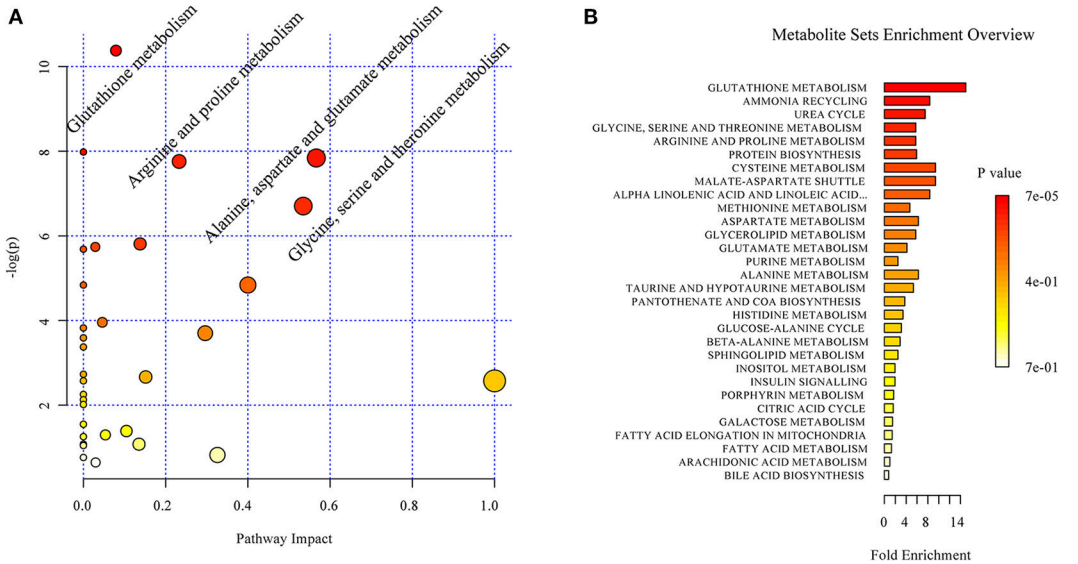

FIGURE 5 | Overview of the impact of acoustic trauma on brain metabolites. (A) The pathway impact of acoustic trauma on metabolites. The y axis shows the *p*-values and the x axis, the pathway impact values; the node color is based on its *p*-value and the node size reflects the pathway impact values. **(B)** The enrichment overview of the pathway-associated metabolite sets perturbed by acoustic trauma.

become one of the major "omics" tools in understanding disease pathology, identifying biomarkers, improving diagnosis and developing personalized therapy (see Botas et al., 2015; Shah et al., 2015 for reviews). In addition, brain metabolomics has been used to understand the pathology and identify potential markers for neurodegenerative diseases in both animal models and human post-mortem tissues due to the fact that metabolic changes in the brain are more likely to reflect disease etiology than those in peripheral biofluids (Pears et al., 2005; Salek et al., 2010; Graham et al., 2013; Fauvelle et al., 2015; Xu et al., 2016). In the present study, 107 small molecules were detected from 12 different brain regions and 88 of them were authentically identified. The metabolic models developed were able to accurately distinguish acoustic trauma animals from sham controls in selected brain regions. The predictive accuracies of these models ranged from 45 to 70.2%, which suggests a reasonably good level of predictive power when compared with 41% accuracy in predicting Batten's disease (Pears et al., 2005) and 54–60% in predicting Alzheimer's disease using animal brain tissues (Salek et al., 2010), although not as good as that reported using human post-mortem brain tissues for Alzheimer's disease (91–97%) (Graham et al., 2013).

Although it has neither been extensively studied and nor is well understood, hearing loss and/or tinnitus has been linked to changes in the homeostasis of body metabolism. For example, it has long been noticed that chronic kidney disease (Ikeda et al., 1987; Govender et al., 2013; Renda et al., 2015) or diabetes (Kurien et al., 1989; Sasso et al., 1999) that cause metabolic disturbances are often accompanied by impaired hearing. Moreover, metabolite changes have been associated with presbycusis (Profant et al., 2013), congenital sensorineural hearing loss (Wu et al., 2016) and idiopathic sudden sensorineural hearing loss (Dinc et al., 2016). In the present study, among the 88 small molecules identified, 17 of them had their levels significantly altered by acoustic trauma in

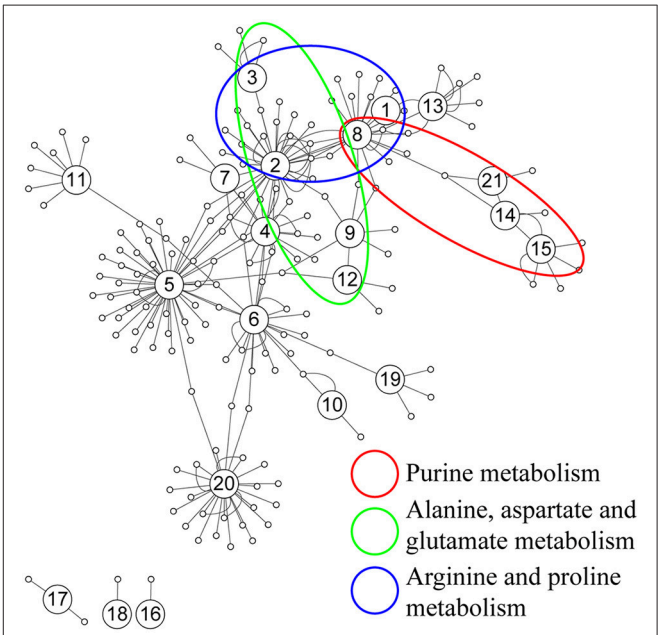

FIGURE 6 | Correlation network analysis of differential metabolites. (1) N-Acetylaspartate, (2) Glutamic acid, (3) γ-aminobutyric acid, (4) Cysteine, (5) Glycine, (6) Serine, (7) Pyroglutamic acid, (8) Aspartic acid, (9) Ornithine, (10) Glyceric acid, (11) Myo-Inositol, (12) Urea, (13) Citric acid, (14) Hypoxanthine, (15) Xanthine, (16) 3-Hydroxybutyric acid, (17) Oleic acid, (18) Stearic acid, (19) Palmitic acid, (20) Arachidonic acid, (21) Inosine.

at least one of the brain regions examined. These metabolites are predominantly involved in amino acid metabolism, the urea cycle as well as oxidation-reduction reactions. The long-term effects of acoustic trauma on amino acid levels in auditory brain regions have been investigated in a previous study, which reported a significant increase in glutamate, GABA and aspartate and a decrease in taurine (Godfrey et al., 2012). This is in a general agreement with the present results except that our study extended the finding into some non-auditory areas, such as the CB and VNC, which may suggest a more widespread effect of acoustic trauma in disturbing the balance between excitatory and inhibitory amino acids. It is of note that Bauer et al. (2013) have recently reported a link between the paraflocculus and tinnitus perception in rats. Using the network impact analysis, glutathione metabolism, the ammonia cycle and the urea cycle appeared to be affected the most by the acoustic trauma. While glutathione is an important antioxidant in cells, ammonia and urea are crucial to amino acid synthesis and metabolism. Studies have shown that a low protein diet resulting in a reduced cochlear glutathione level increased cisplatin-induced ototoxicity (Lautermann et al., 1995) and N-acetylcysteine (NAC), a precursor of glutathione, attenuated hearing loss in a number of animal models (Wu et al., 2010; Ding et al., 2016). Urea tests have long been used to aid the diagnosis of vestibular and hearing impairment in patients with Ménière's disease (Babin and Bumsted, 1980; Imoto and Stahle, 1983) and urea has also been used to temporarily improve hearing in these patients (Angelborg et al., 1977). This effect was thought to be partially due to the changes in perilymph osmolality caused by urea (Juhn et al., 1979). Interestingly, the urea cycle was altered in multiple brain regions in neurodegenerative diseases such as Huntington's disease (Patassini et al., 2015) and Alzheimer's disease (Xu et al., 2016), which suggests that brain urea metabolism may play an important role in maintaining neuronal function. Therefore, the altered brain glutathione and urea metabolism observed in the present study may contribute to acoustic trauma-induced hearing loss. It is worth mentioning that although hearing levels were not measured prior to sacrificing the animals in this study, we found in a separate study that hearing loss had recovered at 6 months following the same acoustic trauma exposure in rats (Zheng et al., 2015). Therefore, it is conceivable that hearing loss had also recovered by 6 months in the present study, hence, the metabolic changes were not directly attributable to hearing loss. It has been shown that acoustic trauma has variable effects on ABR thresholds in individual animals and the degree of hearing loss is not correlated with tinnitus perception in these mice (Longenecker et al., 2014; Longenecker and Galazyuk, 2016), which suggests that the impact of acoustic trauma on the auditory system is not limited to the peripheral damage, but involves damage to the higher centers of the brain. Our results are in agreement with this view and extend the evidence to include changes in metabolic pathways. In addition, the present study investigated metabolic changes in both auditory and non-auditory brain regions, which may provide insights into the wide range of emotional and cognitive impairments associated with acoustic trauma.

One of the limitations that prevented the current results being directly linked to tinnitus is the lack of behavioral evidence of tinnitus in these animals at the time of sample collection. As described in our previous publication (Zheng et al., 2012), the acoustic trauma-exposed animals developed tinnitus at 1 month post-exposure, which was confirmed by the conditioned lick suppression test. Both sham and exposed animals then received saline and 3 different doses of L-baclofen treatment and were tested for their behavioral signs of tinnitus after each treatment. At the conclusion of the experiment, the exposed animals did not have significant behavioral signs of tinnitus compared with sham animals. Therefore, it is not possible to relate the metabolic changes to tinnitus in this study, only to acoustic trauma. However, it is reasonable to attribute the changes to acoustic trauma since both the sham and acoustic trauma animals received exactly the same L-baclofen treatment. Nonetheless, this is the first study using the powerful metabolomics technique to investigate brain metabolite shifts in relation to acoustic trauma at the network level. This is a proof of concept study that established a method sensitive enough to detect metabolic changes in discrete brain regions.

CONCLUSIONS

In this study, we optimized a metabolomics approach to predict acoustic trauma using brain tissues, with comparable accuracy to studies predicting other neurodegenerative diseases in animal models (Pears et al., 2005; Salek et al., 2010). Further studies are needed to identify brain metabolic changes specifically related to tinnitus and to correlate changes in the brain with those in the blood for future clinical translation. This will provide a powerful tool toward a better understanding of tinnitus heterogeneity and the development of personalized therapies.

ETHICS STATEMENT

All experiments were carried out in accordance with the New Zealand Animal Welfare Act 1999 and the University of Otago Code of Ethical Conduct for the Use of Animals. Formal approval to conduct the experiments described has been obtained from the University of Otago Committee for the Care and Use of Laboratory Animals.

AUTHOR CONTRIBUTIONS

YZheng Corresponding author, secured the funding in New Zealand, designed the experiment, supervised the acoustic trauma model, collected the brain tissues, coordinated the collaboration, played a key role in the results interpretation and manuscript writing. GW 2nd corresponding author, secured the funding in China, contributed to the results interpretation and manuscript writing. DD Contributed to the results interpretation and manuscript writing. PS Secured the funding in New Zealand, contributed to the results interpretation and manuscript writing. JA Supervised the metabolomics experiments, analyzed the results and contributed to the results interpretation and manuscript writing. YZhu Performed the metabolomics experiments and contributed to data analysis. JH Performed the metabolomics experiments and contributed to data analysis.

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REFERENCES

Angelborg, C., Klockhoff, I., and Stahle, J. (1977). Urea and hearing in patients with Meniere's disease. *Scand. Audiol.* 6, 143–146. doi: 10.3109/01050397709043115

Babin, R. W., and Bumsted, R. M. (1980). Urea test and vestibular dysfunction in suspected Meniere's disease. *J. Otolaryngol.* 9, 202–206.

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Bauer, C. A., Wisner, K. W., Baizer, J. S., and Brozoski, T. J. (2013). Tinnitus, unipolar brush cells, and cerebellar glutamatergic function in an animal model. *PLoS ONE* 8:e64726. doi: 10.1371/journal.pone.0064726

Botas, A., Campbell, H. M., Han, X., and Maletic-Savatic, M. (2015). Metabolomics of neurodegenerative diseases. *Int. Rev. Neurobiol.* 122, 53–80. doi: 10.1016/bs.irn.2015.05.006

Boyen, K., de Kleine, E., van Dijk, P., and Langers, D. R. (2014). M. Tinnitus-related dissociation between cortical and subcortical neural activity in humans with mild to moderate sensorineural hearing loss. *Hear. Res.* 312, 48–59. doi: 10.1016/j.heares.2014.03.001

Brozoski, T. J., Spires, T. J. D., and Bauer, C. A. (2007). Vigabatrin, a GABA transaminase inhibitor, reversibly eliminates tinnitus in an animal model. *J. Assoc. Res. Otolarnygol.* 8, 105–118. doi: 10.1007/s10162-006-0067-2

Brozoski, T. J., Wisner, K. W., Sybert, L. T., and Bauer, C. A. (2012). Bilateral dorsal cochlear nucleus lesions prevent acoustic-trauma induced tinnitus in an animal model. *J. Assoc. Res. Otolaryngol.* 13, 55–66. doi: 10.1007/s10162-011-0290-3

Chen, Y.-C., Wang, J., Jiao, Y., Zang, F.-C., Yang, M., Tong, J.-X., et al. (2014). "Changes in resting-state fMRI activity during salicylate-induced tinnitus and sound stimulation," in *8th International TRI Tinnitus Conference* (Auckland), Abstract, 106.

Chen, Y. Q., Zhu, J., Lum, P. Y., Yang, X., Pinto, S., MacNeil, D. J., et al. (2008). Variations in DNA elucidate molecular networks that cause disease. *Nature* 452, 429–435. doi: 10.1038/nature06757

Cooper, J. C. Jr. (1994). Health and nutrition examination survey of 1971–75: Part II. Tinnitus subjective hearing loss and well-being. *J. Am. Acad. Audiol.* 5, 37–43.

Dinc, M. E., Ulusoy, S., Is, A., Ayan, N. N., Avincsal, M. O., Bicer, C., et al. (2016). Thiol/disulphide homeostasis as a novel indicator of oxidative stress in sudden sensorineural hearing loss. *J. Laryngol. Otol.* 130, 447–452. doi: 10.1017/S002221511600092X

Ding, D., Jiang, H., Chen, G. D., Longo-Guess, C., Muthaiah, V. P., Tian, C., et al. (2016). N-acetyl-cysteine prevents age-related hearing loss and the progressive loss of inner hair cells in gamma-glutamyl transferase 1 deficient mice. *Aging* 8, 730–750. doi: 10.18632/aging.100927

Dobie, R. A. (2008). The burdens of age-related and occupational noise-induced hearing loss in the United States. *Ear Hear.* 29, 565–577. doi: 10.1097/AUD.0b013e31817349ec

Dong, S., Rodger, J., Mulders, W. H., and Robertson, D. (2010). Tonotopic changes in GABA receptor expression in guinea pig inferior colliculus after partial unilateral hearing loss. *Brain Res.* 1342, 24–32. doi: 10.1016/j.brainres.2010.04.067

Ebhardt, H. A., Root, A., Sander, C., and Aebersold, R. (2015). Applications of targeted proteomics in systems biology and translational medicine. *Proteomics* 15, 3193–3208. doi: 10.1002/pmic.201500004

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Elgoyhen, A. B., Langguth, B., Nowak, W., Schecklmann, M., De Ridder, D., and Vanneste, S. (2014). Identifying tinnitus-related genes based on a side-effect network analysis. *CPT Pharmacometrics Syst. Pharmacol.* 3:e97. doi: 10.1038/psp.2013.75

Fauvelle, F., Boccard, J., Cavarec, F., Depaulis, A., and Deransart, C. (2015). Assessing susceptibility to epilepsy in three rat strains using brain metabolic profiling based on HRMAS NMR spectroscopy and chemometrics. *J. Proteome Res.* 14, 2177–2189. doi: 10.1021/pr501309b

Godfrey, D. A., Kaltenbach, J. A., Chen, K., Ilyas, O., Liu, X., Licari, F., et al. (2012). Amino acid concentrations in the hamster central auditory system and long-term effects of intense tone exposure. *J. Neurosci. Res.* 90, 2214–2224. doi: 10.1002/jnr.23095

Govender, S. M., Govender, C. D., and Matthews, G. (2013). Cochlear function in patients with chronic kidney disease. *S. Afr. J. Commun. Disord.* 60, 44–49. doi: 10.7196/sajcd.243

Graham, S. F., Chevallier, O. P., Elliott, C. T., Holscher, C., Johnston, J., McGuinness, B., et al. (2015). Untargeted metabolomic analysis of human plasma indicates differentially affected polyamine and L-arginine metabolism in mild cognitive impairment subjects converting to Alzheimer's disease. *PLoS ONE* 10:e0119452. doi: 10.1371/journal.pone.0119452

Graham, S. F., Chevallier, O. P., Roberts, D., Holscher, C., Elliott, C. T., and Green, B. D. (2013). Investigation of the human brain metabolome to identify potential markers for early diagnosis and therapeutic targets of Alzheimer's disease. *Anal. Chem.* 85, 1803–1811. doi: 10.1021/ac303163f

Gray, E., Larkin, J. R., Claridge, T. D., Talbot, K., Sibson, N. R., and Turner, M. R. (2015). The longitudinal cerebrospinal fluid metabolomic profile of amyotrophic lateral sclerosis. *Amyotroph. Lateral Scler. Front. Degener.* 16, 456–463. doi: 10.3109/21678421.2015.1053490

Henry, J. A., Dennis, K. C., and Schechter, M. A. (2005). General review of tinnitus: prevalence, mechanisms, effects, and management. *J. Speech Lang. Hear. Res.* 48, 1204–1235. doi: 10.1044/1092-4388(2005/084)

Henry, J. A., Roberts, L. E., Caspary, D. M., Theodoroff, S. M., and Salvi, R. J. (2014). Underlying mechanisms of tinnitus: review and clinical implications. *J. Am. Acad. Audiol.* 25, 5–22; quiz 126. doi: 10.3766/jaaa.25.1.2

Hesser, H., Weise, C., Westin, V. Z., and Andersson, G. (2011). A systematic review and meta-analysis of randomized controlled trials of cognitive-behavioral therapy for tinnitus distress. *Clin. Psychol. Rev.* 31, 545–553. doi: 10.1016/j.cpr.2010.12.006

Hoare, D. J., Kowalkowski, V. L., Kang, S., and Hall, D. A. (2011). Systematic review and meta-analyses of randomized controlled trials examining tinnitus management. *Laryngoscope* 121, 1555–1564. doi: 10.1002/lary.21825

Hong, O., Chin, D. L., Phelps, S., and Joo, Y. (2016). Double jeopardy hearing loss and tinnitus among noise-exposed workers. *Workplace Health Saf.* 64, 235–242. doi: 10.1177/2165079916629975

Hopkins, A. L. (2007). Network pharmacology. *Nat. Biotechnol.* 25, 1110–1111. doi: 10.1038/nbt1007-1110

Hopkins, A. L. (2008). Network pharmacology: the next paradigm in drug discovery. *Nat. Chem. Biol.* 4, 682–690. doi: 10.1038/nchembio.118

Huang, T. L., and Lin, C. C. (2015). Advances in biomarkers of major depressive disorder. *Adv. Clin. Chem.* 68, 177–204. doi: 10.1016/bs.acc.2014.11.003

Husain, F. T., and Schmidt, S. A. (2014). Using resting state functional connectivity to unravel networks of tinnitus. *Hear. Res.* 307, 153–162. doi: 10.1016/j.heares.2013.07.010

Iancu, O. D., Colville, A., Darakjian, P., and Hitzemann, R. (2014). Coexpression and cosplicing network approaches for the study of mammalian brain transcriptomes. *Int. Rev. Neurobiol.* 116, 73–93. doi: 10.1016/B978-0-12-801105-8.00004-7

Ikeda, K., Kusakari, J., Arakawa, E., Ohyama, K., Inamura, N., and Kawamoto, K. (1987). Cochlear potentials of guinea pigs with experimentally induced renal failure. *Acta Otolaryngol. Suppl.* 435, 40–45. doi: 10.3109/00016488709107349

Imoto, T., and Stahle, J. (1983). The clinical picture of Meniere's disease in the light of glycerin and urea tests. *Acta Otolaryngol.* 95, 247–256. doi: 10.3109/00016488309130941

Juhn, S. K., Prado, S., and Rybak, L. (1979). Effect of urea on osmolality of perilymph. *Arch. Otolaryngol.* 105, 538–541. doi: 10.1001/archotol.1979.00790210036008

Kalappa, B. I., Brozoski, T. J., Turner, J. G., and Caspary, D. M. (2014). Single unit hyperactivity and bursting in the auditory thalamus of awake rats directly correlates with behavioural evidence of tinnitus. *J. Physiol.* 592, 5065–5078. doi: 10.1113/jphysiol.2014.278572

Kaltenbach, J. A., and Godfrey, D. A. (2008). Dorsal cochlear nucleus hyperactivity and tinnitus: are they related? *Am. J. Audiol.* 17, S148–S161. doi: 10.1044/1059-0889(2008/08-0004)

Kell, D. B., and Goodacre, R. (2014). Metabolomics and systems pharmacology: why and how to model the human metabolic network for drug discovery. *Drug Discov. Today* 19, 171–182. doi: 10.1016/j.drudis.2013.07.014

Kola, I., and Landis, J. (2004). Can the pharmaceutical industry reduce attrition rates? *Nat. Rev. Drug Discov.* 3, 711–715. doi: 10.1038/nrd1470

Kraus, K. S., and Canlon, B. (2012). Neuronal connectivity and interactions between the auditory and limbic systems. Effects of noise and tinnitus. *Hear. Res.* 288, 34–46. doi: 10.1016/j.heares.2012.02.009

Kurien, M., Thomas, K., and Bhanu, T. S. (1989). Hearing threshold in patients with diabetes mellitus. *J. Laryngol. Otol.* 103, 164–168. doi: 10.1017/S0022215100108345

Langenbach, M., Olderog, M., Michel, O., Albus, C., and Köhle, K. (2005). Psychosocial and personality predictors of tinnitus-related distress. *Gen. Hosp. Psychiatry* 27, 73–77. doi: 10.1016/j.genhosppsych.2004.08.008

Langguth, B., and Elgoyhen, A. B. (2012). Current pharmacological treatments for tinnitus. *Expert Opin. Pharmacother.* 13, 2495–2509. doi: 10.1517/14656566.2012.739608

Langguth, B., Kleinjung, T., Fischer, B., Hajak, G., Eichhammer, P., and Sand, P. G. (2007). Tinnitus severity, depression, and the big five personality traits. *Prog. Brain Res.* 166, 221–225. doi: 10.1016/S0079-6123(07)66020-8

Lautermann, J., Song, B., McLaren, J., and Schacht, J. (1995). Diet is a risk factor in cisplatin ototoxicity. *Hear. Res.* 88, 47–53. doi: 10.1016/0378-5955(95)00097-N

Leaver, A. M., Renier, L., Chevillet, M. A., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2011). Dysregulation of limbic and auditory networks in tinnitus. *Neuron* 69, 33–43. doi: 10.1016/j.neuron.2010.12.002

Leaver, A. M., Seydell-Greenwald, A., and Rauschecker, J. P. (2016). Auditory-limbic interactions in chronic tinnitus: challenges for neuroimaging research. *Hear. Res.* 334, 49–57. doi: 10.1016/j.heares.2015.08.005

Li, M., Wang, X., Aa, J., Qin, W., Zha, W., Ge, Y., et al. (2013). GC/TOFMS analysis of metabolites in serum and urine reveals metabolic perturbation of TCA cycle in db/db mice involved in diabetic nephropathy. *Am. J. Physiol. Renal Physiol.* 304, F1317–F1324. doi: 10.1152/ajprenal.00536.2012

Liu, J., Li, X., Wang, L., Dong, Y., Han, H., and Liu, G. (2003). Effects of salicylate on serotoninergic activities in rat inferior colliculus and auditory cortex. *Hear. Res.* 175, 45–53. doi: 10.1016/S0378-5955(02)00708-6

Loeb, J. A. (2011). Identifying targets for preventing epilepsy using systems biology. *Neurosci. Lett.* 497, 205–212. doi: 10.1016/j.neulet.2011.02.041

Longenecker, R. J., Chonko, K. T., Maricich, S. M., and Galazyuk, A. V. (2014). Age effects on tinnitus and hearing loss in CBA/CaJ mice following sound exposure. *Springerplus* 3:542. doi: 10.1186/2193-1801-3-542

Longenecker, R. J., and Galazyuk, A. V. (2016). Variable effects of acoustic trauma on behavioral and neural correlates of tinnitus in individual animals. *Front. Behav. Neurosci.* 10:207. doi: 10.3389/fnbeh.2016.00207

Manly, B. F. J. (2005). *Multivariate Statistical Methods. A Primer, 3rd Edn.* Boca Raton, FL: Chapman and Hall.

Maudoux, A., Lefebvre, P., Cabay, J. E., Demertzi, A., Vanhaudenhuyse, A., Laureys, S., et al. (2012). Connectivity graph analysis of the auditory resting state network in tinnitus. *Brain Res.* 1485, 10–21. doi: 10.1016/j.brainres.2012.05.006

McCormack, A., Edmondson-Jones, M., Fortnum, H., Dawes, P., Middleton, H., Munro, K. J., et al. (2014). The prevalence of tinnitus and the relationship with neuroticism in a middle-aged UK population. *J. Psychosom. Res.* 76, 56–60. doi: 10.1016/j.jpsychores.2013.08.018

Middleton, J. W., Kiritani, T., Pedersen, C., Turner, J. G., Shepherd, G. M., and Tzounopoulos, T. (2011). Mice with behavioral evidence of tinnitus exhibit dorsal cochlear nucleus hyperactivity because of decreased GABAergic inhibition. *Proc. Natl. Acad. Sci. U.S.A.* 108, 7601–7606. doi: 10.1073/pnas.1100223108

Milbrandt, J. C., Holder, T. M., Wilson, M. C., Salvi, R. J., and Caspary, D. M. (2000). GAD levels and muscimol binding in rat inferior colliculus following acoustic trauma. *Hear. Res.* 147, 251–260. doi: 10.1016/S0378-5955(00)00135-0

Nelson, D. I., Nelson, R. Y., Concha-Barrientos, M., and Fingerhut, M. (2005). The global burden of occupational noise-induced hearing loss. *Am. J. Ind. Med.* 48, 446–458. doi: 10.1002/ajim.20223

Olivares, O., Dabritz, J. H., King, A., Gottlieb, E., and Halsey, C. (2015). Research into cancer metabolomics: towards a clinical metamorphosis. *Semin. Cell Dev. Biol.* 43, 52–64. doi: 10.1016/j.semcdb.2015.09.008

Patassini, S., Begley, P., Reid, S. J., Xu, J. S., Church, S. J., Curtis, M., et al. (2015). Identification of elevated urea as a severe, ubiquitous metabolic defect in the brain of patients with Huntington's disease. *Biochem. Biophys. Res. Commun.* 468, 161–166. doi: 10.1016/j.bbrc.2015.10.140

Paxinos, G., and Watson, C. (2008). *The Rat Brain: In Stereotaxic Coordinates, 6th Edn.* New York, NY: Academic Press.

Pears, M. R., Cooper, J. D., Mitchison, H. M., Mortishire-Smith, R. J., Pearce, D. A., and Griffin, J. L. (2005). High resolution 1H NMR-based metabolomics indicates a neurotransmitter cycling deficit in cerebral tissue from a mouse model of Batten disease. *J. Biol. Chem.* 280, 42508–42514. doi: 10.1074/jbc.M507380200

Profant, O., Balogova, Z., Dezortova, M., Wagnerova, D., Hajek, M., and Syka, J. (2013). Metabolic changes in the auditory cortex in presbycusis demonstrated by MR spectroscopy. *Exp. Gerontol.* 48, 795–800. doi: 10.1016/j.exger.2013.04.012

Rankin, N. J., Preiss, D., Welsh, P., Burgess, K. E. V., Nelson, S. M., Lawlor, D. A., et al. (2014). The emergence of proton nuclear magnetic resonance metabolomics in the cardiovascular arena as viewed from a clinical perspective. *Atherosclerosis* 237, 287–300. doi: 10.1016/j.atherosclerosis.2014.09.024

R Core Team (2013). *A Language and Environment for Statistical Computing*. Vienna: R Foundation for Statistical Computing. Available online at: http://www.R-project.org/.

Renda, R., Renda, L., Selcuk, O. T., Eyigor, H., Yilmaz, M. D., and Osma, U. (2015). Cochlear sensitivity in children with chronic kidney disease and end-stage renal disease undergoing hemodialysis. *Int. J. Pediatr. Otorhinolaryngol.* 79, 2378–2383. doi: 10.1016/j.ijporl.2015.10.048

Richardson, B. D., Brozoski, T. J., Ling, L. L., and Caspary, D. M. (2012). Targeting inhibitory neurotransmission in tinnitus. *Brain Res.* 1485, 77–87. doi: 10.1016/j.brainres.2012.02.014

Roberts, L. E., Eggermont, J. J., Caspary, D. M., Shore, S. E., Melcher, J. R., and Kaltenbach, J. A. (2010). Ringing ears: the neuroscience of tinnitus. *J. Neurosci.* 30, 14972–14979. doi: 10.1523/JNEUROSCI.4028-10.2010

Roth, B. L., Sheffler, D. J., and Kroeze, W. K. (2004). Magic shotguns versus magic bullets: selectively non-selective drugs for mood disorders and schizophrenia. *Nat. Rev. Drug Discov.* 3, 353–359. doi: 10.1038/nrd1346

Salek, R. M., Xia, J., Innes, A., Sweatman, B. C., Adalbert, R., Randle, S., et al. (2010). A metabolomic study of the CRND8 transgenic mouse model of Alzheimer's disease. *Neurochem. Int.* 56, 937–947. doi: 10.1016/j.neuint.2010.04.001

Sasso, F. C., Salvatore, T., Tranchino, G., Cozzolino, D., Caruso, A. A., Persico, M., et al. (1999). Cochlear dysfunction in type 2 diabetes: a complication independent of neuropathy and acute hyperglycemia. *Metabolism* 48, 1346–1350. doi: 10.1016/S0026-0495(99)90141-5

Schulz, T. Y. (2004). Troops return with alarming rates of hearing loss. *Hearing Health* 21, 18–21.

Sedghipour, M. R., and Homayoun Sadeghi-Bazargani, H. (2012). Applicability of supervised discriminant analysis models to analyse astigmatism clinical trial data. *Clin. Ophthalmol.* 6, 1499–1506. doi: 10.2147/OPTH.S34907

Sethi, S., and Brietzke, E. (2016). Omics-Based Biomarkers: application of metabolomics in neuropsychiatric disorders. *Int. J. Neuropsychopharmacol.* 19:pyv096. doi: 10.1093/ijnp/pyv096

Shah, A., Ayala, M., Capra, G., Fox, D., and Hoffer, M. (2014). Otologic assessment of blast and nonblast injury in returning Middle East-deployed service members. *Laryngoscope* 124, 272–277. doi: 10.1002/lary.24169

Shah, N. J., Sureshkumar, S., and Shewade, D. G. (2015). Metabolomics: a tool ahead for understanding molecular mechanisms of drugs and diseases. *Indian J. Clin. Biochem.* 30, 247–254. doi: 10.1007/s12291-014-0455-z

Shargorodsky, J., Curhan, G. C., and Farwell, W. R. (2010). Prevalence and characteristics of tinnitus among US adults. *Am. J. Med.* 123, 711–718. doi: 10.1016/j.amjmed.2010.02.015

Simonetti, P., and Oiticica, J. (2015). Tinnitus neural mechanisms and structural changes in the brain: the contribution of neuroimaging research. *Int. Arch. Otorhinolaryngol.* 19, 259–265. doi: 10.1055/s-0035-1548671

Song, J. J., De Ridder, D., Van de Heyning, P., and Vanneste, S. (2012). Mapping tinnitus-related brain activation: an activation-likelihood estimation metaanalysis of PET studies. *J. Nucl. Med.* 53, 1550–1557. doi: 10.2967/jnumed.112.102939

Tang, L., Peng, S., Bi, Y., Shan, P., and Hu, X. (2014). A new method combining LDA and PLS for dimension reduction. *PLoS ONE* 9:e96944. doi: 10.1371/journal.pone.0096944

Vanneste, S., de Heyning, P. V., and De Ridder, D. (2011). The neural network of phantom sound changes over time: a comparison between recent-onset and chronic tinnitus patients. *Eur. J. Neurosci.* 34, 718–731. doi: 10.1111/j.1460-9568.2011.07793.x

Vanneste, S., and De Ridder, D. (2012). The auditory and non-auditory brain areas involved in tinnitus. An emergent property of multiple parallel overlapping subnetworks. *Front. Syst. Neurosci.* 6:31. doi: 10.3389/fnsys.2012.00031

Vio, M. M., and Holme, R. H. (2005). Hearing loss and tinnitus: 250 million people and a US$10 billion potential market. *Drug Discov. Today* 10, 1263–1265. doi: 10.1016/S1359-6446(05)03594-4

Vogel, I., van de Looij-Jansen, P. M., Mieloo, C. L., Burdorf, A., and de Waart, F. (2014). Risky music listening, permanent tinnitus and depression, anxiety, thoughts about suicide and adverse general health. *PLoS ONE* 9:e98912. doi: 10.1371/journal.pone.0098912

Wiklund, S., Johansson, E., Sjostrom, L., Mellerowicz, E. J., Edlund, U., Shockcor, J. P., et al. (2008). Visualization of GC/TOF-MS-based metabolomics data for identification of biochemically interesting compounds using OPLS class models. *Anal. Chem.* 80, 115–122. doi: 10.1021/ac0713510

Wu, B. P., Searchfield, G., Exeter, D. J., and Lee, A. (2015). Tinnitus prevalence in New Zealand. *N. Z. Med. J.* 128, 24–34. Available online at: https://www.nzma.org.nz/journal/read-the-journal/all-issues/2010-2019/2015/vol-128-no-1423/6683

Wu, C., Huang, L., Tan, H., Wang, Y., Zheng, H., Kong, L., et al. (2016). Diffusion tensor imaging and MR spectroscopy of microstructural alterations and metabolite concentration changes in the auditory neural pathway of pediatric congenital sensorineural hearing loss patients. *Brain Res.* 1639, 228–234. doi: 10.1016/j.brainres.2014.12.025

Wu, H. P., Hsu, C. J., Cheng, T. J., and Guo, Y. L. (2010). N-acetylcysteine attenuates noise-induced permanent hearing loss in diabetic rats. *Hear. Res.* 267, 71–77. doi: 10.1016/j.heares.2010.03.082

Xu, J., Begley, P., Church, S. J., Patassini, S., Hollywood, K. A., Jullig, M., et al. (2016). Graded perturbations of metabolism in multiple regions of human brain in Alzheimer's disease: Snapshot of a pervasive metabolic disorder. *Biochim. Biophys. Acta* 1862, 1084–1092. doi: 10.1016/j.bbadis.2016.03.001

Zhang, J., Zhang, Y., and Zhang, X. (2011). Auditory cortex electrical stimulation suppresses tinnitus in rats. *J. Assoc. Res. Otolaryngol.* 12, 185–201. doi: 10.1007/s10162-010-0246-z

Zheng, Y., Reid, P., and Smith, P. F. (2015). Cannabinoid CB1 receptor agonists do not decrease, but may increase acoustic trauma-induced tinnitus in rats. *Front. Neurol.* 6:60. doi: 10.3389/fneur.2015.00060

Zheng, Y., Vagal, S., Hamilton, E., Darlington, C. L., and Smith, P. F. (2012). A dose-response analysis of the effects of L-baclofen on chronic tinnitus caused by acoustic trauma in rats. *Neuropharmacology* 62, 940–946. doi: 10.1016/j.neuropharm.2011.09.027

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CORRECTION
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Corrigendum: Brain Metabolic Changes in Rats following Acoustic Trauma

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In the original article, the same graph was used for **Figures 3A**, **3E** and **3I** by mistake. The corrected **Figure 3** appears below.

The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way.

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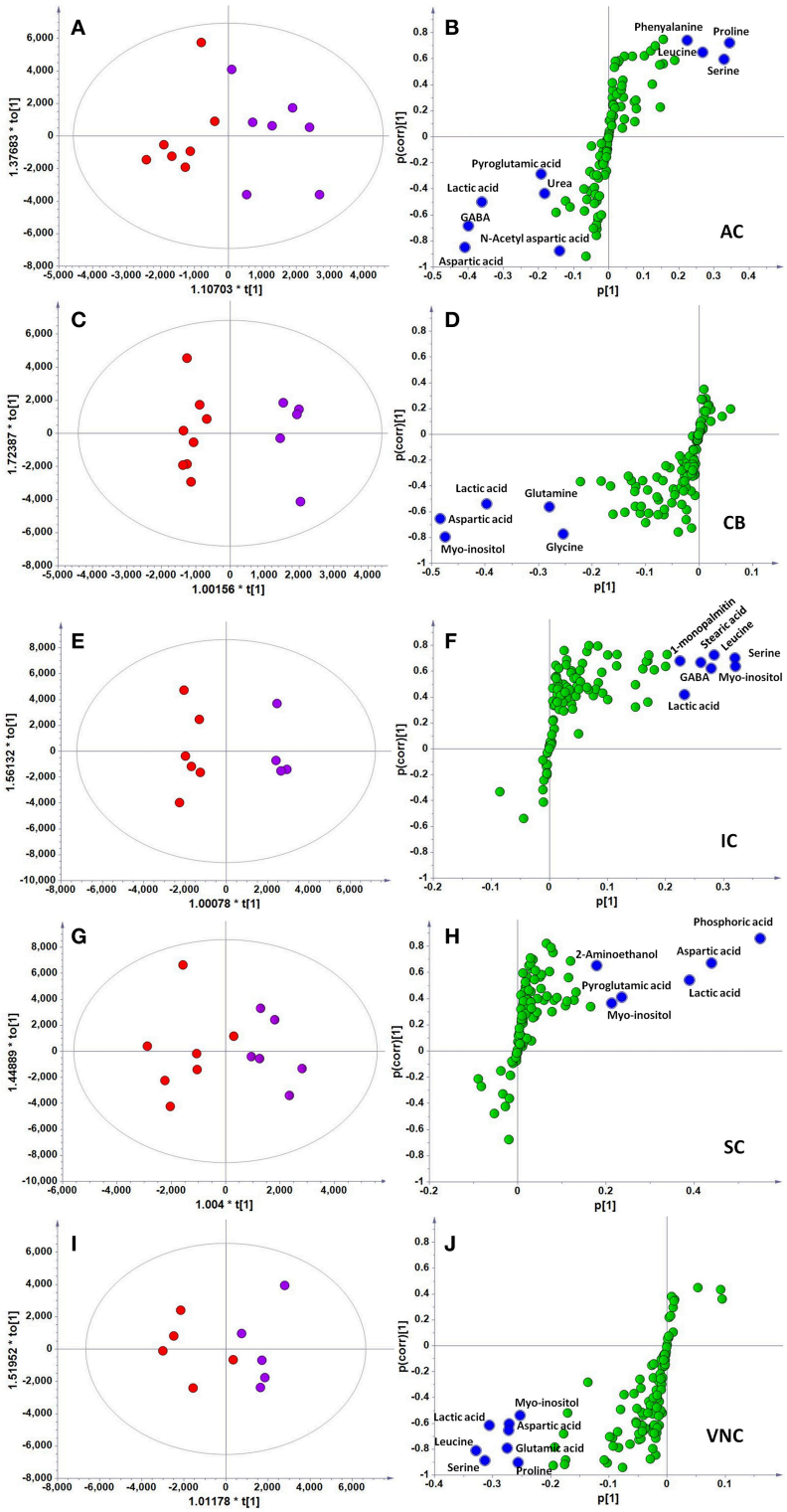

FIGURE 3 | OPLS-DA and S-plot analysis comparing the OPLSDA scores between sham and acoustic trauma animals in different brain regions. Left panel, OPLSDA scores plots, red dots: Sham, purple dots: Acoustic trauma; Right panel, S-plots. **(A,B)** AC (Predictive component: $R^2X = 0.194$, $R^2Y = 0.76$, Q2 = 0.45; Orthogonal component 1: $R^2X = 0.446$; All components: R^2X (cum) = 0.64); **(C,D)** CB (Predictive component: $R^2X = 0.152$, $R^2Y = 0.973$, Q2 = 0.68; Orthogonal component 1: $R^2X = 0.364$; All components: R^2X (cum) = 0.927); **(E,F)** IC (Predictive component: $R^2X = 0.293$, $R^2Y = 0.978$, Q2 = 0.702; Orthogonal component 1: $R^2X = 0.417$; All components: R^2X (cum) = 0.905); **(G,H)** SC (Predictive component: $R^2X = 0.238$, $R^2Y = 0.791$, Q2 = 0.691; Orthogonal component: $R^2X = 0.562$; All components: R^2X (cum) = 0.8); **(I,J)** VNC(Predictive component: $R^2X = 0.403$, $R^2Y = 0.779$, Q2 = 0.445; Orthogonal component: $R^2X = 0.389$; All components: R^2X (cum) = 0.792). AC, auditory cortex; CB, cerebellum; IC, inferior colliculus; CN, cochlear nucleus; VCN, vestibular nucleus complex.

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METHODS
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A New Statistical Approach for the Evaluation of Gap-prepulse Inhibition of the Acoustic Startle Reflex (GPIAS) for Tinnitus Assessment

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Background: An increasingly used behavioral paradigm for the objective assessment of a possible tinnitus percept in animal models has been proposed by Turner and coworkers in 2006. It is based on gap-prepulse inhibition (PPI) of the acoustic startle reflex (ASR) and usually referred to as GPIAS. As it does not require conditioning it became the method of choice to study neuroplastic phenomena associated with the development of tinnitus.

Objective: It is still controversial if GPIAS is really appropriate for tinnitus screening, as the hypothesis that a tinnitus percept impairs the gap detection ability ("filling-in interpretation" is still questioned. Furthermore, a wide range of criteria for positive tinnitus detection in GPIAS have been used across different laboratories and there still is no consensus on a best practice for statistical evaluation of GPIAS results. Current approaches are often based on simple averaging of measured PPI values and comparisons on a population level without the possibility to perform valid statistics on the level of the single animal.

Methods: A total number of 32 animals were measured using the standard GPIAS paradigm with varying number of measurement repetitions. Based on this data further statistical considerations were performed.

Results: We here present a new statistical approach to overcome the methodological limitations of GPIAS. In a first step we show that ASR amplitudes are not normally distributed. Next we estimate the distribution of the measured PPI values by exploiting the full combinatorial power of all measured ASR amplitudes. We demonstrate that the amplitude ratios (1-PPI) are approximately lognormally distributed, allowing for parametrical testing of the logarithmized values and present a new statistical approach allowing for a valid and reliable statistical assessment of PPI changes in GPIAS.

Conclusion: Based on our statistical approach we recommend using a constant criterion, which does not systematically depend on the number of measurement

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repetitions, in order to divide animals into a tinnitus and a non-tinnitus group. In particular, we recommend using a constant threshold based on the effect size as criterion, as the effect size, in contrast to the *p*-value, does not systematically depend on the number of measurement repetitions.

Keywords: hearing loss, Bonferroni correction, tinnitus screening, Turner paradigm, effect size

INTRODUCTION

In western societies up to 15% of the general population suffer from subjective tinnitus (Heller, 2003), the perception of a sound in the absence of any acoustic stimulus. Despite this high prevalence and the tinnitus-associated distress of affected patients, which in severe cases may experience insomnia, psychological disorders like depression, the inability to work, or even commit suicide (Coles, 1984; Lewis et al., 1994; Langguth et al., 2011), there still is no effective cure for the condition, because all tinnitus research faces one central problem: Whereas the existence of a tinnitus percept can unequivocally be determined in human patients (one can simply ask them; cf. e.g., Pantev et al., 2012; Elgoyhen et al., 2015; Husain, 2016; Leaver et al., 2016), this is only unsatisfactorily possible in animal models for tinnitus (Von Der Behrens, 2014; Zhang et al., 2014; Galazyuk and Hebert, 2015; Brozoski and Bauer, 2016). On the other hand, the exact mechanisms within the auditory system that lead to the development of tinnitus are still unknown and hard to identify, since invasive neurophysiological methods that are essential for such research are only available in animal models but not in humans. Therefore, we still lack a mechanistic understanding of the tinnitus phenomenon which would indeed be crucial for the development of an effective cure. Accordingly, current tinnitus therapies mainly aim to help patients to cope with the condition rather than to cure it (e.g., Goebel et al., 1999; Zachriat and Kröner-Herwig, 2004; Westin et al., 2011). Consequently, what is needed most in tinnitus research is a reliable animal model suited to unravel the neurophysiological mechanisms of tinnitus development.

Currently, a number of different mechanistic models for the development of tinnitus do exist. To date, these models are mainly based on animal research (despite the above described fundamental problem of tinnitus research), are still only able to explain a subset of tinnitus phenomena like tonal tinnitus, and in addition, are discussed controversially (Gerken, 1996; Eggermont, 2003; Eggermont and Roberts, 2004; Engineer et al., 2011; Knipper et al., 2011; Schaette and Mcalpine, 2011; Wang et al., 2011; Yang et al., 2011; Ahlf et al., 2012; Ruttiger et al., 2013; Tziridis et al., 2015; Krauss et al., 2016b).

Originally, the assessment of tinnitus in animal models was based on some kind of conditioning where the animal learned to distinguish between conditions of sound vs. silence (Jastreboff et al., 1988a,b; Heffner and Harrington, 2002; Ruttiger et al., 2003). After training and induction of tinnitus (either by salicylate or noise trauma) the animals were expected to show sound-related behavior during the silence condition, and such behavior would then be considered indicative for the existence of a tinnitus percept. A major drawback of all conditioning approaches for tinnitus research is that any conditioning paradigm itself would trigger neuroplastic changes in auditory processing (Weinberger, 1993; Ohl et al., 2001; Ohl and Scheich, 2005) that potentially would interfere with neuroplastic phenomena that are related to tinnitus development. Hence, for any study that aims to unravel the neurophysiological mechanisms that underlie the development of tinnitus, conditioning paradigms for the assessment of tinnitus could lead to misinterpretations as it could be difficult to distinguish between learning induced and tinnitus induced neuroplastic changes in the auditory system (Norena et al., 2010).

Turner et al. (2006) proposed a new model for tinnitus assessment in animals that was based on gap-prepulse inhibition of the acoustic startle reflex (GPIAS). The ASR is a reflex to a loud acoustic stimulus in animals (Koch, 1999) and humans (e.g., Fournier and Hébert, 2016) and can be reduced by the perception of a pre-stimulus—here a gap in a continuous noise background. The reflex amplitude remains unchanged if the pre-stimulus is not perceived, and it is gradually decreased with the increase of the strength of the perception. Under the assumption that a possible tinnitus percept may fill the gap and thereby reduces the PPI of the ASR, it has even been tried to probe different frequency ranges (by using band-pass noise of different spectra as background) and identify the possible pitch range of the animals' tinnitus percept (Turner et al., 2006; Yang et al., 2007; Nowotny et al., 2011; Ahlf et al., 2012; Turner and Larsen, 2012; Tziridis et al., 2014, 2015; Liberman et al., 2015).

This new approach enjoys increasing popularity among the community of animal tinnitus researchers as it is much less time consuming than the aforementioned conditioning paradigms and seemingly simple (cf. Galazyuk and Hebert, 2015). Furthermore, the fact that it requires no conditioning prior to tinnitus testing, no conditioning-related plasticity is induced. Therefore, GPIAS seems well suited for studies that investigate mechanisms of tinnitus development.

Nevertheless, despite these obvious advantages it is still controversial if the method in general is appropriate for tinnitus screening, as the "filling-in" interpretation has been questioned (Campolo et al., 2013; Radziwon et al., 2015). Furthermore, a wide range of criteria for positive tinnitus detection have been used across different laboratories and there still is no consensus on a "best practice" for statistical evaluation of GPIAS results, as it exists for other behavioral paradigms (cf. Hinkle et al., 2003). Current approaches are often based on simple averaging of measured PPI values and comparisons on a population level without the possibility to perform valid statistics on the level of the single animal.

In this study we propose a straight forward, statistical stringent approach that could be used to harmonize and standardize GPIAS data analysis in future tinnitus research.

METHODS

Animals and Ethical Statement

Mongolian gerbils (*Meriones unguiculatus*) were housed in standard animal racks (Bio A.S. Vent Light, Ehret Labor- und Pharmatechnik, Emmendingen, Germany) in groups of 2–3 animals per cage with free access to water and food at 20–24°C room temperature under 12/12 h dark/light cycle. The use and care of animals was approved by the state of Bavaria (Regierungspräsidium Mittelfranken, Ansbach, Germany, No. 54-2532.1-02/13). A total of 32 male gerbils aged 10–12 weeks were purchased from Janvier Laboratories Inc. and used in this study after acclimatization in our animal facility.

Acoustic Trauma

The pure tone acoustic trauma for tinnitus induction is applied under deep ketamine xylazine anesthesia as described in detail earlier (Ahlf et al., 2012; Walter et al., 2012; Tziridis et al., 2014, 2015; Krauss et al., 2016a). In a nutshell, the anesthetized animals were placed on a regulated heating pad with a temperature of 37°C central in front of a Loudspeaker (Canton Plus X Series 2; Canton, Weilrod, Germany). Using a signal generator (hp 33120A, HP, Böblingen, Germany) connected to an audio amplifier (Amp 75, Thomas Wulf, Frankfurt, Germany), a 2 kHz pure tone was presented at a sound pressure level of 115 dB SPL for 75 min.

PPI of ASR Measurements

For ASR measurements, as described earlier (e.g., Ahlf et al., 2012; Tziridis et al., 2012), animals were placed in a transparent acrylic tube (length 10 cm, inner diameter 4.3 cm) which was positioned at a distance of 10 cm in front of a loudspeaker (Canton Plus X Series 2), on a low-vibration table (TMC, Peabody, MA, USA). The whole setup was placed in an acoustic chamber (Industrial Acoustics Company GmbH, Niederkrüchten, Germany). The startle response was measured by a piezo force sensor (Honeywell FSG15N1A; sensitivity 0.24 mV/g; null shift at 25°C is ±1 mV; force range 0–1,500 g) attached underneath the tube. The front end of the tube was closed with a stainless steel grate (wire mesh, width 0.5 mm) allowing for acoustic stimulation with no detectable distortion within the used stimulation range of 250–8,000 Hz (signal-to-noise ratio at least 70 dB). Sound pressure level was calibrated using a condenser microphone (B and K Type 4190) via a preamplifier (B and K Type 2669) and measuring amplifier (B and K Type 2610). Stimulus generation and data acquisition was performed using custom-made programs (Matlab 2008, MathWorks, Natick, MA, USA). As startle amplitudes tend to be higher for the first few trials, five startle stimuli were presented before the beginning of each measurement to rule out strong habituation effects (Turner et al., 2006; Valsamis and Schmid, 2011).

The standard gap-startle protocol to measure behavioral correlates of tinnitus in rodents consists of several trials using a 20 ms long 115 dB SPL loud noise burst as startle stimulus presented in a continuous background band pass noise with a spectral width of half an octave centered on a given frequency. In half of the trials the "no-gap" condition, i.e., without any silent period within the background noise, was presented. In the other half of the trials—the "gap" condition—the band pass noise was interrupted by a 50 ms interval of silence, presented 100 ms before the startle stimulus. The stimulation was chosen according to the protocol used by Turner et al. (2006), but the stimulus frequency range was adapted to our animal model (cf. below). The response of the animals to the startle pulse was measured with the piezo force sensor described above.

Invalid trials (trials where the animal moved before the startle stimulus) were discarded by thresholding of the signal in the time interval 550 ms before the startle stimulus. The threshold was set to 0.5 mV. As the signal is superposed by high frequency measurement noise a low pass filter (butterworth 6th order, cutoff: 40 Hz was applied). The complete procedure is explained in detail in Supplementary Figure 1.

In a first protocol, we presented 200 trials with and 200 trials without a gap in a background noise centered at 2,000 Hz (± half an octave) to evaluate the distributions of the different responses of the animals to the two different stimulus conditions (in depth analysis of habituation effects is shown in Supplementary Figure 2). In other words all together 400 trials were presented to each animal (two stimulus conditions and 200 repetitions of each stimulus). In a second protocol we analyzed frequency dependent effects of the background noise as used in the standard protocol (cf. Turner et al., 2006): Here, only 15 trials with and without gap for each of 9 different center frequencies were presented (center frequencies: 500, 707, 1,000, 1,414, 2,000, 2,828, 4,000, 5,657, 8,000 Hz, all together 270 stimuli were presented). (For tinnitus testing, this protocol was measured before and after a pure tone acoustic trauma.) In both protocols the inter-stimulus intervals were randomized (10 ± 2 s) to exclude any possible adaptation or habituation of the animals to fixed time intervals (Joober et al., 2002; Ahlf et al., 2012; Krauss et al., 2016a,b).

Typically the startle reflex amplitude (A) is defined as the peak-to-peak amplitude of the reflexive response of the animal. According to Joober et al. (2002) and Jovanovic et al. (2004), the prepulse inhibition is defined as 1 minus the amplitude ratio of A_{gap} vs. A_{nogap}:

$$PPI = 1 - \frac{A_{gap}}{A_{nogap}} \tag{1}$$

where A_{gap} and A_{nogap} are the peak-to-peak response amplitudes for the gap and no-gap condition, respectively. Hence, the PPI value is always from the interval$]-\infty,1]$.

Evaluation and Statistics

The complete evaluation software including the applied statistical tests is written in Python 2.7 using the Pylab, Numpy and SciPy library, for scientific research (Hunter, 2007; Oliphant,

2007; Millman and Aivazis, 2011; Walt et al., 2011). All calculations were performed on a standard desktop PC. The statistical distributions were fitted using a maximum likelihood estimator provided by the stats library included in SciPy. Bootstrapped data sets were drawn using a self-written Python program based on the Numpy (random) library (Walt et al., 2011).

RESULTS

Distribution of ASR Peak-to-Peak Amplitudes

For any proper selection of statistical tests to be applied to a certain data set, knowledge about the distribution of values within the data set is crucial. Therefore, to obtain a valuable estimation of the distributions of startle reflex amplitudes and the PPI values, data from $n = 6$ animals were collected, with 200 gap and 200 no-gap condition measurement repetitions each. The stimulation paradigm for these measurements was a narrow band noise centered around 2 kHz with a spectral width of half an octave (cf. section Methods).

For processing the raw data, a fully automated procedure based on a MATLAB program has been applied. This fully automated evaluation of the startle reflex amplitudes provides the advantage that the evaluation is not influenced by any subjective bias. The program applies a low-pass (Butterworth, 6th order) filter with a cutoff frequency of 40 Hz to remove any high frequency background noise. The cutoff frequency was chosen not to distort the startle reflex amplitudes (cf. Supplementary Figure 1). Invalid trials, i.e., trials where the animal moved during the 550 ms time interval before the startle stimulus, were detected using an empirically determined threshold criterion (force > 0.2 mN $= 0.5$ mV) and discarded from further analysis (**Figures 1A,B**). The peak-to-peak amplitudes of the valid ASRs were calculated from the 150 ms time interval starting at stimulus onset (**Figures 1C,D**).

The distributions of the ASR amplitudes for gap (A_{gap}) stimuli are compared to those of the no-gap condition (A_{nogap}), as this is critical for any statistical testing of PPI changes. Obviously, the peak-to-peak ASR amplitudes (**Figure 1E**) were not Gaussian-like distributed, indicating that standard parametric testing procedures, (such as *t*-testing) cannot be applied to ASR amplitudes, ratios of ASR amplitudes or PPI values. Or in other words, the mean of the peak-to-peak ASR amplitude is highly influenced by outliers and therefore, may not be considered a good statistical measure. This is further demonstrated with 10,000 bootstrapped data sets drawn from the measured no-gap ASR amplitudes (of the shown animal) as shown in **Figure 1F**, where the distributions of the medians as well as the means are given. The accompanying boxplots provide evidence that the variance of the means is significantly higher than the variance of the medians. Taken together, any statistical analysis of raw ASR data must be based on non-parametrical testing. For example, Mann-Whitney U-statistics could be applied to test if presentation of a gap in noise before the startle stimulus led to a significant PPI (as evident in **Figure 1C** vs. **Figure 1D**: $p < 0.001$) which represents the basis for GPIAS behavioral testing for tinnitus assessment.

Distribution of PPI Values

As demonstrated in the previous section, ASR amplitudes are broadly distributed and skewed. Therefore, comparing the ASR amplitudes with and without gap cannot simply be achieved by using the mean and standard deviation, but the whole distribution has to be taken into account. From our dataset (200 measurement repetitions at 2 kHz band noise gap and no-gap, respectively), the ratio of all combinations of gap and no-gap ASR amplitudes was calculated (cf. Supplementary Figures 3, 4). The full combinatorial number N_{PPI} of all possibly ASR amplitude combinations is given by:

$$N_{PPI} = N_{A_{gap}} \cdot N_{A_{nogap}} \tag{2}$$

Hence, if all measurements are valid, a maximal number of 40,000 PPI values can be calculated. The calculation of all combinatorial PPI values is a valid estimator for the distribution of the compound variable (combination of several variables, Poe et al., 2005).

To compare the histogram of the PPI values to standard stochastic distributions, ASR amplitude ratios (= 1-PPI; cf. Equation 1) were used to shift the value range from $]-\infty, 1]$ (**Figure 2A2**) to $[0, \infty[$ (**Figure 2A1**). Fitting different distributions to the data using a maximum likelihood estimator revealed that the lognormal distribution provides the highest likelihood and therefore estimates the true data distribution best. Consequently, the calculated logarithm of the ASR amplitude ratios was approximately normally distributed (**Figure 2B**). The finding that the ratio distribution can be approximated with a lognormal distribution has been reported for standard startle paradigms in humans and mice (Csomor et al., 2008). Additionally, the Akaike information criterion was used to quantify which distribution fits best. This criterion introduces a penalty for the number of used fit parameters preventing overfitting (Akaike, 1974; Saffron et al., 2006). This criterion also leads to the result that the lognormal distribution fits best to the observed data.

Therefore, although parametric statistics may not be applied to raw ASR amplitude ratio data, parametric statistics may be applied to logarithmized ASR amplitude ratio data.

To further examine the underlying statistical distribution, a quantile analysis was performed, allowing for the evaluation of how well a given distribution describes the data (q-q plot and p-p plot, Michael, 1983; Gan and Koehler, 1990; Holmgren, 1995). The cumulated distribution (integral function of probability density) of the fitted distributions and of the data (ratio histogram) were calculated (**Figure 2C**).

Figures 2D,E show so called p-p and q-q analysis respectively. These plots indicate how well the histogram could be described by the fitted distributions, with a perfect fit resulting in all

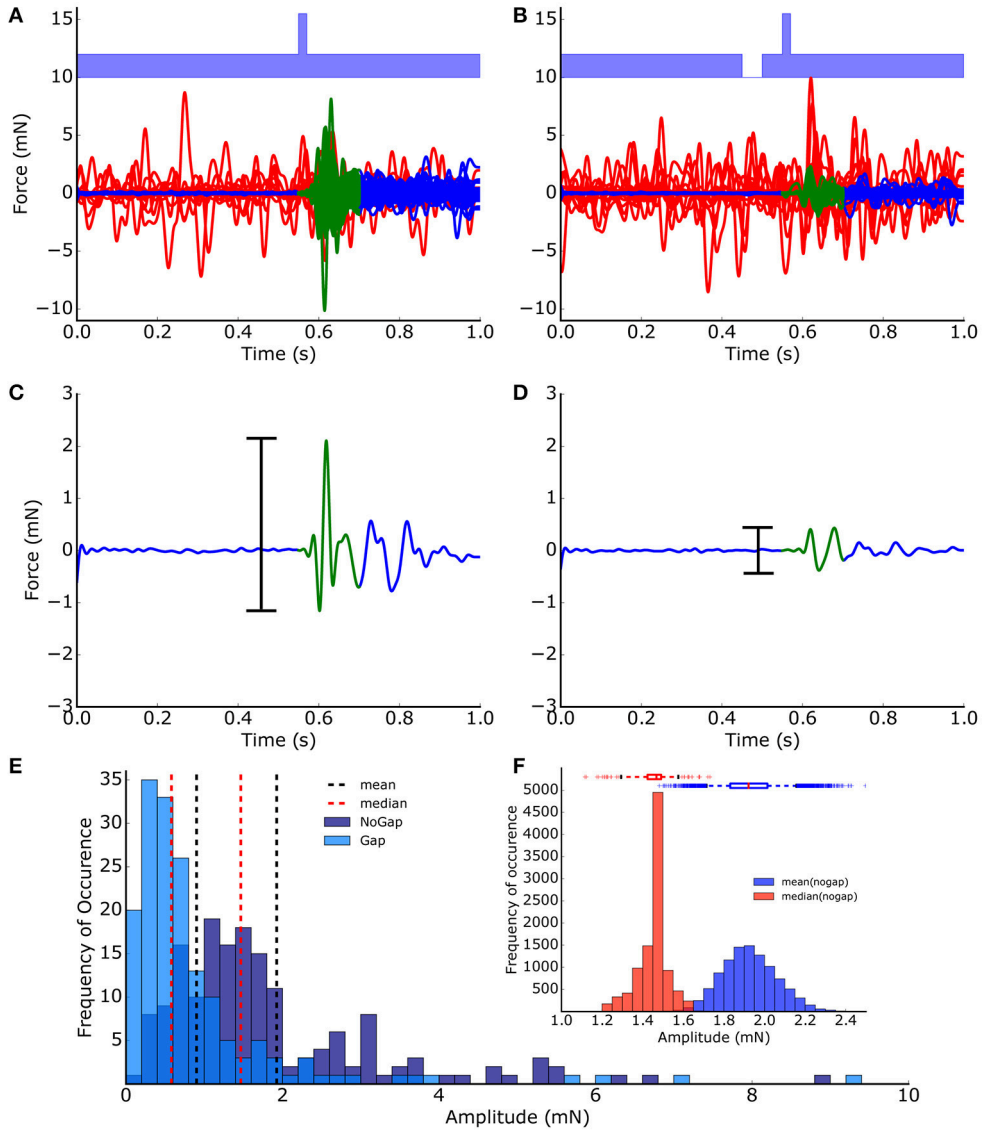

FIGURE 1 | ASR peak-to-peak amplitude extraction and distribution. Startle reflexes ($N = 200$) of one animal from no-gap **(A)** and gap **(B)** condition measurements (light blue, band-noise, 2 kHz). Valid trials (blue/green lines) and invalid trials (red lines) were classified automatically based on a low-pass filtering and threshold procedure. **(C,D)** The peak-to-peak-amplitudes (black bars) of the 150 ms time intervals directly after the stimulus (green) was used as a measure of the strength of the startle reflex (startle reflex amplitude). **(E)** Frequency distribution of startle reflex amplitudes. **(F)** Distribution of means (blue) and medians (red) calculated from 10,000 bootstrapped data sets (no-gap ASR amplitudes, boxes: quartiles, whisker: 5–95% quantiles); The bootstrap procedure provides evidence that the median is the more robust measure for the startle amplitudes compared to the mean.

supporting points lying on the identity line for p-p (**Figure 2D**) as well as for q-q plots (**Figure 2E**).

For q-q analysis the quantiles of the data are plotted as a function of the quantiles of the fitted distribution. The q-q analysis emphasizes the edges of the distributions. One scale invariant representation is the so called p-p plot (Holmgren, 1995) comparing the cumulated probabilities (cannot exceed 1). Thus, the upper limit of a p-p plot is always one. This q-q plot shows that the ratio distribution could be nicely described by a lognormal distribution up to the 95% percentile (further animals are shown in Supplementary Figure 5).

As the standard procedure for tinnitus detection in animal models is the analysis of the PPI decrease due to a treatment (in most cases an acoustic trauma, Bauer and Brozoski, 2001; Norena and Eggermont, 2003; Yang et al., 2011; Ahlf et al., 2012; Tziridis et al., 2015; Krauss et al., 2016b) the next section discusses possible measures for PPI change and valid inferential statistical tests.

GPIAS Statistics

As demonstrated above, the ASR amplitude ratios (1-PPI) can be described by lognormal distributions up to the 95% quantile, so

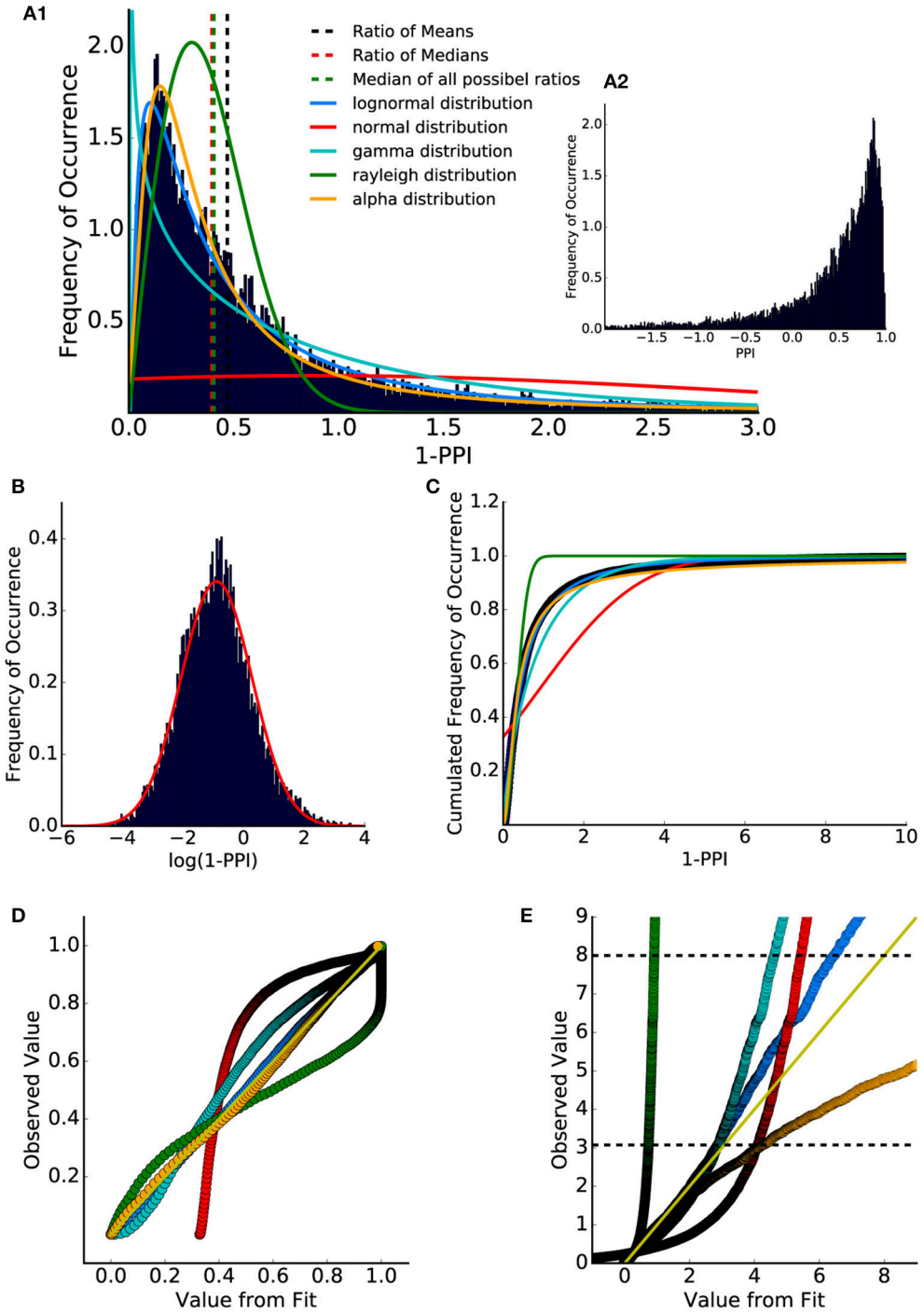

FIGURE 2 | Distribution of ASR amplitude ratios and PPI values. **(A1)** Ratio between all combinations of gap and no-gap ASR amplitudes (1-PPI). Data were fitted with lognormal (solid blue), gamma (solid cyan), Rayleigh (solid green), and alpha (solid orange) distribution (normed probability densities). Ratio of means of gap and no-gap ASR amplitudes (dashed black) differed from the ratio of medians of gap and no-gap ASR amplitudes (dashed red), which is similar to the median of the (full combinatorial) ratio distribution (dashed green). **(A2)** Distribution of PPI values (1-A_gap/A_no-gap). The histogram shows that the PPI values cover a wide codomain including a considerable number of negative values (]−∞,1]). **(B)** Histogram of the logarithmized (base e) ASR amplitude ratios. When plotted this way, the data are almost Gaussian-like distributed. **(C)** Cumulative distribution of the ASR amplitude ratios (dark blue dots) and cumulative distribution function of the fitted distributions: lognormal (blue) and Gaussian (red), gamma (cyan), Rayleigh (green) and alpha (orange). **(D)** p-p plot shows that the lognormal distribution describes the data best. However, the q-q plot **(E)** provides evidence that for percentiles higher than 95% (lower black dashed line) the lognormal distribution slightly differs from the measured values.

that parametric statistics may be applied if data are logarithmized. To test if the ASR amplitude ratio distributions changed significantly post trauma relative to pre trauma conditions, the combined standard error of the logarithmized ASR amplitude ratios was calculated. The logarithmized ratio of the ASR amplitudes is given by the difference:

$$\log\left(\frac{A_{gap}}{A_{nogap}}\right) = \log\left(A_{gap}\right) - \log\left(A_{nogap}\right) = L_{gap} - L_{nogap} \quad (3)$$

As the logarithmized ASR amplitude ratios are Gaussian-like distributed, and the difference of two Gaussian-like distributed random variables is again Gaussian-like distributed (Eisenberg and Sullivan, 2008; Kersting and Wakolbinger, 2008), one may infer that also the logarithmized ASR amplitude values for both the gap and the no-gap condition could be Gaussian-like distributed (however it is not crucial that they are Gaussian distributed). That this is at least the case for our data set could be confirmed using the Shapiro-Wilk test (Shapiro and Wilk, 1965) (**Figure 3**, $p > 0.1$ for both the gap and the no-gap condition). The standard error for the distribution of the logarithmized ASR amplitude ratios can therefore be calculated using error propagation.

$$\Delta L_{ges}^2 = \Delta L_{gap}^2 + \Delta L_{nogap}^2 \quad (4)$$

(ΔL_{ges}: standard error of means of the logarithmized ratios, $\Delta L_{gap}/\Delta L_{nogap}$: standard error of means of logarithmized gap and no-gap amplitudes)

Furthermore, the variance of a compound Gaussian-like distributed measure is simply the sum of the variances (Satterthwaite, 1941). The effective number of independent samples of the compound variable can be calculated using the variance and the standard error

$$n = \frac{Var(L_{ges})}{\Delta {L_{ges}}^2} \quad (5)$$

where $Var(L_{ges})$ is the variance of the compound distribution and ΔL_{ges} the standard error of the compound distribution. However, this effective n is only an approximation and may also be replaced by a more conservative estimation (cf. Supplementary Figures 7D–F). The information on the standard error of the compound variable (logarithmized ratios) and an effective n makes it possible to test if the pure tone acoustic trauma leads to a significant change of the logarithmized ratios. In other words, the null hypothesis (H_0) can be formulated as follows:

The logarithmized ratios before (L_{ges}^{pre}) and after (L_{ges}^{post}) the acoustic trauma arise from the same distribution. (Note that this test is done for each stimulus center frequency individually).

Calculating the values $Mean(L_{ges})$ and ΔL_{ges}^2 for pre- and post-trauma conditions allows calculating the T statistics for the comparison between pre- and post-trauma conditions:

$$T = \frac{Mean\left(L_{ges}^{pre}\right) - Mean\left(L_{ges}^{post}\right)}{\sqrt{\Delta {L_{ges}^{pre}}^2 + \Delta {L_{ges}^{post}}^2}} \quad (6)$$

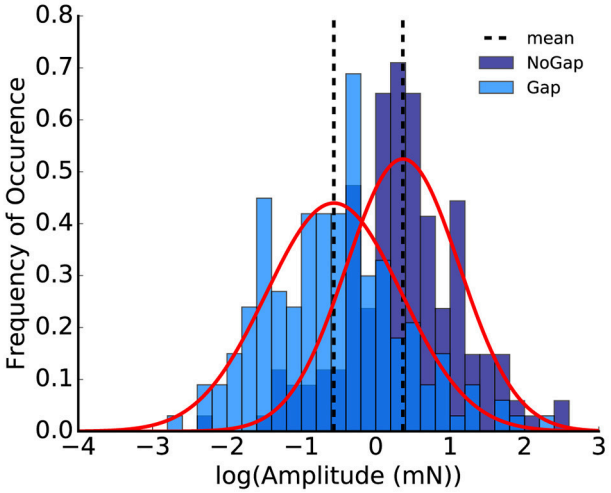

FIGURE 3 | Histogram of the logarithmized peak-to-peak amplitudes; the plot shows the animal replot of the data from Figure 1E. Shapiro-Wilk-test for normality emphasizes that the logarithmized amplitudes are Gaussian-like distributed ($p = 0.11$ for no-gap and $p = 0.21$ for gap, normalized probability density).

The mean of the logarithmized ratios ($Mean\left(L_{ges}^{pre/\ post}\right)$) is the difference of the means of the logarithmized gap and no-gap amplitudes. However, it can also be regarded as the mean of the full combinatorial difference of logarithmized gap and no-gap amplitudes. It can be shown that these two possibilities are equal (cf. Supplements, Equation 2).

Finally, using the T-statistics, a p-value can be calculated:

$$p = 2 \cdot \int_{-\infty}^{-|T|} Stud\left(t, df\ (n)\right)\ dt \quad (7)$$

Where T refers to the test statistics, Stud to the students T-distribution and df(n) are the degrees of freedom (Monte Carlo simulation used to prove validity of statistics cf. simulation study Supplementary Figures 6, 7).

In summary, the paragraph shows how to calculate the p-value when comparing the logarithmized ASR-ratios before and after an acoustic trauma for one specified stimulus frequency (band noise center frequency) and one animal. The p-value is a measure for the probability that the null hypothesis (pure tone trauma has no effect on ASR amplitude ratios) is falsely rejected. In other words, this p-value indicates if the effect of a change of the average ratio of gap and no-gap amplitudes is by chance (sampling error) or if there exists a real effect. However, this measure only indirectly gives information on the size of the effect. Furthermore, it has to be considered that not significant ($p > 0.05$) does not mean that the trauma did not lead to any effect (e.g., development of a tinnitus percept) but that the sample size is too small to detect the effect or that there is no effect. The p-value makes no statement about the second order error.

Effect Size as a Novel and Normed Measure for the PPI Change (ΔPPI)

Furthermore, a novel measure for the ΔPPI (until now: ΔPPI = PPI_{post} - PPI_{pre}) and hence for the tinnitus percept can be defined using the effect size (**Figure 4D**). This measure for the PPI change is normalized and not dependent on the dimension of the measured variables. This measure is based on two Gaussian distributions and represents the difference of the means in terms of standard deviations (**Figures 4A–C**).

As the sample sizes are not equal, the definition by Hedges (Zimmermann et al., 2005; Nakagawa and Cuthill, 2007; Hofmann and Smits, 2008) has to be used.

$$g = \left(Mean(L_{ges}^{pre}) - Mean(L_{ges}^{post})\right)/s \quad (8)$$

$$s = \sqrt{\frac{\left(n_{pre}-1\right)\cdot\ Var(L_{ges}^{pre}) + \left(n_{post}-1\right)\ \cdot\ Var(L_{ges}^{post})}{(n_{pre}+n_{post}-2)}} \quad (9)$$

(s: combined, weighted standard deviation, $L_{ges}^{pre/post}$: logarithmized ratios for pre and post condition, respectively, $n_{pre/post}$ sample size same as for Welch-*t*-test, as variance estimator of $L_{ges}^{pre/post}$ the sum of the variences of logarithmized gap and no-gap amplitudes are used).

Furthermore, the effect size (Hedges g) can be corrected for small sample sizes (Hedges, 1982; Nakagawa and Cuthill, 2007):

$$g^* = \left(1 - \frac{3}{4\cdot(n_{pre}+n_{post})-9}\right)\cdot g \quad (10)$$

In the following the effect size is used as synonym for the sample size-corrected version of Hedges g (g*).

Figure 4D gives the effect size (corrected Hedges g) of one exemplary animal as a function of the frequency spectrum (center frequency) of the band noise presented. This animal showed a clear effect (ASR amplitude ratio decrease, PPI increase) at a center frequency of 1 kHz and a PPI decrease at a frequency of 5.7 kHz, indicating a potential tinnitus percept there.

Figure 5 summarizes the GPIAS results for all 26 animals to which the standard tinnitus paradigm was applied. It shows the effects size as a function of the median of the classical ΔPPI (calculated from the full combinatorial of all pre and post PPI values, error bars: quartiles, complete procedure of full combinatorial calculation shown in Supplements, cf. Supplementary Figures 3, 4), significant PPI changes are either colored in red (PPI decrease) or green (PPI increase). Trivially, effect size and ΔPPI are highly correlated, but the effect size is normed by the standard deviations of the logarithmized ASR amplitude ratio distributions. Using this new statistical criterion, only three animals show a significant PPI decrease indicating that the number of measured trials for the standard Turner paradigm might be too low to see small effects.

DISCUSSION

To date, there are no proper, universally accepted and used statistics for the determination of a significant change of the PPI as an indicator for a tinnitus percept in animals. With this study we attempted to provide such a statistical approach for variance estimation of PPIs and for reliably testing if PPI changes in a GPIAS paradigm, e.g., after trauma, are significant. The method is robust and does not require any removal of outliers, which otherwise is a common procedure (e.g., Longenecker and Galazyuk, 2011), and therefore can be applied fully automated.

The basis of that analysis is that the ASR amplitude ratios (1-PPI) of gap and no-gap ASR amplitudes are lognormally distributed for percentiles lower than 95%, estimated by calculating the full combinatorial of the gap and no-gap ASR amplitudes and q-q-analysis and p-p-analysis. In addition, the Shapiro-Wilk-test for normality provides evidence that the logarithmized ASR amplitude ratios are well described by a normal distribution. Hence, the effect size can be used as a normalized measure for the PPI change. Finally, the Welch-*T*-test, used on the propagated error, provides a measure for the significance value of that change.

In contrast to the statistics proposed here, earlier evaluation procedures calculated PPI values by simply combining the averaged gap and no-gap ASR amplitudes (Lehmann et al., 2000; Joober et al., 2002; Jovanovic et al., 2004; Wolff and Bilkey, 2010) and therefore the information about measurement uncertainties (variance) was removed. As a result of such procedures, it is impossible to provide information on the variance of the PPI values as well as the *p*-value of a possible PPI change. Some approaches tried to overcome these limitations by averaging the ASR amplitudes during the gap condition and dividing it by all ASR amplitudes during the no-gap condition (Longenecker and Galazyuk, 2011; Tziridis et al., 2012). However, thereby the *t*-test was erroneously used on the averaged variables, which are not normally distributed and therefore, may not be tested parametrically. Furthermore, there is no clear rule which ASR amplitudes (gap or no-gap) should be averaged and consequently the methods are ambiguous. The results of dividing all gap amplitudes by the averaged no-gap amplitudes obviously leads to different results than dividing the averaged gap amplitude by all no-gap amplitudes.

Additionally, it should be noted that averaging of one variable amplitude (gap or no-gap) leads to an underestimation of the error of the compound variable (ratio) and therefore applying inferential statistics to this data leads to an overestimation of the *p*-value.

All these calculations are usually performed to obtain information about the existence of a possible tinnitus percept in the animal tested. In tinnitus research, animals are often divided into a tinnitus group (T; based on significant PPI decrease in the GPIAS paradigm) and a second, no-tinnitus group (NT) containing the animals showing no significant PPI decrease after an acoustic trauma. The criterion for T animals is usually that a significant PPI decrease (Ahlf et al., 2012; Tziridis et al., 2015; Krauss et al., 2016a) at least in one specific frequency can be observed. Thereby the significance level α has to be corrected as one false positive value, out of all tested frequencies would lead to a false positive T-animal status. By using the Bonferroni-correction, the significance level α has to be adapted to the number of measured stimulus frequencies by dividing the value

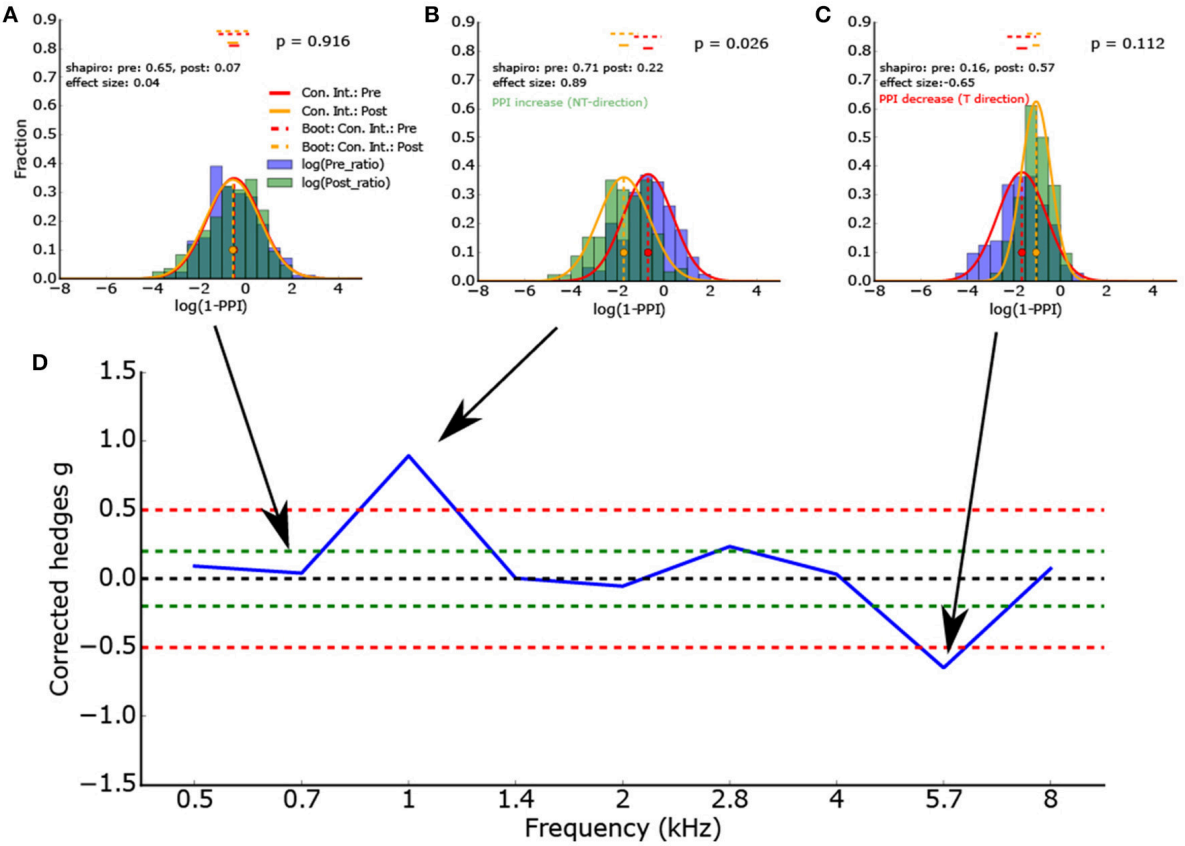

FIGURE 4 | (A–C) Histogram of the logarithmized ratios of gap and no-gap amplitudes (full combinatorial) for different center frequencies (0.7, 1.0, and 5.7 kHz) of the band noise presented. As the ASR amplitude ratios are almost lognormal distributed, the logarithmized values are Gaussian-like distributed. Application of the Shapiro-Wilk test proves that the normal distribution is a valid description of the data. The red and orange vertical lines show the full combinatorial 95% confidence intervals. The dashed vertical line show the 2.5–97.5% quantile of the means calculated via bootstrapping. The size of the bootstrapped data sets was the minimum of the number of peak-to peak amplitudes of gap and no-gap measurement. In other words the bootstrapped data sets provide the upper limit of the variance of the determined means (the confidence intervals). **(D)** To determine the PPI change, the corrected effect size is used (n1, n2 same sample size used for statistics). To test if the distributions differ significantly, inferential statistics were applied. However, as the ASR amplitude ratios arise from all combinations of gap and no-gap amplitudes, it is possible that the number of independent ASR amplitude ratios is overestimated.

by the number of measured frequencies, but this has not been done in many studies using GPIAS. In our approach proposed here, the PPI change at one stimulus frequency is significant if the calculated significance level is lower than $\alpha = 0.05/9 = 0.0056$. This correction is only used to identify T-animals, if the stimulation frequencies are treated individually and the α-value is set to 0.05. A reduction of the α-value for single frequencies would lead to a higher second order error. Additionally, by performing this analysis it has to be considered that classification as NT does not mean that the animals definitely have no tinnitus, but only that no significant PPI change for any tested stimulus frequency could be determined.

Despite these statistical considerations further criteria for the separation of T and NT animals should be taken into account.

In classical GPIAS approaches, as the *p*-value is highly dependent on the number of measured trials (measurement repetitions), the fraction of T animals will rise systematically with increasing measurement repetitions (number of repetitions of the same stimulus to better estimate the underlying distribution of the startle amplitudes).

Hence, altering of the number of applied stimulus repetitions leads to a systematic shift of the number of T classified animals (cf. Supplements: "Classification of T animals: significance criterion compared to effect size threshold" and Supplementary Figure 8).

One modern method to compare the distributions of test statistics of two groups, even if these distributions are not Gaussian, would be Bayesian statistics (Kruschke, 2013). However, even this approach does not solve the problem of systematically rising number of T classified animals for increasing number of measurement repetitions. We therefore refrained from elaborating it here.

Therefore, we here propose to choose a criterion independent from the number of trials, such as the effect size. Trivially, an increase of measurement repetitions will then lead to a more exact estimation of the effect size (cf. Supplementary Figure 8).

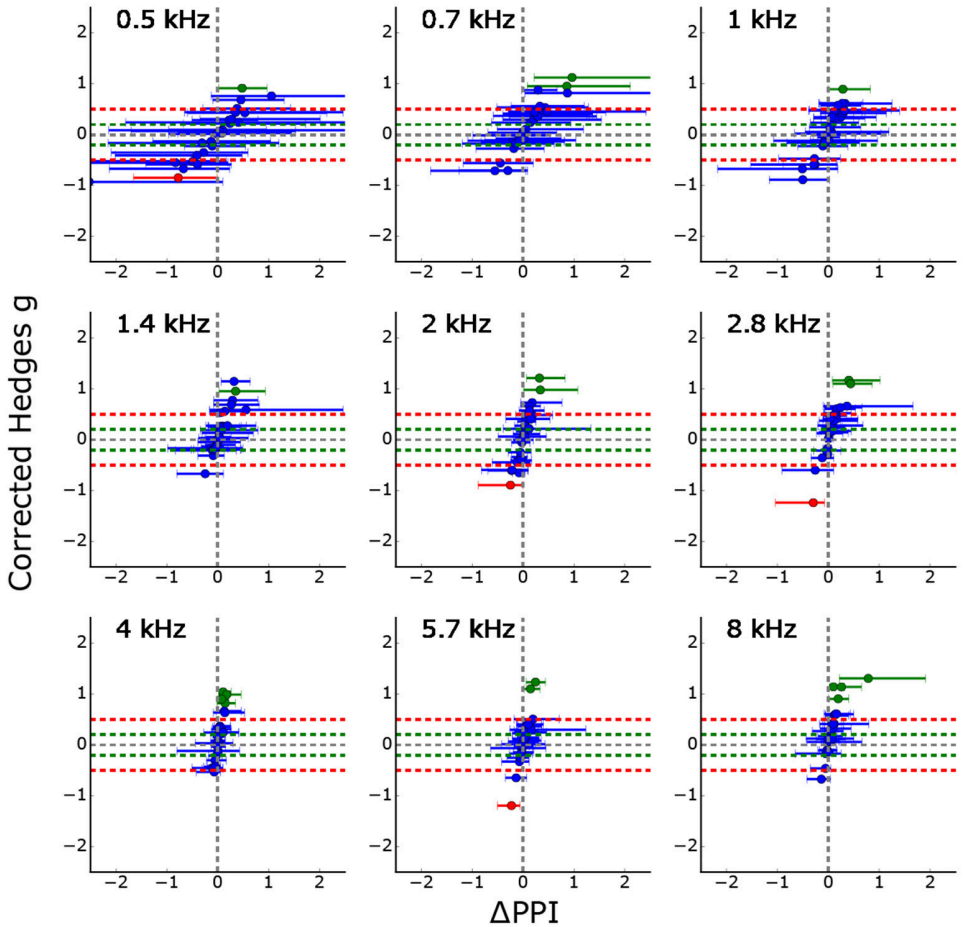

FIGURE 5 | Corrected effect size as a function of the median ΔPPIs for all 26 animals (error bars: quartiles). Significant PPI decrease (based on Welch-Test cf. section GPIAS statistics, $p < 0.05$) is marked in red whereas significant PPI increase is marked in green. Trivially, the effect size and the median ΔPPI are highly correlated, but the effect size is additionally normed by the standard deviation of the logarithmized ASR amplitude ratio distributions. The figure shows that only three PPI changes become significantly smaller (red) for all 26 animals and 9 stimulus frequencies each.

However, no systematic deviation of the effect size depending on the number of measurement repetitions is observable. In other words, we believe that a separation of T and NT animals based on the effect size of PPI change in GPIAS represents a more reliable approach for tinnitus assessment in animals than the commonly used significance criteria.

In summary, the study demonstrates that ASR amplitudes for single animals are not Gaussian-like distributed. Furthermore, the ratios of ASR amplitudes during gap and no-gap conditions are well (although not perfectly) described by lognormal distributions. Based on this insight it is possible to estimate a *p*-value specifying if any observed PPI change after an acoustic trauma is significant. Alternatively, it is possible to calculate a normed measure, the effect size, which can be used to divide animals in T and NT animals as it is not systematically influenced by the number of applied measurement repetitions but should saturate at a certain value (cf. Supplementary Figure 8).

AUTHOR CONTRIBUTIONS

AS, HS, KT, and PK designed the study; AS performed the measurements and implemented the computer simulations; AS, RG, PK, CM developed and discussed the statistics; AS, HS, KT, RG wrote the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnbeh.2017.00198/full#supplementary-material

REFERENCES

Ahlf, S., Tziridis, K., Korn, S., Strohmeyer, I., and Schulze, H. (2012). Predisposition for and prevention of subjective tinnitus development. *PLoS ONE* 7:e44519. doi: 10.1371/journal.pone.0044519

Akaike, H. (1974). A new look at the statistical model identification. *IEEE Trans. Automat. Contr.* 19, 716–723. doi: 10.1109/TAC.1974.1100705

Bauer, C. A., and Brozoski, T. J. (2001). Assessing tinnitus and prospective tinnitus therapeutics using a psychophysical animal model. *J. Assoc. Res. Otolaryngol.* 2, 54–64. doi: 10.1007/s101620010030

Brozoski, T. J., and Bauer, C. A. (2016). Animal models of tinnitus. *Hear. Res.* 338, 88–97. doi: 10.1016/j.heares.2015.10.011

Campolo, J., Lobarinas, E., and Salvi, R. (2013). Does tinnitus "fill in" the silent gaps? *Noise Health* 15, 398–405. doi: 10.4103/1463-1741.121232

Coles, R. R. (1984). Epidemiology of tinnitus: (1) prevalence. *J. Laryngol. Otol. Suppl.* 9, 7–15. doi: 10.1017/S1755146300090041

Csomor, P. A., Yee, B. K., Vollenweider, F. X., Feldon, J., Nicolet, T., and Quednow, B. B. (2008). On the influence of baseline startle reactivity on the indexation of prepulse inhibition. *Behav. Neurosci.* 122, 885–900. doi: 10.1037/0735-7044.122.4.885

Eggermont, J. J. (2003). Central tinnitus. *Auris Nasus Larynx* 30(Suppl.), S7–S12. doi: 10.1016/S0385-8146(02)00122-0

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Eisenberg, B., and Sullivan, R. (2008). Why is the sum of independent normal random variables normal? *Math. Mag.* 81, 362–366.

Elgoyhen, A. B., Langguth, B., De Ridder, D., and Vanneste, S. (2015). Tinnitus: perspectives from human neuroimaging. *Nat. Rev. Neurosci.* 16, 632–642. doi: 10.1038/nrn4003

Engineer, N. D., Riley, J. R., Seale, J. D., Vrana, W. A., Shetake, J. A., Sudanagunta, S. P., et al. (2011). Reversing pathological neural activity using targeted plasticity. *Nature* 470, 101–104. doi: 10.1038/nature09656

Fournier, P., and Hébert, S. (2016). The gap-startle paradigm to assess auditory temporal processing: bridging animal and human research. *Psychophysiology* 53, 759–766. doi: 10.1111/psyp.12620

Galazyuk, A., and Hebert, S. (2015). Gap-Prepulse Inhibition of the Acoustic Startle Reflex (GPIAS) for tinnitus assessment: current status and future directions. *Front. Neurol.* 6:88. doi: 10.3389/fneur.2015.00088

Gan, F., and Koehler, K. (1990). Goodness-of-fit tests based on p-p probability plots. *Technometrics* 32, 289–303. doi: 10.2307/1269106

Gerken, G. M. (1996). Central tinnitus and lateral inhibition: an auditory brainstem model. *Hear. Res.* 97, 75–83. doi: 10.1016/S0378-5955(96)80009-8

Goebel, G., Rübler, D., Stepputat, F., Hiller, W., Heuser, J., and Fichter, M. (1999). "Controlled prospective study of tinnitus retraining therapy compared to tinnitus coping therapy and broad-band noise generator therapy," in *Proceedings of the Sixth International Tinnitus Seminar* (Cambridge: Citeseer), 302–306.

Hedges, L. V. (1982). Estimation of effect size from a series of independent experiments. *Psychol. Bull.* 92:490. doi: 10.1037/0033-2909.92.2.490

Heffner, H. E., and Harrington, I. A. (2002). Tinnitus in hamsters following exposure to intense sound. *Hear. Res.* 170, 83–95. doi: 10.1016/S0378-5955(02)00343-X

Heller, A. J. (2003). Classification and epidemiology of tinnitus. *Otolaryngol. Clin. North Am.* 36, 239–248. doi: 10.1016/S0030-6665(02)00160-3

Hinkle, D. E., Wiersma, W., and Jurs, S. G. (2003). *Applied Statistics for the Behavioral Sciences*. Washington, DC: American Educational Research Association and American Statistical Association.

Hofmann, S. G., and Smits, J. A. (2008). Cognitive-behavioral therapy for adult anxiety disorders: a meta-analysis of randomized placebo-controlled trials. *J. Clin. Psychiatry* 69:621. doi: 10.4088/JCP.v69n0415

Holmgren, E. B. (1995). The PP plot as a method for comparing treatment effects. *J. Am. Stat. Assoc.* 90, 360–365. doi: 10.1080/01621459.1995.10476520

Hunter, J. D. (2007). Matplotlib: a 2D graphics environment. *Comput. Sci. Eng.* 9, 90–95. doi: 10.1109/MCSE.2007.55

Husain, F. T. (2016). Neural networks of tinnitus in humans: elucidating severity and habituation. *Hear. Res.* 334, 37–48. doi: 10.1016/j.heares.2015.09.010

Jastreboff, P. J., Brennan, J. F., Coleman, J. K., and Sasaki, C. T. (1988a). Phantom auditory sensation in rats: an animal model for tinnitus. *Behav. Neurosci.* 102, 811–822. doi: 10.1037/0735-7044.102.6.811

Jastreboff, P. J., Brennan, J. F., and Sasaki, C. T. (1988b). An animal model for tinnitus. *Laryngoscope* 98, 280–286. doi: 10.1288/00005537-198803000-00008

Joober, R., Zarate, J.-M., Rouleau, G.-A., Skamene, E., and Boksa, P. (2002). Provisional mapping of quantitative trait loci modulating the acoustic startle response and prepulse inhibition of acoustic startle. *Neuropsychopharmacology* 27, 765–781. doi: 10.1016/S0893-133X(02)00333-0

Jovanovic, T., Szilagyi, S., Chakravorty, S., Fiallos, A. M., Lewison, B. J., Parwani, A., et al. (2004). Menstrual cycle phase effects on prepulse inhibition of acoustic startle. *Psychophysiology* 41, 401–406. doi: 10.1111/1469-8986.2004.00166.x

Kersting, G., and Wakolbinger, A. (2008). Prinzipien des Schätzens. *Elementare Stochastik* 115–119. doi: 10.1007/978-3-7643-8431-9_19

Knipper, M., Ruettiger, L., Schick, B., and Dlugaiczyk, J. (2011). Glycine *Receptor Agonists* for the *Treatment* of *Phantom Phenomena*. Google Patents.

Koch, M. (1999). The neurobiology of startle. *Prog. Neurobiol.* 59, 107–128. doi: 10.1016/S0301-0082(98)00098-7

Krauss, P., Tziridis, K., Buerbank, S., Schilling, A., and Schulze, H. (2016a). Therapeutic value of Ginkgo biloba extract EGb 761(R) in an animal model (*Meriones unguiculatus*) for noise trauma induced hearing loss and tinnitus. *PLoS ONE* 11:e0157574. doi: 10.1371/journal.pone.0157574

Krauss, P., Tziridis, K., Metzner, C., Schilling, A., Hoppe, U., and Schulze, H. (2016b). Stochastic resonance controlled upregulation of internal noise after hearing loss as a putative cause of tinnitus-related neuronal hyperactivity. *Front. Neurosci.* 10:597. doi: 10.3389/fnins.2016.00597

Kruschke, J. K. (2013). Bayesian estimation supersedes the t test. *J. Exp. Psychol. Gen.* 142, 573–603. doi: 10.1037/a0029146

Langguth, B., Landgrebe, M., Kleinjung, T., Sand, G. P., and Hajak, G. (2011). Tinnitus and depression. *World J. Biol. Psychiatry* 12, 489–500. doi: 10.3109/15622975.2011.575178

Leaver, A. M., Turesky, T. K., Seydell-Greenwald, A., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2016). Intrinsic network activity in tinnitus investigated using functional MRI. *Hum. Brain Mapp.* 37, 2717–2735. doi: 10.1002/hbm.23204

Lehmann, J., Pryce, C. R., and Feldon, J. (2000). Lack of effect of an early stressful life event on sensorimotor gating in adult rats. *Schizophr. Res.* 41, 365–371. doi: 10.1016/S0920-9964(99)00080-8

Lewis, J. E., Stephens, S. D., and Mckenna, L. (1994). Tinnitus and suicide. *Clin. Otolaryngol. Allied Sci.* 19, 50–54. doi: 10.1111/j.1365-2273.1994.tb01147.x

Liberman, L. D., Suzuki, J., and Liberman, M. C. (2015). Dynamics of cochlear synaptopathy after acoustic overexposure. *J. Assoc. Res. Otolaryngol.* 16, 205–219. doi: 10.1007/s10162-015-0510-3

Longenecker, R. J., and Galazyuk, A. V. (2011). Development of tinnitus in CBA/CaJ mice following sound exposure. *J. Assoc. Res. Otolaryngol.* 12, 647–658. doi: 10.1007/s10162-011-0276-1

Michael, J. R. (1983). The stabilized probability plot. *Biometrika* 70, 11–17. doi: 10.1093/biomet/70.1.11

Millman, K. J., and Aivazis, M. (2011). Python for scientists and engineers. *Comput. Sci. Eng.* 13, 9–12. doi: 10.1109/MCSE.2011.36

Nakagawa, S., and Cuthill, I. C. (2007). Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biol. Rev. Camb. Philos. Soc.* 82, 591–605. doi: 10.1111/j.1469-185X.2007.00027.x

Norena, A. J., and Eggermont, J. J. (2003). Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. *Hear. Res.* 183, 137–153. doi: 10.1016/S0378-5955(03)00225-9

Norena, A. J., Moffat, G., Blanc, J. L., Pezard, L., and Cazals, Y. (2010). Neural changes in the auditory cortex of awake guinea pigs after two tinnitus inducers: salicylate and acoustic trauma. *Neuroscience* 166, 1194–1209. doi: 10.1016/j.neuroscience.2009.12.063

Nowotny, M., Remus, M., Kossl, M., and Gaese, B. H. (2011). Characterization of the perceived sound of trauma-induced tinnitus in gerbils. *J. Acoust. Soc. Am.* 130, 2827–2834. doi: 10.1121/1.3646902

Ohl, F. W., and Scheich, H. (2005). Learning-induced plasticity in animal and human auditory cortex. *Curr. Opin. Neurobiol.* 15, 470–477. doi: 10.1016/j.conb.2005.07.002

Ohl, F. W., Scheich, H., and Freeman, W. J. (2001). Change in pattern of ongoing cortical activity with auditory category learning. *Nature* 412, 733–736. doi: 10.1038/35089076

Oliphant, T. E. (2007). Python for scientific computing. *Comput. Sci. Eng.* 9, 10–20. doi: 10.1109/MCSE.2007.58

Pantev, C., Okamoto, H., and Teismann, H. (2012). Music-induced cortical plasticity and lateral inhibition in the human auditory cortex as foundations for tonal tinnitus treatment. *Front. Syst. Neurosci.* 6:50. doi: 10.3389/fnsys.2012.00050

Poe, G. L., Giraud, K. L., and Loomis, J. B. (2005). Computational methods for measuring the difference of empirical distributions. *Am. J. Agric. Econ.* 87, 353–365. doi: 10.1111/j.1467-8276.2005.00727.x

Radziwon, K. E., Stolzberg, D. J., Urban, M. E., Bowler, R. A., and Salvi, R. J. (2015). Salicylate-induced hearing loss and gap detection deficits in rats. *Front. Neurol.* 6:31. doi: 10.3389/fneur.2015.00031

Ruttiger, L., Ciuffani, J., Zenner, H. P., and Knipper, M. (2003). A behavioral paradigm to judge acute sodium salicylate-induced sound experience in rats: a new approach for an animal model on tinnitus. *Hear. Res.* 180, 39–50. doi: 10.1016/S0378-5955(03)00075-3

Ruttiger, L., Singer, W., Panford-Walsh, R., Matsumoto, M., Lee, S. C., Zuccotti, A., et al. (2013). The reduced cochlear output and the failure to adapt the central auditory response causes tinnitus in noise exposed rats. *PLoS ONE* 8:e57247. doi: 10.1371/journal.pone.0057247

Saffron, C. M., Park, J. H., Dale, B. E., and Voice, T. C. (2006). Kinetics of contaminant desorption from soil: comparison of model formulations using the Akaike information criterion. *Environ. Sci. Technol.* 40, 7662–7667. doi: 10.1021/es0603610

Satterthwaite, F. E. (1941). Synthesis of variance. *Psychometrika* 6, 309–316. doi: 10.1007/BF02288586

Schaette, R., and Mcalpine, D. (2011). Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J. Neurosci.* 31, 13452–13457. doi: 10.1523/JNEUROSCI.2156-11.2011

Shapiro, S. S., and Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika* 52, 591–611. doi: 10.1093/biomet/52.3-4.591

Turner, J. G., Brozoski, T. J., Bauer, C. A., Parrish, J. L., Myers, K., Hughes, L. F., et al. (2006). Gap detection deficits in rats with tinnitus: a potential novel screening tool. *Behav. Neurosci.* 120, 188–195. doi: 10.1037/0735-7044.120.1.188

Turner, J., and Larsen, D. (2012). "Relationship between noise exposure stimulus properties and tinnitus in rats: results of a 12-month longitudinal study," in *ARO. Abs.*, 594.

Tziridis, K., Ahlf, S., Jeschke, M., Happel, M. F., Ohl, F. W., and Schulze, H. (2015). Noise trauma induced neural plasticity throughout the auditory system of mongolian gerbils: differences between tinnitus developing and non-developing animals. *Front. Neurol.* 6:22. doi: 10.3389/fneur.2015.00022

Tziridis, K., Ahlf, S., and Schulze, H. (2012). A low cost setup for behavioral audiometry in rodents. *J. Vis. Exp.* e4433. doi: 10.3791/4433

Tziridis, K., Korn, S., Ahlf, S., and Schulze, H. (2014). Protective effects of Ginkgo biloba extract EGb 761 against noise trauma-induced hearing loss and tinnitus development. *Neural Plast.* 2014:427298. doi: 10.1155/2014/427298

Valsamis, B., and Schmid, S. (2011). Habituation and prepulse inhibition of acoustic startle in rodents. *J. Vis. Exp.* 55:e3446. doi: 10.3791/3446

Von Der Behrens, W. (2014). Animal models of subjective tinnitus. *Neural Plast.* 2014:741452. doi: 10.1155/2014/741452

Walt, S. V. D., Colbert, S. C., and Varoquaux, G. (2011). The NumPy array: a structure for efficient numerical computation. *Comput. Sci. Eng.* 13, 22–30. doi: 10.1109/MCSE.2011.37

Walter, M., Tziridis, K., Ahlf, S., and Schulze, H. (2012). Context dependent auditory threshold determined by brainstem audiometry and prepulse inhibition in mongolian gerbils. *Open J. Acoustics* 2, 34–49. doi: 10.4236/oja.2012.21004

Wang, H., Brozoski, T. J., and Caspary, D. M. (2011). Inhibitory neurotransmission in animal models of tinnitus: maladaptive plasticity. *Hear. Res.* 279, 111–117. doi: 10.1016/j.heares.2011.04.004

Weinberger, N. M. (1993). Learning-induced changes of auditory receptive fields. *Curr. Opin. Neurobiol.* 3, 570–577. doi: 10.1016/0959-4388(93)90058-7

Westin, V. Z., Schulin, M., Hesser, H., Karlsson, M., Noe, R. Z., Olofsson, U., et al. (2011). Acceptance and commitment therapy versus tinnitus retraining therapy in the treatment of tinnitus: a randomised controlled trial. *Behav. Res. Ther.* 49, 737–747. doi: 10.1016/j.brat.2011.08.001

Wolff, A. R., and Bilkey, D. K. (2010). The maternal immune activation (MIA) model of schizophrenia produces pre-pulse inhibition (PPI) deficits in both juvenile and adult rats but these effects are not associated with maternal weight loss. *Behav. Brain Res.* 213, 323–327. doi: 10.1016/j.bbr.2010.05.008

Yang, G., Lobarinas, E., Zhang, L., Turner, J., Stolzberg, D., Salvi, R., et al. (2007). Salicylate induced tinnitus: behavioral measures and neural activity in auditory cortex of awake rats. *Hear. Res.* 226, 244–253. doi: 10.1016/j.heares.2006.06.013

Yang, S., Weiner, B. D., Zhang, L. S., Cho, S., and Bao, S. (2011). Homeostatic plasticity drives tinnitus perception in an animal model. *Proc. Natl. Acad. Sci. U.S.A.* 108, 14974–14979. doi: 10.1073/pnas.1107998108

Zachriat, C., and Kröner-Herwig, B. (2004). Treating chronic tinnitus: comparison of cognitive-behavioural and habituation-based treatments. *Cogn. Behav. Ther.* 33, 187–198. doi: 10.1080/16506070410029568

Zhang, F. Y., Xue, Y. X., Liu, W. J., Yao, Y. L., Ma, J., Chen, L., et al. (2014). Changes in the numbers of ribbon synapses and expression of RIBEYE in salicylate-induced tinnitus. *Cell. Physiol. Biochem.* 34, 753–767. doi: 10.1159/000363040

Zimmermann, G., Favrod, J., Trieu, V., and Pomini, V. (2005). The effect of cognitive behavioral treatment on the positive symptoms of schizophrenia spectrum disorders: a meta-analysis. *Schizophr. Res.* 77, 1–9. doi: 10.1016/j.schres.2005.02.018

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frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
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Variable Effects of Acoustic Trauma on Behavioral and Neural Correlates of Tinnitus In Individual Animals

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The etiology of tinnitus is known to be diverse in the human population. An appropriate animal model of tinnitus should incorporate this pathological diversity. Previous studies evaluating the effect of acoustic over exposure (AOE) have found that animals typically display increased spontaneous firing rates and bursting activity of auditory neurons, which often has been linked to behavioral evidence of tinnitus. However, only a subset of studies directly associated these neural correlates to individual animals. Furthermore, the vast majority of tinnitus studies were conducted on anesthetized animals. The goal of this study was to test for a possible relationship between tinnitus, hearing loss, hyperactivity and bursting activity in the auditory system of individual unanesthetized animals following AOE. Sixteen mice were unilaterally exposed to 116 dB SPL narrowband noise (centered at 12.5 kHz) for 1 h under ketamine/xylazine anesthesia. Gap-induced prepulse inhibition of the acoustic startle reflex (GPIAS) was used to assess behavioral evidence of tinnitus whereas hearing performance was evaluated by measurements of auditory brainstem response (ABR) thresholds and prepulse inhibition PPI audiometry. Following behavioral assessments, single neuron firing activity was recorded from the inferior colliculus (IC) of four awake animals and compared to recordings from four unexposed controls. We found that AOE increased spontaneous activity in all mice tested, independently of tinnitus behavior or severity of threshold shifts. Bursting activity did not increase in two animals identified as tinnitus positive (T+), but did so in a tinnitus negative (T−) animal with severe hearing loss (SHL). Hyperactivity does not appear to be a reliable biomarker of tinnitus. Our data suggest that multidisciplinary assessments on individual animals following AOE could offer a powerful experimental tool to investigate mechanisms of tinnitus.

Keywords: gap-induced prepulse inhibition of the acoustic startle reflex, prepulse audiometry, single unit recording, inferior colliculus, tinnitus, hearing loss

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors; ANOVA, analysis of variance; AOE, acoustic over exposure; Arc, activity-regulated cytoskeletal immediate early gene; ASR, acoustic startle reflex; CV, coefficient of variation; GAP, Gap + Startle; GPIAS, gap-induced prepulse inhibition of the acoustic startle reflex; HB, high bursting activity; HL, Hearing Loss; IC, inferior colliculus; ISI, inter spike interval; ITI, inter trial interval; LB, low bursting activity; LSD, least significant difference *post hoc* test; mGluRs, metabotropic glutamate receptors; NB, no bursting activity; NMDA, N-methyl-D-aspartate receptors; NoHL, no hearing loss; PPI, prepulse inhibition; SHL, Severe Hearing Loss; SO, startle only; T+, tinnitus positive; T−, tinnitus negative.

INTRODUCTION

Tinnitus, the perception of sound in the absence of an external sound source, is often developed after acoustic over exposure (AOE; Hoffman and Reed, 2004; Møller, 2011; Baguley et al., 2013). Animal studies have shown that AOE leads to cochlear damage and subsequent threshold shifts (Liberman and Kiang, 1978; Kujawa and Liberman, 2009). Following this damage, the central auditory system increases its gain to compensate for the reduced sensorineural input from the cochlea, which can lead to tinnitus (Salvi et al., 2000; Schaette and McAlpine, 2011; Auerbach et al., 2014). In most clinical cases, there is a strong correlation between hearing loss and tinnitus (Lockwood et al., 2002), but interestingly, this is not always true, as some patients with clinically normal thresholds have tinnitus (Weisz et al., 2006; Job et al., 2007). Just as in humans (Hall et al., 2016), the extent of peripheral damage and central plasticity in individual animals of the AOE model differs greatly, leading to a heterogeneous population of hearing loss (HL) and/or tinnitus pathology (Longenecker and Galazyuk, 2011; Singer et al., 2013; Hickox and Liberman, 2014; Knipper et al., 2015).

Ever since the first animal model of tinnitus (Jastreboff et al., 1988), research has focused on being able to separate animals into T+ and T− groups to reach conclusions concerning the underlying pathology related to AOE. Following AOE animals typically demonstrate increased spontaneous firing, bursting, and neural synchrony at multiple levels of the central auditory system (see Roberts et al., 2010; Kaltenbach, 2011; Wang et al., 2011). Recordings form anesthetized animals have revealed coincidence of hyperactivity and increased burst firing in the cochlear nucleus (Kaltenbach and Afman, 2000; Chang et al., 2002; Brozoski and Bauer, 2005; Finlayson and Kaltenbach, 2009; Pilati et al., 2012), inferior colliculus (IC; Wang et al., 1996; Ma et al., 2006; Bauer et al., 2008; Coomber et al., 2014), medial geniculate body (Kalappa et al., 2014), and auditory cortex (Syka and Rybalko, 2000; Noreña and Eggermont, 2003). It is thought that hyperactivity could manifest as the phantom sound of tinnitus (Gerken, 1996; Salvi et al., 2000; Eggermont and Roberts, 2004). In contrast to many of these pioneer works, recent studies have found that hyperactivity is not always linked to tinnitus because only a fraction of animals in these studies exhibited behavioral signs of tinnitus after AOE (Ropp et al., 2014: Coomber et al., 2014). Similarly, increased bursting activity was elevated in the IC of all noise-exposed guinea pigs, but there was no difference found between T+ and T− groups (Coomber et al., 2014). However, excluding hyperactivity and increased bursting as neural correlates of tinnitus would be inappropriate because there is no compelling evidence that these abnormalities are absent in T+ animals. It is possible that more than one, or a specific combination of these neural correlates is required for tinnitus percept.

The vast majority of tinnitus studies have been conducted on anesthetized animals, which might alter experimental results and lead to erroneous conclusions. Ketamine, perhaps the most used anesthetic drug in animals, is known to affect many molecular targets which include: disrupting N-methyl-D-aspartate (NMDA) receptors, Ih currents, nicotinic acetyl-choline channels, nitric oxide, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors, and metabotropic glutamate receptors (mGluRs; Sleigh et al., 2014). Ketamine also has long-term effects on suppression of many immediate early genes, NMDA receptor phosphorylation, and reduced astrocyte and microglial function (Sleigh et al., 2014). While awake recordings can be challenging, the data gathered from the relatively unaltered nervous system might elucidate new information about the neural correlates of tinnitus.

In the present study, we investigate the effects of AOE on behavioral hearing thresholds, auditory brainstem response (ABR) thresholds, and behavioral signs of tinnitus assessed by gap-induced prepulse inhibition of the acoustic startle reflex (GPIAS) on 16 CBA/CaJ mice. To investigate several suspected neural correlates of tinnitus in detail, we performed single unit recordings in 4 mice out of the 16 behaviorally tested mice. To eliminate a possible effect of anesthesia, all single unit recordings were performed in awake animals. We found that each of the four animals studied in depth demonstrated a unique phenotype following near-identical AOE. All of these mice showed some degree of increase spontaneous activity, while only one showed increased burst firing. While results should be considered in light of the small sample size presented here, future tinnitus studies might benefit from studying individual animals to elucidate the neural mechanisms of tinnitus.

MATERIALS AND METHODS

Subjects

A total of 20 male CBA/CBJ mice were used. Sixteen mice were used for the experimental group which included behavioral testing pre exposure and 3–4 months post exposure. Four mice from the experimental group were randomly selected for single unit recordings. The remaining four mice were also used for single unit recordings as controls (age-matched, without AOE). Mice were obtained from Jackson Laboratories, Bar Harbor, ME, USA and were approximately 12 weeks old with a mean weight of 27.5 g at the beginning of behavioral testing. Mice were housed in pairs within a colony room with a 12-h light–dark cycle at 25°C. Procedures used in this study were approved by the Institutional Animal Care and Use Committee at the Northeast Ohio Medical University.

Acoustic Trauma

Sixteen mice were anesthetized with an intraperitoneal injection of a ketamine/xylazine mixture (100/10 mg/kg). Mice were at least 5 months old at the time of exposure. An additional injection (50% of the initial dose) was given intramuscularly 30 min after the initial injection. Mice were exposed to a one octave band noise centered at 12.5 kHz (∼8–17 kHz) unilaterally for 1 h. This noise was generated using a waveform generator (Wavetek model 395), amplified (Sherwood RX-4109) to 116 dB Sound Pressure Level (SPL), and played through a loudspeaker (Fostex FT17H). The output of the

loudspeaker was calibrated with a 0.25-inch microphone (Brüel and Kjaer, 4135) and found to be ±4 dB between 10 and 60 kHz. The left external ear canal was obstructed with a cotton plug and a Kwik-Sil silicone elastomer plug (World Precision Instruments), a manipulation which typically reduces sound levels by 30–50 dB SPL (Turner et al., 2006; Ropp et al., 2014).

Auditory Brainstem Response (ABR) Thresholds

Mice were anesthetized with ketamine/xylazine. ABR thresholds were obtained by presenting tone bursts at 4, 12.5, 16, 20, 25 and 31.5 kHz at increasing sound intensities ranging from 10–80 dB SPL in 10 dB steps. Tones were 5 ms in duration, with 0.5 ms rise/fall time and delivered at the rate of 50/s. ABR thresholds were obtained before, directly following, and 3 months after acoustic trauma. Sterile stainless-steel electrodes were placed subdermally, one behind the right pinna of the sound exposed ear and the other along the vertex. The unexposed ear was obstructed with a cotton plug. Evoked potentials were averaged over 300 repetitions. These potentials were amplified (Dagan 2400A preamplifier), filtered (100–3,000 Hz bandpass), digitized (HEKA Elektronik), and stored on a computer hard drive. Thresholds, the smallest sound amplitude that evoked a visible ABR, were determined by visually examining the averaged ABR waveforms in response to every sound frequency presented at different sound levels.

Behavioral Assessments of Tinnitus and Hearing Loss

Prior to exposure, the 16 experimental mice were behaviorally tested with GPIAS and prepulse inhibition (PPI) to obtain baseline values for gap detection and hearing thresholds. Mice were assessed for tinnitus/threshold shifts 3 months after exposure. Four mice were randomly selected to highlight the importance of individual differences.

Acoustic Startle Hardware/Software

The equipment used to collect all acoustic startle reflex (ASR) data has been described in detail previously (Longenecker and Galazyuk, 2012). Briefly, commercial hardware/software equipment from Kinder Scientific, Inc. Poway, CA, USA was used. Each behavioral testing station was lined with anechoic foam to prevent sound reflection and wave cancelling sound echoes (Sonex foam from Pinta Acoustics, Minneapolis, MN, USA). Mice restrainers were open walled to allow for maximum sound penetration (Figure 3 in Longenecker and Galazyuk, 2012). Background sound levels within each testing chamber were calibrated with a 0.25-inch microphone (Brüel and Kjaer 4135) attached to a measuring amplifier (Brüel and Kjaer 2525) and found to be less than 40 dB SPL between 4 and 60 kHz. Startle waveforms were recorded using load-cell platforms which measure actual force changes during an animal's startle. Each load cell was calibrated with a 100 g weight which corresponds to 1 newton of force. Offline waveform analysis converted these forces into animals' center of mass displacement (in mm; Grimsley et al., 2015).

Startle Waveform Identification and Measurement

All waveforms collected during testing sessions were analyzed offline using a recently developed automatic method of startle waveform identification via a template matching paradigm (Grimsley et al., 2015). In this study, we used high-speed video recordings (1,000 frames/s) to visualize animal startles in order to identify stereotyped waveforms associated with a startle. This allowed us to develop custom software which automatically separates data into either startles or non-startle-related movements. Based on this separation, we have only included trials that resulted in successful startle responses in our data analysis. We also used a mathematical approach to normalize startle response magnitudes of individual animals to their body mass (Grimsley et al., 2015). This mathematical conversion allows legitimate comparisons between animals of different mass.

GPIAS for Tinnitus Assessment

The ability of mice to detect a gap of silence preceding the startle stimulus was determined using a startle stimulus presented alone (startle only: SO) and a startle stimulus paired with a gap (Gap + startle: GAP) both embedded into continuous background noise. The gap had a 20 ms duration and 1 ms rise/fall time. Background for all these trials was presented as a narrow band (1/3 octave) noise centered at six different frequencies (8, 10, 12.5, 16, 20 and 25 kHz). This background noise level was constant (65 dB SPL) throughout the session. The startle stimulus was presented at 105 dB SPL (white noise, 1 ms rise/fall, 20 ms duration). The gap was presented 100 ms before (onset to onset) the startle stimulus. The testing session started with an acclimation period lasting 3 min. Immediately afterwards, animals received five SO trials in order to habituate their startle responses to a steady state level. For each of six background frequencies, we presented five SO trials and five GAP trials. The SO and GAP trials were pseudo-randomized. The inter-trial intervals were also pseudo-randomized between 7 and 15 s. After we completed testing all six background frequencies, the entire session was repeated two more times. Thus, during this testing for each background frequency we obtained 15 data points for both the SO and GAP trials.

Tinnitus was classified in the same way as in previous studies (Longenecker and Galazyuk, 2011; Dehmel et al., 2012; Coomber et al., 2014). A two-way repeated measures analysis of variance (ANOVA) was applied to the behavioral data to determine whether there were any significant changes ($p < 0.05$) in gap detection levels before vs. 3 months after exposure. A Least Significant Difference (LSD) *post hoc* test was used to determine the specific frequency of any deficits. Importantly, this allowed each animal to act as its own control. Mice that exhibited a significant reduction in gap detection ability at one or two background frequencies after noise exposure were categorized as

"tinnitus positive" (T+) animals while those that did not were assigned to a "tinnitus negative" (T−) group.

PPI Audiometry for Hearing Assessments

PPI audiometry was described in detail previously (Longenecker et al., 2016), and heavily relied on the advanced startle waveform analysis mentioned above (Grimsley et al., 2015). Hearing thresholds assessed by PPI audiometry were conducted at the same experimental epochs as GPIAS tests to ensure behavioral data were comparable and relevant. Testing sessions contained two types of stimuli. The SO stimulus was identical to the GPIAS tests except it was presented at 100 dB SPL. The second stimulus type consisted of a congruent startle stimulus preceded by a prepulse. Prepulse stimuli were 20 ms pure tones with a 1 ms rise/fall time presented at six different frequencies (4, 12.5, 16, 20, 25 and 31.5 kHz) 100 ms before the startle stimulus. Prepulses were presented pseudo-randomized by intensity (10–80 dB SPL, 10 dB step) for each sound frequency (each frequency was one block of testing; six blocks total). Each frequency/intensity combination was presented 39 times. SOs were pseudo-randomly mixed throughout each testing session. In each session, the magnitudes of the SOs were compared to the magnitudes of startles preceded by prepulses with various frequencies and intensities. A significant reduction in the magnitude of the startle response by the prepulse compared to the SO was defined as the prepulse detection threshold.

Identifying this threshold involved several steps. First, we examined the distribution of the raw startle magnitudes. Startle magnitude is known to be quite variable in mice, and indeed the startle magnitude typically was strongly positively skewed. This positive skew could allow aberrantly high values to obscure reliable changes in startle magnitude across stimulus parameters. Furthermore, a normal distribution of magnitudes is an assumption underlying the method for threshold determination that we use here. Thus we transformed the data (Tukey, 1977). A square root transform was found to best generate a normal distribution of startle responses within each trial type, as assessed using the Anderson-Darling test. This standard statistical transform reduces skew by enhancing the lesser and reducing the larger startle magnitudes. Second, for each animal, the transformed SO data was bootstrapped to determine 95% confidence intervals for the SO response magnitudes. Then we calculated medians of transformed magnitudes for each frequency and intensity, as the median value is a better measure of central tendency than the mean for skewed distributions. For each frequency, a detection function was calculated by fitting a cubic spline from the median transformed SO magnitude through the median transformed magnitudes for prepulses presented at various intensities. Detection threshold was defined as the sound level at which the fitted detection function crossed the lower 95% confidence interval.

Each animal was classified as having hearing loss (HL), severe hearing loss (SHL), or no hearing loss (NoHL) based on the number of frequencies at which PPI thresholds were elevated. HL was defined as at least a 30 dB threshold increase in at least two frequencies, while SHL was similarly defined, but with more than two frequencies affected. These classifications can be seen in **Figure 2**.

Electrophysiological Recordings

Surgery

Four mice from the control and four mice from the sound exposed groups were used for extracellular recordings. Each mouse was anesthetized using isoflurane (1.5–2.0%) prior to surgery. A midline incision of the skin over the cranium was made. The tissue overlying the skull then was removed and a small metal rod was glued to the skull using glass ionomer cement (3 M ESPE, Germany). Following 2 days of recovery, each mouse was trained to stay inside a small plastic tube, to be used as a holding device during recording sessions. The metal rod on the head of the mouse was secured to a small holder designed to restrain the head of the animal without causing distress, while the ears were unobstructed for free-field acoustic stimulation.

Extracellular IC Recordings

Recordings were made from both the ipsi- and contra-lateral IC (compared to the side of exposure) in awake mice inside a single-walled sound attenuating chamber (Industrial Acoustics Company, Inc., North Aurora, IL, USA). Throughout the recording session (2–3 h), the animal was offered water periodically and monitored for signs of discomfort. After a recording session, the exposed skull was covered with sterile bone wax and the animal was returned to its holding cage. Experiments were conducted every day for 6 days after which the animal was sacrificed with an IP injection of FatalPlus. No sedative drugs were used during recording sessions. If the animal showed any signs of discomfort, the recording session was terminated and the mouse was returned to its cage.

Recording electrodes were inserted through a small (50 μm) hole drilled in the skull and dura overlying the IC. Extracellular single-unit recordings were made with quartz glass micropipettes (10–20 MΩ impedance, 2–3 μm tip) filled with 0.5 M sodium acetate. The electrode was positioned into the drilled hole by means of a precision (1 μm) digital micromanipulator using a surgical microscope (Leica MZ9.5). The relative position of each electrode was monitored from the readouts of digital micrometers using a common reference point on the skull.

Extracellular recordings were limited to the central nucleus of the IC based on the depth of recordings. Vertical advancement of the electrode was made by a precision piezoelectric microdrive (Model 660, KOPF Instruments) from outside the sound-attenuating chamber. Recorded action potentials were amplified (Dagan 2400A preamplifier), monitored audio-visually on a digital oscilloscope (DL1640, YOKOGAWA), digitized and then stored on a computer hard drive using EPC-10 digital interface and PULSE software from HEKA Elektronik at a bandwidth of 100 kHz.

The search stimulus consisted of a train of tone bursts (20 ms duration, 5–50 kHz, 5 kHz step). This train was repeated while the recording electrode was advanced in 3 μm steps. To ensure that the sample of neurons we recorded was unbiased based

on frequency tuning, the characteristic frequency of recorded neurons was assessed manually by presenting tone pips 100 ms in duration using a wide range of sound frequencies (4–50 kHz, 2 kHz step) and different sound levels (20, 40, 60 dB SPL). The spontaneous rate (SR) of neuronal firing was assessed during a 40 s recording (without stimulus). No sound-evoked recordings are reported.

RESULTS

Behavioral Signs of Tinnitus After Sound Exposure

Behavioral assessments using GPIAS methodology were conducted on 16 mice before vs. 3 months after sound exposure. Six of 16 mice developed significant gap detection deficits at frequencies between 12.5 and 25 kHz. Since gap detection deficits have been associated with tinnitus (see Galzyuk and Hébert, 2015), these mice constituted the T+ group. The remaining 10 mice did not show gap detection deficits and they were considered as T−. Data from two representative mice from each group are shown in **Figure 1**. Comparison of the gap detection performance before vs. 3 months after exposure with a repeated measures ANOVA indicated a significant effect of AOE in mouse #13 ($F_{(1,75)} = 666.11$, $p < 0.001$) and mouse #1 ($F_{(1,61)} = 661.73$, $p < 0.001$). A LSD *post hoc* test revealed a frequency-specific deficit at 20 kHz for mouse #13 ($p = 0.05$) and 16 kHz for mouse #1 ($p = 0.012$). Two representative mice from T− group (#10 and #12) did show significant overall differences in gap detection performance before vs. 3 months after exposure (mouse #10 ($F_{(1,57)} = 326.40$, $p > 0.001$); mouse #12 ($F_{(1,50)} = 435.22$, $p > 0.001$), but LSD *post hoc* tests did not reveal any frequency specific differences (**Figure 1**). When data from all six T+ mice were averaged, a significant effect of exposure was seen ($F_{(1,30)} = 1562.12$, $p < 0.000$). However, LSD *post hoc* tests did not determine any specific tinnitus frequencies, although the deficits at 16 kHz were close to significance ($p = 0.053$). The 10 animals determined to be T− did not show any significant gap detection deficits after exposure, but interestingly, did tend (not significant) to demonstrate improved gap detection performance at frequencies above the sound exposure.

Effect of Sound Exposure on Hearing Performance

Hearing thresholds assessed by PPI audiometry revealed inter-animal differences in the severity of threshold shifts in AOE mice (**Figure 2**), and each animal was classified based on the criteria explained above in "PPI Audiometry for Hearing Assessments" Section. Threshold elevations were seen in 13 of the 16 exposed mice. Six of these 16 mice showed narrowband frequency deficits. Examples of this type of deficit are shown in mouse #13 (at 25 kHz) and mouse #1 (at 16 kHz) in **Figure 2**. Seven out of 16 mice exhibited severe deficits spanning a wider frequency range. A representative mouse from this population (mouse #10) showed elevated thresholds from 12.5 kHz to 25 kHz (**Figure 2**). Finally, 3 out of 16 mice did not show PPI audiometric threshold changes after noise exposure (**Figure 2**, representative mouse #12). When animals were grouped together by T+ and T− classifications (as in **Figure 1**), differences in threshold elevations were observed (**Figure 2**). The T+ group demonstrated increased thresholds following sound exposure ($F_{(1,30)} = 316.56$, $p < 0.001$), LSD *post hoc* tests showed a significant threshold shift only at 16 kHz ($p = 0.01$). The T− group by comparison demonstrated further exaggerated thresholds after sound exposure ($F_{(1,54)} = 385.37$, $p < 0.001$). This group of mice, which comprised more animals with the "SHL" classification, showed significant threshold increases at 12.5 kHz ($p = 0.035$), 16 kHz ($p = 0.003$), 20 kHz ($p < 0.001$) and 25 kHz ($p < 0.001$).

ABRs were collected to serve as a control for PPI hearing assessments following sound exposure (**Figure 3**), however these measures were compared extensively in our previous work (Longenecker et al., 2016). Thresholds of the T+ group nearly returned to baseline levels 3 months following exposure, with an average increase across frequencies of 6.11 dB SPL ($F_{(1,30)} = 478.47$, $p < 0.001$). *Post hoc* LSD tests revealed that only 31.5 kHz significantly differed from control levels ($p = 0.021$), with a 13.33 dB SPL shift. These results contrasted ABR threshold shifts from the T− group, which as mentioned previously, encompassed more animals determined to have SHL measure by PPI. This group showed an average threshold increase of 7.33 dB SPL across frequencies after exposure ($F_{(1,54)} = 1366.24$, $p < 0.001$). Specific deficits were seen at 12.5 kHz ($p = 0.037$), 16 kHz ($p = 0.001$), 20 kHz ($p = 0.001$), and 25 kHz ($p = 0.001$). Not surprisingly, the range of frequencies showing a deficit with ABR (**Figure 3**) closely followed the deficits seen by PPI audiometry (**Figure 2**), although ABR deficits were not nearly as pronounced.

Increased Spontaneous Activity Following Sound Exposure

To determine how spontaneous firing rates of IC neurons changed for each behavioral AOE outcome single unit recordings were conducted in awake mice. Behavioral AOE outcomes included classifications for T+ and T− groups as well as the severity of hearing loss (based on number of elevated thresholds; 0 = NoHL; 1–2 = HL; 3–6 = SHL). Spontaneous activity from 118 neurons recorded in four control (unexposed) mice was compared to the activity of 384 neurons in the four exposed mice described above. In the exposed mice, the ipsi- and contra-lateral ICs were considered separately in an attempt to differentiate the possible effects of unilateral sound exposure. A one-way ANOVA was used to compare spontaneous firing rates of IC neurons between control and exposed (both ipsi and contra exposed IC's) mice ($F_{(500,8)} = 5.34$, $p < 0.001$; **Figure 4**). The Tukey HSD *post hoc* tests revealed significant increases in neural firing rates following sound trauma in the following IC's: #13contra ($p < 0.001$), #13ipsi ($p = 0.012$), #10contra ($p = 0.043$), #1ipsi ($p = 0.030$). Although not all statistically significant, seven

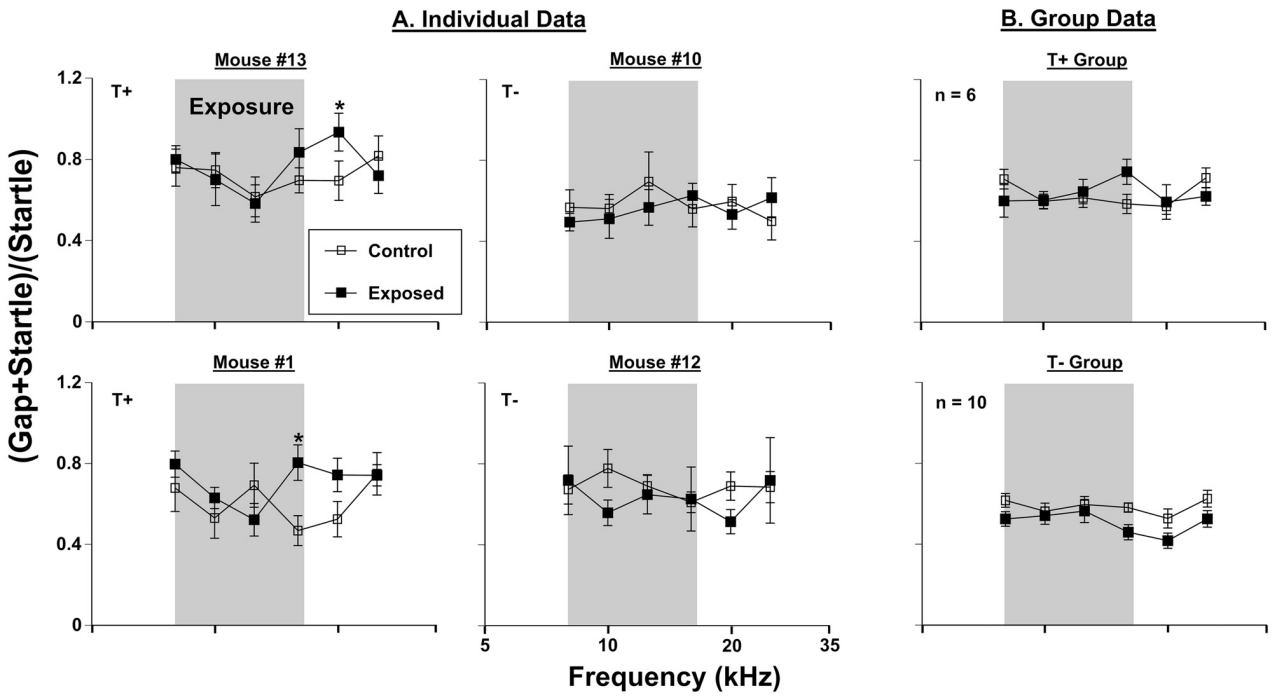

FIGURE 1 | Gap-induced prepulse inhibition of the acoustic startle reflex (GPIAS) assessment for tinnitus. (A) Four individual mice were tested before (control) and 3 months (exposed 3M) after sound exposure. **(B)** Group GPIAS data for T+ mice (n = 6) and T− mice (n = 10), in the same conditions as **(A)**. The narrow band exposure stimulus is represented by a gray box. Data are represented by ratio means and standard errors. Tinnitus was identified as a significant (*) difference between control and exposed conditions. Tinnitus positive and negative mice are labeled as (T+) and (T−), respectively.

out of eight ICs demonstrated increased mean spontaneous rates. Interestingly, mouse #10's ipsilateral IC demonstrated a non-significant slight decrease in activity compared to control rates (p = 1.00), however, the ipsi IC had significantly higher neural firing rates than the contralateral IC ($t_{(64)}$ = 6.23, $p < 0.001$).

Effect of Sound Exposure on Burst Firing in IC Neurons

To determine the effect of sound exposure on bursting activity in IC neurons, the spontaneous firing of 118 neurons from control (unexposed) mice and 384 neurons from exposed mice was analyzed and compared. These are the same pool of neurons that have been used for assessing spontaneous firing rate changes described above.

Bursting activity has been linked to tinnitus-like behavior in many studies (review by Wang et al., 2011). In our study, we adopted the following burst classifications from Bauer et al. (2008) to define a bursting event: (1) maximum allowable burst duration: 310 ms; (2) maximum ISI at burst start: 500 ms; (3) maximum within-burst ISI: 10 ms; (4) minimum interval between bursts: 50 ms; (5) minimum burst duration: 5 ms; and (6) minimum number of spikes comprising a burst: 2 ms. The distribution of burst firing neurons (**Figure 5**) was not bimodal as determined by the Kolmogorov-Smirnov test of normality ($D_{(178)}$ = 0.176, $p < 0.001$). However, if the data were separated into low bursting and high bursting units at the 35% mark, the two divisions became normally distributed (Low Bursting (LB): $D_{(53)}$ = 0.97, p = 0.09; High Bursting (HB): $D_{(48)}$ = 0.106, p = 0.20; **Figure 5**). Neurons with no bursting (NB) were not include in this distribution. Therefore, we developed a new classification system to further separate bursting activity into three categories: high bursting, low bursting, or non-bursting based on the percentage of total spikes within each bursting event (**Figure 5**).

The percentage of units exhibiting bursting activity was plotted based on the degree of bursting activity (**Figure 6**). In control, unexposed mice, 31% of neurons demonstrated bursting activity (24% LB, 7% HB), while 69% of the neurons did not show bursting activity. This observation is similar to the bursting rate distributions found in sound exposed guinea pigs (Coomber et al., 2014). The percentage of bursting neurons in mouse #13, #1, and #12 were similar to the control (unexposed) distribution. Furthermore, the differences between ipsi- and contralateral IC's were surprisingly small. In contrast, mouse #10 demonstrated a unique change in bursting activity following sound exposure. Eighty-three percentage of the neurons in the ipsilateral IC of this mouse showed some degree of bursting activity (55% LB, 28% HB). Interestingly, the contralateral IC of mouse #10 had only 19% of its neurons displaying bursting, which was the lowest value among all IC's tested.

To quantify changes in bursting activity, we applied the coefficient of variation (CV) analysis, a statistic that is derived by

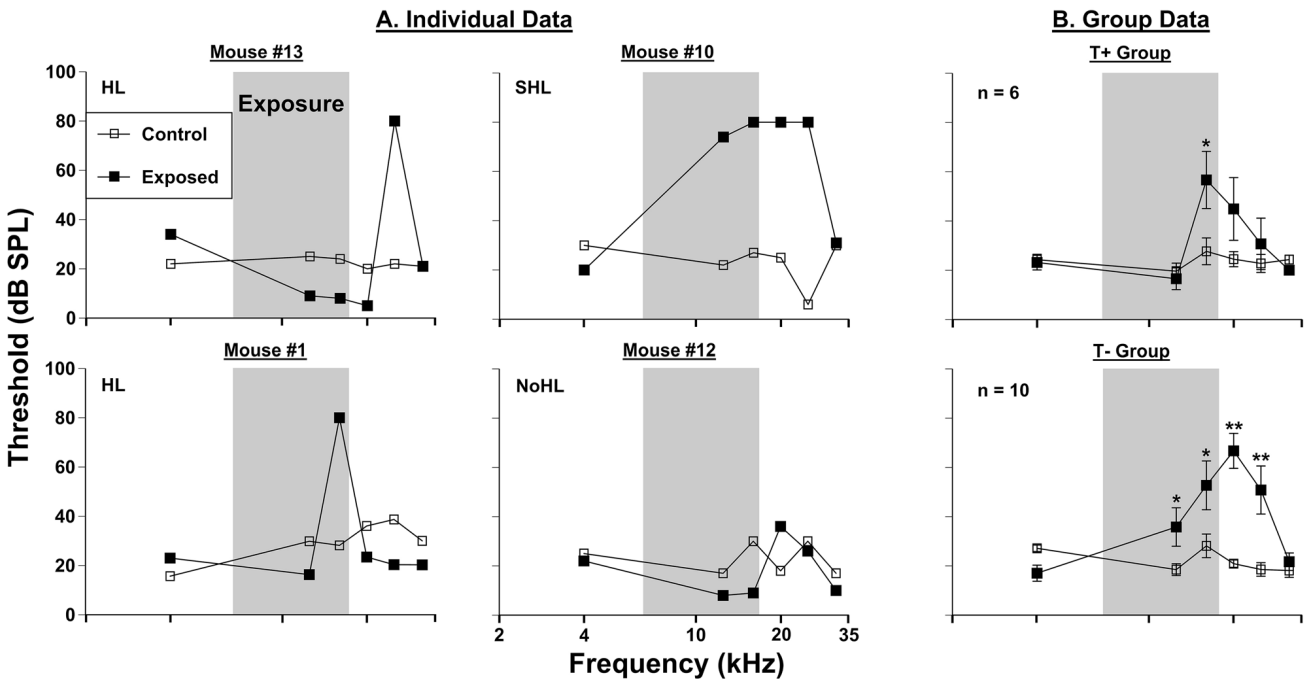

FIGURE 2 | Auditory thresholds assessed via prepulse inhibition (PPI) audiometry. (A) Four individual mice were tested before (control) and 3 months (exposed 3M) after sound exposure. **(B)** Group PPI data for T+ mice (n = 6) and T− mice (n = 10; from **Figure 1B**), in the same conditions as **(A)**. The narrow band exposure stimulus is represented by a gray box. Each animal was classified as having hearing loss (HL), severe hearing loss (SHL), or no hearing loss (NoHL) based on the number of frequencies at which PPI thresholds were elevated. Significant threshold shifts indicated with (*) at p = 0.05 level or (**) at p = 0.001 level.

normalizing the standard deviation of each unit's ISI distribution by its mean (Ma et al., 2006; Kalappa et al., 2014). Units with high CVs had more irregular ISIs, which suggests that they had more bursting activity (**Figure 7**). The normal physiological range for CVs has been reported as 0.5 to 1 in cortical neurons (Christodoulou and Bugmann, 2001), however recent studies have shown values of 1.2 or higher in the dorsal cochlear nucleus (DCN) following acoustic trauma (Pilati et al., 2012). Statistical comparisons of the mean distribution of CVs across each IC of four exposed mice were tested with a Wilcoxon Signed Rank Test to evaluate whether sound exposure changed the bursting patterns in IC neurons (**Figure 7**). Small but significant CV decreases were observed in the contra IC of mouse #13 ($Z = -2.67$, $p = 0.007$), mouse #1 ($Z = 2.15$, $p = 0.049$) and mouse #12 ($Z = 2.92$, $p = 0.003$). A much more significant CV increase was only observed for the ipsilateral IC for mouse #10 ($Z = -4.24$, $p < 0.001$), and a large significant decrease was found in the contralateral IC for mouse #10 ($Z = -3.06$, $p = 0.002$).

DISCUSSION

The important finding of this study was that despite identical sound exposure parameters, behavioral and neural assessments revealed a diverse set of pathologies among mice. Behavioral evidence of tinnitus was observed in just under half of mice exposed, which was often accompanied with some degree of threshold shifts measured by PPI audiometry. The burst firing rate in animals classified as T+ was not significantly different from the control animals. The bursting activity, however, greatly increased in one animal with significant PPI threshold shifts. Increases in spontaneous activity following sound exposure were observed regardless of behavioral evidence of tinnitus or hearing loss in the small sample of mice tested.

To this point, most animal model tinnitus studies have grouped T+ and T− animals together in order to explain differences in any number of physiological factors that change after AOE. While this is a reasonable approach to make conclusions about sample populations that underwent the identical experimental manipulations, it does not lend credence to the significant variability of peripheral, central auditory, emotional, and cognitive aspects of the human tinnitus population (Langers et al., 2012). Clinically, this sort of problem might be best addressed by increased data sharing and a systematic review of all clinical studies (Hall et al., 2016). However, in animal studies both group and individual results should be included in order to parse apart the various factors that could influence tinnitus manifestation. Below, we discuss the individual results of a small sample of mice that underwent several behavioral and electrophysiological evaluations.

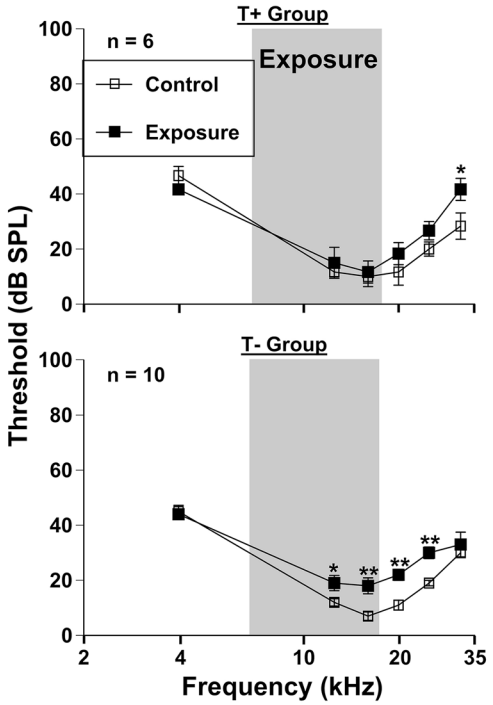

FIGURE 3 | Auditory thresholds assessed via auditory brainstem response (ABR) grouped by T + (*n* = 6) and T− (*n* = 10) animals (from Figure 1B). Mice were tested before (control) and 3 months (exposed 3M) after sound exposure. The narrow band exposure stimulus is represented by a gray box.

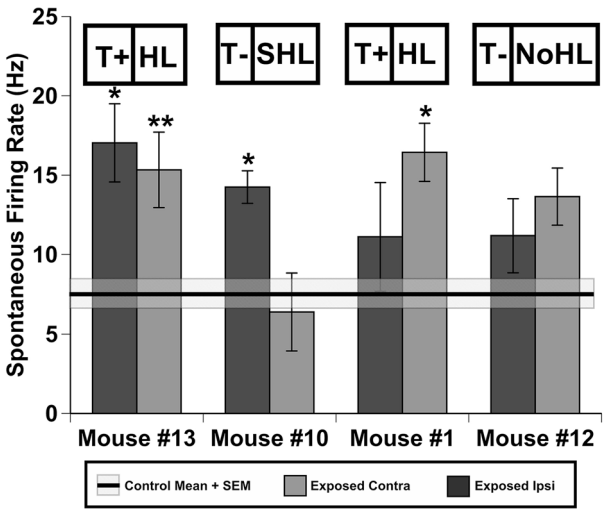

FIGURE 4 | Spontaneous firing of inferior colliculus (IC) neurons in four individual mice after sound exposure. Data represents means and standard errors of spontaneous rate (SR)s of contra- and ipsi-lateral ICs (relatively to the exposed ear) for each mouse. Signifcant differences (*0.05, **0.001) between each IC of exposed mice and averaged values across four control (unexposed) animals (black line = mean, shaded region = std. error). Tinnitus and hearing loss abbreviations are taken from **Figures 1**, **2** respectively.

Behavioral Data: Group vs. Individual Analysis

We found that 6 out of 16 mice developed GPIAS deficits consistent with behavioral evidence of tinnitus 3 months following AOE. This percentage of T+ animals fits within the range of other studies that report between 30% and 75% rates of tinnitus among animals tested (see Galzyuk and Hébert, 2015). The T+ group (n = 6) showed a non-significant, although close (p = 0.053), GPIAS deficit at 16 kHz, whereas the T− group (n = 10) did not show deficits at any frequencies, and in fact showed non-significant improvements in high frequency gap detection following AOE (**Figure 1**). It is not surprising that GPIAS results assessed in the T+ group did not show a significant deficit because the frequency of the gap detection deficit varies among mice, although usually manifests at, or above the center frequency of exposure (Longenecker and Galazyuk, 2011; Turner et al., 2012; Coomber et al., 2014; Ropp et al., 2014). Thus, 16 kHz would fall within this expected deficit range following an AOE with a 12.5 kHz centered noise. Indeed, all individual animal GPIAS deficits ranged from 16 kHz to 25 kHz, of which two examples can be seen in **Figure 1**.

PPI audiometry can provide behavioral thresholds in a matter of a couple hours compared with months of training for behavioral audiograms (Heffner et al., 2008; Radziwon et al., 2009). Because behavioral thresholds are considered the "gold standard" for auditory evaluations, it is important that they are used in conjunction with tinnitus assessments. With the same 16 mice tested for behavioral evidence of tinnitus, we found that thirteen showed increased behavioral thresholds when assessed by PPI audiometry 3 months after AOE. Of those 13 with increased thresholds, six showed narrowband frequency deficits restricted to one or two neighboring frequencies tested, while wide-band deficits were seen in seven mice. The animals with narrowband deficits, had PPI threshold increases within the same 16–25 kHz band as GPIAS deficits were usually seen. This finding does bring into question whether the animals with specific deficits (like mouse #1) have tinnitus. If GPIAS (**Figure 1**) and PPI deficits (**Figure 2**) match in frequency it is possible that the mouse could not hear the background noise during the GPIAS testing and thus showed a gap detection deficit at that frequency. Seven mice however showed broadband deficits, exemplified by mouse #10 (**Figure 2**). If the dramatic PPI audiometric threshold elevations are reflecting peripheral damage, it would imply that many of these animals could not hear well. Future experiments should identify the level of cochlear damage at which GPIAS could not be used for tinnitus assessment.

Thresholds assessed by ABRs were nearly identical for all animals before and 3 months after AOE (**Figure 3**), with only minor permanent threshold increases. Although this does not exclude "hidden hearing loss" as a result of deafferentation at the ribbon synapses of the cochlea (Schaette and McAlpine, 2011). ABR wave one amplitudes would better assess these changes (Kujawa and Liberman, 2009; Lin et al., 2011). In a

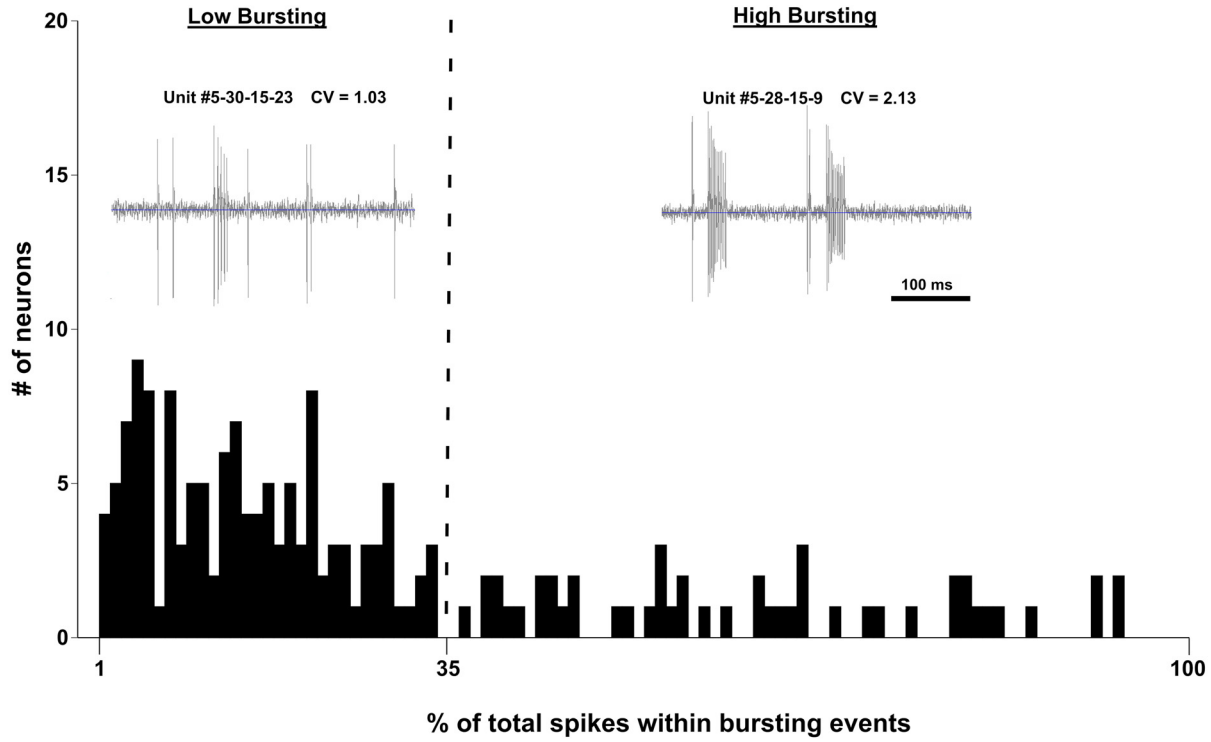

FIGURE 5 | Histogram of the percentage of spikes that occurred in bursting events of 502 IC neurons recorded from four control mice and four exposed mice. Units were separated into Low Bursting (LB) and High Bursting (HB) units using a 35% cutoff point (vertical dashed line) which was determined statistically. Examplar units for each bursting classification are shown in the upper part of the figure. Each unit includes its coefficient of varation (CV) which represents a measure of spike regularity. Higher CV values mean that a given neuron is bursting more.

mirror study with the same AOE parameters we found that wave one amplitudes were dramatically decreased, especially at suprathreshold levels, and more importantly, that these deficits corresponded to PPI audiometric deficits (Longenecker et al., 2016). Therefore, we expect that these animals also experience a decrease in ABR wave one amplitudes, signifying peripheral deafferentation (Kujawa and Liberman, 2015). Only small threshold shifts were seen at 31.5 kHz in the T+ group, which contrasted with the more significant deficits seen in the T− group between 12.5 and 25 kHz (**Figure 3**). This ABR data correlates well with PPI threshold data (**Figure 2**), which suggests that T− animals might have a tendency towards more wide spread peripheral and/or central damage (Roberts et al., 2012). A similar finding demonstrated that non-tinnitus rats had a higher expression of activity-regulated cytoskeletal (Arc) immediate early gene measured across the hippocampus, amygdala, and auditory cortex (Singer et al., 2013). This means more central plasticity occurred in animals with SHL, and more importantly that tinnitus generation might require a more focused peripheral/central damage.

Relationship Between Hyperactivity and Tinnitus

AOE causes peripheral damage that gradually leads to central plastic changes, possibly leading to tinnitus in some individuals, but not others. Possible reasons for this variability are discussed below. Regardless of the behavioral variability of tinnitus, some neural correlates have consistently been seen after AOE (see Eggermont, 2016), many in the IC (see Berger and Coomber, 2015). For several decades animal studies have suggested hyperactive neuronal activity is a neural correlate of tinnitus (see review by Eggermont, 2005, 2013). Our study demonstrated hyperactivity is developed in all mice regardless of behavioral evidence of tinnitus (**Figure 4**). A similar finding has been reported for rats (Ropp et al., 2014) and guinea pigs in IC neurons (Coomber et al., 2014) as well as in neurons of the auditory cortex (Engineer et al., 2011). Interestingly, mouse #10 in our study demonstrated decreased spontaneous activity in the ipsilateral IC. This is particularly interesting when considering the bursting activity in this ipsilateral IC was dramatically increased compared to all other exposed ICs tested as discussed below. This might suggest that increased bursting activity in the IC could be an alternative neural coding strategy following decreased peripheral input (see Roberts et al., 2010). When combining the behavioral data with the single unit recordings, the results of this study would suggest that hyperactivity should be considered as a generalized outcome of sound exposure rather than a specific neural correlate of tinnitus. Our study also suggests that there does not seem to be a specific effect of being T+ and symmetry of neural firing in contra/ipsi ICs following AOE. While both

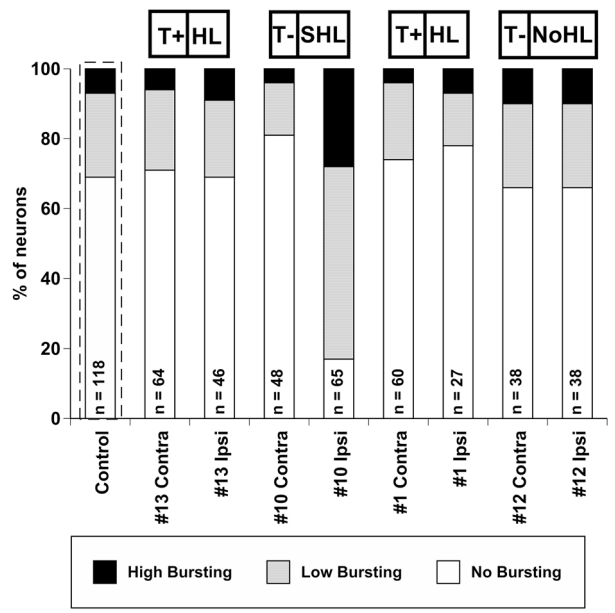

FIGURE 6 | Percent distribution of auditory neurons having different levels of bursting activity (high bursting, low bursting, and no bursting) in conta- and ipsilateral ICs of four exposed mice. The average distribution from four control (unexposed) mice is outlined by a dashed line. The number of neurons collected is labeled for each exposed IC (or all ICs for control). Tinnitus and hearing loss abbreviations are taken from **Figures 1**, **2** respectively.

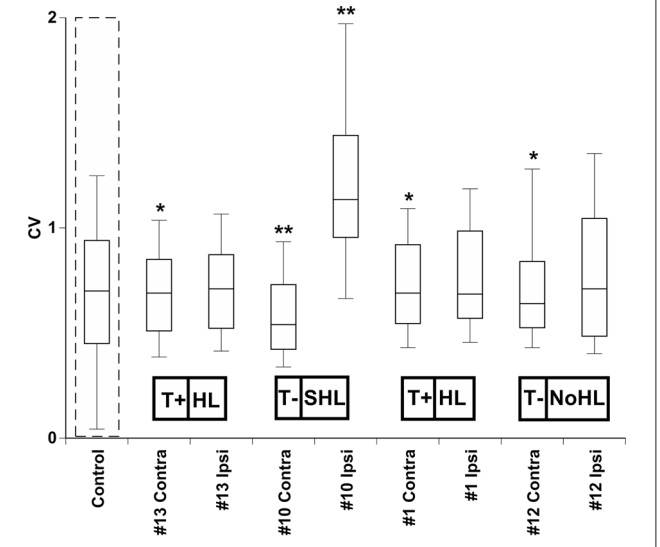

FIGURE 7 | Distribution of CVs across conta- and ipsilateral ICs of four exposed mice. The average CV from four control (unexposed) mice is outlined by a dashed line. Significant differences between control and each exposed IC was determined by a paired *t*-test (*0.05, **0.001). Tinnitus and hearing loss abbreviations are taken from **Figures 1**, **2** respectively.

the ipsi and contra ICs had significantly elevated neural activity for mouse #13 (T+ group), only the ipsi IC showed this effect in mouse #1 (T+ group; **Figure 4**). Mouse #10 which was T− and had SHL when measured with PPI audiometry, demonstrated a bias of hyperactivity skewed toward the contra IC. While the overall results of hyperactivity correlate well with previous studies on different model species (Coomber et al., 2014; Ropp et al., 2014), specific details of increased spontaneous activity laterality should be further studied before robust conclusions can be made.

Bursting Activity and Tinnitus

Many studies have implicated increased bursting activity as one of several possible neural correlates of tinnitus (e.g., Ma et al., 2006; Bauer et al., 2008). Although estimates of bursting are somewhat dependent on distinct definitions of what constitutes a burst, our results clearly suggest that bursting activity decreases in the vast majority of exposed mice (**Figures 6**, **7**) and does not correlate with behavioral evidence of tinnitus (**Figure 1**). There are several possible explanations for such discrepancy: (1) The effect of anesthesia. In our study extracellular recordings were conducted without the use of any anesthetics, whereas other studies were conducted on ketamine-anesthetized animals which is known to affect NMDA receptors, AMPA receptors, mGluRs, and most modulatory neurotransmitter systems which in turn might alter bursting activity (Sleigh et al., 2014). (2) The difference in timing after sound exposure. Studies assessing bursting activity have started physiological recordings a few days (Noreña and Eggermont, 2003; Finlayson and Kaltenbach, 2009), 2–3 months, (Ma et al., 2006; Coomber et al., 2014) up to a maximum of a 9 months (Bauer et al., 2008) after sound exposure. We began testing our mice at least 2 months following exposure. This matches previous behavioral (Turner et al., 2012; Longenecker et al., 2014) and physiological (Mulders and Robertson, 2011) timelines for tinnitus development. It is possible that bursting activity during the acute phase of tinnitus is very different from the chronic tinnitus. (3) The difference in sound exposure. In our study animals were exposed to a 12.5 kHz octave band noise for 1 h presented unilaterally at 116 dB SPL. Unfortunately, nearly every hearing loss/tinnitus study has adapted unique sound exposure parameters, leading to possible differential exposure effects (Galzyuk and Hébert, 2015). Thus, direct comparisons between studies are extremely tenuous, especially considering the difficulty of tinnitus assessment and internal confounds arising from various levels of hearing loss resulting from sound exposure. Any or all of these factors could explain differences in burst firing activity observed in this study.

A unique pattern of neuronal activity was observed in the one mouse with severe widespread threshold deficits. This was the only animal which showed decreased bursting contralaterally and significant increased bursting ipsilaterally (**Figure 6**). In line with this observation, the CV value and percentage of spikes within bursts were also increased in the ipsilateral IC while decreasing in the contralateral IC (**Figure 7**). These results appear paradoxical when considering the spontaneous firing rates mentioned above because the spontaneous firing rate contralaterally was much higher than control levels while

the ipsilateral IC had somewhat decreased firing rates. This increased bursting activity without increasing the firing rate could represent an alternative neural strategy in compensating for the putative decreased peripheral signals. Further research is necessary to clarify neuronal mechanisms underlying changes in bursting activity following sound exposure.

Different Pathologies Following Acoustic Exposure

Perhaps the most significant question that is left unanswered in both the literature and this study is why some animals develop tinnitus after sound exposure and others do not. This phenomenon has been indirectly reported in the vast majority tinnitus related publications but has not been studied systematically. For example, most clinical cases reported a strong correlation between hearing loss and tinnitus (Weisz et al., 2006), whereas other studies described tinnitus cases where tinnitus patients demonstrated no audiometric deficits (Schmuziger et al., 2006; Job et al., 2007; Langers et al., 2012). The diversity of tinnitus pathologies seen in the current study and other animal studies (Longenecker and Galazyuk, 2011; Coomber et al., 2014; Hickox and Liberman, 2014; Ropp et al., 2014) could be explained by a number of phenotypic factors including differences in individual animals in stress levels, unintentional noise exposure, differential peripheral damage, or unique animal-specific maladaptive neuroplasticity pattern caused by sound exposure.

Possible Causes of Phenotypic Diversity

Stress is known to play an important role in many disease states clinically, including tinnitus (Hébert and Lupien, 2007). Some literature suggests that stress is also an important factor for the etiology of tinnitus in animals (Singer et al., 2013). Many common housing and handling procedures can cause an animal's stress levels to increase dramatically (for review see Balcombe et al., 2004). Stress is known to impair cognitive function (Arnsten, 2009), and thus it can alter the results of behavioral tests (Kaneto, 1997; Dawood et al., 2004; Graham et al., 2011). It has been shown that loud unexpected sounds can raise levels of stress-related hormones (Burow et al., 2005). Both restraint and social stress are common in laboratory animals (Stone and Quartermain, 1997; Ma et al., 2015). Simple handling of an animal, putting it in a new environment, and cage changes can also raise levels of stress in animals (Seggie and Brown, 1975; Duke et al., 2001). Interestingly, it was found that mice subjected to restraint stress had less dramatic permanent threshold shifts after noise exposure (Wang and Liberman, 2002). All animals in our study were subjected to restraint stress before exposure when they were placed into small restrainers during behavioral testing. Although all animals were tested the same number of times, the stress of restraint could vary between animals, leading to the differential pathologies we observed. Animals housed alone show much higher stress levels than animals housed in pairs or groups (Sharp et al., 2003). However, dominance struggles between pairs can cause a great deal of stress to the subordinate animal (Makinson et al., 2015). Any or all of these stressing factors could lead to a potential difference in the outcome of sound exposure, especially since it is known that sound exposure itself leads to increased levels of stress hormones (Kozlovsky et al., 2009).

Unintentional exposure to sound could occur in animal housing facilities (see Turner et al., 2007). A recent study recorded sounds from an animal care facility and found that weekday sound levels could easily reach the level of 70 dB SPL, but varied in intensity throughout the day (Liberman et al., 2014). Even if sound levels are not damaging, each animal would be subjected to different auditory experience which may lead to animal specific plastic changes over the length of a longitudinal study. CBA/CaJ mice conditioned to moderate levels of sound (81–89 dB SPL) before exposure demonstrated less permanent threshold shifts compared to mice that were just exposed (Yoshida and Liberman, 2000). Sound conditioning can also lead to permanent central changes as well (Turner et al., 2013). These changes can lead to changes in ABRs, prepulse behavioral measures, and startle reflex magnitudes (Turner and Willott, 1998). This suggests that an animal facility, if not closely monitored, could be greatly influencing an animal's auditory experience and thus the results of sound exposure. Specific to the conclusions of this study, it is important to consider that each mouse could be in a slightly different area in reference to given unintentional sound source, so it is likely that sound levels were not even for every animal. Animals can also be unintentionally exposed during transportation to and from a lab or in the lab itself. These discrepancies could explain why certain mice develop tinnitus, hearing loss, or show the absence of such maladies.

Additionally, the actual conditions of exposure for each animal might differ slightly resulting in various degrees of damage. Such factors could include the exact animal placement in relation to the sound source, the time of day the animal was exposed (Meltser et al., 2014), the exact amount of anesthetic that is absorbed into the blood, as well as all stress-related factors listed above. Even among heterogeneous genotypes of guinea pig and inbred strain mice the variability of ABR threshold shifts after unanesthetized AOE can be dramatic (Wang et al., 2002). Similarly, ABR and ASR amplitudes were shown to be quite variable between mice after AOE in a recent tinnitus assessment study (Hickox and Liberman, 2014). Current theories of how this peripheral damage leads to the manifestation of tinnitus is still debated. However, it is known that exposure to loud sounds leads to permanent damage to cochlear nerve fibers, even without direct damage to inner or outer hair cells (Kujawa and Liberman, 2009; Lin et al., 2011). This was confirmed in human work, which suggested that tinnitus in patients with clinically normal audiograms may be correlated with a peripheral neuropathy, which is typically patient specific (Schaette and McAlpine, 2011; Gu et al., 2012). The resulting decrease of central input leads to maladaptive up regulation of firing in the lower auditory brainstem (for review see Roberts et al., 2010). A recent study has suggested that rats behaviorally

positive for tinnitus demonstrated the greatest degree of ribbon synapse degeneration at the cochlear nerve terminal (Singer et al., 2013). Regardless of what peripheral damage occurs, the strongest evidence of the manifestation of the tinnitus percept is explained by peripherally-driven central auditory plasticity.

Central neural plastic changes resulting from AOE have been abundantly studied (see Eggermont, 2016) but how these changes result in tinnitus remains poorly understood. We know that the exposure increases the rate of peripheral degradation, assessed by threshold (Kujawa and Liberman, 2006) and suprathreshold (Fernandez et al., 2015) ABR measures. Further, it is known that unilateral peripheral damage, like the AOE in this study, drives central plasticity more significantly than bilateral lesions (Rubio, 2006). Here we describe common neuronal changes associated with tinnitus, hyperactivity and bursting activity in the IC in animals assumed to have some degree of unilateral peripheral deafferentation. The conclusions here match other recent studies that suggest hyperactivity is not associated with tinnitus but sound exposure in general (Coomber et al., 2014; Ropp et al., 2014). Additionally, a new finding in this work, albeit with a low sample size, advocates that bursting activity (**Figures 6**, **7**) is more prevalent animals with the most significant auditory threshold shifts (**Figure 2**), but less so in T+ animals, with less significant threshold shifts (**Figure 2**). Although this finding requires more validation, due to the low sample size.

In agreement with our findings, it was found that the activity-regulated cytoskeletal protein, Arc was downregulated in the amygdala, hippocampus, and auditory cortex in mice with behavioral evidence of tinnitus but upregulated in animals with possible hearing loss (Singer et al., 2013). A similar finding in the cochlear nucleus of rats demonstrated that GAP-43 (a synaptic plasticity associated protein) was upregulated in rats with SHL but not in rats with tinnitus (Kraus et al., 2011). This suggests that certain neural activity might be downregulated in tinnitus animals but upregulated in animals with greater degrees of hearing loss. Human imaging studies have found neural plastic changes in many brain regions (see review by Simonetti and Oiticica, 2015). Most studies find that tinnitus patients show increased cerebral gray matter in the auditory pathways, and a decreased cerebral gray matter outside the auditory pathways (i.e., Limbic system, cerebellum, basal ganglia) in comparison to non-tinnitus controls. Although comparing changes in neuronal signatures between humans and animal models of tinnitus is difficult, future studies should work towards more relatable comparisons.

AUTHOR CONTRIBUTIONS

RJL: planned and conducted all experiments and manuscript production; AVG: planned experiments and manuscript production.

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REFERENCES

Arnsten, A. F. T. (2009). Stress signaling pathways that impair prefrontal cortex structure and function. *Nat. Rev. Neurosci.* 10, 410–422. doi: 10.1038/nrn2648

Auerbach, B. D., Rodrigues, P. V., and Salvi, R. J. (2014). Central gain control in tinnitus and hyperacusis. *Front. Neurol.* 5:206. doi: 10.3389/fneur.2014.00206

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Balcombe, J. P., Bernard, N. D., and Sandusky, C. (2004). Laboratory routines cause animal stress. *Contemp. Top. Lab. Anim. Sci.* 43, 42–51.

Bauer, C. A., Turner, J. G., Caspary, D. M., Myers, K. S., and Brozoski, T. J. (2008). Tinnitus and inferior colliculus activity in chinchillas related to three distinct patterns of cochlear trauma. *J. Neurosci. Res.* 88, 2564–2578. doi: 10.1002/jnr.21699

Berger, J. I., and Coomber, B. (2015). Tinnitus-related changes in the inferior colliculus. *Front. Neurol.* 6:61. doi: 10.3389/fneur.2015.00061

Brozoski, T. J., and Bauer, C. A. (2005). The effect of dorsal cochlear nucleus ablation on tinnitus in rats. *Hear. Res.* 206, 227–236. doi: 10.1016/j.heares.2004.12.013

Burow, A., Day, H. E. W., and Campeau, S. (2005). A detailed characterization of loud noise stress: intensity analysis of hypothalamo–pituitary–adrenocortical axis and brain activation. *Brain Res.* 1062, 63–73. doi: 10.1016/j.brainres.2005.09.031

Chang, H., Chen, K., Kaltenbach, J. A., Zhang, J., and Godfrey, D. A. (2002). Effects of acoustic trauma on dorsal cochlear nucleus neuron activity in slices. *Hear. Res.* 164, 59–68. doi: 10.1016/s0378-5955(01)00410-5

Christodoulou, C., and Bugmann, G. (2001). Coefficient of variation vs. mean interspike interval curves: What do they tell us about the brain? *Neurocomputing* 38, 1141–1149. doi: 10.1016/s0925-2312(01)00480-5

Coomber, B., Berger, J. I., Kowalkowski, V. L., Shackleton, T. M., Palmer, A. R., and Wallace, M. N. (2014). Neural changes accompanying tinnitus following unilateral acoustic trauma in the guinea pig. *Eur. J. Neurosci.* 40, 2427–2441 doi: 10.1111/ejn.12580

Dawood, M. Y., Lumley, L. A., Robison, C. L., Saviolakis, G. A., and Meyerhoff, J. L. (2004). Accelerated Barnes maze test in mice for assessment of stress effects on memory. *Ann. N Y Acad. Sci.* 1032, 304–307. doi: 10.1196/annals.1314.047

Dehmel, S., Eisinger, D., and Shore, S. E. (2012). Gap prepulse inhibition and auditory brainstem-evoked potentials as objective measures for tinnitus in guinea pigs. *Front. Syst. Neurosci.* 6:42. doi: 10.3389/fnsys.2012.00042

Duke, J. L., Zammit, T. G., and Lawson, D. M. (2001). The effects of routine cage-changing on cardiovascular and behavioral parameters in male Sprague-Dawley rats. *Contemp. Top. Lab. Anim. Sci.* 40, 17–20.

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Eggermont, J. J. (2005). Tinnitus: neurobiological substrates. *Drug Discov. Today* 10, 1283–1290. doi: 10.1016/s1359-6446(05)03542-7

Eggermont, J. J. (2013). Hearing loss, hyperacusis, or tinnitus: what is modeled in animal research? *Hear. Res.* 295, 140–149 doi: 10.1016/j.heares.2012.01.005

Eggermont, J. J. (2016). Acquired hearing loss and brain plastisity. *Hear. Res.* 1–15. doi: 10.1016/j.heares.2016.05.008

Engineer, N. D., Riley, J. R., Seale, J. D., Vrana, W. A., Shetake, J. A., Sudanagunta, S. P., et al. (2011). Reversing pathological neural activity using targeted plasticity. *Nature* 470, 101–104. doi: 10.1038/nature09656

Fernandez, K. A., Jeffers, P. W. C., Lall, K., Liberman, M. C., and Kujawa, S. G. (2015). Aging after noise exposure: Acceration of cochlear synaptopathy in "recovered" ears. *J. Neurosci.* 35, 7509–7520. doi: 10.1523/JNEUROSCI.5138-14.2015

Finlayson, P. G., and Kaltenbach, J. A. (2009). Alterations in the spontaneous discharge patterns of single units in the dorsal cochlear nucleus following intense sound exposure. *Hear. Res.* 256, 104–117. doi: 10.1016/j.heares.2009.07.006

Galzyuk, A., and Hébert, S. (2015). Gap-prepulse inhibition of the acoustic startle reflex (GPIAS) for tinnitus assessment: current status and future directions. *Front. Neurol.* 6:88. doi: 10.3389/fneur.2015.00088

Gerken, G. M. (1996). Central tinnitus and lateral inhibition: an audiotry brainstem model. *Hear. Res.* 97, 75–83. doi: 10.1016/s0378-5955(96)80009-8

Graham, L. K., Yoon, T., and Kim, J. J. (2011). Stress impairs optimal behavior in a water foraging choice task in rats. *Learn Mem.* 17, 1–4 doi: 10.1101/lm.1605510

Grimsley, C. A., Longenecker, R. J., Rosen, M. J., Young, J. W., Grimsley, J. M., and Galazyuk, A. V. (2015). An improved approach to separating startle data from noise. *J. Neurosci. Methods* 253, 206–217. doi: 10.1016/j.jneumeth.2015.07.001

Gu, J. W., Herrmann, B. S., Levine, R. A., and Melcher, J. R. (2012). Brainstem auditory evoked potentials suggest a role for the ventral cochlear nucleus in tinnitus. *J. Assoc. Res. Otolaryngol.* 13, 819–833. doi: 10.1007/s10162-012-0344-1

Hall, D. A., Haider, H., Szczepek, A. J., Lau, P., Rabau, S., Jones-Diette, J., et al. (2016). Systemic review of outcome domains and instruments used in clincial trials of tinnitus treatments in adults. *Trials* 17:120. doi: 10.1186/s13063-016-1399-9

Hébert, S., and Lupien, S. J. (2007). The sound of stress: Blunted cortisol reactivity to psychosocial stress in tinnitus sufferers. *Neuros. Lett.* 411, 138–142. doi: 10.1016/j.neulet.2006.10.028

Heffner, H. E., Koay, G., and Heffner, R. S. (2008). Comparison of behavioral and auditory brainstem response measures of threshold shift in rats exposed to loud sound. *J. Acoust. Soc. Am.* 124, 1093–1104 doi: 10.1121/1.2949518

Hickox, A. E., and Liberman, M. C. (2014). Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus? *J. Neurophysiol.* 111, 552–564. doi: 10.1152/jn.00184.2013

Hoffman, H. J., and Reed, G. W. (2004). "Epidemiology of tinnitus," in *Tinnitus Theory and Management* ed. J. Snow (Hamilton, ON: BC Decker), 16–41.

Jastreboff, P. J., Brennan, J. F., Coleman, J. K., and Sasaki, C. T. (1988). Phantom audiotry sensation in rats: An animal model for tinnitus. *Behav. Neurosci.* 102, 811–822. doi: 10.1037/0735-7044.102.6.811

Job, A., Raynal, M., and Kossowski, M. (2007). Susceptiblity to tinnitus revealed at 2 kHz range by bilateral lower DPOAEs in normal hearing subjects with noise exposure. *Audiol Neurotol.* 12, 137–144. doi: 10.1159/000099025

Kalappa, B. I., Brozoski, T. J., Turner, J. G., and Caspary, D. M. (2014). Single unit hyperactivity and bursting in the auditory thalamus of awake rats directly correlates with behavioral evidence of tinnitus. *J. Physiol.* 592, 5065–5078. doi: 10.1113/jphysiol.2014.278572

Kaltenbach, J. A., and Afman, C. E. (2000). Hyperactivity in the dorsal cochlear nucleus after intense sound exposure and its resemblance to tone-evoked activity: a physiological model for tinnitus. *Hear. Res.* 140, 165–172 doi: 10.1016/s0378-5955(99)00197-5

Kaltenbach, J. A. (2011). Tinnitus: Models and mechanisms. *Hear. Res.* 276, 52–60. doi: 10.1016/j.heares.2010.12.003

Kaneto, H. (1997). Learning/memory processes under stress conditions. *Behav. Brain Res.* 83, 71–74. doi: 10.1016/s0166-4328(97)86048-2

Knipper, M., Panford-Walsh, R., Singer, W., Rüttiger, L., and Zimmermann, U. (2015). Specific synaptopathies diversify brain responses and hearing disorders: you lose the gain from early life. *Cell Tissue Res.* 361, 77–93. doi: 10.1007/s00441-015-2168-x

Kozlovsky, N., Matar, M. A., Kaplan, Z., Zohar, J., and Cohen, H. (2009). A distinct pattern of intracellular glucocorticoid-related responses is associated with extreme behaviroal response to stress in an animal model of post-traumatic stress disorder. *Eur. Neuropsychopharm.* 19, 759–771. doi: 10.1016/j.euroneuro.2009.04.009

Kraus, K. S., Ding, D., Jiang, H., Lobarinas, E., Sun, W., and Salvi, J. R. (2011). Relationship between noise-induced hearing-loss, persistent tinnitus and growthassociated protein-43 expression in the rat cochlear nucleus: Does synaptic plasticity in ventral cochlear nucleus suppress tinnitus? *Neuroscience* 194, 309–325 doi: 10.1016/j.neuroscience.2011.07.056

Kujawa, S. G., and Liberman, M. C. (2006). Acceration of age-related hearing loss by early noise exposure: evidence of a misspent youth. *J. Neurosci.* 26, 2115–2123. doi: 10.1523/JNEUROSCI.4985-05.2006

Kujawa, S. G., and Liberman, M. C. (2009). Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. *J. Neurosci.* 29, 14077–14085 doi: 10.1523/JNEUROSCI.2845-09.2009

Kujawa, S. G., and Liberman, M. C. (2015). Synaptopathy in the noise-exposed and aging cochlea: Primary neural degeneration in aquired sensorineural hearing loss. *Hear. Res.* 330, 191–199. doi: 10.1016/j.heares.2015.02.009

Langers, D. R. M., de Kleine, E., and van Dijk, P. (2012). Tinnitus does not require macroscopic tonotopic map reorganization. *Front Syst Neurosci.* 6:2. doi: 10.3389/fnsys.2012.00002

Liberman, M. C., and Kiang, N. Y. S. (1978). Acoustic trauma in cats. Cochlear pathlogy and auditory-nerve activity. *Acta. Otolaryngol Suppl.* 358, 1–63.

Liberman, M. C., Liberman, L. D., and Maison, S. F. (2014). Efferent feedback slows cochlear aging. *J Neurosci.* 34, 4599–4607. doi: 10.1523/JNEUROSCI.4923-13.2014

Lin, H. W., Furman, A. C., Kujawa, S. G., and Liberman, M. C. (2011). Primary neural degeneration in the guinea pig cochlea after reversible noise-induced threshold shift. *J. Assoc. Res. Otolaryngol.* 12, 605–616. doi: 10.1007/s10162-011-0277-0

Lockwood, A. H., Salvi, R. J., and Burkard, R. F. (2002). Tinnitus. *N. Engl. J. Med.* 347, 904–910. doi: 10.1056/NEJMra013395

Longenecker, R. J., and Galazyuk, A. V. (2011). Development of tinnitus in CBA/CaJ mice following sound exposure. *J. Assoc. Res. Otolaryngol.* 12, 647–658 doi: 10.1007/s10162-011-0276-1

Longenecker, R. J., and Galazyuk, A. V. (2012). Methodological optimization of tinnitus assessment using prepulse inhibition of the acoustic startle reflex. *Brain Res.* 1485, 54–62 doi: 10.1016/j.brainres.2012.02.067

Longenecker, R. J., Chonko, K. T., Maricich, S. M., and Galazyuk, A. V. (2014). Age effects on tinnitus and hearing loss in CBA/CaJ mice following sound exposure. *Springerplus* 3, 542–554. doi: 10.1186/2193-1801-3-542

Longenecker, R. J. (2015). *Differential Pathologies Resulting from Sound Exposure: Tinnitus vs. Hearing Loss*. Kent, OH: Kent State University (Dissertation).

Longenecker, R. J., Alghamdi, F., Rosen, M. J., and Galazyuk, A. V. (2016). Prepulse inhibition of the acoustic startle reflex vs. auditory brainstem response for hearing assessment. *Hear. Res.* 339, 80–93. doi: 10.1016/j.heares.2016.06.006

Ma, W.-L. D., Hidaka, H., and May, B. J. (2006). Spontaneous activity in the inferior colliculus of CBA/J mice after manipulations that induce tinnitus. *Hear. Res.* 212, 9–21 doi: 10.1016/j.heares.2005.10.003

Ma, Z., Wang, G., Cui, L., and Wang, Q. (2015). Myricetin attenuates depressant-like behavior in mice subjected to repeated restraint stress. *Int. J. Mol. Sci.* 16, 28377–28385. doi: 10.3390/ijms161226102

Makinson, R., Lundgren, K. H., Seroogy, K. B., and Herman, J. P. (2015). Chronic social subordination stress modulates glutamic acid decarboxylase (GAD) 67 mRNA expression in central stress circuits. *Physiol. Behav.* 146, 7–15. doi: 10.1016/j.physbeh.2015.04.025

Meltser, I., Cederroth, C. R., Basinou, V., Savelyev, S., Lundkvist, G. S., and Canlon, B. (2014). TrkB-mediated protection against ciradian sensitivity to noise trauma in the murine cochlea. *Curr. Biol.* 24, 658–663. doi: 10.1016/j.cub.2014.01.047

Møller, A. R. (2011). "Epidemiology of tinnitus in adults," in *Textbook of Tinnitus*. New York, NY: Springer Science + Business Media, LLC

Mulders, W. H. A. M., and Robertson, D. (2011). Progressive Centralization of midbrain hyperacitivty after acoustic trauma. *Neurosci.* 192, 1–17. doi: 10.1016/j.neuroscience.2011.06.046

Noreña, A.J., and Eggermont, J. J. (2003). Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. *Hear. Res.* 183, 137–153 doi: 10.1016/s0378-5955(03)00225-9

Pilati, N., Large, C., Forsythe, I. D., and Hamann, M. (2012). Acoustic over-exposure triggers burst firing in dorsal cochlear nucleus fusiform cells. *Hear. Res.* 283, 98–106. doi: 10.1016/j.heares.2011.10.008

Pilati, N., Ison, M. J., Barker, M., Mulheran, M., Large, C. H., Forsythe, I. D., et al. (2012). Mechanisms contributing to central excitability changes during hearing loss. *Proc. Natl. Acad. Sci. U S A* 109, 8292–8297. doi: 10.1073/pnas.1116981109

Radziwon, K. E., June, K. M., Stolzberg, D. J., Xu-Friedman, M. A., Salvi, R. J., and Dent, M. L. (2009). Behaviorally measured audiograms and gap detection thresholds in CBA/CaJ mice. *J. Comp. Physiol. A. Neuroethol. Sens. Neural. Behav. Physiol.* 195, 961–969. doi: 10.1007/s00359-009-0472-1

Roberts, L. E., Eggermont, J. J., Caspary, D. M., Shore, S. E., Melcher, J. R., and Kaltenbach, J. A. (2010). Ringing ears: The neuroscience of tinnitus. *J. Neurosci.* 30, 14972–14979. doi: 10.1523/JNEUROSCI.4028-10.2010

Roberts, L. E., Bosnyak, D. J., and Thompson, D.C. (2012). Neural plasticity expressed in central auditory structures with and without tinnitus. *Front. Syst. Neurosci.* 6:40. doi: 10.3389/fnsys.2012.00040

Ropp, T.-J. F., Tiedemann, K. L., Young, E. D., and May, B. J. (2014). Effects of unilateral acoustic trauma on tinnitus-related spontaneous activity in the inferior colliculus. *J. Assoc. Res. Otolaryngol.* 15, 1007–1022. doi: 10.1007/s10162-014-0488-2

Rubio, M. E. (2006). Redistribution of synaptic AMPA receptors at glutamatergic synapses in the dorsal cochlear nucleus as an early response to cochlear ablation in rats. *Hear. Res.* 216–217, 154–167. doi: 10.1016/j.heares.2006.03.007

Salvi, R. J., Wang, J., and Ding, D. (2000). Audiotry plasticity and hyperactivity following cochlear damage. *Hear. Res.* 147, 261–274. doi: 10.1016/s0378-5955(00)00136-2

Schaette, R., and McAlpine, D. (2011). Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J. Neurosci.* 31, 13452–13457. doi: 10.1523/JNEUROSCI.2156-11.2011

Schmuziger, N., Fostiropoulos, K., and Probst, R. (2006). Long-term assessment of auditory changes resulting from a single noise exposure associated with nonoccupational activities. *Int. J. Audiol.* 45, 46–54. doi: 10.1080/14992020500377089

Seggie, J. A., and Brown, G. M. (1975). Stress response patterns of plasma corticosterone, prolactin, and growth hormone in the rat, following handling or exposure to novel environment. *Can. J. Physiol. Pharmacol.* 53, 629–637.

Sharp, J., Zammit, T., Azar, T., and Lawson, D. (2003). Stress-like responses to common procedures in individually and group-housed female rats. *Contemp. Top. Lab. Anim. Sci.* 42, 9–18.

Simonetti, P., and Oiticica, J. (2015). Tinnitus neural mechanisms and structural changes in the brain: The contribution of neuroimaging research. *Int. Arch. Otorhinolaryngol.* 19, 259–265. doi: 10.1055/s-0035-1548671

Singer, W., Zuccotti, A., Jaumann, M., Lee, S. C., Panford-Walsh, R., Xiong, H., et al. (2013). Noise-induced inner hair cell ribbon loss disturbs central arc mobilization: a novel molecular paradigm for understanding tinnitus. *Mol. Neurobiol.* 47, 261–279. doi: 10.1007/s12035-012-8372-8

Sleigh, J., Harvey, M., Voss, L., and Denny, B. (2014). Ketamine—More mechanisms of action than just NMDA blockade. *Curr. Anaesth. Crit. Care* 4, 76–81. doi: 10.1016/j.tacc.2014.03.002

Stone, E. A., and Quartermain, D. (1997). Greater behavioral effects of stress in immature as compared to mature male mice. *Physiol. Behav.* 63, 143–145. doi: 10.1016/s0031-9384(97)00366-1

Syka, J., and Rybalko, N. (2000). Threshold shifts and enhancement of cortical evoked responses after noise exposure in rats. *Hear. Res.* 139, 59–68. doi: 10.1016/s0378-5955(99)00175-6

Tukey, J. W. (1977). *Exploratory Data Analysis*. Boston, MA: Addison-Wesley Publishing Company.

Turner, J. G., Brozoski, T. J., Bauer, C. A., Parrish, J. L., Myers, K., Hughes, L. F., et al. (2006). Gap detection deficits in rats with tinnitus: a potential novel screening tool. Behav. *Neurosci.* 120, 188–195. doi: 10.1037/0735-7044.120.1.188

Turner, J. G., Bauer, C. A., and Rybak, L. P. (2007). Noise in animal facilities: why it matters. *J. Am. Assoc. Lab. Anim. Sci.* 46, 10–13.

Turner, J. G., Larsen, D., Hughes, L., Moechars, D., and Shore, S. (2012). Time course of tinnitus development following noise exposure in mice. *J. Neurosci. Res.* 90, 1480–1488. doi: 10.1002/jnr.22827

Turner, J. G., Parrish, J. L., Zuiderveld, L., Darr, S., Hughes, L. F., Caspary, D. M., et al. (2013). Acoustic experience alters the aged auditory system. *Ear Hear.* 34, 151–159. doi: 10.1097/AUD.0b013e318269ca5b

Turner, J. G., and Willott, J. F. (1998). Exposure to an augmented acoustic environment alters auditory function in hearing-impaired DBA/2J mice. *Hear. Res.* 118, 101–113. doi: 10.1016/s0378-5955(98)00024-0

Wang, Y., and Liberman, M. C. (2002). Restraint stress and protection from acoustic injury in mice. *Hear. Res.* 165, 96–102. doi: 10.1016/s0378-5955(02)00289-7

Wang, J., Salvi, R. J., and Powers, N. (1996). Plasticity of response properties of inferior colliculus neurons following acute cochlear damage. *J. Neurophysiol.* 75, 171–183.

Wang, Y., Hirose, K., and Liberman, M. C. (2002). Dynamics of noise-induced cellular injury and repair in the mouse cochlea. *J. Assoc. Res. Otolaryngol.* 3, 248–268. doi: 10.1007/s101620020028

Wang, H., Brozoski, T. J., and Caspary, D. M. (2011). Inhibitory neurotransmition in animal models of tinnitus: Maladaptive plasticity. *Hear. Res.* 279, 111–117. doi: 10.1016/j.heares.2011.04.004

Weisz, N., Hartmann, T., Dohrmann, K., Schlee, W., and Noreña, A. (2006). High-frequency tinnitus without hearing loss does not mean absence of deafferentation. *Hear. Res.* 222, 108–114. doi: 10.1016/j.heares.2006.09.003

Yoshida, N., and Liberman, M. C. (2000). Sound conditioning reduces noise-induced permanent threshold shift in mice. *Hear. Res.* 148, 213–219. doi: 10.1016/s0378-5955(00)00161-1

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GLAST Deficiency in Mice Exacerbates Gap Detection Deficits in a Model of Salicylate-Induced Tinnitus

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Gap detection or gap pre-pulse inhibition of the acoustic startle (GPIAS) has been successfully used in rat and guinea pig models of tinnitus, yet this system has been proven to have low efficacy in CBA mice, with low basal GPIAS and subtle tinnitus-like effects. Here, we tested five mouse strains (CBA, BalbC, CD-1, C57BL/6 and 129sv) for pre-pulse inhibition (PPI) and gap detection with varying interstimulus intervals (ISI) and found that mice from a CBA genetic background had the poorest capacities of suppressing the startle response in the presence of a pre-pulse or a gap. CD-1 mice displayed variable responses throughout all ISI. Interestingly, C57BL/6, 129sv and BalbC showed efficient suppression with either pre-pulses or gaps with shorter ISI. The glutamate aspartate transporter (GLAST) is expressed in support cells from the cochlea and buffers the excess of glutamate. We hypothesized that loss of GLAST function could sensitize the ear to tinnitus-inducing agents, such as salicylate. Using shorter ISI to obtain a greater dynamic range to assess tinnitus-like effects, we found that disruption of gap detection by salicylate was exacerbated across various intensities of a 32-kHz narrow band noise gap carrier in GLAST knockout (KO) mice when compared to their wild-type (WT) littermates. Auditory brainstem responses (ABR) and distortion-product otoacoustic emission (DPOAE) were performed to evaluate the effects on hearing functions. Salicylate caused greater auditory threshold shifts (near 15 dB) in GLAST KO mice than in WT mice across all tested frequencies, despite similarly reduced DPOAE. Despite these changes, inhibition using broad-band gap carriers and 32 kHz pre-pulses were not affected. Our study suggests that GLAST deficiency could become a useful experimental model to decipher the mechanisms underlying drug-induced tinnitus. Future studies addressing the neurological correlates of tinnitus in this model could provide additional insights into the mechanisms of tinnitus.

Keywords: hearing loss, salicylate, tinnitus, gap detection, pre-pulse inhibition, startle response, mouse, disease models

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INTRODUCTION

Tinnitus remains an untreatable condition frequently associated with stress, anxiety or depression (Baguley et al., 2013), and affects 10–15% of the population. In spite of increasing attention towards the understanding and treatment of tinnitus, experimental efforts in the field remain relatively limited in comparison to the numerous clinical reports (Cederroth et al., 2013). Experimentally, the induction of tinnitus is achieved through noise overexposure or the administration of ototoxic drugs (e.g., salicylate, quinine or cisplatin; Stolzberg et al., 2012; von der Behrens, 2014).

Advances in the field of tinnitus have been made due to the development of behavioral methods to objectively assess the perception of non-existing sounds. Gap pre-pulse inhibition of the acoustic startle reflex (GPIAS), validated in a rat model of tinnitus using the operant conditioning paradigm (Turner et al., 2006; Turner and Parrish, 2008), resembles pre-pulse inhibition of the acoustic startle reflex (PPI or PPIAS), whereby a reduction in the response to an intense stimulus is observed when preceded by a subthreshold intensity pre-pulse (Ison et al., 1973; Graham, 1975). Animal models or humans with schizophrenia or bipolarity disorders have deficits in inhibiting the startle reflex with a pre-pulse as a consequence of a dysfunction in the sensorimotor gating mechanism (Braff et al., 1978; DelPezzo and Hoffman, 1980). In contrast to PPI, which uses a low-intensity pre-pulse to inhibit the startle reflex, GPIAS presents a silent gap embedded in a continuous carrier noise. Despite startle suppression being calculated similarly for both GPIAS and PPI, these use different neural pathways to regulate inhibition. Lesion studies have shown that the auditory cortex regulates GPIAS but not PPI (Bowen et al., 2003). When used in the context of tinnitus, as an animal's tinnitus closely matches the background noise, the startle reflex is less suppressed by the pre-pulse gap because tinnitus interferes with the optimal inhibition of the startle reflex mediated by the gap. As a consequence, affected animals display greater startle response (meaning less inhibited) than in the absence of tinnitus. The use of GPIAS for the assessment of tinnitus has been supported by additional neuronal correlates of tinnitus such as increased spontaneous firing rates in the dorsal cochlear nucleus (DCN; Li et al., 2013), hyperactivity in the inferior colliculus (Holt et al., 2010) and remapping of the auditory cortex (Engineer et al., 2011).

CBA mice have been conventionally used in auditory research for their excellent hearing abilities. In contrast, C57BL/6, in which mutant strains have been traditionally developed, display age-related hearing loss due to a point mutation on the *Cdh23* gene (Ohlemiller and Gagnon, 2004) and thus have been less accepted in the auditory field. However, in the absence of tinnitus, suppression of the startle response with the presence of a gap in narrow band carriers remains highly inefficient in CBA mice (10–50%; Middleton et al., 2011; Llano et al., 2012; Hickox and Liberman, 2014) when compared to that in rats (40–60%; Turner et al., 2006; Turner and Parrish, 2008; Engineer et al., 2011; Su et al., 2012; Yi et al., 2016), consequently offering a small dynamic range to distinguish tinnitus-like effects. Thus, improving the basal suppression of gap detection in mice would increase the confidence window for detecting tinnitus. Curiously, mice appear to be resistant to developing tinnitus, as only 20–50% of the mice that are exposed to noise display behavioral evidence of tinnitus (Middleton et al., 2011; Li et al., 2013), compared to 70–75% of that in rats (Wang et al., 2009; Zhang et al., 2011; Ruttiger et al., 2013). These observations are consistent with the notion that mice are more resilient than guinea pigs to drug-induced hearing loss by, for instance, aminoglycoside antibiotics or the anti-cancer drug cisplatin (Poirrier et al., 2010). Overall, a smaller number of mice display noise-induced changes in GPIAS than do rats, and this species appears more resistant to drug-induced hearing loss than are guinea pigs.

The glutamate aspartate transporter (GLAST) appears as a potential candidate to explain such differences. GLAST belongs to the family of glutamate transporters that stabilize the extracellular environment and maintain cell-to-cell communication. GLAST, but not GLT1, has been identified in the hearing organ, the cochlea (Jin et al., 2003). GLAST is present in the inner phalangeal cells (IPCs) that surround the sensory inner hair cells (IHCs) and afferent neuron synapse (Ruel et al., 2007), where it pumps back excessive glutamate released by sensory cells (Glowatzki et al., 2006) during noise exposure (Hakuba et al., 2000; Chen et al., 2010). As a consequence, mice lacking GLAST show greater hearing threshold shifts and synaptic damage than do wild-type (WT) mice after noise exposure (Hakuba et al., 2000; Chen et al., 2010). GLAST is hardly detectable in the cochlea of rats or guinea pigs and is abundant in the mouse cochlea (Jin et al., 2003). This is why we hypothesized that GLAST abundance in the murine cochlea could underlie the resilience of mice to inner ear insults by drugs, and potentially tinnitus.

Research on tinnitus would benefit from the use of mice, whose species offers facilitated genetic manipulations for understanding the mechanisms that are related to this auditory disorder. In the present study, we address the limitations of using mice by: (i) increasing the dynamic range of startle suppression in the presence of a gap; and (ii) identifying a protein whose disruption exacerbates gap detection deficits induced by salicylate. By combining these two advances, we propose a new mouse model for investigating tinnitus.

MATERIALS AND METHODS

Experimental Animals

Experimental procedures on animals performed at the Karolinska Institutet were in accordance with the guidelines and regulations set out by the University and *Stockholm's Norra Djurförsöksetiska Nämnd*. Experiments performed at the Rockefeller University were approved by the Institutional Animal Care and Use Committee of The Rockefeller University. GLAST knockout (KO) mice on a C57BL/6 background (Watase et al., 1998) and the other listed strains (from Charles Rivers) were maintained at 19–21°C in a 50%–50% light-dark cycle.

GLAST KO mice and their WT littermates were obtained from heterozygous crosses. We observed a non-

mendelian distribution in the progeny of crosses between heterozygous KO mice ($n = 145$): 28% WT animals, 61% heterozygous KO animals, and only 10% homozygous KO animals. When crossing male homozygotes with female heterozygotes, we obtained 80% heterozygous and 20% homozygous KO animals among the progeny. In addition, homozygous females appeared highly anxious and displayed pup-killing behavior. Only male mice between 2 and 4 months of age were used in this study. Baseline auditory thresholds of GLAST KO mice measured by auditory brainstem responses (ABR) within this age range were similar to those of WT littermates (8 kHz = 35.71 ± 2; 16 kHz = 19.29 ± 0.7; 32 kHz = 29.64 ± 1.7; KO: 8 kHz = 38 ± 1.7; 16 kHz = 21 ± 1; 32 kHz = 32 ± 1.33; $p = 0.97$ by a 2-way ANOVA).

The animals had free access to water and food. They were injected daily with intraperitoneal (i.p.) sodium salicylate (Sigma, S3007) at 300 mg/kg diluted in 0.9% NaCl for three consecutive days. Hearing threshold measures and behavior tests were performed 2 h after the last injection, as previously described (Guitton et al., 2003).

Auditory Brainstem Responses

Mice were anesthetized with 80 mg/kg ketamine and 12 mg/kg xylazine for measurements of ABR. The positive needle electrode was inserted subdermally at the vertex, the negative electrode was placed beneath the pinna of the left ear, and the ground electrode was located near the tail. ABR were evoked by tone bursts of 8, 16 and 32 kHz, produced by a closed-field electrostatic speaker connected to a driver (EC-1 and ED-1, Tucker-Davis Technologies). The 5-ms signals were presented 33.3 times per second; their 0.5-ms onsets and offsets were tapered with a squared cosine function. The speaker's audio output was transmitted into the ear through a custom acoustic coupler. Sound pressure levels were measured with a calibrated microphone and preamplifier, connected to a conditioning amplifier (4939-A-011 and 2690-A-0S1, Brüel and Kjær). The response was amplified 10,000× and bandpass filtered at 0.3–3 kHz (P55, Natus Neurology Inc.). The amplified response was then digitally sampled at 10-μs intervals with a data acquisition device (USB-6210, National Instruments), controlled by custom software (LabVIEW 2010, National Instruments). The responses to 1000 bursts were averaged at each intensity level to determine the threshold; the threshold is defined as the lowest level at which a response peak is distinctly and reproducibly present. For each frequency, the sound pressure level was decreased from 100 dB SPL in 5 dB steps, until the threshold was reached and confirmed with one replicate measure. Threshold shifts were measured against individual's baseline values.

Distortion-Product Otoacoustic Emissions

Distortion-product otoacoustic emissions were elicited with an acoustic assembly, consisting of two electrostatic speakers (EC-1, Tucker-Davis Technologies), to generate primary tones and a miniature microphone (EK-23103, Knowles) to measure ear-canal sound pressure. The speakers and the microphone were both calibrated with the calibration microphone described above. The $2f_1 - f_2$ distortion product was measured with f_1 = 6–24 kHz, $f_2/f_1 = 1.25$ and the stimulus levels $L_1 = L_2 = 75$ dB SPL. The acoustic signal was amplified by a preamplifier (ER-10B+, Etymotic Research), and the sound pressure measured in the ear canal was digitally sampled at 10-μs intervals with the data acquisition system described above. Each frequency pair was presented for 1 s. After computing fast Fourier transforms and averaging them over 10 consecutive traces, we determined for each frequency pair the amplitudes of the $2f_1 - f_2$ distortion product and of the noise floor measured at ±100 Hz from $2f_1 - f_2$; this procedure required 17 s of data acquisition and processing.

Testing of the Interstimulus Interval

Interstimulus intervals (ISI) were tested using the SR-Lab startle response system from San Diego Instruments as previously described with minor modifications (Lowry et al., 2013). Animals were acclimated to the procedure on day 1, and were tested for PPI on day 2 and GPIAS on day 5. Background sound level (unfiltered white noise) was 65 dB SPL for PPI sessions, with pre-pulses of 75 dB SPL. For GPIAS, carrier level was 80 dB SPL and silent gaps (absence of stimulus) went down to the noise floor of the SR-Lab chamber. Startle pulses were presented at 115 dB SPL. Trials varied pseudo-randomly in their ISI by 10 ms, from 150 to 0 ms. Ten trials per ISI were tested. Inter-trial time interval varied randomly from 8 to 15 ms. Twenty startle-only trials were presented before the session, and five startle-only trials at the end in order to assess habituation. One whole session of PPI or GPIAS lasted 1 h.

Tinnitus Evaluation by the Gap Detection Method

To test frequency-specific gap detection deficits, we developed a custom-made set-up. Tests were performed in a sound-attenuating chamber (ENV-022S, Med Associates, Inc.). whose noise floor was 55 ± 0.5 dB SPL. In the case of PPI tests, which were used to verify the normal sensorimotor gating, white noise was generated by a function generator (DS340, Stanford Research Systems) and filtered (Wavetek 852 Dual HI/LO Variable Analog Filter, Butterworth filtered, 48 dB/octave roll-off) to 1 kHz-wide narrowband noise centered at a given frequency or broadband noise (BBN) and presented at a given sound intensity (60, 65, 70, 75, 80 dB SPL) in a silent environment for a duration of 50 ms through a speaker (NX-6, Power Acoustik) positioned in front of the animal. To startle an animal, a 20-ms white noise startle pulse of 115 dB SPL was delivered from a second speaker positioned above the animal, 70 ms after the pre-pulse. Calibration was done using the microphone, preamplifier and conditioning amplifier mentioned above.

For the gap detection tests that were used to evaluate tinnitus perception, the same set-up was used. The gap carrier was filtered as above into a broadband or 1 kHz-wide narrowband noise

centered at a given frequency and presented at a given sound intensity (60, 65, 70, 75, 80 dB SPL). A relay switch was used to silence the noise for 50 ms, a gap with 0.1 ms rise and fall times (RT/FT), which was followed 15 ms later by a 20-ms white noise startle pulse of 115 dB SPL. The startle response was captured through an electromagnetic coil, bandpass filtered at 3–100 Hz and amplified 10× (P55, Natus Neurology Inc.). Gap detection or PPI was quantified as the percentage decrease in the peak amplitude of the startle response, when a warning gap or pre-pulse preceded the startling noise in comparison to the amplitude when no gap or pre-pulse was present [(1 − the ratio) × 100] (Engineer et al., 2011), using a similar representation as used in PPI studies (greater suppression of the startle reflex closer to 100%). The more tinnitus fills the gap, the less inhibition of the startle is observed. Only naïve animals were used in this study.

Experimental Procedure for the Screening of the Putative Tinnitus Frequency

This procedure was performed on a small group of animals ($n = 3$) to identify the putative tinnitus frequency at which a deeper analysis could be performed with a greater number of animals (see below). Here, PPI was not performed in order to avoid excessive habituation to the startle stimulus.

In order to acclimatize the animals to the testing procedure, a 30-min preliminary test was performed on day 1. On the following day (day 2), the experiment comprised a session of three consecutive blocks.

Block 1

The first block started with a 5-min acclimatization to silence, followed by 20 startle pulses; each of the pulses was 20 ms in duration and at 115 dB SPL. The time between each trial was random and varied between 8 and 12 s.

Block 2

The second block consisted in testing gap detection at various carrier frequencies (BBN, 8, 10, 12, 16, 20, 24, 32-kHz) of 80 dB SPL with 20 trials per frequency, with or without silent gaps (50 ms). Each frequency was tested sequentially with 20 trials. Each trial was performed with 20-ms startle pulses and an ISI of 15 ms.

Block 3

The final block comprised five trials only with startle pulses to be compared with those of the first block to assess habituation. A total of 185 trials was presented in approximately 40 min.

One day after the testing (day 3), animals were treated with salicylate daily for 2 days and tested on the third day of administration (day 5) with the same procedure.

Experimental Procedure for the Validation of the Putative Tinnitus Frequency

This procedure was performed to validate the findings from the frequency screening performed above. In order to acclimatize the animals to the testing procedure, a 30-min preliminary test was performed on day 1. On the following day (day 2), the experiment comprised a session of four consecutive blocks.

Block 1

The first block started with a 5-min acclimation to silence, followed by 20 startle pulses; each of the pulses was 20 ms in duration and at 115 dB SPL. The time between each trial was random and between 8 and 12 s.

Block 2

A second block of stimuli consisted in testing PPI with 20 trials with or without pre-pulses (50 ms), first consisting of a BBN of 75 dB SPL, and then consisting of a 32 kHz centered narrowband noise of successive intensities of 60, 65, 70, 75 and 80 dB SPL in a quiet background. Each trial was performed with 20-ms startle pulses and an ISI of 70 ms.

Block 3

The third block consisted in testing gap detection with 20 trials, with or without silent gaps (50 ms), first embedded in a broadband carrier noise at 75 dB SPL and then embedded in a 32-kHz centered narrowband noise of successive intensities of 60, 65, 70, 75 and 80 dB SPL. Each trial was performed with 20-ms startle pulses and an ISI of 15 ms.

Block 4

The final block comprised five trials only with startle pulses to be compared with those of the first block to assess habituation. A total of 265 trials were presented in approximately 55 min.

One day after the testing (day 3), animals were treated with salicylate daily for 2 days and tested on the third day of administration (day 5) with the same procedure.

Quantitative Real Time-PCR

SybrGreen qRT-PCR assays from cochlear extracts were performed as previously described (Meltser et al., 2014; Vikhe Patil et al., 2015). A mean quantity was calculated from triplicate PCR for each sample, and this quantity was normalized with the geometric mean of the three most stable genes out of six reference genes (tubulin β, Tubb; glyceraldehyde-3-phosphate dehydrogenase, G3pdh; transferring receptor 1, Trf1R; Tubulin α2, Tuba2; hypoxanthine phosphoribosyltransferase, HPRT; and Cyclophilin B) selected using the geNorm algorithm as described (Vandesompele et al., 2002). Normalized quantities were averaged for three technical replicates for each data point and represented as mean ± SD. The highest normalized relative quantity was arbitrarily designated 1.0. Fold changes were calculated from the quotient of means of these normalized quantities and reported as ± SEM. The primers for *eaat1* used are F: 5′-GGGAAGATGGGGATGCGAG-3′ and R: 5′-GCCGAAGCACATGGAGAAG-3′.

Statistical Analysis

Two-way ANOVA and a Bonferroni *post hoc* were used for statistical analysis (Prism version 4.0, GraphPad software). Differences were considered significant if $P < 0.05$. Animals that

failed to respond to the startle (any peak-to-peak response above noise floor was considered a startle) or failed to inhibit the startle in the presence of a pre-pulse before salicylate treatment (any decrease in startle amplitude during pre-pulse trials vs. startle only trials) were excluded from the analysis (near 5%). When performing PPI tests using a pre-pulse of 80 dB SPL, nearly 10% of the pre-pulses elicited a startle, which then completely suppressed the startle response. The greater the intensity of the pre-pulse, more efficient was the inhibition of the startle response. As a consequence, trials in which the 80 dB pre-pulse induced a startle response before the startle pulse (10% of the 80 dB pre-pulse trials) were excluded from the analysis.

RESULTS

Shorter Interstimulus Interval Improves GPIAS in Specific Mouse Strains

When developing a custom-made gap detection set-up, we found that the ability of C57BL/6 mice to inhibit the startle response in the presence of a gap was almost null when using similar settings to those used for PPI (duration of the pre-pulse: 50 ms; ISI: 70 ms; startle duration: 20 ms; startle intensity: 115 dB). This particular paradigm only reduced the startle response by 20% (data not shown). We found that GPIAS was particularly sensitive to modifications of the ISI. We observed that in both C57BL/6 and 129sv mice, unlike for PPI, the shorter the ISI, the greater the inhibition of startle response, achieving up to 80% in C57BL/6 and 129sv mice (**Figures 1B,C**). Measures of the startle amplitude before and after each PPIAS or GPIAS session confirmed the lack of habituation during this test (data not shown). While this pattern was present in CBA mice, it did not exceed more than 50% at the lowest ISI, strongly suggesting that the inhibition of the startle reflex in this strain is not efficient (**Figure 1D**). This trend was not observed when using other genetic backgrounds such as CD-1 and Balb-C mice (**Figures 1E,F**). In Balb-C, PPI and GPIAS followed a similar course over varying ISIs, and CD-1 had highly variable responses, suggesting they would require additional acclimatization sessions (as typically performed with rats) to obtain a more robust reflex response. Overall, it is concluded that ISI is a critical parameter for improving gap detection in C57BL/6 and 129sv mice, and that shorter ISIs can be used to increase the basal level of suppression of the startle response in these strains in order to provide greater dynamic range to detect deficits in gap detection.

Salicylate Causes Severe Gap Detection Deficits in GLAST KO Mice

The basal level of startle suppression in the presence of a gap was further improved using Longenecker and Galazyuk (2012) recommendations by: (i) adjusting speaker non-linearity by calibrating the intensity output for each filtered narrowband noise; (ii) suppressing echo and reverberation by covering the interior walls with sound-insulating foam; and (iii) replacing the acrylic animal restrainer with a non-resonating perforated plastic pipette box. Because noise is effective in inducing tinnitus in nearly 50% of animals, we used salicylate, which is the most commonly used drug in animal models of tinnitus (Cazals, 2000; von der Behrens, 2014). Salicylate has an advantage over noise in that it has previously been used in humans to induce tinnitus

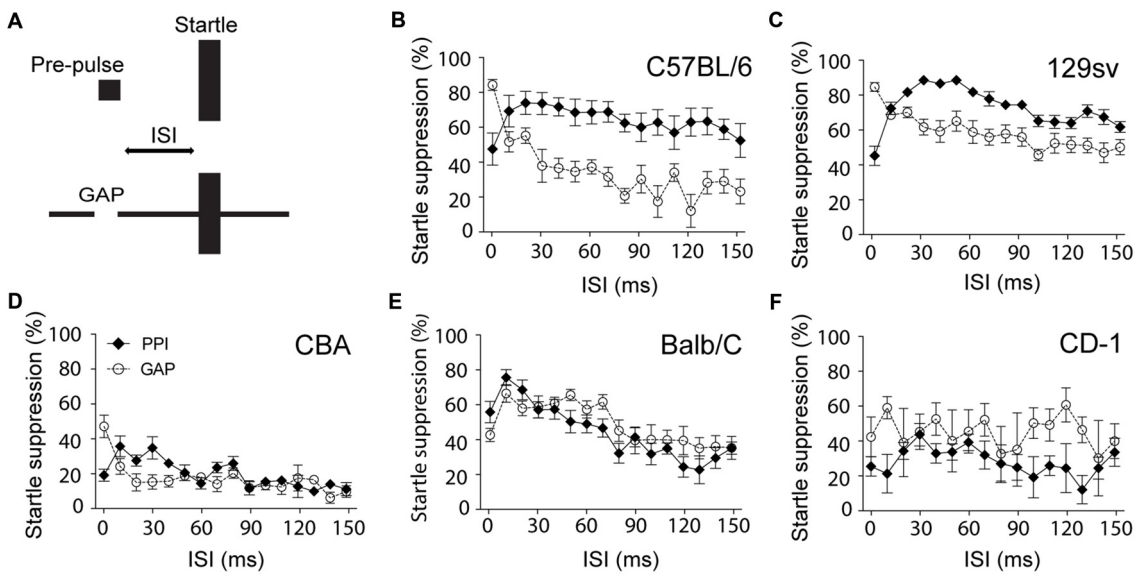

FIGURE 1 | Shorter interstimulus intervals (ISI) improves gap-mediated suppression of the startle response. (A) Schematic model of the experiment. Male C57BL/6 **(B)**, 129sv **(C)**, CBA **(D)**, Balb/C **(E)** and CD-1 **(F)** male mice (2–4 months old) were exposed to pre-pulse inhibition of the acoustic startle reflex (PPIAS) (black diamonds) and gap pre-pulse inhibition (PPI) of the acoustic startle (GPIAS; open circles) sequentially. ISI was modified by steps of 10 ms. Average startle amplitudes before and after each paradigm were not affected. Data represent means ± SEM ($n = 8$).

(3.9 g salicylate/day for 5 days; Mongan et al., 1973; McFadden et al., 1984).

We screened various 1-kHz narrowband frequencies (from 8 to 32 kHz) in a small group of animals ($n = 3$) to identify the putative tinnitus frequency, meaning the frequency at which GPIAS would be most affected by salicylate treatment according to a previous model (300 mg/kg/day for 3 days, Guitton et al., 2003). We used a carrier noise of high intensity (80 dB SPL) to maximize the inhibition of the startle by the gap and identify the frequencies with the greatest changes. This initial screening showed that both WT and KO mice treated with salicylate exhibit greater deficits in sensing the gap at 32 kHz (two-way ANOVA, Genotype and Treatment Factor: $F_{(7,64)} = 6.108$, $p < 0.0001$; Frequency Factor: $F_{(3,64)} = 11.44$, $p < 0.0001$, data not shown).

We next focused on the 32-kHz narrowband noise to perform both GPIAS, using different carrier intensities, and PPI, using increasing pre-pulse intensities. PPI was used as a control for normal temporal processing or sensory motor gating. Before salicylate administration, WT animals increasingly detected the gap with increasing intensities of a narrowband carrier noise centered at 32 kHz, inhibiting their startle reflexes up to 91% (**Figure 2A**). After 3 days of salicylate administration, the ability of WT mice to repress their startle reflexes decreased by 25% at all carrier intensities tested ($p < 0.0001$ by a two-way ANOVA with Bonferroni *post hoc* test, Treatment Factor, $F_{(1,110)} = 40.46$, $p < 0.0001$; Carrier Intensity Factor, $F_{(4,110)} = 35.36$, $p < 0.0001$; **Figure 2A**). Salicylate did not affect PPI with the exception of the lowest intensity of pre-pulse tested, suggestive of hyperacusis ($p = 0.03$ by a two-way ANOVA with Bonferroni *post hoc* test, Treatment Factor, $F_{(1,111)} = 4.64$, $p = 0.0333$; Pre-pulse Intensity Factor, $F_{(4,111)} = 25.40$, $p < 0.0001$; **Figure 2B**). The overall gap detection deficits, although significant, appeared to be relatively small.

Before salicylate treatment, GLAST KO mice displayed GPIAS as efficiently as WT mice (two-way ANOVA, Genotype Factor: $F_{(1,79)} = 0.4351$, $p = 0.5114$; Carrier Intensity Factor: $F_{(4,79)} = 57.0$, $p < 0.0001$). However, after salicylate treatment, the ability of GLAST KO mice to detect the gap was severely impaired throughout all carrier intensities tested (two-way ANOVA with Bonferroni *post hoc* test, Treatment Factor: $F_{(1,89)} = 117.5$, $p < 0.0001$; Carrier Intensity Factor: $F_{(4,89)} = 16.02$, $p < 0.0001$; **Figure 2C**). Although PPIAS at 32 kHz appeared to be lower in GLAST KO mice than in WT mice (two-way ANOVA, Genotype Factor: $F_{(1,105)} = 17.19$, $p < 0.0001$; Pre-pulse Intensity Factor: $F_{(4,105)} = 27.54$, $p < 0.0001$), it was not affected by salicylate administration (two-way ANOVA, Treatment Factor: $F_{(1,105)} = 0.2299$, $p = 0.6326$; Pre-pulse Intensity Factor: $F_{(4,105)} = 15.72$, $p < 0.0001$, **Figure 2D**), suggesting that the disruption of gap detection is more likely a tinnitus effect rather than a deficit in temporal processing or in auditory sensitivity (hyperacusis-like phenomenon).

To confirm the frequency-specific effects on GPIAS observed after the treatment with salicylate, we used a BBN Gap or pre-pulse (BBN PPI) at 75 dB SPL, and next verified whether inhibition of the startle response was affected before and after salicylate administration in both WT and GLAST KO mice. GPIAS in a BBN carrier was equally efficient before or after salicylate treatment in both genotypes (two-way ANOVA, Genotype Factor: $F_{(1,40)} = 0.7098$, $p = 0.4045$; Treatment Factor: $F_{(1,40)} = 0.2667$, $p = 0.6084$, **Figure 2E**). Similarly, the efficacy of BBN PPI, which is also used to assess normal temporal processing (Turner et al., 2006; Turner and Parrish, 2008; Middleton et al., 2011; Llano et al., 2012), was identical in WT and mutant animals both before and after the administration of salicylate (two-way ANOVA, Genotype Factor: $F_{(1,46)} = 0.4673$, $p = 0.4976$; Treatment Factor: $F_{(1,46)} = 0.009994$, $p = 0.9208$, **Figure 2F**). These control experiments confirmed that BBN PPIAS and GPIAS are not affected by salicylate in both WT and KO mice and support the notion that salicylate causes greater gap detection deficits at 32 kHz in GLAST KO than it does in WT mice.

We also assessed how basal startle amplitudes were affected in WT and GLAST KO mice by salicylate. Salicylate treatment increased the startle amplitude in response to startle pulses alone in KO mice (two-way ANOVA, Genotype Factor: $F_{(1,56)} = 37.90$, $p < 0.0001$; Treatment Factor: $F_{(1,56)} = 17.86$, $p < 0.0001$,

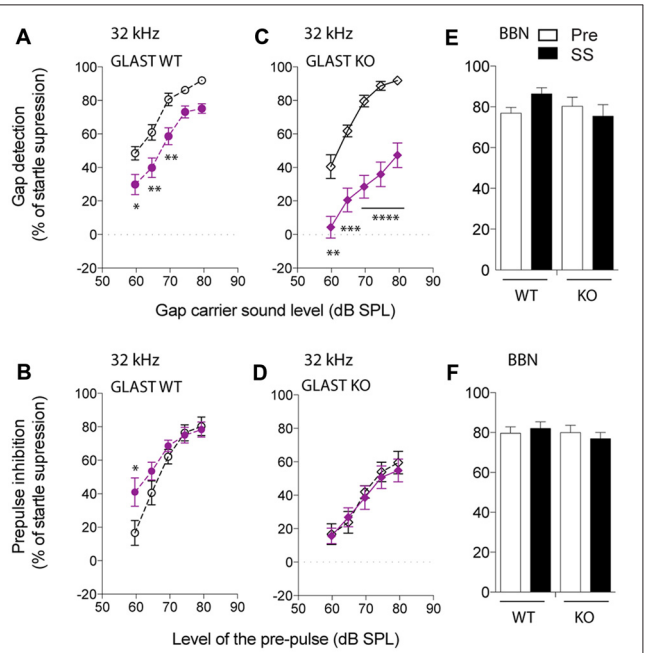

FIGURE 2 | Glutamate aspartate transporter (GLAST) deficiency facilitates the induction of tinnitus by salicylate. GPIAS in wild-type (WT) **(A)** and GLAST knockout (KO) **(C)** mice before (dark) and 2 h after the last salicylate (SS) administration (purple) using a 32-kHz narrowband carrier noise presented with a 5 dB step increase in intensity. PPIAS in WT **(B)** and GLAST KO **(D)** mice before (dark) and after (purple) salicylate treatment using a 32-kHz narrowband pre-pulse presented with a 5 dB step increase in intensity. Normal gap **(E)** and pre-pulse sensing **(F)** in WT and GLAST KO mice before (white) and after (black) the injection of SS using a broadband noise (BBN) at 75 dB SPL carrying a silent gap (GPIAS session) or a 75 dB SPL broadband pre-pulse presented in a silent environment (PPI session). Pre-treatment values are shown in dark, and SS values are shown in filled symbols in purple. WT mice are shown with circles and GLAST KO mice are shown with diamonds. Data represent means ± SEM ($n = 8$–14). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$, by a two-way ANOVA with *post hoc* Bonferroni test.

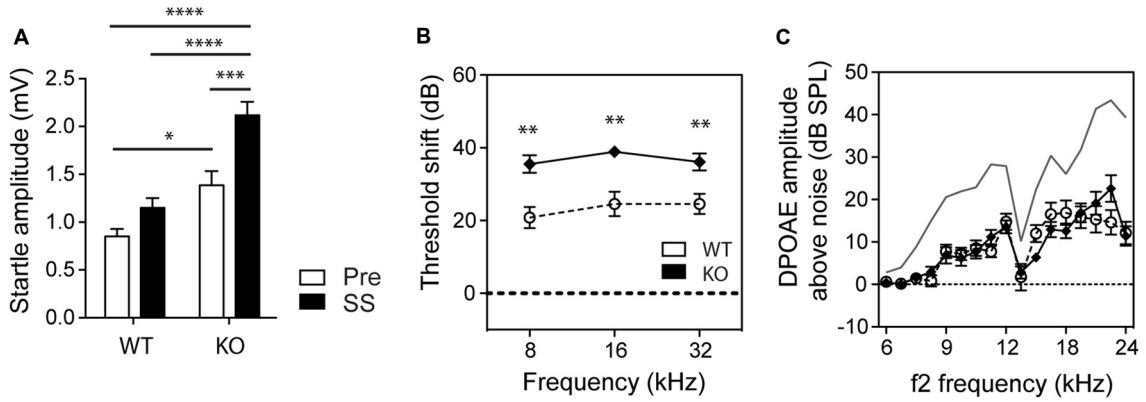

FIGURE 3 | GLAST deficiency sensitizes mice to salicylate-induced hearing loss but without affecting outer hair cell function. (A) GLAST KO mice subjected to startle pulses showed significantly larger startle responses than did WT mice. Salicylate (black, SS) enhanced the startle reflexes of GLAST KO mice. Pre-treatment values are shown in white. Data represent means ± SEM (n = 8–14). Auditory threshold shifts **(B)** and distortion-product otoacoustic emissions (DPOAEs **C)** of GLAST WT (white circles) and homozygous (black diamonds) mice with sodium salicylate at 300 mg/kg/day for 3 days, measured 2 h after the last injection. The uppermost curve (in gray) represents the mean for saline-treated WT control animals analyzed on the same day. Data of the auditory measures represent means ± SEM (n = 10–16). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ and $^{****}P < 0.0001$, by a two-way ANOVA with *post hoc* Bonferroni test.

Figure 3A). Changes in hearing thresholds could account for differences in gap detection as well as in PPI. It is worth noting that salicylate caused a loss of hearing threshold by 20 dB across all frequencies (from 8 to 32 kHz) in WT mice and 35 dB in GLAST KO mice (two-way ANOVA, Genotype Factor, $F_{(1,57)} = 35.03$, $p < 0.0001$; Frequency Factor, $F_{(2,57)} = 0.8140$, $p = 0.4482$, $n = 10$–16, **Figure 3B**) with distortion-product otoacoustic emissions (DPOAEs) reduced by half, and in a similar way in WT and KO mice (Genotype Factor, $F_{(16,340)} = 19.05$, $p = 0.9188$; Frequency Factor, $F_{(16,340)} = 1.248$, $p < 0.0001$, **Figure 3C**). However, DPOAE measures were only performed at suprathreshold levels, and potential differences at threshold could have occurred. Still, the lack of differences in DPOAEs between WT and KO mice suggests that GLAST does not regulate outer hair cell function; rather GLAST could potentiate the effects of salicylate at the afferent synapse as suggested by the greater threshold shifts in GLAST KO mice. These findings are in agreement with previous work showing that salicylate potentiates glutamate-evoked responses in spiral ganglion neurons *ex vivo* (Ruel et al., 2008).

Such differences in salicylate-induced threshold shifts between WT and KO should have equally affected the perception of the pre-pulse or the perception of the gap and subsequent PPIAS and GPIAS. However, the findings indicate that this is not the case since PPI remained completely unaffected by salicylate treatment at 32 kHz where gap detection deficits were the greatest (**Figures 2B,D**). Second, we observed gap sensing deficits in GLAST WT and KO mice at 32 kHz but not when using a BBN as a carrier (**Figure 2E**), although similar hearing threshold shifts have been found across all frequencies tested (**Figure 3B**). Overall, our results suggest that the gap detection deficits observed here are reminiscent of tinnitus perception in the high-frequency area and are not due to hearing loss or defective temporal processing.

DISCUSSION

The present study shows that suppression of the startle response in the presence of a gap can be improved in C57BL/6 and 129sv mice by shortening the ISI and that constitutive loss of glutamate transporter function likely exacerbates the tinnitus-inducing effects of salicylate.

In some strains of mice, the efficacy of the startle suppression by the gap requires shorter ISI than with PPI. With a 15-ms ISI, gap detection suppressed the startle response by nearly 80% in C57BL/6 mice. Our findings contrast with those in the previous studies in rats by Ison and Bowen (2000), in which a biphasic response in the ability of the gap to suppress the startle was observed when varying the ISI . It is likely that these differences are related to species differences. Our results also underline the importance of the genetic background when performing GPIAS experiments. Previous studies have shown that PPI is highly influenced by genetic background and that the C57BL/6 strain displays rather efficient abilities to suppress the startle response in the presence of a pre-pulse (Willott et al., 2003). It would thus be interesting to evaluate how GPIAS is affected by varying ISIs in humans and how genetic background (e.g., different ethnicities) affects the efficacy of GPIAS.

In spite of the successful use of gap detection to identify physiological and molecular pathways involved in tinnitus (Engineer et al., 2011; Li et al., 2013, 2015; Kalappa et al., 2014, 2015), there has been a lot of debate regarding the use of gap detection for tinnitus evaluation (Campolo et al., 2013; Fournier and Hébert, 2013; Boyen et al., 2015; Galazyuk and Hébert, 2015). First, the low level of startle suppression in the presence of a gap reported in tinnitus studies that have used CBA mice leaves little margin to identify gap detection deficits and infer the presence of tinnitus (Longenecker and Galazyuk, 2011; Hickox and Liberman, 2014; Longenecker et al., 2014). Methods to discriminate between tinnitus and non-tinnitus

animals have proven useful (Li et al., 2013, 2015) but an increase in the basal suppression level of the startle response offers a greater dynamic range for identifying tinnitus with greater confidence. Second, Hickox and Liberman (2014) performed a tinnitus study in which they adjusted the gap such that some of the trials were done with a gap closer to the startle stimulus (ISI = 0 ms) and some were done with a gap presented at greater lead times (ISI = 70 ms). Consistent with our findings (**Figure 1**), differences in gap detection between the two ISI conditions were found (being less efficient with greater lead times), however the authors interpreted that tinnitus "was not filling" the gap, since deficits in GPIAS were not observed at both ISIs (Hickox and Liberman, 2014). We believe the experimental conditions used were not optimal for inferring the presence of tinnitus as they used CBA mice, whose basal level of startle suppression in the presence of the gap was inefficient and even more variable with greater ISI. In addition, it has been shown that scopolamine, a muscarinic receptor blocker that disrupts cholinergic function in the brain, affects gap detection when the ISI is larger and not when the gap is closer to the startle stimulus (Ison and Bowen, 2000), meaning that different mechanisms operate GPIAS depending on the ISI. As a consequence, since GPIAS is a reflex response that relies on temporal processing and not on a conscious percept (meaning tinnitus does not fill the gap but interferes with the reflex response *per se*), gap detection deficits caused by tinnitus cannot be expected to be equally efficient at various lead times. The mechanism with which salicylate interferes solely with GPIAS and not PPI is not well understood, however it has been shown that gap detection operates in the auditory cortex, which is not the case of PPI (Bowen et al., 2003). Recent optogenetic studies in mice have shown that cortical inhibition is important in controlling perceptual gap detection (Weible et al., 2014). Since salicylate has been shown to alter the activity in the auditory cortex (Wang et al., 2006; Sun et al., 2009), we postulate that salicylate alters perceptual gap detection at the level of the auditory cortex. Additional behavioral methods in mice would be needed to confirm that the GPIAS deficits observed here correlate with a tinnitus percept.

Previous studies have shown that the ability to suppress the startle response in the presence of a gap improves with experience or with repeated pre-acclimatization sessions (Crofton et al., 1990; Ison and Bowen, 2000), and can reach 50% of startle suppression in CBA mice (Ison et al., 2002). The same applies to PPI (Plappert et al., 2006). We believe that the CBA strain, which is commonly used in the auditory field because of the well-preserved hearing, is not appropriate for PPIAS and GPIAS studies, unless longer acclimatization sessions are performed to achieve efficient gap processing. Instead, C57BL/6 or C57BL/6 × 129sv mixed mice (typical of most mouse mutant models available) would be most appropriate at an age when PPIAS/GPIAS responses are maximized and hearing deficits not yet detectable (between 3 and 4 months of age). Longenecker and Galazyuk (2012) also improved gap detection by decreasing the startle stimulus intensity to non-saturating levels (around 105 dB SPL), which has not been implemented in the current experiment. The differences we observe may also be due to differences in sound quality. With the exception of the initial study by Turner and Parrish (2008); Hickox and Liberman (2014), who describe a 48 dB/octave roll-off in their sound filtering like the one used in the present report, none of the other studies that used GPIAS describe the frequency filtering slope of their narrowband noises. Typically, when the shape of the filter is too narrow (e.g., when the slope of the filter is steep), perceptual artifacts are generated and could interfere with the ability of tinnitus to disrupt GPIAS. These artifacts decrease when using a 48 dB/octave roll-off, and could contribute to improved "detectability" of tinnitus. How the contour of the narrowband filtering affects the ability of tinnitus to interfere with GPIAS remains to be addressed. We believe that the appropriate selection of background strain, startle impulse level and ISI can provide robust PPI and gap detection responses when combined with adequate acclimatization sessions. Such parameters should be taken into account when testing gap detection for the assessment of tinnitus in humans.

The identification of GPIAS deficits in the 32-kHz frequency region when using salicylate in GLAST KO mice contrasts with previous research. A recent review article by Galazyuk and Hébert (2015) summarizes the results obtained with different tinnitus models using GPIAS. It appears that the generation of tinnitus by salicylate varies in terms of frequency depending on species and strains (i.e., Wistar rats get broader-range tinnitus, Brown Norways display tinnitus at 10, 12 and 24 kHz, and Sprague Dawleys at 16 kHz). The overall conclusion is that tinnitus does not seem to be focal and its frequency depends on the strain used. It is possible that we may have missed some frequencies in our screening that would have been otherwise revealed using lower carrier sound intensities and a greater number of animals throughout the procedure. Conversely, the protocols using GPIAS to assess tinnitus are very diverse and none of the salicylate studies (with the exception of the article by Turner and Parrish, 2008) have tested potential GPIAS deficits at 32 kHz (Galazyuk and Hébert, 2015). It is thus hard to determine whether our findings are specific to our model, or whether salicylate-induced tinnitus typically triggers high-frequency tinnitus, or simply whether the deficits in GPIAS are truly triggered by tinnitus *per se* and do not result from confounding effects of hearing loss. The control experiments we have performed rule out the potential bias of high-frequency hearing loss in the disrupted GPIAS observed in GLAST KO mice since: (i) auditory thresholds were equally affected by salicylate in WT and KO mice at all frequencies; (ii) salicylate did not affect GPIAS using a BBN carrier sound; (iii) basal auditory thresholds were normal and equivalent in both WT and KO mice at the time of the test; and (iv) salicylate treatment did not disrupt PPI in either WT or KO mice at 32 khZ, which otherwise would have been altered if hearing loss had contributed to the lower GPIAS at 32 kHz found in GLAST KO mice treated with salicylate. However, we do not rule out the possibility that in spite of the significant changes in GPIAS (in either genotype), the lack of changes in PPI upon salicylate administration could arise from salicylate-induced loudness recruitment, which may maintain suprathreshold PPI behavior.

Our study proposes the first gene potentially involved in tinnitus. GLASTs are mainly present in astrocytes in the central nervous system, buffering the excess of glutamate released at the synaptic cleft, converting it into glutamine, which is then transported back to pre-synaptic terminals to be recycled to glutamate. Two major glutamate transporters, namely GLAST (predominant in the cortex and hippocampus) and the glial GLutamate Transporter GLT1 (predominant in the cerebellum), are responsible for more than 80% of the glutamate uptake in the brain. Loss of glutamate transporters with subsequently uncontrolled extracellular glutamate levels has been associated with neurotoxic effects during seizures, amyotrophic lateral sclerosis, epilepsy and now, possibly, tinnitus. Although species comparison in gene expression levels is difficult to evaluate, given the broad changes in endogenous normalizing components (e.g., ubiquitously expressed genes), it appears likely that the resistance of mice to auditory insults could result, at least in part, from the higher expression of cochlear GLAST. On the other hand, a low abundance of cochlear GLAST would predict higher sensitivity to noise and drug-induced tinnitus. Consistent with this notion, we found that GLAST mRNA abundance was greater in CBA mouse cochleae than in those from CD-1 mice (CBA: 1.02 ± 0.06 relative expression level; CD-1: 0.76 ± 0.04; $p = 0.008$ by an unpaired two-tailed t-test, $n = 5$ per strain), which correlates with these strains' known auditory sensitivity and development of age-related hearing loss. In this regard, Shimizu et al. (2005) found that GLAST KO mice are more vulnerable to kanamycin ototoxicity. Ongoing data collection in our laboratory indicates that this is also the case for cisplatin (unpublished observations). In humans, whose susceptibility to ototoxic medications and noise varies from one individual to another, it remains unknown how cochlear GLAST levels correlate with tinnitus predisposition. A recent study failed to detect GLAST protein in support cells from the human cochlea (Ahmed et al., 2013), suggesting that the human cochlea expresses very low levels in GLAST at the afferent synapse. As a consequence, we predict that humans would show less glutamate-buffering capacity and thus greater vulnerability to noise and ototoxic medications. Genetic analyses of the human homolog of GLAST (*EAAT1*) in subjects with and without tinnitus could bring new knowledge about the mechanisms underlying the vulnerability and resilience to tinnitus.

It is rather widely accepted that peripherally generated tinnitus arises from a lack of cochlear output, rather than an increase in cochlear output. There are clear cochlear differences between salicylate and noise insults whereby spontaneous activity of the auditory nerve (AN) is increased after salicylate but decreased after noise (Eggermont and Roberts, 2004). However, most measures in noise-traumatized animals are performed after noise exposure and not *during* noise exposure. Thus, we would predict an early phase of increased AN activity during noise exposure (Searchfield et al., 2004) and a decrease in AN activity resulting from permanent synaptic damage. In contrast to noise, measures on salicylate-treated animals are performed shortly after the administration of salicylate—presumably when salicylate bioavailability peaks in the cochlea. Although there are to our knowledge no studies that report salicylate bioavailability in the cochlea after intraperitoneal injections, a peak is observed in other tissues after 30–60 min, with blood clearance after 8 h (Sturman et al., 1968). As salicylate potentiates glutamate-evoked responses in primary afferent neurons (Ruel et al., 2008), and lack of GLAST results in increased excess glutamate at the IHC-cochlear nerve synapse (Hakuba et al., 2000; Glowatzki et al., 2006), the overall cochlear output in GLAST KO mice would be expected to increase before salicylate is cleared out. Whether repeated administration of salicylate would cause permanent damage is unclear, but a recent rat model of chronic salicylate-administration leads tinnitus (Yi et al., 2016) and could prove useful to investigate whether more permanent damages would occur at the synapse, and reconcile salicylate-induced tinnitus with the decreased cochlear output theory.

The tinnitus effects observed here could also underlie central actions of salicylate and/or loss of GLAST function in the brain. Indeed, systemic administration of salicylate could activate non-auditory structures (Stolzberg et al., 2012; Chen et al., 2015) translating into broader central impacts than those happening after noise exposure (Holt et al., 2010). However, GLAST is predominant in the cortex and the hippocampus, and whether it is present in structures of the auditory pathway is unknown. The higher baseline startle amplitudes observed in GLAST KO mice could reflect hyperacusis. These findings are also consistent with the known higher anxiety levels in this model (Karlsson et al., 2009): increased locomotor activity in the open field, decreased sociability and social novelty preference, and poor nesting behavior. In addition, (Karlsson et al., 2009) found that pairwise discrimination learning is also affected in GLAST KO mice. The startle responses we obtain are however inconsistent with Karlsson et al.'s (2009) findings, which were not controlled—as we did with ABR—for hearing levels (as acknowledged by the authors), known to be affected at 6 months of age in GLAST KO mice (Hakuba et al., 2000). Importantly, unlike the auditory field, which uses startle response as an indicator of hearing abilities, all biological psychiatry textbooks mention the use of the startle response as a gauge of fear or anxiety. For instance, people with post-traumatic stress disorder have greater startle responses (Grillon et al., 1998). After an animal has learned to associate a specific stimulus with fear, such as light being paired with shocks, greater startle response is observed after presenting a fear signal just before the startle stimulus. Conversely, signals associated with pleasure decrease the startle amplitude response (Schmid et al., 1995). Fear or pleasure signals fail to modulate the startle response in amygdala-lesioned animals, showing that the amygdala is involved in the startle response system (Hitchcock and Davis, 1991). We thus believe that the increased startle response seen in GLAST KO mice in the presence of salicylate is an indicator of amygdala-mediated effects. The recent evidence linking tinnitus with the amygdala suggests that central-GLAST deficiency in the amygdala could contribute to the increased tinnitus severity observed in GLAST KO mice. Nonetheless, Karlsson et al. (2009) also found that PPI was not affected in GLAST KO mice and hence reasoned that these mice display *some* of the

multiple and complex symptoms belonging to schizophrenia (e.g., GLAST KO mice would belong to a subgroup of schizophrenia of lesser severity). Salicylate, which is known to be anxiogenic in high doses (Puel and Guitton, 2007; Guitton, 2009), exacerbated this anxiety effect by increasing startle amplitude responses in KO animals (**Figure 3A**), something that we were able to qualitatively observe when handling the animals. It is thus possible that the higher basal anxiety levels of GLAST KO mice facilitated the tinnitus-inducing effects of salicylate, thereby increasing tinnitus intensity. Specific deletion of GLAST either in the brain or in the ear should enable discrimination of the contribution of all of these factors.

CONCLUSION

Our results suggest a potential role for GLAST in the vulnerability to salicylate-induced tinnitus. Given the magnitude of the disruption in gap detection observed in GLAST KO mice treated with salicylate, we propose that GLAST deficiency may serve as a useful model to distinguish more subtle, yet unidentified mechanisms on how tinnitus is triggered and maintained. Finally, optimizing parameters in gap detection in humans may uncover a potential use of this technology in the objective diagnosis of tinnitus (Galazyuk and Hébert, 2015).

AUTHOR CONTRIBUTIONS

HY, KVP, CH, BF and CRC carried out the experiments; HY, KVP and CRC analyzed the results; CRC designed and directed the research; BC, SS and CRC discussed the results and wrote the manuscript; all authors reviewed the manuscript.

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REFERENCES

Ahmed, S., Vorasubin, N., Lopez, I. A., Hosokawa, S., Ishiyama, G., and Ishiyama, A. (2013). The expression of glutamate aspartate transporter (GLAST) within the human cochlea and its distribution in various patient populations. *Brain Res.* 1529, 134–142. doi: 10.1016/j.brainres.2013.06.040

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Bowen, G. P., Lin, D., Taylor, M. K., and Ison, J. R. (2003). Auditory cortex lesions in the rat impair both temporal acuity and noise increment thresholds, revealing a common neural substrate. *Cereb. Cortex* 13, 815–822. doi: 10.1093/cercor/13.8.815

Boyen, K., Başkent, D., and van Dijk, P. (2015). The gap detection test: can it be used to diagnose tinnitus? *Ear Hear.* 36, e138–e145. doi: 10.1097/aud.0000000000000156

Braff, D., Stone, C., Callaway, E., Geyer, M., Glick, I., and Bali, L. (1978). Prestimulus effects on human startle reflex in normals and schizophrenics. *Psychophysiology* 15, 339–343. doi: 10.1111/j.1469-8986.1978.tb01390.x

Campolo, J., Lobarinas, E., and Salvi, R. (2013). Does tinnitus "fill in" the silent gaps? *Noise Health* 15, 398–405. doi: 10.4103/1463-1741.121232

Cazals, Y. (2000). Auditory sensori-neural alterations induced by salicylate. *Prog. Neurobiol.* 62, 583–631. doi: 10.1016/s0301-0082(00)00027-7

Cederroth, C. R., Canlon, B., and Langguth, B. (2013). Hearing loss and tinnitus–are funders and industry listening? *Nat. Biotechnol.* 31, 972–974. doi: 10.1038/nbt.2736

Chen, Z., Kujawa, S. G., and Sewell, W. F. (2010). Functional roles of high-affinity glutamate transporters in cochlear afferent synaptic transmission in the mouse. *J. Neurophysiol.* 103, 2581–2586. doi: 10.1152/jn.00018.2010

Chen, Y. C., Li, X., Liu, L., Wang, J., Lu, C. Q., Yang, M., et al. (2015). Tinnitus and hyperacusis involve hyperactivity and enhanced connectivity in auditory-limbic-arousal-cerebellar network. *Elife* 4:e06576. doi: 10.7554/elife.06576

Crofton, K. M., Dean, K. F., Sheets, L. P., and Peele, D. B. (1990). Evidence for an involvement of associative conditioning in reflex modification of the acoustic startle response with gaps in background noise. *Psychobiology* 18, 467–474.

DelPezzo, E. M., and Hoffman, H. S. (1980). Attentional factors in the inhibition of a reflex by a visual stimulus. *Science* 210, 673–674. doi: 10.1126/science.7433993

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Engineer, N. D., Riley, J. R., Seale, J. D., Vrana, W. A., Shetake, J. A., Sudanagunta, S. P., et al. (2011). Reversing pathological neural activity using targeted plasticity. *Nature* 470, 101–104. doi: 10.1038/nature09656

Fournier, P., and Hébert, S. (2013). Gap detection deficits in humans with tinnitus as assessed with the acoustic startle paradigm: does tinnitus fill in the gap? *Hear. Res.* 295, 16–23. doi: 10.1016/j.heares.2012.05.011

Galazyuk, A., and Hébert, S. (2015). Gap-Prepulse Inhibition of the Acoustic Startle Reflex (GPIAS) for tinnitus assessment: current status and future directions. *Front. Neurol.* 6:88. doi: 10.3389/fneur.2015.00088

Glowatzki, E., Cheng, N., Hiel, H., Yi, E., Tanaka, K., Ellis-Davies, G. C., et al. (2006). The glutamate-aspartate transporter GLAST mediates glutamate uptake at inner hair cell afferent synapses in the mammalian cochlea. *J. Neurosci.* 26, 7659–7664. doi: 10.1523/JNEUROSCI.1545-06.2006

Graham, F. K. (1975). Presidential Address, 1974. The more or less startling effects of weak prestimulation. *Psychophysiology* 12, 238–248. doi: 10.1111/j.1469-8986.1975.tb01284.x

Grillon, C., Morgan, C. A. III, Davis, M., and Southwick, S. M. (1998). Effects of experimental context and explicit threat cues on acoustic startle in Vietnam veterans with posttraumatic stress disorder. *Biol. Psychiatry* 44, 1027–1036. doi: 10.1016/s0006-3223(98)00034-1

Guitton, M. J. (2009). Tinnitus-provoking salicylate treatment triggers social impairments in mice. *J. Psychosom. Res.* 67, 273–276. doi: 10.1016/j.jpsychores.2008.10.017

Guitton, M. J., Caston, J., Ruel, J., Johnson, R. M., Pujol, R., and Puel, J. L. (2003). Salicylate induces tinnitus through activation of cochlear NMDA receptors. *J. Neurosci.* 23, 3944–3952.

Hakuba, N., Koga, K., Gyo, K., Usami, S. I., and Tanaka, K. (2000). Exacerbation of noise-induced hearing loss in mice lacking the glutamate transporter GLAST. *J. Neurosci.* 20, 8750–8753.

Hickox, A. E., and Liberman, M. C. (2014). Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus? *J. Neurophysiol.* 111, 552–564. doi: 10.1152/jn.00184.2013

Hitchcock, J. M., and Davis, M. (1991). Efferent pathway of the amygdala involved in conditioned fear as measured with the fear-potentiated startle paradigm. *Behav. Neurosci.* 105, 826–842. doi: 10.1037/0735-7044.105.6.826

Holt, A. G., Bissig, D., Mirza, N., Rajah, G., and Berkowitz, B. (2010). Evidence of key tinnitus-related brain regions documented by a unique combination of manganese-enhanced MRI and acoustic startle reflex testing. *PLoS One* 5:e14260. doi: 10.1371/journal.pone.0014260

Ison, J. R., and Bowen, G. P. (2000). Scopolamine reduces sensitivity to auditory gaps in the rat, suggesting a cholinergic contribution to temporal acuity. *Hear. Res.* 145, 169–176. doi: 10.1016/s0378-5955(00)00088-5

Ison, J. R., Castro, J., Allen, P., Virag, T. M., and Walton, J. P. (2002). The relative detectability for mice of gaps having different ramp durations at their onset and offset boundaries. *J. Acoust. Soc. Am.* 112, 740–747. doi: 10.1121/1.1490352

Ison, J. R., McAdam, D. W., and Hammond, G. R. (1973). Latency and amplitude changes in the acoustic startle reflex of the rat produced by variation in auditory prestimulation. *Physiol. Behav.* 10, 1035–1039. doi: 10.1016/0031-9384(73)90185-6

Jin, Z. H., Kikuchi, T., Tanaka, K., and Kobayashi, T. (2003). Expression of glutamate transporter GLAST in the developing mouse cochlea. *Tohoku J. Exp. Med.* 200, 137–144. doi: 10.1620/tjem.200.137

Kalappa, B. I., Brozoski, T. J., Turner, J. G., and Caspary, D. M. (2014). Single unit hyperactivity and bursting in the auditory thalamus of awake rats directly correlates with behavioural evidence of tinnitus. *J. Physiol.* 592, 5065–5078. doi: 10.1113/jphysiol.2014.278572

Kalappa, B. I., Soh, H., Duignan, K. M., Furuya, T., Edwards, S., Tzingounis, A. V., et al. (2015). Potent KCNQ2/3-specific channel activator suppresses *in vivo* epileptic activity and prevents the development of tinnitus. *J. Neurosci.* 35, 8829–8842. doi: 10.1523/JNEUROSCI.5176-14.2015

Karlsson, R. M., Tanaka, K., Saksida, L. M., Bussey, T. J., Heilig, M., and Holmes, A. (2009). Assessment of glutamate transporter GLAST (EAAT1)-deficient mice for phenotypes relevant to the negative and executive/cognitive symptoms of schizophrenia. *Neuropsychopharmacology* 34, 1578–1589. doi: 10.1038/npp.2008.215

Li, S., Choi, V., and Tzounopoulos, T. (2013). Pathogenic plasticity of Kv7.2/3 channel activity is essential for the induction of tinnitus. *Proc. Natl. Acad. Sci. U S A* 110, 9980–9985. doi: 10.1073/pnas.1302770110

Li, S., Kalappa, B. I., and Tzounopoulos, T. (2015). Noise-induced plasticity of KCNQ2/3 and HCN channels underlies vulnerability and resilience to tinnitus. *Elife* 4:e07242. doi: 10.7554/eLife.07242

Llano, D. A., Turner, J., and Caspary, D. M. (2012). Diminished cortical inhibition in an aging mouse model of chronic tinnitus. *J. Neurosci.* 32, 16141–16148. doi: 10.1523/JNEUROSCI.2499-12.2012

Longenecker, R. J., and Galazyuk, A. V. (2011). Development of tinnitus in CBA/CaJ mice following sound exposure. *J. Assoc. Res. Otolaryngol.* 12, 647–658. doi: 10.1007/s10162-011-0276-1

Longenecker, R. J., and Galazyuk, A. V. (2012). Methodological optimization of tinnitus assessment using prepulse inhibition of the acoustic startle reflex. *Brain Res.* 1485, 54–62. doi: 10.1016/j.brainres.2012.02.067

Longenecker, R. J., Chonko, K. T., Maricich, S. M., and Galazyuk, A. V. (2014). Age effects on tinnitus and hearing loss in CBA/CaJ mice following sound exposure. *Springerplus* 3:542. doi: 10.1186/2193-1801-3-542

Lowry, E. R., Kruyer, A., Norris, E. H., Cederroth, C. R., and Strickland, S. (2013). The GluK4 kainate receptor subunit regulates memory, mood and excitotoxic neurodegeneration. *Neuroscience* 235, 215–225. doi: 10.1016/j.neuroscience.2013.01.029

McFadden, D., Plattsmier, H. S., and Pasanen, E. G. (1984). Aspirin-induced hearing loss as a model of sensorineural hearing loss. *Hear. Res.* 16, 251–260. doi: 10.1016/0378-5955(84)90114-x

Meltser, I., Cederroth, C. R., Basinou, V., Savelyev, S., Lundkvist, G. S., and Canlon, B. (2014). TrkB-mediated protection against circadian sensitivity to noise trauma in the murine cochlea. *Curr. Biol.* 24, 658–663. doi: 10.1016/j.cub.2014.01.047

Middleton, J. W., Kiritani, T., Pedersen, C., Turner, J. G., Shepherd, G. M., and Tzounopoulos, T. (2011). Mice with behavioral evidence of tinnitus exhibit dorsal cochlear nucleus hyperactivity because of decreased GABAergic inhibition. *Proc. Natl. Acad. Sci. U S A* 108, 7601–7606. doi: 10.1073/pnas.1100223108

Mongan, E., Kelly, P., Nies, K., Porter, W. W., and Paulus, H. E. (1973). Tinnitus as an indication of therapeutic serum salicylate levels. *JAMA* 226, 142–145. doi: 10.1001/jama.1973.03230020014004

Ohlemiller, K. K., and Gagnon, P. M. (2004). Apical-to-basal gradients in age-related cochlear degeneration and their relationship to "primary" loss of cochlear neurons. *J Comp Neurol* 479, 103–116. doi: 10.1002/cne.20326

Plappert, C. F., Kuhn, S., Schnitzler, H. U., and Pilz, P. K. (2006). Experience increases the prepulse inhibition of the acoustic startle response in mice. *Behav. Neurosci.* 120, 16–23. doi: 10.1037/0735-7044.120.1.16

Poirrier, A. L., Van den Ackerveken, P., Kim, T. S., Vandenbosch, R., Nguyen, L., Lefebvre, P. P., et al. (2010). Ototoxic drugs: difference in sensitivity between mice and guinea pigs. *Toxicol. Lett.* 193, 41–49. doi: 10.1016/j.toxlet.2009.12.003

Puel, J. L., and Guitton, M. J. (2007). Salicylate-induced tinnitus: molecular mechanisms and modulation by anxiety. *Prog. Brain Res.* 166, 141–146. doi: 10.1016/s0079-6123(07)66012-9

Ruel, J., Chabbert, C., Nouvian, R., Bendris, R., Eybalin, M., Leger, C. L., et al. (2008). Salicylate enables cochlear arachidonic-acid-sensitive NMDA receptor responses. *J. Neurosci.* 28, 7313–7323. doi: 10.1523/JNEUROSCI.5335-07.2008

Ruel, J., Wang, J., Rebillard, G., Eybalin, M., Lloyd, R., Pujol, R., et al. (2007). Physiology, pharmacology and plasticity at the inner hair cell synaptic complex. *Hear. Res.* 227, 19–27. doi: 10.1016/j.heares.2006.08.017

Ruttiger, L., Singer, W., Panford-Walsh, R., Matsumoto, M., Lee, S. C., Zuccotti, A., et al. (2013). The reduced cochlear output and the failure to adapt the central auditory response causes tinnitus in noise exposed rats. *PLoS One* 8:e57247. doi: 10.1371/journal.pone.0057247

Schmid, A., Koch, M., and Schnitzler, H. U. (1995). Conditioned pleasure attenuates the startle response in rats. *Neurobiol. Learn. Mem.* 64, 1–3. doi: 10.1006/nlme.1995.1037

Searchfield, G. D., Munoz, D. J., and Thorne, P. R. (2004). Ensemble spontaneous activity in the guinea-pig cochlear nerve. *Hear. Res.* 192, 23–35. doi: 10.1016/j.heares.2004.02.006

Shimizu, Y., Hakuba, N., Hyodo, J., Taniguchi, M., and Gyo, K. (2005). Kanamycin ototoxicity in glutamate transporter knockout mice. *Neurosci. Lett.* 380, 243–246. doi: 10.1016/j.neulet.2005.01.066

Stolzberg, D., Salvi, R. J., and Allman, B. L. (2012). Salicylate toxicity model of tinnitus. *Front. Syst. Neurosci.* 6:28. doi: 10.3389/fnsys.2012.00028

Sturman, J. A., Dawkins, P. D., McArthur, N., and Smith, M. J. (1968). The distribution of salicylate in mouse tissues after intraperitoneal injection. *J. Pharm. Pharmacol.* 20, 58–63. doi: 10.1111/j.2042-7158.1968.tb09619.x

Su, Y. Y., Luo, B., Jin, Y., Wu, S. H., Lobarinas, E., Salvi, R. J., et al. (2012). Altered neuronal intrinsic properties and reduced synaptic transmission of the rat's medial geniculate body in salicylate-induced tinnitus. *PLoS One* 7:e46969. doi: 10.1371/journal.pone.0046969

Sun, W., Lu, J., Stolzberg, D., Gray, L., Deng, A., Lobarinas, E., et al. (2009). Salicylate increases the gain of the central auditory system. *Neuroscience* 159, 325–334. doi: 10.1016/j.neuroscience.2008.12.024

Turner, J. G., Brozoski, T. J., Bauer, C. A., Parrish, J. L., Myers, K., Hughes, L. F., et al. (2006). Gap detection deficits in rats with tinnitus: a potential

novel screening tool. *Behav. Neurosci.* 120, 188–195. doi: 10.1037/0735-7044.120.1.188

Turner, J. G., and Parrish, J. (2008). Gap detection methods for assessing salicylate-induced tinnitus and hyperacusis in rats. *Am. J. Audiol.* 17, S185–S192. doi: 10.1044/1059-0889(2008/08-0006)

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., et al. (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol.* 3:RESEARCH0034. doi: 10.1186/gb-2002-3-7-research0034

Vikhe Patil, K., Canlon, B., and Cederroth, C. R. (2015). High quality RNA extraction of the mammalian cochlea for qRT-PCR and transcriptome analyses. *Hear. Res.* 325, 42–48. doi: 10.1016/j.heares.2015.03.008

von der Behrens, W. (2014). Animal models of subjective tinnitus. *Neural Plast.* 2014:741452. doi: 10.1155/2014/741452

Wang, H. T., Luo, B., Zhou, K. Q., Xu, T. L., and Chen, L. (2006). Sodium salicylate reduces inhibitory postsynaptic currents in neurons of rat auditory cortex. *Hear. Res.* 215, 77–83. doi: 10.1016/j.heares.2006.03.004

Wang, H., Brozoski, T. J., Turner, J. G., Ling, L., Parrish, J. L., Hughes, L. F., et al. (2009). Plasticity at glycinergic synapses in dorsal cochlear nucleus of rats with behavioral evidence of tinnitus. *Neuroscience* 164, 747–759. doi: 10.1016/j.neuroscience.2009.08.026

Watase, K., Hashimoto, K., Kano, M., Yamada, K., Watanabe, M., Inoue, Y., et al. (1998). Motor discoordination and increased susceptibility to cerebellar injury in GLAST mutant mice. *Eur. J. Neurosci.* 10, 976–988. doi: 10.1046/j.1460-9568.1998.00108.x

Weible, A. P., Moore, A. K., Liu, C., DeBlander, L., Wu, H., Kentros, C., et al. (2014). Perceptual gap detection is mediated by gap termination responses in auditory cortex. *Curr. Biol.* 24, 1447–1455. doi: 10.1016/j.cub.2014.05.031

Willott, J. F., Tanner, L., O'Steen, J., Johnson, K. R., Bogue, M. A., and Gagnon, L. (2003). Acoustic startle and prepulse inhibition in 40 inbred strains of mice. *Behav. Neurosci.* 117, 716–727. doi: 10.1037/0735-7044.117.4.716

Yi, B., Hu, S., Zuo, C., Jiao, F., Lv, J., Chen, D., et al. (2016). Effects of long-term salicylate administration on synaptic ultrastructure and metabolic activity in the rat CNS. *Sci. Rep.* 6:24428. doi: 10.1038/srep24428

Zhang, J., Zhang, Y., and Zhang, X. (2011). Auditory cortex electrical stimulation suppresses tinnitus in rats. *J. Assoc. Res. Otolaryngol.* 12, 185–201. doi: 10.1007/s10162-010-0246-z

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Differential Neural Responses Underlying the Inhibition of the Startle Response by Pre-Pulses or Gaps in Mice

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Gap pre-pulse inhibition of the acoustic startle (GPIAS) is a behavioral paradigm used for inferring the presence of tinnitus in animal models as well as humans. In contrast to pre-pulse inhibition (PPI), the neural circuitry controlling GPIAS is poorly understood. To increase our knowledge on GPIAS, a comparative study with PPI was performed in mice combining these behavioral tests and c-Fos activity mapping in brain areas involved in the inhibition of the acoustic startle reflex (ASR). Both pre-pulses and gaps efficiently inhibited the ASR and abolished the induction of c-Fos in the pontine reticular nucleus. Differential c-Fos activation was found between PPI and GPIAS in the forebrain whereby PPI activated the lateral globus pallidus and GPIAS activated the primary auditory cortex. Thus, different neural maps are regulating the inhibition of the startle response by pre-pulses or gaps. To further investigate this differential response to PPI and GPIAS, we pharmacologically disrupted PPI and GPIAS with D-amphetamine or Dizocilpine (MK-801) to target dopamine efflux and to block NMDA receptors, respectively. Both D-amp and MK-801 efficiently decreased PPI and GPIAS. We administered Baclofen, an agonist $GABA_B$ receptor, but failed to detect any robust rescue of the effects of D-amp and MK-801 suggesting that PPI and GPIAS are $GABA_B$-independent. These novel findings demonstrate that the inhibition of the ASR by pre-pulses or gaps is orchestrated by different neural pathways.

Keywords: tinnitus, pre-pulse inhibition, gap detection, auditory cortex, hearing loss, c-fos, neural mapping

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INTRODUCTION

Pre-pulse inhibition (PPI) is a quantitative measure of the sensorimotor gating where a pre-pulse attenuates the motor reflex that is induced by a subsequent acoustic startle. The acoustic startle reflex (ASR) is a primitive survival reaction relying on the dorsal cochlear nucleus (DCN), the caudal pontine reticular nucleus (PnC), and spinal motor neurons (Koch, 1999). Higher order nuclei including the limbic system and the prefrontal cortex regulate the inhibition of the ASR during PPI. It is known that the activation of dopamine and the blockade of NMDA receptors can disrupt the function of the pre-frontal cortex and the nucleus accumbens and lead to impaired PPI (Koch and Schnitzler, 1997). Altered PPI responses are found in a variety of psychiatric disorders including schizophrenia, obsessive–compulsive disorder, and Tourette's syndrome (Braff et al., 2001; Swerdlow et al., 2008, 2016). Enhancing GABAergic

inhibition has been used as strategy to circumvent the disrupted dopamine/glutamate circuitry and to restore the inhibitory inputs to the PnC and PPI (Braff et al., 2001; Swerdlow et al., 2008, 2016).

A variant to PPI is gap pre-pulse inhibition of the acoustic startle response (GPIAS), which has emerged as a potential tool for the assessment of tinnitus in animals and humans (Galazyuk and Hebert, 2015). Conceived by Turner et al. (2006) and Turner and Parrish (2008) it was validated against a model of operant conditioning and was further supported by additional neuronal correlates of tinnitus including increased spontaneous firing rates (SFRs) in the DCN (Li et al., 2013, 2015), hyperactivity in the inferior colliculus (IC) (Holt et al., 2010) and remapping of the auditory cortex (AC) (Engineer et al., 2011). Unlike PPI, GPIAS uses a silent gap embedded in a carrier noise as a pre-stimulus to decrease a subsequent ASR. When the carrier frequency closely matches that of the tinnitus, it interferes with the optimal inhibition caused by the silent gap. Lowe and Walton (2015) adapted the GPIAS to assess neuronal responses instead of startle responses using a paradigm named auditory brainstem response gap-in-noise (ABR GIN), which showed similar efficacy in detecting tinnitus. How GPIAS and PPI, which are elicited by different auditory cues, differ in terms of neural mapping and pharmacological regulation, remains to be determined.

Some of the temporal characteristics and neural circuitry used to elicit inhibition with gaps or pre-pulses differ. For instance, PPI is stable within a large range of inter-stimulus intervals (ISI), whereas GPIAS improves with shorter lead times in mice depending on the strain (Yu et al., 2016). Moreover, the AC appears as an important regulator of GPIAS, but not PPI, as shown by surgical ablation studies (Bowen et al., 2003). Recent optogenetic studies in mice have shown that GABAergic interneuron activity in the AC is important in controlling perceptual gap detection (Weible et al., 2014). With the exception of the AC, little is known about the neural structures controlling GPIAS. In this study, we performed behavioral tests and histological evaluations based on c-Fos induction to compare GPIAS and PPI responses in the mouse.

MATERIALS AND METHODS

Experimental Animals

Experimental procedures on animals were performed in accordance with the guidelines and regulations set out by *Stockholm's Norra Djurförsöksetiska Nämnd* (N156/14). Male mice from 3 to 4 months of age in a C57BL/6JRj background (Janvier Labs, France), were group-housed (4–5 per cage) and maintained at 19–21°C in a 50–50% light-dark cycle (lights on at 06:00). Animals had free access water and food (Lactamin R34, Lantmännen) and were given a minimum of 1 week acclimatization upon their arrival prior to any manipulation. All behavioral experiments were carried out between 09:00 and 16:00 h.

Gap-Pre-pulse Inhibition of the Startle Eesponse (GPIAS) and Pre-pulse Inhibition (PPI)

A SR-Lab startle response system from San Diego Instruments was used. A background carrier sound level consisted of an unfiltered white noise at 65 dB sound pressure level (SPL) for PPI. Pre-pulses of 3, 6, and 12 dB SPL above the carrier noise were used. For GPIAS, the gaps were 6, 11 and 16 dB SPL below the carrier noise, and the lowest level of the gaps was 65 dB SPL (noise floor). Both pre-pulses and gaps were 50 ms in duration and had 0.1-ms rise and fall times. Startle pulses of 20 ms were presented at 114 dB SPL. Calibration was performed before each procedure and SPL were measured with a calibrated microphone and preamplifier (4939-A-011 and 2633, Brüel & Kjær), connected to an amplifier (Brüel & Kjær, type 2636). The ISI was set at 70 ms for pre-pulses and 15 ms for silent gaps according to Yu et al. (2016). Gap detection or pre-pulse inhibition was quantified as [(1 – (startle amplitude during pre-pulse or gap + pulse)/(startle amplitude during pulse alone)) × 100)] (Yu et al., 2016), using a similar representation as used in pre-pulse inhibition studies (greater suppression of the startle reflex closer to 100%).

Experimental Procedure for PPI and GPIAS

A 10 min acclimatization to the procedure was conducted on day 1. On the following day (day 2), a baseline experiment comprised a complete test with both PPI and GPIAS sessions, and the same scheme was used on day 5 for the evaluation of drug effects. PPI sessions were performed first, immediately followed by GPIAS sessions. A pilot test showed that inverting PPI and GPIAS did not alter the outcome of the study in presence or absence of drugs. The PPI and GPIAS sessions were initiated after a 5 min acclimatization to the environment, followed by 5 min in a 65 dB SPL continuous white noise.

PPI session

The PPI session started with five startle pulses; each pulse was 20 ms in duration and at 114 dB SPL. PPI was tested at a single carrier intensity (white noise) of 65 dB SPL with trials containing pre-pulses of 3, 6, and 12 dB SPL above the carrier level. Each pre-pulse intensity was tested pseudo-randomly 10 times with eight no stimulus trials randomly inserted. The time between each trial was random and between 9 and 15 s. The session ended with five trials containing only startle pulses to be compared with those of the beginning of the session to assess habituation.

GPIAS session

The GPIAS session started with five startle pulses 20 ms in duration and at 114 dB SPL. GPIAS was then tested at a different carrier intensities (white noise) with trials containing gaps of 6, 11, and 16 dB SPL below the carrier level and reaching a floor of 65 dB SPL. Each intensity was tested pseudo-randomly 10 times with eight no stimulus trials randomly inserted. The session ended with five trials containing only startle pulses to be compared with those of the beginning to assess habituation.

A total of 118 trials were presented in approximately 40 min. A schematic diagram illustrating the sequence of the sessions performed on day 5 is presented in **Figure 1A**. The sequence

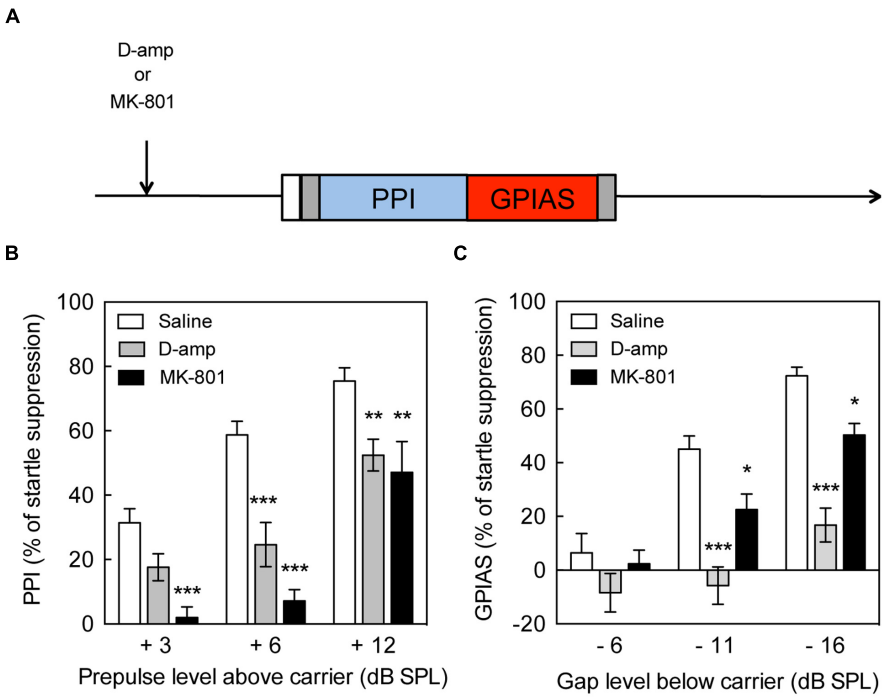

FIGURE 1 | Impairment of pre-pulse inhibition and gap pre-pulse inhibition of the acoustic startle by D-amp and MK-801. (A) Schematic diagram of the behavioral tests performed to evaluate the effects of drugs on pre-pulse inhibition (PPI) and gap pre-pulse inhibition of the acoustic startle (GPIAS). Effect of acute D-amp (10 mg/kg, gray) and MK-801 (0.5 mg/kg, dark) administration on PPI **(B)** and GPIAS **(C)**. Data represent mean ± SEM ($n = 13$–19). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.0001$.

of PPI and GPIAS trials for these sessions are provided in the supplementary method (Supplementary Data Sheet 1).

PPI and GPIAS Sessions for c-FOS Evaluation in the Brain

For the evaluation of c-Fos induction in the brain, mice were acclimatized to the procedure 10 min per day, for 3 consecutive days. On day 4, mice were randomly allocated to six test groups, which lasted approximately 20 min. Each session started with a 10 min acclimatization (5 min silence and 5 min background noise) followed by one session of 60 trials with a random inter-trial time interval from 10 to 20 s. The six sessions were as follows: (i) carrier noise only (65 dB SPL), (ii) startle only (60 startle pulses), (iii) pre-pulses (60 pre-pulses), (iv) PPI (60 pre-pulses followed by startle pulses), (v) gaps (60 gaps), and (vi) GPIAS groups (60 gaps followed by startle pulses). Pre-pulses used here were +12 dB SPL above carrier noise (65 dB SPL), gaps were −16 dB SPL below carrier noise (81 dB SPL), startle pulses were 20 ms in duration and 114 dB SPL. A schematic of the trials presented for the evaluation of c-Fos is shown in **Figure 2A**. The sequence of PPI and GPIAS trials used for the evaluation of c-Fos induction are provided in the supplementary method (Supplementary Data Sheet 2).

Immunohistochemistry

Animals were anesthetized 2 h after the end of the behavioral procedure with a mixture of ketamine/xylazine (100/10 mg/kg) and underwent transcardiac perfusion with phosphate-buffered saline (PBS) and then with 4% paraformaldehyde. A pilot study found a 2 h time point to be the most effective in revealing c-Fos induction when compared to 1 or 3 h after the end of the experimental procedure. Brains were post-fixed (1 h) and then cryoprotected in 30% sucrose in PBS, mounted in NEG 50 (#6502, Thermo Scientific) and frozen prior to serial sectioning at 14 μm thickness. Sections from four animals per conditions were immunostained with rabbit-antibodies to c-Fos (#4384; 1:250; Cell Signaling technology) then incubated with biotinylated goat anti-rabbit antibodies (BA-1000; Vector Laboratories, Burlingame, CA, USA) and the avidin/biotin system (SP-2001; Vector Laboratories, Burlingame, CA, USA) and visualized using 3, 3′-diaminobenzidine (DAB) solution (SK-4100; Vector Laboratories). Negative controls were performed by omitting the primary antibody yielding no staining. Images were obtained using DP Controller software (Olympus, Tokyo, Japan) and immunopositive nuclei were counted automatically using Image-Pro Plus 6.2 software (Media Cybernetics, Rockville, MD, USA). Pilot studies were performed using counterstaining with hematoxylin QS (H-3404, Vector Laboratories) to confirm the different brain regions.

Quantitative Analysis of c-Fos Immunohistochemistry

Serial coronal sections were examined at 10× magnification using a Zeiss Axioskop (Zeiss, Germany). Photographs were

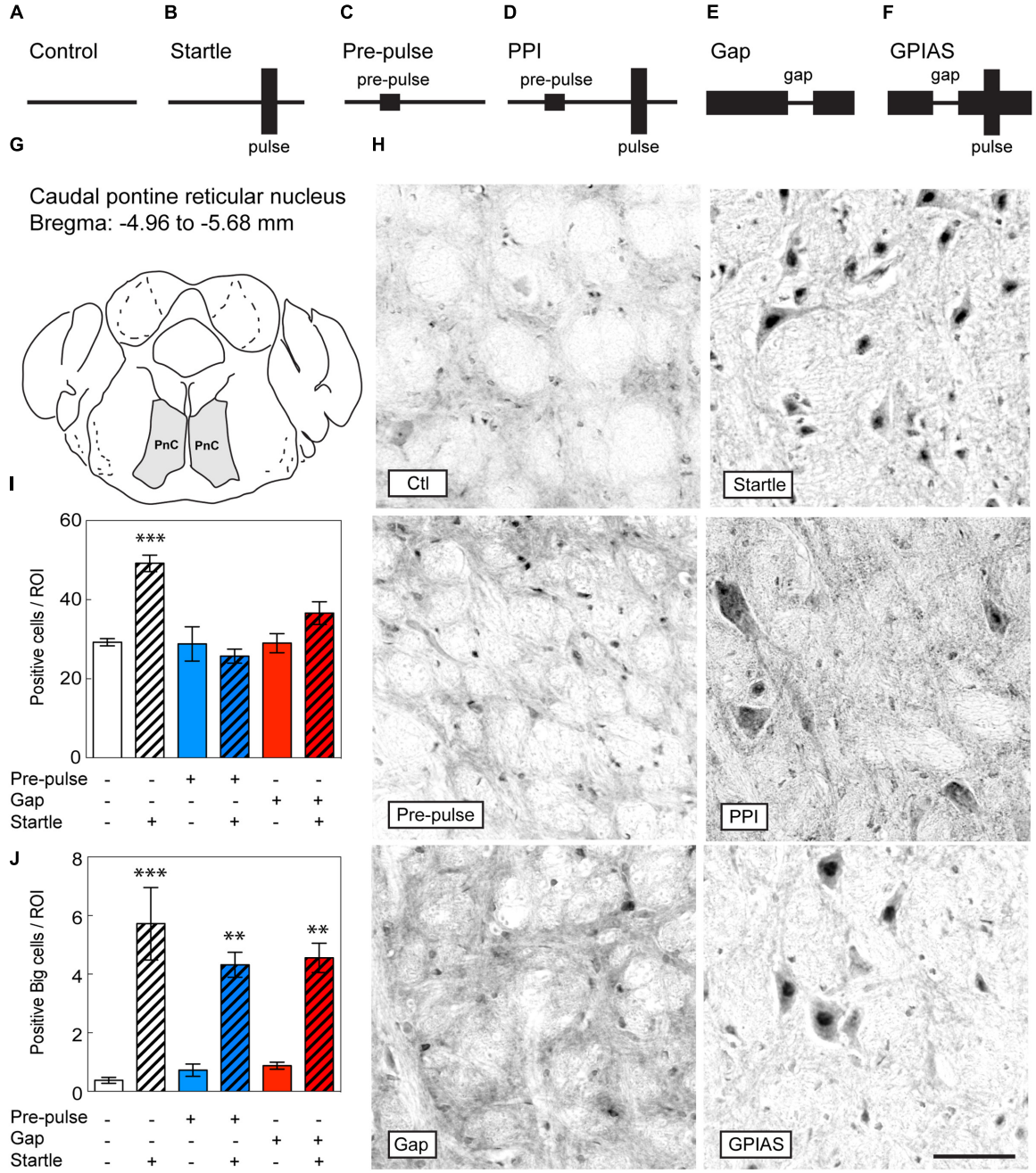

FIGURE 2 | Lack of c-Fos induction in the PnC upon inhibition by pre-pulses or gaps. Schematic diagram of the behavioral tests performed to evaluate c-Fos induction in the brain under **(A)** only carrier noise (control), **(B)** pulses only (startle), **(C)** pre-pulses only (pre-pulse), **(D)** pre-pulses in presence of startle stimuli (PPI), **(E)** gaps only (gap), and **(F)** gaps in presence of startle stimuli (GPIAS). Sections were taken from the PnC **(G)**, which is located caudally from the bregma along the rostrocaudal axis between −4.96 and −5.68 mm. **(H)** Representative photomicrographs of c-Fos-immunostaining in the PnC under the six conditions described in **(A–F)** Scale bar, 50 μm. Quantification of c-Fos positive cells **(I)** and big neurons **(J)** in the PnC of the six groups. Groups with startle pulses are in hatched bars. Those with pre-pulses are in blue and those with gaps are in red. Data represent means ± SEM ($n = 4$). $^{**}P < 0.01$, $^{***}P < 0.0001$.

taken with an Olympus DP71 digital camera (resolution of 4140 pixels × 3086 pixels). Franklin and Paxinos (2008) stereotaxic coordinates were used to define specific brain regions: (i) the caudal pontine reticular nucleus (PnC), between −4.96 and −5.70 mm; (ii) the lateral globus pallidus (LGP), −0.10 and −1.06 mm; and the auditory cortex (AC), in the region between −2.18 and −3.64 mm. Both right and left hemispheres of three different sections separated by at least 140 μm for each area were examined. Within each section 3 regions of interest (ROI) were analyzed. The size of the three ROI was

200 × 200 μm. Using Image-pro 6.2.1 (Media Cybernetics) and ImageJ 1.50i (NIH), cells with threshold above background were counted. The researcher performing the analysis was blind to the experimental conditions. This procedure resulted in a total of 18 determinations of the number of cells stained with c-Fos within a specified area for each brain.

Pharmacological Procedures

For the pharmacological evaluation of drugs on PPI and GPIAS, animals were acclimatized to the procedure on day 1, baseline levels were tested on day 2 and drug treatment effects were tested on day 5. Mice were injected subcutaneously (s.c.) with 7.5 mg/kg (±) baclofen (B5399; Sigma–Aldrich), followed 15 min after with 10 mg/kg D-amphetamine hemisulfate salt (A5880, Sigma–Aldrich) or 0.5 mg/kg MK-801 (M107; Sigma–Aldrich). For the use of MK-801, we initially tested a 1 mg/kg dose as previously reported in the literature (Arai et al., 2008), however, mice appeared lethargic and therefore used a 0.5 mg/kg dose, which was tolerated better (qualitative observations). Drugs were dissolved in physiological saline and administered at a volume of 0.2 ml/30 g body weight. The PPI/GAP test was performed 30 or 15 min after the last administration of D-Amp or MK-801, respectively (**Figure 1A**). Saline was used as vehicle and control treatment.

Statistical Analyses

One-way ANOVA and a Tukey *post hoc* test were used for all histological quantifications, and a two-way ANOVA and a Bonferroni *post hoc* test were used in the context of GPIAS and PPI (Prism version 4.0, GraphPad software). Differences were considered significant if $p < 0.05$. Animals that failed to respond to the startle (any peak-to-peak response above noise floor) or failed to inhibit the startle in the presence of a pre-pulse before treatment (any decrease in startle amplitude during pre-pulse trials versus startle only trials) were excluded from the analysis (near 5%).

RESULTS

Disruption of PPI and GAP by D-Amp and MK-801

The paradigm used for the drug treatment is illustrated (**Figure 1A**) and shows the sequence of drug administration and behavioral tests. PPI increased up to 75% suppression of the startle response in presence of a +12 dB pre-pulse, whereas GPIAS achieved near 72% suppression with a −16 dB gap. These results are illustrating that the paradigm between the two tests is relatively equal, and a progressive increase in inhibition with increasing pre-pulse or carrier levels was achieved. D-amp suppressed PPI by 23–36% in the +6 and +12 dB SPL pre-pulse intensities [Treatment Factor, $F(1,102) = 36.35$, $p < 0.0001$; Carrier Intensity Factor, $F(2,102) = 30.83$, $p < 0.0001$; **Figure 1B**]. Similarly, D-amp suppressed GPIAS by 49–54% in the −11 and −16 dB SPL gaps [Treatment Factor, $F(1,96) = 54.54$, $p < 0.0001$; Carrier Intensity Factor, $F(2,96) = 25.43$, $p < 0.0001$; **Figure 1C**]. MK-801 suppressed PPI by 53% in the +6 and +12 dB SPL pre-pulse intensities [Treatment Factor, $F(1,87) = 75.95$, $p < 0.0001$; Carrier Intensity Factor, $F(2,87) = 38.75$, $p < 0.0001$; **Figure 1B**]. Similarly, MK-801 suppressed GPIAS by 41–50% in the −11 and −16 dB SPL gaps [Treatment Factor, $F(1,96) = 54.54$, $p < 0.0001$; Carrier Intensity Factor, $F(2,96) = 25.43$, $p < 0.0001$; **Figure 1C**]. These findings show that −16 dB SPL gaps inhibit the startle response to a level equivalent to a +12 dB SPL pre-pulses, and that both PPI and GPIAS are disrupted by the rise in available dopamine and NMDA receptor antagonism. Moreover, the data suggest that GPIAS is more vulnerable to D-Amp than PPI and this was confirmed by measuring the change caused by D-Amp versus the individual's baseline values [$q(180) = 4.453$, $p = 0.0103$, data not shown].

PPI and GPIAS Elicit Similar c-FOS Responses in the PnC

To identify the differential neural circuits underlying PPI and GPIAS, we evaluated c-Fos induction in the brain by immunohistochemistry from mice under the following conditions: (i) control (carrier noise only), (ii) startle only, (iii) pre-pulses only, (iv) PPI (pre-pulses in presence of startle stimuli), (v) gaps only, and (vi) GPIAS (gaps in presence of startle stimuli) (**Figures 2A–F**). We hypothesized that regions that specifically regulating PPI and GPIAS would show a differential induction of c-Fos. When first assessing the PnC (**Figures 2G,H**), the difference across the six groups was significant [Stimulus Factor, $F(5,18) = 10.95$, $p < 0.0001$]. We found that startle pulses increased by 1.7-fold in the number of c-Fos positive cells in comparison to the control group [$t(18) = 5.382$, $p = 0.0006$, $n = 4$ per group; **Figure 2I**]. Inhibition of the startle by the optimal gaps (−16 dB SPL) and optimal pre-pulses (+12 dB SPL) caused a significant decrease of c-Fos induction, down to control levels [in comparison to startle pulses only – PPI: $t(18) = 6.338$, $p < 0.0001$; GPIAS: $t(18) = 3.399$, $p = 0.0479$; $n = 4$ per group]. The c-Fos expression of the giant nuclei in the PnC was triggered after the startle stimulus while pre-pulses or gaps did not alter their activation, suggesting that they are not involved in the motor output, but instead only responsive to the startle pulse [in comparison to startle pulses only with an average of six cells per ROI – PPI: $t(18) = 1.705$, $p > 0.99$; GPIAS: $t(18) = 1.418$, $p > 0.99$; $n = 4$ per group, **Figure 2J**]. These findings indicate that the induction of c-Fos in the PnC by startle stimuli is effectively prevented by both pre-pulses and gaps.

Lack of c-FOS Induction in the LGP after GPIAS

We next investigated the induction of c-Fos in the LGP (**Figure 3A**), known to be involved in the inhibitory pathway of pre-pulse inhibition (Takahashi et al., 2007). In the LGP, the difference across the six groups was significant [Stimulus Factor, $F(5,18) = 35.44$, $p < 0.0001$]. We found that both pre-pulses and PPI increased the number of c-Fos positive cells in the LGP by near twofold in comparison to startle-only groups [pre-pulses: $t(18) = 9.986$, PPI: $t(18) = 6.624$, $p < 0.0001$, $n = 4$ per

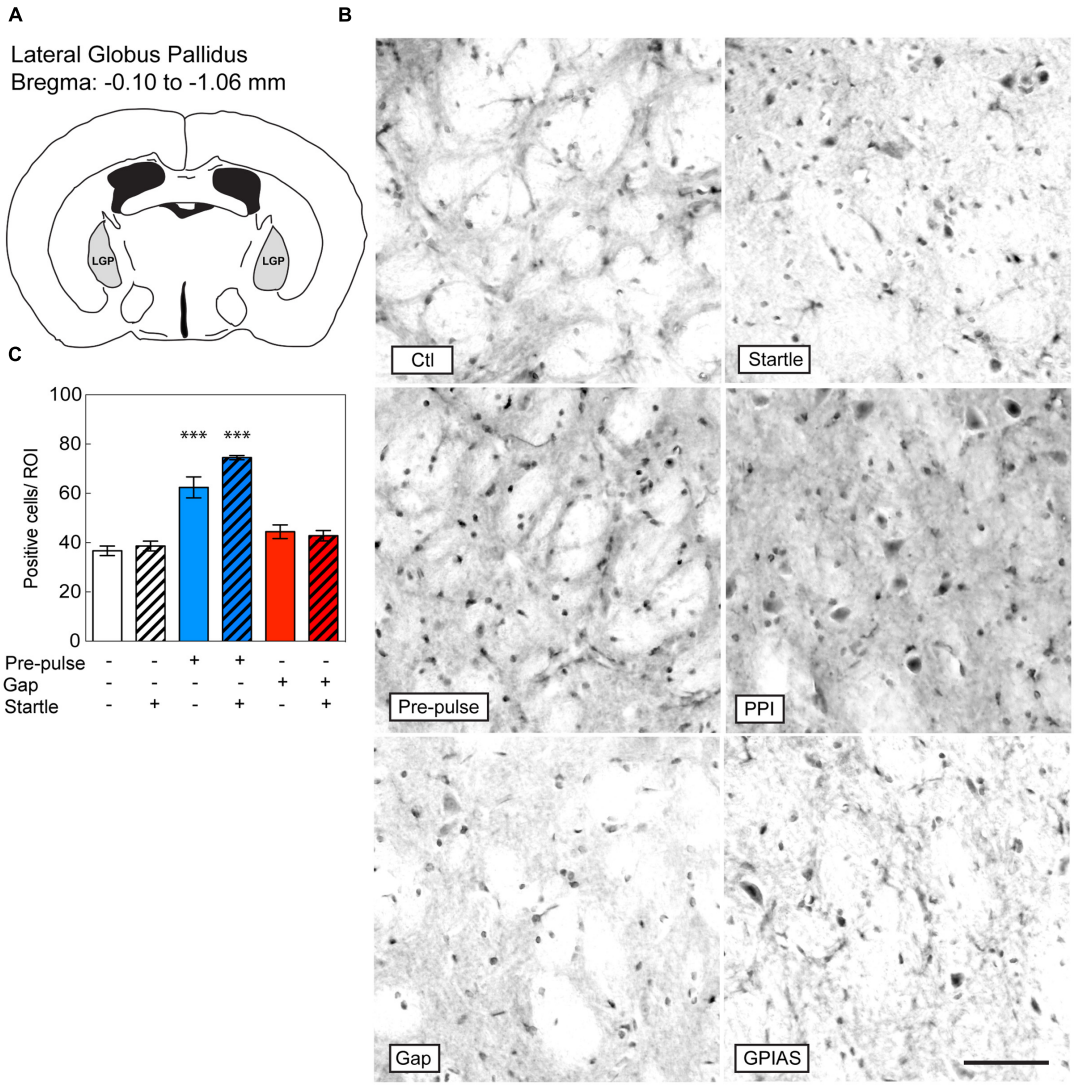

FIGURE 3 | Induction of c-Fos in the LGP by pre-pulses but not gaps. Sections were taken from the LGP **(A)**, which is located caudally from the bregma along the rostrocaudal axis between −0.10 and −1.06 μm. **(B)** Representative photomicrographs of c-Fos-immunostaining in the LGP under the six conditions listed in **Figure 2A**. Scale bar, 50 μm. **(C)** Quantification of c-Fos positive cells in the LGP in the six groups. Groups with startle pulses are in hatched bars. Those with pre-pulses are in blue and those with gaps are in red. Data represent means ± SEM ($n = 4$). ***$P < 0.0001$.

group; **Figures 3B,C**]. In contrast, gaps or GPIAS did not trigger any additional c-Fos staining when compared to the control or startle-only groups [GAP16: $t(18) = 1.623$, $p > 0.99$; GPIAS: $t(18) = 1.171$, $p > 0.99$; $n = 4$ per group]. These findings strongly suggest that the LGP is not involved in the inhibitory effects caused by gaps on startle suppression.

GPIAS Triggers c-Fos Expression in the AC unlike PPI

Based on studies that have shown the important contribution of the AC in gap detection (Ison et al., 1991; Bowen et al., 2003; Weible et al., 2014), we evaluated the changes in c-Fos positive cells in the primary AC under the six conditions and the difference across the six groups was significant [Stimulus Factor, $F(5,18) = 5.253$, $p = 0.0038$; **Figure 4A**]. With the exception of GPIAS, none of the other conditions triggered c-Fos staining [GPIAS: $t(18) = 3.864$, $p = 0.0171$, $n = 4$ per group; **Figures 4B,C**]. Varying the carrier sound intensities of the control session (e.g., 77, 81 or 100 dB SPL) had no effect on c-Fos activation in the primary AC (data not shown). These results indicate that only the inhibition of the startle pulse by gaps triggers c-Fos induction in the primary AC.

Baclofen Does Not Rescue from D-amp or MK-801 Disruption of PPI and GPIAS

In an attempt to restore the disruption of PPI and GPIAS caused by D-amp and MK-801, we pre-administered baclofen,

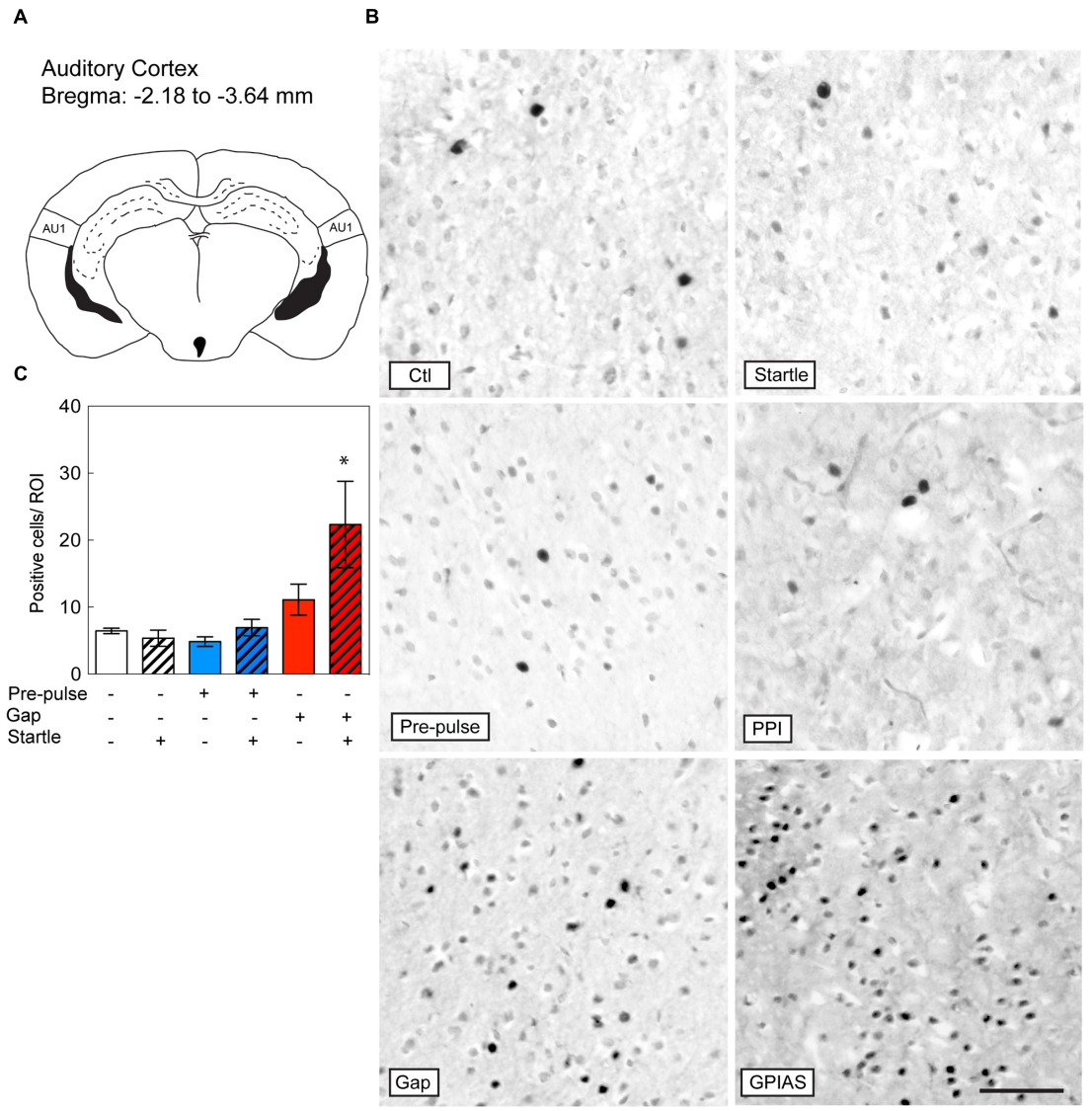

FIGURE 4 | Induction of c-Fos in the Auditory Cortex by GPIAS only. Sections were taken from the primary auditory cortex (AC) **(A)**, which is located caudally from the bregma along the rostrocaudal axis between −2.18 and −3.64 mm. **(B)** Representative photomicrographs of c-Fos-immunostaining in the primary AC under the six conditions listed in **Figure 2A**. Scale bar, 50 μm. **(C)** Quantification of c-Fos positive cells in the LGP in the six groups. Groups with startle pulses are in hatched bars. Those with pre-pulses are in blue and those with gaps are in red. Data represent means ± SEM ($n = 4$). $^*P < 0.05$.

a $GABA_B$ receptor agonist that has been previously shown to block the effects of methamphetamine on PPI (Arai et al., 2008). Since both D-Amp and MK-801 appeared as effective disruptors of PPI and GPIAS (**Figures 1B,C**), we sought to determine whether pharmacological treatment could restore normal PPI and GPIAS. We pre-treated animals with baclofen 15 min prior the administration of D-Amp or MK-801 and performed serial PPI and GPIAS sessions. To exclude the potential influence of differences in startle responses, we performed an exclusion based on a criteria previously described by Stadlbauer et al. (2013). Here, both saline or baclofen groups served as controls. Animals from any of the drug-administered groups for which a startle amplitude was lower than the startle response from control groups were excluded. Animals from the control group for which a startle amplitude was higher than any drug-administered group were excluded as well (**Figures 5A,B**). Analysis of the average pre-pulses and gaps of the greatest differences against the carrier noise (+6 and +12 db SPL in PPI and −11 and −16 dB SPL in GPIAS) stimuli allowed to summarize the differences as a whole and establish that baclofen had no effect on D-amp- or MK-801-induced PPI and GPIAS disruption [PPI MK80-1: $t(172) = 1.329$, $p > 0.9999$; PPI D-Amp: $t(172) = 1.150$, $p > 0.9999$; GPIAS MK80-1: $t(172) = 2.350$, $p = 0.2986$; GPIAS D-Amp: $t(172) = 1.852$, $p = 0.9854$; $n = 10$–18 per group; **Figures 5C,D**].

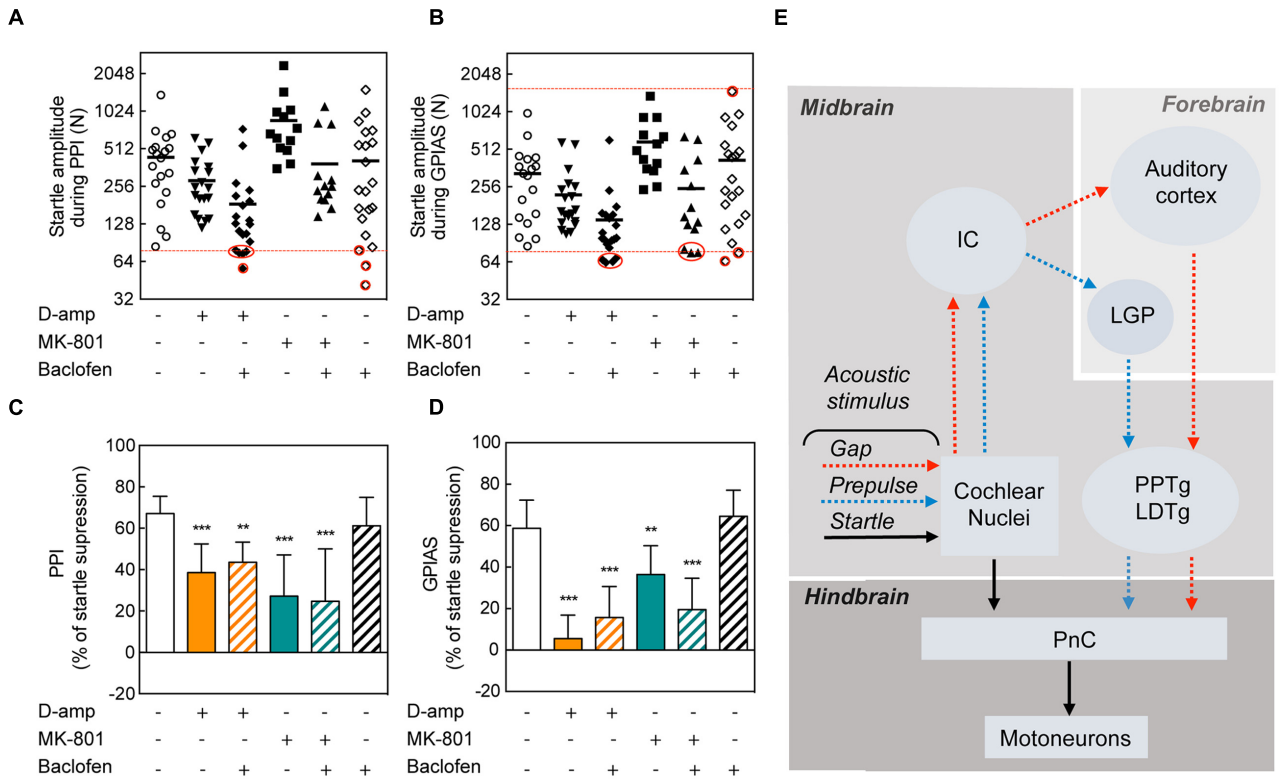

FIGURE 5 | Lack of rescuing effects of baclofen on PPI and GPIAS. Startle amplitude during PPI **(A)** and GPIAS **(B)** sessions under different drug conditions. The dots represent each individual's average startle response during pulse-alone trials for each drug-treatment condition. The dotted red line determines the higher amplitude limit (targeted by the greatest startle amplitude in the drug-administered groups) and/or the lower amplitude limit (targeted by the lowest startle amplitude in the control group). Red circles indicate the excluded animals. Average PPI **(C)** and GPIAS **(D)** scores of the two highest pre-pulse (+6 and +12 dB SPL) and gap (−11 and −16 dB SPL) stimuli. Data represent means ± SEM ($n = 10$–19). $^{**}P < 0.01$, $^{***}P < 0.0001$. **(E)** Schematic of the neuronal pathways involved in PPI and GPIAS. The acoustic startle reflex (arrows in black) is composed of three neurons: a neuron in the PnC that transfers acoustic stimulus from the cochlear nucleus to a muscular reaction. Pre-pulses (blue arrows) or gaps (red arrows) are relayed from the cochlear nuclei to the inferior colliculus (IC). In the forebrain, pre-pulses will be directed to the LGP whereas gaps are directed to the AC. Both pre-pulse and gaps will modulate the pedunculopontine nucleus (PPTg), which will inhibit the PnC.

DISCUSSION

The salient features of this study reveal that the processing of information during a GPIAS test involves the AC but not the LGP. This indicates that distinct neural nuclei are recruited during GPIAS or PPI to inhibit the ASR. It is interesting to note that a gap within a carrier noise (decreasing intensity) triggers a similar inhibitory response as a pre-pulse (increased intensity) but the two paradigms rely on two different brain regions. This study establishes a framework to better understand the networks involved in GPIAS and therefore improve the understanding of its applicability to temporal processing disorders as well as auditory processing disorders and tinnitus.

We have used c-Fos mapping to compare brain regions involved in GPIAS and PPI. This method has been successfully applied to identify regions involved in PPI (Takahashi et al., 2007; Arai et al., 2008). We found that, in contrast to pre-pulses, gaps embedded in a carrier background do not elicit c-Fos activity in the LGP. This is giving a clear neuroanatomical distinction between these two behavioral paradigms and could help better understand the regulation of temporal information encoded by sounds. Corroborating our findings, it has been found that the LGP is involved in PPI (Takahashi et al., 2007). Electrolytic ablations or local inactivation of the LGP by lidocaine have shown to affect the response to pre-pulses (Takahashi et al., 2007). The involvement of $GABA_B$ receptors in the control of PPI has been suggested with the local injection of phaclofen (a $GABA_B$ receptors antagonist) in the PPTg. Since GABAergic neurons from the LGP project directly to the PPTg to control the startle response (Takahashi et al., 2007), it has been hypothesized that the LGP controls PPTg function through GABAergic modulation. In contrast to our expectations, baclofen administration, which enhances GABAergic function via $GABA_B$ receptors, was not able to restore D-amp- or MK-801-induced PPI or GPIAS disruption. Even though we tested a range of doses (2.5, 5, 7.5, and 10 mg/kg), none were successful in rescuing the phenotype (data not shown). The effects of baclofen are complicated because different studies have shown mixed results in the rescue of PPI (Bortolato et al., 2004; Arai et al., 2008; Frau et al., 2014). This could be due to several reasons including the species or strain used, the analog of amphetamine (e.g., methamphetamine or D-amphetamine), and the dose. For

instance, rats have been shown to be responsive to the rescuing effects of baclofen on MK-801-mediated PPI disruption unlike C57BL/6J mice. Nonetheless, our findings suggest that PPI and GPIAS are $GABA_B$-independent in the C57 strain.

We found that GPIAS triggered c-Fos activation in the AC. Pioneering work from Ison et al. (1991) has demonstrated in rats the involvement of the AC in GPIAS but not in PPI (Bowen et al., 2003). Temporary and reversible inhibition of cortical activity via the application of high concentrations of potassium chloride (which does not affect the startle response amplitude) disrupted GPIAS but not PPI (Ison et al., 1991). However, when the end of the gap signal is coupled to the onset of the startle stimulus, Ison et al. (1991) were able to reveal that the noise offset itself does not require cortical control. Lesioning of the AC yielded similar conclusions (Bowen et al., 2003). It is possible that gaps presented at different lead times recruit different operating mechanisms. For instance, in rats gap detection at distal lead times (>40 ms) requires muscarinic receptor function, which is not the case at shorter lead times (Ison and Bowen, 2000). Whether this also applies to mice is unknown. Previous work from Ison et al. (1991) identified in rats a biphasic response on startle suppression depending on the interstimulus interval, which is something we did not observe in mice (Yu et al., 2016). In the present study, we used short gaps of 20 ms, presented at 15 ms lead times and showed these could trigger c-Fos staining in the AC in presence of startle stimuli. It is thus possible that the recruitment of cortical function differs between species. Another possibility is that pre-pulse inhibition of the startle reflex could have been sensed by the AC if shorter lead times would have been used. This possibility will require further evaluations in the future.

Studies in rodents have suggested a number of tinnitus neuronal correlates in the AC that translate into (i) an increase in SFR, (ii) neuronal synchrony, (iii) tonotopic reorganization (Elgoyhen et al., 2015; Shore et al., 2016). Such changes could be the underlying cause of the inability of tinnitus-experiencing rodents to have efficient startle suppression by gaps. Weible et al. (2014) identified that comparisons between pre- and post-gap neural activity determines the efficacy in GPIAS. In the AC, parvalbumin-expressing and somatostatin-expressing GABAergic interneurons exert their inhibitory activity on CaMKII-expressing pyramidal neurons to regulate GPIAS (Weible et al., 2014). Interestingly, the optogenetic inactivation of inhibitory interneurons in the AC before or after the gap enhances GPIAS, however, when this is performed throughout pre-, during and post-gap, GPIAS could not be altered (Weible et al., 2014). We thus believe that the continuous pharmacological action of baclofen on the AC throughout the entire trial (pre-, during and post-gap) could not restore GPIAS. This illustrates the limitations of pharmacological approaches in testing the mechanisms of gap detection when compared to the accuracy of optogenetic approaches. We propose an initial model of the regulation of PPI and GPIAS, where differences in the modulation of the inhibitory path occur at the level of the forebrain, in the LGP and the AU, respectively (**Figure 5E**).

A potential application of the present findings to humans would be that, in the event such modulation of AC activity would be detectable during GPIAS for instance by using electroencephalography (EEG) or other non-invasive neuroimaging techniques, such as positron emission tomography (PET), we would predict responses to be altered in presence of tinnitus. As the ASR in humans is highly variable (Braff et al., 2001), and its reliability in presence of tinnitus questioned (Fournier and Hebert, 2013), such measures could become an interesting alternative to blinking responses. Since ABR GIN responses closely reproduce behavioral startle responses in presence of gaps (Lowe and Walton, 2015), a focus on the AC response during gaps in noise appears as a feasible path.

Whereas the role of the AC in GPIAS has been evidenced here, it remains to be determined whether GPIAS has similar or additional nuclei involved in the inhibitory pathway compared to PPI. The neural pathways that control the inhibition of the ASR by pre-pulses is established in higher order regions such as the ventral hippocampus-medial prefrontal cortex (mPFC), as well as the "CSPP" circuitry involving the limbic cortex, striatum, pallidal or pontine tegmentum (Swerdlow et al., 2008, 2016). Acoustic pre-pulses are also regulated via the IC, superior colliculus (SC), pedunculopontine tegmental nucleus (PPTg) and substantia nigra pars compacta (Leitner and Cohen, 1985; Koch et al., 1993; Li et al., 1998; Koch et al., 2000). The large connectivity network involved in PPI emphasizes the need of performing similar studies for GPIAS in order to better understand the factors that modulate the inhibition by gaps and how reliable this paradigm is in the context of assessment of psychiatric and auditory processing disorders as well as tinnitus. The recent findings that tinnitus is involving a large number of non-auditory brain areas (Chen et al., 2015; Sedley et al., 2015) predicts that other brain regions [e.g., amygdala, hippocampus, cerebellum, medial geniculate body, and the reticular formation (Eggermont, 2016)] may alter the efficacy of startle suppression by gaps.

CONCLUSION

Gap pre-pulse inhibition of the acoustic startle and PPI both rely on sound cues but utilize different neural pathways to regulate the inhibition of the startle response. These different sound cues, characterized by either an increase (pre-pulses) or a decrease (gaps) in intensity, are recruiting different brain regions. Pre-pulses are activating the LGP to inhibit the startle response, while gaps bypass the LGP to activate the AC. These results are establishing a neuroanatomical foundation for understanding gap detection and its applicability in the context of neurological disorders including auditory processing disorders and tinnitus.

ETHICS STATEMENT

Experimental procedures on animals were performed in accordance with the guidelines and regulations set out by Stockholm's Norra Djurförsöksetiska Nämnd (N156/14).

AUTHOR CONTRIBUTIONS

RM-P carried out the experiments. RM-P, BC, and CC analyzed the results and designed the research. BC and CC directed the research. RM-P, BC, and CC discussed the results and wrote the manuscript. All authors reviewed the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fncel.2017.00019/full#supplementary-material

REFERENCES

Arai, S., Takuma, K., Mizoguchi, H., Ibi, D., Nagai, T., Takahashi, K., et al. (2008). Involvement of pallidotegmental neurons in methamphetamine- and MK-801-induced impairment of prepulse inhibition of the acoustic startle reflex in mice: reversal by GABAB receptor agonist baclofen. *Neuropsychopharmacology* 33, 3164–3175. doi: 10.1038/npp.2008.41

Bortolato, M., Frau, R., Aru, G. N., Orru, M., and Gessa, G. L. (2004). Baclofen reverses the reduction in prepulse inhibition of the acoustic startle response induced by dizocilpine, but not by apomorphine. *Psychopharmacology (Berl.)* 171, 322–330. doi: 10.1007/s00213-003-1589-5

Bowen, G. P., Lin, D., Taylor, M. K., and Ison, J. R. (2003). Auditory cortex lesions in the rat impair both temporal acuity and noise increment thresholds, revealing a common neural substrate. *Cereb. Cortex* 13, 815–822. doi: 10.1093/cercor/13.8.815

Braff, D. L., Geyer, M. A., and Swerdlow, N. R. (2001). Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl.)* 156, 234–258. doi: 10.1007/s002130100810

Chen, Y. C., Li, X., Liu, L., Wang, J., Lu, C. Q., Yang, M., et al. (2015). Tinnitus and hyperacusis involve hyperactivity and enhanced connectivity in auditory-limbic-arousal-cerebellar network. *Elife* 4:e06576. doi: 10.7554/eLife.06576

Eggermont, J. J. (2016). Can animal models contribute to understanding tinnitus heterogeneity in humans? *Front. Aging Neurosci.* 8:265. doi: 10.3389/fnagi.2016.00265

Elgoyhen, A. B., Langguth, B., De Ridder, D., and Vanneste, S. (2015). Tinnitus: perspectives from human neuroimaging. *Nat. Rev. Neurosci.* 16, 632–642. doi: 10.1038/nrn4003

Engineer, N. D., Riley, J. R., Seale, J. D., Vrana, W. A., Shetake, J. A., Sudanagunta, S. P., et al. (2011). Reversing pathological neural activity using targeted plasticity. *Nature* 470, 101–104. doi: 10.1038/nature09656

Fournier, P., and Hebert, S. (2013). Gap detection deficits in humans with tinnitus as assessed with the acoustic startle paradigm: does tinnitus fill in the gap? *Hear. Res.* 295, 16–23. doi: 10.1016/j.heares.2012.05.011

Franklin, K. B. J., and Paxinos, G. (2008). *The Mouse Brain*. Cambridge: Elsevier Inc.

Frau, R., Bini, V., Pillolla, G., Malherbe, P., Pardu, A., Thomas, A. W., et al. (2014). Positive allosteric modulation of GABAB receptors ameliorates sensorimotor gating in rodent models. *CNS Neurosci. Ther.* 20, 679–684. doi: 10.1111/cns.12261

Galazyuk, A., and Hebert, S. (2015). Gap-prepulse inhibition of the acoustic startle reflex (GPIAS) for tinnitus assessment: current status and future directions. *Front. Neurol.* 6:88. doi: 10.3389/fneur.2015.00088

Holt, A. G., Bissig, D., Mirza, N., Rajah, G., and Berkowitz, B. (2010). Evidence of key tinnitus-related brain regions documented by a unique combination of manganese-enhanced MRI and acoustic startle reflex testing. *PLoS ONE* 5:e14260. doi: 10.1371/journal.pone.0014260

Ison, J. R., and Bowen, G. P. (2000). Scopolamine reduces sensitivity to auditory gaps in the rat, suggesting a cholinergic contribution to temporal acuity. *Hear. Res.* 145, 169–176. doi: 10.1016/S0378-5955(00)00088-5

Ison, J. R., O'Connor, K., Bowen, G. P., and Bocirnea, A. (1991). Temporal resolution of gaps in noise by the rat is lost with functional decortication. *Behav. Neurosci.* 105, 33–40. doi: 10.1037/0735-7044.105.1.33

Koch, M. (1999). The neurobiology of startle. *Prog. Neurobiol.* 59, 107–128. doi: 10.1016/S0301-0082(98)00098-7

Koch, M., Fendt, M., and Kretschmer, B. D. (2000). Role of the substantia nigra pars reticulata in sensorimotor gating, measured by prepulse inhibition of startle in rats. *Behav. Brain Res.* 117, 153–162. doi: 10.1016/S0166-4328(00)00299-0

Koch, M., Kungel, M., and Herbert, H. (1993). Cholinergic neurons in the pedunculopontine tegmental nucleus are involved in the mediation of prepulse inhibition of the acoustic startle response in the rat. *Exp. Brain Res.* 97, 71–82. doi: 10.1007/BF00228818

Koch, M., and Schnitzler, H. U. (1997). The acoustic startle response in rats–circuits mediating evocation, inhibition and potentiation. *Behav. Brain Res.* 89, 35–49. doi: 10.1016/S0166-4328(97)02296-1

Leitner, D. S., and Cohen, M. E. (1985). Role of the inferior colliculus in the inhibition of acoustic startle in the rat. *Physiol. Behav.* 34, 65–70. doi: 10.1016/0031-9384(85)90079-4

Li, L., Priebe, R. P., and Yeomans, J. S. (1998). Prepulse inhibition of acoustic or trigeminal startle of rats by unilateral electrical stimulation of the inferior colliculus. *Behav. Neurosci.* 112, 1187–1198. doi: 10.1037/0735-7044.112.5.1187

Li, S., Choi, V., and Tzounopoulos, T. (2013). Pathogenic plasticity of Kv7.2/3 channel activity is essential for the induction of tinnitus. *Proc. Natl. Acad. Sci. U.S.A.* 110, 9980–9985. doi: 10.1073/pnas.1302770110

Li, S., Kalappa, B. I., and Tzounopoulos, T. (2015). Noise-induced plasticity of KCNQ2/3 and HCN channels underlies vulnerability and resilience to tinnitus. *Elife* 4. doi: 10.7554/eLife.07242

Lowe, A. S., and Walton, J. P. (2015). Alterations in peripheral and central components of the auditory brainstem response: a neural assay of tinnitus. *PLoS ONE* 10:e0117228. doi: 10.1371/journal.pone.0117228

Sedley, W., Gander, P. E., Kumar, S., Oya, H., Kovach, C. K., Nourski, K. V., et al. (2015). Intracranial mapping of a cortical tinnitus system using residual inhibition. *Curr. Biol.* 25, 1208–1214. doi: 10.1016/j.cub.2015.02.075

Shore, S. E., Roberts, L. E., and Langguth, B. (2016). Maladaptive plasticity in tinnitus–triggers, mechanisms and treatment. *Nat. Rev. Neurol.* 12, 150–160. doi: 10.1038/nrneurol.2016.12

Stadlbauer, U., Langhans, W., and Meyer, U. (2013). Administration of the Y2 receptor agonist PYY3-36 in mice induces multiple behavioral changes relevant to schizophrenia. *Neuropsychopharmacology* 38, 2446–2455. doi: 10.1038/npp.2013.146

Swerdlow, N. R., Braff, D. L., and Geyer, M. A. (2016). Sensorimotor gating of the startle reflex: what we said 25 years ago, what has happened since then, and what comes next. *J. Psychopharmacol.* 30, 1072–1081. doi: 10.1177/0269881116661075

Swerdlow, N. R., Weber, M., Qu, Y., Light, G. A., and Braff, D. L. (2008). Realistic expectations of prepulse inhibition in translational models for schizophrenia research. *Psychopharmacology (Berl.)* 199, 331–388. doi: 10.1007/s00213-008-1072-4

Takahashi, K., Nagai, T., Kamei, H., Maeda, K., Matsuya, T., Arai, S., et al. (2007). Neural circuits containing pallidotegmental GABAergic neurons are involved in

the prepulse inhibition of the startle reflex in mice. *Biol. Psychiatry* 62, 148–157. doi: 10.1016/j.biopsych.2006.06.035

Turner, J. G., Brozoski, T. J., Bauer, C. A., Parrish, J. L., Myers, K., Hughes, L. F., et al. (2006). Gap detection deficits in rats with tinnitus: a potential novel screening tool. *Behav. Neurosci.* 120, 188–195. doi: 10.1037/0735-7044.120.1.188

Turner, J. G., and Parrish, J. (2008). Gap detection methods for assessing salicylate-induced tinnitus and hyperacusis in rats. *Am. J. Audiol.* 17, S185–S192. doi: 10.1044/1059-0889(2008/08-0006)

Weible, A. P., Moore, A. K., Liu, C., DeBlander, L., Wu, H., Kentros, C., et al. (2014). Perceptual gap detection is mediated by gap termination responses in auditory cortex. *Curr. Biol.* 24, 1447–1455. doi: 10.1016/j.cub.2014.05.031

Yu, H., Vikhe, Patil K, Han, C., Fabella, B., Canlon, B., Someya, S., et al. (2016). GLAST deficiency in mice exacerbates gap detection deficits in a model of salicylate-induced tinnitus. *Front. Behav. Neurosci.* 10:158. doi: 10.3389/fnbeh.2016.00158

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REVIEW
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Genetics of Tinnitus: An Emerging Area for Molecular Diagnosis and Drug Development

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Subjective tinnitus is the perception of sound in the absence of external or bodily-generated sounds. Chronic tinnitus is a highly prevalent condition affecting over 70 million people in Europe. A wide variety of comorbidities, including hearing loss, psychiatric disorders, neurodegenerative disorders, and temporomandibular joint (TMJ) dysfunction, have been suggested to contribute to the onset or progression of tinnitus; however, the precise molecular mechanisms of tinnitus are not well understood and the contribution of genetic and epigenetic factors remains unknown. Human genetic studies could enable the identification of novel molecular therapeutic targets, possibly leading to the development of novel pharmaceutical therapeutics. In this article, we briefly discuss the available evidence for a role of genetics in tinnitus and consider potential hurdles in designing genetic studies for tinnitus. Since multiple diseases have tinnitus as a symptom and the supporting genetic evidence is sparse, we propose various strategies to investigate the genetic underpinnings of tinnitus, first by showing evidence of heritability using concordance studies in twins, and second by improving patient selection according to phenotype and/or etiology in order to control potential biases and optimize genetic data output. The increased knowledge resulting from this endeavor could ultimately improve the drug development process and lead to the preventive or curative treatment of tinnitus.

Keywords: epidemiology, genetic, hearing loss, tinnitus, meniere's disease, phenotyping, subtype

INTRODUCTION

Tinnitus, the perception of a phantom sound, affects nearly 15% of the population. It can severely affect quality of life in 3–6% of the population, becoming chronically bothersome, and incapacitating (Davis and Refaie, 2000). From the social perspective, tinnitus leads to a loss of productivity and increases the risk of receiving a disability pension (Friberg et al., 2012).

Tinnitus varies on the perceptional level, ranging from beeping, hissing, ringing, and buzzing to drumming sounds. Tinnitus can be objective (generated by the ear and perceived by external people) or subjective (only perceived by the concerned individual), pulsatile (synchronous or asynchronous), constant or intermittent, loud or faint, perceived in one or both ears, or within the head. Despite the fact that noise overexposure is most frequently associated with tinnitus (15%) (Nicolas-Puel et al., 2006), tinnitus may be associated with many conditions other than dysfunction of the auditory system (e.g., obesity, diabetes, smoking, alcohol consumption, neck pain, allergies, thyroid dysfunction, brain tumors, temporomandibular joint (TMJ) dysfunction and as a side effect of several medications) (Baguley et al., 2013). Tinnitus often coincides with severe psychological dysfunction. Anxiety, depression, and disruptions in the execution of cognitive and attention tasks are frequently reported. Another symptom commonly associated with tinnitus is a decreased tolerance to loud sounds (hyperacusis), which is observed in 40–55% of patients with tinnitus (Baguley, 2003; Schecklmann et al., 2014). Tinnitus can be categorized based on psychoacoustic features and the levels of severity, psychological distress, and daily life disability. According to its duration, tinnitus is often assessed as follows: up to 3 months of duration is considered "acute" between 3 and 12 months "subacute" and more than 1 year is considered "chronic." At present, there are no effective drugs for tinnitus while the need for effective treatments is likely to increase (Cederroth et al., 2013). The lack of treatment success in clinical trials has been attributed to the heterogeneity of clinical conditions associated with tinnitus. Genetic studies would help in identifying diagnostic markers for subgroups of tinnitus patients (subtypes) or markers of resistance to treatment in order to improve the selection of subjects and optimize treatment outcome. In addition, since the current pipeline of drugs to treat tinnitus is rather small (Cederroth et al., 2013), genetic studies could provide additional targets for drug development.

In this article, we briefly present the current evidence regarding heritability in tinnitus, the hypothetical pathophysiological mechanisms of tinnitus and the underlying challenges of tinnitus phenotyping. We next propose different approaches toward the genetic elucidation of tinnitus including the analysis of concordance in twins, familial aggregation studies, exome sequencing in families with multiple cases, and sequencing studies in cohorts of patients with extreme phenotypes. We suggest that tinnitus subtyping strategies based on precise definition of phenotypes would favor the selection of homogeneous groups of tinnitus patients with matching controls that might serve as a solid basis for genetic studies.

GENETIC CONTRIBUTION TO TINNITUS: THE MISSING EVIDENCE

There is a lot of evidence to support a genetic contribution for complex disorders: differences in the prevalence according to the ethnic background, familial aggregation, and higher concordance in monozygotic twins than in dizygotic twins. In this section, we address each of these in the context of tinnitus.

The prevalence of tinnitus ranges from 6 to 30%, while the prevalence of severe tinnitus ranges from 0.7 to 16% in the same studies (Cooper, 1994; Sindhusake et al., 2003; Hasson et al., 2010; Krog et al., 2010; Michikawa et al., 2010; Nondahl et al., 2010; Shargorodsky et al., 2010; Engdahl et al., 2012; McCormack et al., 2014; Park et al., 2014; Gallus et al., 2015). This wide range likely reflects the large number of questions that have been used to define tinnitus, which makes the genetic basis of tinnitus difficult to determine. An age-dependent increase in the prevalence of tinnitus is seen across all studies, with a peak in the seventh decade of life (Gopinath et al., 2010; Shargorodsky et al., 2010; Park et al., 2014). There is no agreement on whether there is a gender bias, but there is a tendency for males to be more affected than women (Cooper, 1994; Sindhusake et al., 2003; Hasson et al., 2010; Krog et al., 2010; Michikawa et al., 2010; Nondahl et al., 2010; Shargorodsky et al., 2010; Engdahl et al., 2012; McCormack et al., 2014; Park et al., 2014; Gallus et al., 2015).

With regard to ethnic differences, studies performed in Egypt (Khedr et al., 2010), Japan (Michikawa et al., 2010), and Nigeria (Lasisi et al., 2010) suggest that the prevalence is broadly the same. However, one study reported a higher prevalence of tinnitus in non-Hispanic whites than in other racial or ethnic groups in the U.S. (Shargorodsky et al., 2010). Additional ethnic studies are needed to infer potential genetic influences on tinnitus.

Table 1 presents a summary of human genetic studies for tinnitus. Most of them were genotyping studies with a small sample size (54–288) on candidate genes including *KCNE1*, *KCNE3*, *GDNF*, *BDNF*, *COCH,* and *SLC12A* (Sand et al., 2010, 2011, 2012a,b; Gallant et al., 2013). Overall, no associations were found with one exception (Pawelczyk et al., 2012). The small sample size and the paucity of patient characterization (tinnitus only being characterized as chronic) could account for these outcomes. For instance, the study by Sand et al. (2010) included 201 German patients with "chronic tinnitus" and no controls. The authors used public genotyping data from other studies as control subjects, without any ancestry-informative markers to prevent population stratification. Pawelczyk et al. (2012) conducted a case-control study in Poland including 626 subjects exposed to occupational noise (128 with tinnitus and 498 without tinnitus). While they reported an association with the SNP rs915539 in normal hearing subjects ($p = 0.005$), no ancestry-informative markers were used and the current standards in genetic association studies require a replication in another association study with an independent population, something that to our knowledge has not been yet reported.

Studies on familial tinnitus are scarce. A large study analyzed the occurrence of familial tinnitus within 198 European families (Hendrickx et al., 2007). The authors found a familial correlation between siblings reaching 0.16, and the finding was independent of differences in age, gender, and hearing threshold. Using a Cox proportional model, the risk of developing tinnitus was estimated to be 1.7 times higher in siblings with tinnitus than that observed in families without tinnitus, after correcting for risk factors (Hendrickx et al., 2007). However, the authors reasoned

TABLE 1 | Available human genetic studies on tinnitus.

Tinnitus property	HL	Size of the population	Reported gene	Design	Associations	References
Tinnitus associated with NIHL	Occupational Noise	$N = 626$ (128 with tinnitus)	KCNE1, SLC12A2	Genotyping	KCNE1 associated with tinnitus independent of HL	Pawelczyk et al., 2012
Chronic	Controlled	$N = 240$	GDNF, BDNF	Genotyping	None	Sand et al., 2012b
Chronic	Controlled	$N = 95$	KCTD12	Genotyping	None	Sand et al., 2012a
Chronic	Not reported	$N = 201$	KCNE1	Genotyping	None	Sand et al., 2010
Unknown	All	$N = 54$	SLC6A4	Genotyping	None	Deniz et al., 2010
Chronic	Not reported	$N = 288$	KCNE3	Sanger sequencing	None	Sand et al., 2011
Tinnitus associated with HFHL	HFHL	$N = 1$ family	COCH	Linkage analysis	Single family study	Gallant et al., 2013
Unknown	All	$N = 28{,}066$	None	Familial aggregation	Population-based study	Kvestad et al., 2010
Unknown	Not reported	$N = 198$ families	None	Familial aggregation	Multiple family study	Hendrickx et al., 2007

HL, Hearing loss.

that this could be simply due to the fact of raising awareness on tinnitus within the family. The selection of multicase families with tinnitus for exome sequencing studies to search for rare variants with a high penetrant effect has not been explored.

To the best of our knowledge, there is no published work on the concordance, or heritability of tinnitus from twin studies. Such studies could appropriately address the issue of sibling influences on awareness and provide solid evidence on whether or not there is a genetic contribution to tinnitus. Heritability is an estimation of the genetic contribution in relation to the phenotypic variability for a particular trait that occurs within populations. The variation in the phenotype for a particular trait in a population arises from differences in the genotype and environmental variation. Falconer's formula for estimating heritability is based on the concordance rates among monozygotic and dizygotic twins:

$$h^2 = 2^*(rMZ - rDZ) \quad (1)$$

where h^2 is the heritability or the proportion of variance due to genetic factors and r is the correlation coefficient between MZ and DZ twins. Heritability values have a theoretical range of 0–1.5. In general, it is considered that a trait has a genetic component if h^2 is between 0.5 and 1. With this approach in mind, we have initiated a study to evaluate the concordance of tinnitus in twins and ongoing data collection is in support of a genetic contribution to some forms of tinnitus.

TINNITUS PHENOTYPING: NEEDLE IN A HAYSTACK?

A major limitation in genetic association studies, whatever the field of research, is the classification of subjects according to a common phenotype. Tinnitus is considered a symptom. It is thought that the large number of clinical conditions associated with chronic tinnitus has contributed to the unsuccessful clinical trials and genetic studies listed above. An initial suggestion of classification into subgroups was proposed by the Tinnitus Research Initiative in 2010 (Landgrebe et al., 2010) followed by the Tinnitus Holistic Simplified Classification (Cianfrone et al., 2015). The Tinnitus Holistic Simplified Classification proposes that tinnitus stems from (i) auditory alterations (Auditory Tinnitus), (ii) complex auditory-somatosensory interactions (Somatosensory Tinnitus), (iii) psychopathological-auditory interactions (Psychopathology-related Tinnitus), and (iv) 2 or all of the previous mechanisms (Combined Tinnitus). Others have classified tinnitus into originating either from the auditory system (usually peripheral, rarely central) or from the somatosensory system (head and neck), or a combination of the two (Levine and Oron, 2015). Recently, another work has revealed that somatic tinnitus may represent a subtype (Ward et al., 2015), being more prevalent in younger groups, unrelated to hearing loss but rather associated with TMJ disorders. Overall, the definition of tinnitus subtypes is still a matter of debate, and no consensus has been found due to the large number of contributing factors, the multitude of etiologies, and the psychoacoustic profiles of tinnitus.

The benefits of subtyping approaches in genetic studies have been shown in a genome-wide association study (GWAS) for major depressive disorders (MDD). The analysis of more than 9000 cases did not yield robustly replicated genetic loci, and it was thought that the heterogeneity contributed to the reduction in the power of the genetic associations. The selection of a severe subtype of MDD with accompanying melancholia allowed the successful mapping of a single gene, namely *SIRT1* (CONVERGE, 2015). Such stratification of diseases into homogeneous subcategories or subtypes has been successful in reducing genetic background noise and clinical heterogeneity, ultimately helping in the identification of genetic variants (Gelernter et al., 2006; Schwartz et al., 2010). Although these approaches may lead to hits that are not applicable to the general population, they may still facilitate (i) the understanding of the mechanisms of specific subcategories of tinnitus, (ii) the development of biological markers of tinnitus subtypes, and (iii) the identification of candidates for drug development.

How can the tinnitus field benefit from genetic studies to improve treatment outcome? While such conceptual approaches are at the forefront of disease treatment, an example can be

provided with ongoing research on a specific subtype of Autism Spectrum Disorder (ASD), namely Phelan-McDermid syndrome, which is a rare disorder with deletions or mutations in the SHANK3 gene. Studies have shown the beneficial use of IGF-1 for neuronal function using cells with the SHANK3 mutations (Bozdagi et al., 2013). This approach has been tested on nine children in a pilot study showing the successful therapeutic effects of IGF-1 treatment in improving social behavior and reducing repetitive behavior (Kolevzon et al., 2014), whose positive outcomes have also been reported in a preclinical mouse model of autism (Bozdagi et al., 2013). Such studies show how genetic studies, coupled with preclinical research, can help in developing targeted treatments for different disease subtypes. Such examples are on rare monogenic disorders, so how can this be applied to tinnitus, which—assuming there is significant heritability—would likely be polygenic? Tinnitus is probably a polygenic condition, however genomic research will reveal whether some subtypes of familial tinnitus, are monogenetically driven.

Several disorders have been categorized into subgroups in order to facilitate the identification of biomarkers and optimize treatment outcomes. Schizophrenia is segregated into subtypes according to the expression of behavioral symptoms (e.g., paranoid, disorganized, catatonic, undifferentiated, and residual). Multiple sclerosis (MS) subtypes, on the other hand, are defined on the basis of time-course development (primary progressive, relapsing-progressive, relapsing-remitting, secondary progressive, transitional progressive), prognosis, and pathogenicity (obtained through the analysis of biopsies) (Bitsch and Brück, 2002). Interestingly, in the case of MS, studies have revealed blood, CSF, and MRI biomarkers associated with particular subgroups. Alzheimers disease is categorized into three subgroups thanks to metabolic profiling (Bredesen, 2015): inflammatory (presence of specific blood markers), noninflammatory (absence of these blood markers), and cortical (no specific Alzheimer gene detected, but normally associated with zinc deficiency). The value of these biomarkers in clinical practice remains to be established due to the large phenotypic variability.

The above examples possess numerous advantages over tinnitus. First, these are diseases whereas tinnitus is considered a symptom. Second, they rely on available biomarkers from blood, CSF, molecular, and histological profiles. An example of the advantage that these biomarkers provide to the refinement of genetic studies has been shown in bipolar disorders. Bipolar disorders are classified into two major subtypes. Kynurenic acid (KYNA) has been recently identified as a CSF biomarker in both subtypes, and has been associated with a greater history of psychosis. This biomarker provided a powerful advantage in a recent GWAS study involving only 76 patients and 46 controls that identified a single nucleotide polymorphism causing a reduction of sorting nexin 7 (*SNX7*) expression in astrocytes, leading to higher IL-1β production, and subsequently increasing KYNA in patients carrying this variant (Sellgren et al., 2015).

The tinnitus field suffers from a lack of such biomarkers. Current subtyping strategies thus rely on the clinical features of tinnitus (acute vs. chronic, objective vs. subjective, pulsatile vs. nonpulsatile, constant vs. intermittent), taking into account cofactors such as hearing loss, vertigo, headache, psychiatric influences, and somatosensory origins, as well as its triggers (e.g., noise trauma, accident, medication, Ménière's disease). A putative list of factors that need to be taken into account is shown in **Table 2**. Which of these are relevant to tinnitus will only emerge in future clinical and genetic studies.

The selection of individuals for genetic studies will have to consider all the above features, including severity, duration, gender, age, age of onset, pitch, intensity, hearing thresholds, psychological burden, and etiology, to reduce clinical heterogeneity, and to control biases. Categorizing tinnitus subtypes according to tinnitus pitch, severity, and hearing profile might be sufficient, however this needs to be tested. In addition, tinnitus perception may change over time and patients might be classified into a different subtype, or even belong to multiple subtypes (e.g., noise trauma causing unilateral deafness, being initially acute, and then transiting to chronic stages, becoming bilateral with the emergence of psychiatric burden but still unilaterally dominant, and pitch decreasing with age). As a consequence, psychoacoustic evaluations should be performed in the first years of the onset of tinnitus to reduce the number of confounding factors. Finally, there is little biological information on the mechanisms underlying each of these subtypes, and this is where genetics may play an important role by defining a subgroup of tinnitus subjects with a defined phenotype. The identification of tinnitus subtypes is thus in the early stages.

TABLE 2 | List of potential factors to take into account in genetic studies on tinnitus.

Forms of tinnitus
Subjective, objective
Pulsatile, nonpulsatile
Constant, intermittent
Unilateral, bilateral
Temporal
Acute, subacute, chronic
Severity
Moderate, severe, catastrophic
Etiology
Noise trauma, medication, post-traumatic stress disorder, Ménière's disease, TMJ
Influencers
Age, sex, ethnicity
Cofactors
Hearing loss, hyperacusis, vertigo, headache, psychiatric (stress, anxiety, depression), somatosensory
Comorbidities
Hypertension, diabetes, cancer, chronic pain, neurological problems,
Response to treatment
Improvement, worsening, none

We propose a nonexhaustive list of factors to take into account when designing genetic studies on tinnitus. A large variety of tinnitus subtypes may thus emerge from the combination of severity, forms of tinnitus, etiology, temporal characteristics, and comorbidities.

PATHOPHYSIOLOGY OF TINNITUS

Mechanisms Mediating Tinnitus Perception

A detailed phenotyping of tinnitus patients is necessary to investigate the genetic and environmental factors contributing to its development. Both auditory and psychological components of tinnitus are important aspects to be evaluated. Studies reveal that tinnitus possesses a dual mechanism that emerges most frequently from (i) peripheral dysfunctions leading to changes in (ii) the activity of the central auditory pathway likely influenced by nonauditory networks that feed tinnitus-related distress and possibly influence its persistence (**Figure 1**). The current knowledge stipulates that the perception of tinnitus resembles the phantom perception of an amputated limb, whereby the loss of sensory input leads to compensation mechanisms in the brain (hyperactivity). Indeed, tinnitus networks are similar to those involved in chronic pain (perception, salience, distress, memory), and could contribute to the maintenance of tinnitus, in the absence of the initial trigger (Langguth et al., 2013). Confirming the idea about the loss of peripheral (cochlear) input causing tinnitus, human subjects that wore a silicone earplug for 7 days experienced tinnitus (Schaette et al., 2012), which disappeared after the earplug was removed, supporting the hypothesis that therapeutic interventions restoring cochlear output to the brain can abolish phantom perception.

The relationship between peripheral damage and tinnitus has been recently reviewed (Schaette, 2014). Patients with conductive hearing loss (e.g., otosclerosis) often complain about tinnitus, which is then completely abolished after surgery (Gersdorff et al., 2000; Ayache et al., 2003; Sobrinho et al., 2004). Similarly, hearing aids, and cochlear implants are capable of improving tinnitus in 50% of patients, and abolishing tinnitus in 20% of cases (Moffat et al., 2009; Olze et al., 2012; Schaette, 2014). Mertens et al. provided the only long-term study that clearly shows a reduction in tinnitus and hypercusis with cochlear implants (Mertens et al., 2016). Interestingly, one study reported lower amplitudes of wave I recorded from click auditory brainstem responses (ABRs) in tinnitus patients with normal hearing thresholds when measured by pure tone audiometry, suggesting the existence of cochlear damage leading to a decreased input toward the brain (Schaette and McAlpine, 2011). Overall, these studies suggest a peripheral (cochlear) contribution in some forms of tinnitus, which supports the inclusion of ABR measurements in patients with normal audiometry. Central mechanisms that compensate for the lack of input (homeoplastic plasticity) could emerge. Interestingly, fMRI studies revealed that people with tinnitus have increased activity in auditory, and nonauditory networks such as the limbic system, including the nucleus accumbens (Rauschecker et al., 2010; Leaver et al., 2011). It was suggested that this increased activity was a result of reduced functional output of the ventromedial prefrontal cortex in tinnitus patients (Leaver et al., 2011). Activation of the nucleus accumbens would lead to increased inhibition of thalamic reticular nucleus neurons, and thus result in increased inhibition of medial

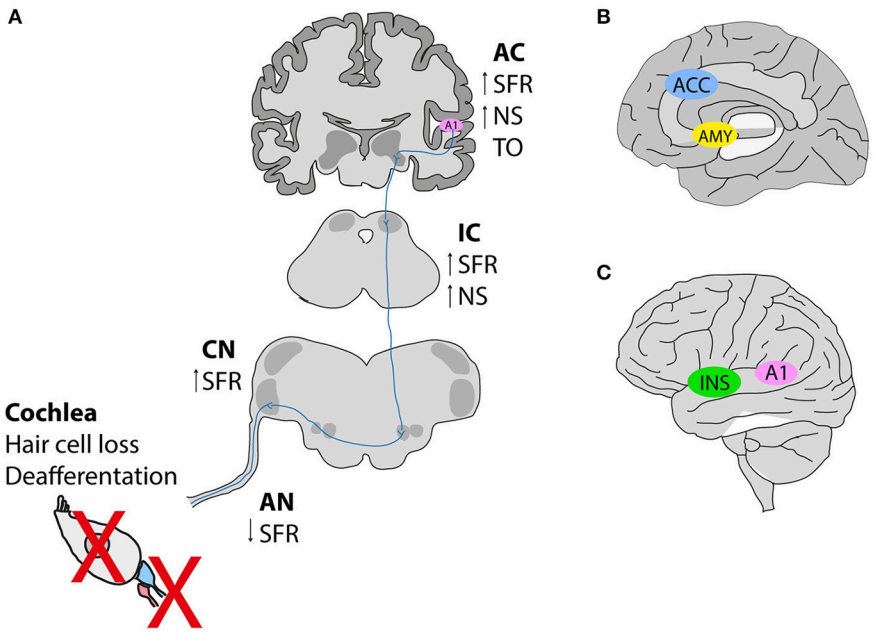

FIGURE 1 | Hypothetical schema of tinnitus pathogenesis. (A) Noise exposure in animal models leads to deafferentation between inner hair cells and afferent neurons, or hair cell loss subsequently causing a reduction in the spontaneous activity of the auditory nerve (AN). In contrast, an increase in the spontaneous firing rates is observed along the auditory pathway [dorsal cochlear nucleus (CN), inferior colliculus (IC) and auditory cortex (A1)]. Tonotopic organization (TO) is also altered in the auditory cortex **(B,C)**. Neuroimaging studies in humans with tinnitus identified the involvement of nonauditory areas such as the salience network [the anterior cingulate cortex (ACC) and the insula (INS)], as well as the emotional components of tinnitus involving an increased connectivity between the amygdala (AMY), and the primary auditory cortex (A1). SFR, spontaneous firing rate; NS, neuronal synchrony. This figure was modified from Elgoyhen et al. (2015) with permission from the Nature Publishing Group (Elgoyhen et al., 2015).

geniculate body neurons. In patients with gaze-induced tinnitus, hypometabolic theta activity, and reduced inhibition in the auditory cortex were found to occur hand in hand with reduced medial geniculate body activity (van Gendt et al., 2012). However, the precision of EEG measures in tinnitus assessment has recently been questioned (Pierzycki et al., 2016). It can be concluded that there is clear evidence of a profound impact of the "emotional brain network" on the generation of manifestation of tinnitus.

Mechanisms Mediating Tinnitus-Related Distress

Most people have probably transiently experienced tinnitus at some point in their life. However, in some cases tinnitus becomes permanent and can seriously impact the quality of life. Interestingly, in individuals with chronic persistent, nonfluctuating tinnitus, the psychoacoustic characteristics of tinnitus (e.g., loudness or pitch) are not unequivocally related to its severity or the treatment outcome (Jastreboff and Hazell, 1993). In chronic tinnitus, *the interpretation of the tinnitus percept* might be more important in impacting the severity of complaints than the sound itself (Jastreboff and Hazell, 1993; Henry and Meikle, 2000; Andersson, 2003; Hiller and Goebel, 2007). Psychological distress, which includes negative attitudes, and cognitions, impaired concentration, insomnia, depression, and anxiety, is a significant predictor for the variability in the quality of life (Erlandsson and Hallberg, 2000). Accumulating evidence suggests that cognitive misinterpretations, negative emotional reactivity and attention processes are crucial in dysfunctional habituation leading to severe tinnitus distress (Erlandsson and Hallberg, 2000; Kröner-Herwig et al., 2003; Zachriat and Kröner-Herwig, 2004; Cima et al., 2012).

The emotional neural networks that possibly influence the peripheral to central circuit in tinnitus patients likely comprise the regions known to be involved in normal emotional behavior. These regions can be altered in mood disorders and involve the medial prefrontal cortex, the medial, and caudolateral orbital cortex (medial prefrontal network), anterior cingulate, amygdala, hippocampus, and ventromedial parts of the basal ganglia (Jastreboff, 1990; Drevets et al., 2008). Indeed, clinical imaging of individuals with tinnitus provides evidence that tinnitus-related and distress-related brain networks overlap, such as the limbic, and paralimbic regions (Rauschecker et al., 2010), the amygdala (Shulman, 1995; Mirz et al., 2000), the hippocampus (Lockwood et al., 1998; Landgrebe et al., 2009), the basal ganglia (Lowry et al., 2004; Cheung and Larson, 2010) and the subcallosal region, including the nucleus accumbens (Mühlau et al., 2006; Leaver et al., 2011). Favoring the possible cross-modal interactions of the limbic system central responsiveness, perhaps related to the peripheral damage after auditory trauma, thalamic/amygdala projections change their activity pattern during tinnitus (Knipper et al., 2013). Overall, it appears to be important to measure emotional components during tinnitus phenotyping.

TINNITUS PHENOTYPING STRATEGIES

Precise phenotyping of patients with tinnitus is the first step in defining clusters of patients based on a few variables that will configure a tinnitus subtype (Tyler et al., 2008). Poor phenotyping can significantly contaminate large epidemiological or genetic studies leading to a loss of power and false-positive results. For instance, not controlling for emotional factors (such as stress, anxiety, or depression) could lead to the identification of genes falsely associated with tinnitus, while they would be truly linked to depression. Hearing profile and tinnitus pitch are minimum requirements, but additional measures—including questionnaires covering psychological aspects—are also needed. The common psychological comorbidities of depression, anxiety, insomnia and cognitive impairment disable 10–50% of patients suffering from tinnitus. Similarly to some tinnitus measures, assessment of tinnitus comorbidities has been neglected in drug development efforts. This gap is currently being addressed in a consensus-driven effort to provide international guidelines on Core Outcome Measures in Tinnitus (COMiT) (Hall et al., 2015), which will define the domains and related instruments necessary to perform tinnitus studies.

An example that illustrates the importance of genetic studies in subtypes of tinnitus patients is the identification of a polymorphism in the serotonin transporter gene (*SLC6A4*), which has been previously shown to be associated with anxiety (Lesch et al., 1996), and is now linked with the severity of the psychological conditions associated with tinnitus (Deniz et al., 2010). As a consequence of these findings, one could envisage that *SLC6A4* variants could become markers of tinnitus distress, and that serotonin reuptake inhibitors could be targeted at subtypes of patients with tinnitus and depression in the presence of the risk allele. However, some of the mechanisms and drug treatments of these tinnitus comorbidities might differ from patients without tinnitus, which would suggest tinnitus-specific mechanisms.

Defining potential tinnitus subtypes will be essential in investigating the heritability for each subtype in familial and twin studies. This strategy will enhance the results in genetic studies, in addition to improving clinical trial outcomes. However, this can be a challenging task since a subtype will also be characterized by either a successful therapeutic intervention or by the identification of a gene associated with, for example, a particular form of tinnitus. In the context of genetics, this conundrum can be potentially addressed with concordance studies in twins by identifying traits that are more prevalent in monozygotic twins than in dizygotic twins.

The assessment of a patient with tinnitus should include a complete audiological evaluation, psychoacoustic measures of tinnitus and several instruments to determine the severity of tinnitus, and its impact on health-related quality of life. However, it is important to note that the exclusion of measures could also lead to the inclusion of nonspecific groups and bias the genetic analysis or treatment outcome. A comprehensive measure of tinnitus features is thus required to characterize each form of tinnitus. To achieve this important classification procedure, considerable thought should be invested in selecting the right tools for measuring tinnitus experience (e.g., validated questionnaires, psychoacoustic measures, audiological measures), and the selection will depend on the aims of the study.

A number of instruments have been recommended by the Tinnitus Research Initiative (Langguth et al., 2007) for the assessment of treatment outcomes in clinical trials. Of

note, we emphasize that conventional pure tone audiometry (PTA), which measures hearing thresholds from 250 Hz to 8 kHz, is no longer adapted to tinnitus cases. A number of tinnitus patients diagnosed with no hearing loss when measured with conventional audiometry tend to be diagnosed with somatosensory tinnitus. However, high-frequency PTA (up to 20 kHz) might reveal an auditory component to tinnitus, thereby completely reallocating a patient into another subtype category. Normal hearing should be considered from <20 dB HL up to 16 kHz in adults. Interestingly, one study reported lower amplitudes of wave I (based on I/V ratio) recorded from click auditory brainstem responses (ABRs) in tinnitus patients with normal hearing thresholds, however the latter were measured with PTA only up to 12 kHz, suggesting that a decreased cochlear input toward the brain causes some forms of tinnitus (Schaette and McAlpine, 2011). ABRs could thus become important in revealing cochlear damage and objectively categorize subjects into a specific peripherally injured tinnitus subtype. However, since ABRs are known to be sensitive at higher frequencies (Don and Eggermont, 1978; Eggermont and Don, 1980), differences in hearing thresholds above 12 kHz could have accounted for these wave I/V differences in amplitude in the tinnitus group. This reinforces the importance of assessing PTA up to at least 16 kHz. Distortion products of otoacoustic emissions (DPOAEs) are measures of outer hair cell function. Often neglected in the assessment of tinnitus patients, DPOAEs can measure both a decreased function and a loss of outer hair cells (OHCs) likely due to cell death, or a gain in OHC function as can sometimes be observed in subgroups of tinnitus patients with hyperacusis (Sztuka et al., 2010). Psychoacoustic measures have been most commonly used to determine the pitch-matched frequency and intensity of the perceived tinnitus. Little is known on how tinnitus pitch can evolve with time and whether patients with different pitches might constitute different subtypes. Neuroimaging studies have been recently reviewed (Elgoyhen et al., 2015) and it has been proposed that tinnitus heterogeneity is the consequence of abnormal activity from specific networks. Neuroimaging techniques, including fMRI, EEG, and MEG, could thus constitute an important set of instruments to help categorize tinnitus patients into different subgroups according to the involvement of specific networks (such as the hippocampal-cortical memory networks, the frontoparietal control system, the salience network, and the autonomic nervous system). Research is currently underway to define which networks specify a given subtype of tinnitus and how relevant these tools can be for characterizing tinnitus.

DESIGNING HUMAN GENETIC STUDIES

Over the last three decades, medical genetic research has focused largely on inherited variation in the human genome. Most of the DNA variability can be explained by single nucleotide variants (SNVs) and small structural variants involving one or a few nucleotides (insertions, deletions), or large structural variants involving hundreds to thousands of nucleotides (copy number variants, CNVs). These variants mostly occur in noncoding regions, which can affect the degree of expression of a given allele, but CNVs may also involve coding regions causing partial or complete loss or gain of function.

There are several complementary approaches to demonstrate an association between genetic variants and tinnitus in humans:

- focusing on patients with a common genetic background (e.g., identical twins or familial aggregation studies) to estimate heritability.
- designing case-control studies (e.g., cases with common etiology or disease such as Ménière's disease) to search for rare variants on monogenic tinnitus families.
- GWAS using large cohorts of sporadic patients to search for common regulatory variants.

All of these designs can be used to identify the most heritable tinnitus phenotype and to find candidate genes. However, the complexity and heterogeneity of tinnitus implies the need for in-depth tinnitus phenotyping, using questionnaires as well as audiological and psychoacoustic measures, to accurately identify genes responsible for tinnitus resilience, or susceptibility.

Methods: Genotyping vs. Sequencing

There are two methods for reading the genome: genotyping and sequencing. Genotyping determines the differences in SNVs in a given individual when their sequence is compared with the reference genome. Sequencing is the process of determining the nucleotide order of a given DNA fragment and is usually performed for short fragments of DNA by the chain termination method developed by Sanger et al. (1977). New sequencing technologies such as pyrosequencing have enabled rapid, large-scale sequencing of human genome, including whole genome sequencing (WGS) and the most popular enrichment approach for coding regions, whole exome sequencing (WES) (Mardis, 2008).

Genotyping larger cohorts of patients with a given disorder using microarrays has been the basis for GWAS during the last 15 years. Trait-associated SNVs have identified regulatory common genetic variants (minor allele frequency—MAF > 0.05) with small genetic effects, but are unlikely to define the causative rare variants in most cases. Although GWAS for complex disorders have resulted in great progress, most of the candidate genes investigated in case-control studies, including candidate genes for chronic tinnitus, could not be replicated. Replication is essential for establishing the credibility of a genotype—phenotype association, whether derived from candidate genes or GWAS (Mardis, 2008). Large-scale genotyping studies are based on the knowledge that SNVs along the entire genome are conserved in specific regions, and SNVs can be used as markers of the sequence in these regions. To generate a map of SNVs in the human genome, the HapMap Project was carried out (International HapMap, 2003). GWAS have identified common SNVs in large-population studies, mostly in noncoding regions with unknown functional significance (Cooper and Shendure, 2011). Furthermore, this design is not suitable for the study of

genetic conditions that are caused by rare or novel mutations (Robinson et al., 2011).

On the other hand, high-throughput sequencing technologies, such as WES, are designed to enrich the sequencing of coding regions, which contain 85% of disease-causing mutations defining rare variants in familial and sporadic patients in 65% of cases (Samuels et al., 2013). Moreover, the cost of WGS or WES studies has been dramatically reduced in recent years, facilitating their implementation for clinical diagnosis (Biesecker and Green, 2014). Although genotyping has been the preferred approach to identify common SNVs with regulatory effects in GWAS, the decreased cost involved in WGS is predicted to lead to genotyping being replaced in a few years. WES and WGS have become the standard in searching for rare variants in any genomic study.

Candidate Gene vs. Genomic Approaches

Several genes have been considered as candidate genes for tinnitus, but replication studies, are missing or have failed to confirm previously reported associations (**Table 1**). The main reason for the lack of reproducibility is population stratification or the systematic ancestry differences between cases and controls, which is a confounder in genetic association studies (Price et al., 2010). Instead, targeted sequencing of candidate genes is considered a suitable method to determine the relevance of a candidate variant previously identified by a genomic approach.

Genotyping microarrays and next-generation sequencing technologies help to overcome the limitations of traditional approaches. Either WGS or WES combined with linkage studies have become the most efficient strategies for discovering causal genes for Mendelian diseases (Zhang, 2014). We have used this approach to identify novel and rare variants in *FAM136A* and *DTNA* genes in autosomal dominant familial Ménière's disease (Requena et al., 2015). We were also able to reveal a missense variant in the *PRKCB* gene in a family with Ménière's disease segregating low-frequency sensorineural hearing loss (Martín-Sierra et al., 2016). The next step will be to investigate rare variants of candidate genes in more families and sporadic cases. This approach can be used for specific forms of familial tinnitus after obtaining a detailed phenotype. To the best of our knowledge, this strategy has not been applied yet to specific tinnitus subtypes. The clinical heterogeneity of tinnitus makes the selection of patients according to the tinnitus phenotype a crucial step in the design of the study.

Sample Selection

There are compelling reasons to focus on tinnitus symptoms that are defined by a common trigger or clinical syndrome. First, the more homogenous the tinnitus phenotype, according to the tinnitus pitch and hearing profile, the better the chance that an allelic variant segregates with the particular phenotype. The reason to classify tinnitus by its frequency is the tonotopic gradient of gene expression in the mammalian cochlea (Yoshimura et al., 2014). The frequency selectivity is maintained along the auditory pathway and precise regulation of this gene expression is required to preserve tonotopy. Individuals with selective low- or high-frequency sensorineural hearing loss could potentially be good candidates for a case-control study. The reduction in error will increase the power to detect a small gene effect. Second, patients with different tinnitus conditions will vary in other ways that increase the variance and reduce the power to detect gene effects. For instance, a completely different set of factors may mediate the onset of chronic tinnitus due to age-related hearing loss vs. an ear injury or a cardiovascular disorder. For this reason, the selection of younger individuals is preferred, since the cumulative effect of different epigenetic and environmental triggers may favor the onset of tinnitus in elderly individuals. Third, completely different measures are needed to adequately characterize a tinnitus phenotype in different conditions, for example, in noise-induced tinnitus vs. stress-induced tinnitus.

There are several limitations when designing genetic studies in patients with chronic tinnitus. First, the clinical heterogeneity observed makes it difficult to select patients with the same phenotype (Sand et al., 2007). A clinically well-defined phenotype is a prerequisite in designing a case-control study. Since most patients with tinnitus also have a certain degree of hearing loss and a number of comorbidities related to tinnitus, the design should control these biases by selecting individuals with the same hearing profile and tinnitus pitch. Since it has been hypothesized that tinnitus subjects will possibly accumulate multiple common and rare variants segregating with the phenotype, it could be advantageous to select younger individuals in multicase families in order to search for highly penetrant rare variants with a large effect size (Requena et al., 2014). In contrast, older subjects with tinnitus will probably reflect the cumulative effect of epigenetic and environmental factors throughout their lives, diluting the effect of genetic variation. Therefore, a possible solution is to reduce the selection to a subset of patients with extreme phenotypes, filtering them according to early age of onset, gender, ethnic background, and for instance, specific clinical features that would show higher concordance in monozygotic twins. Such strategies have proven successful in previous studies, whereby the exclusion of hearing impairment increases the number of twins concordant for noise sensitivity (Heinonen-Guzejev et al., 2005) and the selection of a subtype of severe melancholia increases the concordance of major depressive disorder (CONVERGE, 2015). Furthermore, the selection of a reference population matching for age, gender, ethnicity, comorbidities, emotional burden, and quality of life could also be critical, since many confounder factors may arise.

A second limitation for small-size case-control studies is that tinnitus is a highly prevalent condition, which anticipates that many genetic variants could confer resilience or susceptibility (Veltman and Brunner, 2012). A large genetic heterogeneity is expected for chronic tinnitus, which would possibly complicate the functional interpretation of rare variants in genes encoding, for instance, proteins that are known to have a physiological role in the synapse. Often associated with tinnitus is the high-frequency SNHL that is typically observed in presbycusis and is known to have a significant genetic heterogeneity (Fransen et al., 2015).

Learning from previous research in fields such as pain and schizophrenia, which are very heterogeneous disorders, we

believe that studies should be restricted to the most homogeneous groups in terms of etiology, age, gender balance, severity of tinnitus, audiometric profile, and comorbidities. Moreover, the smaller the variation of the genetic background within a group, the more robust the study will be. In this direction, we favor the following sequence of prioritization: studying twins > multiplex families with tinnitus > Ménière's disease patients with chronic tinnitus, groups with cisplatin-induced tinnitus following chemotherapy, noise overexposure (military training or work exposure), or ARHL (age-related hearing loss) > large health cohorts with undefined etiology.

Design of Tinnitus Sequencing Studies

Let's consider one example for which the initiating trigger is well-defined (i.e., noise trauma or Ménière's disease), and another example where the initiating trigger is not clearly defined. After sensorineural hearing loss, some patients experience short-term tinnitus, but do not develop chronic tinnitus, suggesting some type of resilience. However, a few patients with some intrinsic susceptibility will experience chronic persistent tinnitus.

In a case-control design, the case group is defined as having chronic persistent tinnitus as a consequence of the trigger, and controls must have had the initiating trigger as well, leading to temporary tinnitus, or no tinnitus. Data collected from cases and controls can include previous tinnitus history, history of psychiatric disorders, assessment of traits, exposure to stressors, and actual comorbid conditions relevant to tinnitus domains (hearing loss, hyperacusis, stress, anxiety, or depression). Then, cases and controls are compared at the level of individual domain-specific measures. Measures from different domains can also be compared (e.g., hearing loss and stress) in order to better understand the subgroups. Such comprehensive studies will accelerate the gathered knowledge on the interaction between causative factors and the genetic components underlying a specific tinnitus phenotype.

Obviously, the method chosen for the case-control studies will depend on the incidence, prevalence of the condition and the proportion of those with the phenotype of interest that seek care. Direct ascertainment in the population might differ from the phenotypes assessed in tinnitus clinics (self-selected samples) since the psychiatric conditions and behaviors of those who seek care can be genetically influenced. As an example, treatment resistance, and psychiatric comorbidities are more likely to occur in patients with migraines that get treated by a specialist than in a population-based sample for the same disorder (Lipton et al., 2003; Kolodner et al., 2004; Bigal et al., 2006). In addition, identifying appropriate controls for the tinnitus groups from specialty care centers can be particularly challenging.

Epigenetic Factors Possibly Contributing to Tinnitus

Epigenetics is the discipline that studies changes to the genome that do not involve modifications in the DNA sequence *per se* (Cederroth et al., 2007). Since psychological distress is often associated with tinnitus, and psychosocial stress has been well documented in animals and people as a modifier of epigenetic marks (Franklin et al., 2012; Bohacek and Mansuy, 2015; Vaiserman, 2015), it is tempting to speculate that tinnitus could also emerge from epigenetic modifications. The two main epigenetic mechanisms are gene methylation and histone modifications. DNA methylation typically reduces or even silences the expression of genes encoded by methylated DNA. The modification of histones may either enhance or reduce gene expression, depending on the type of histone and type of modification. Histones, which are structural proteins of chromatin, are responsible for tight packaging of DNA, and their modifications (e.g., acetylation or deacetylation) affect the accessibility of DNA by various enzymes. Changes in methylation occur during embryonic development as early as a few hours in the paternal genome after fertilization, whereas in the maternal genome this is a more passive phenomenon. After the implantation of the embryo, along the differentiation of embryonic tissues, cells become more abundantly methylated—a phenomenon called reprogramming (Jaenisch, 1997; Mayer et al., 2000). In adulthood, the environment can induce changes in specific cell types. Monozygotic twins offer an excellent illustration of this phenomenon, since despite their genetic identity, there are morphological variations and also different susceptibility to diseases. Environmental factors such as psychosocial stress, smoking, physical activity, or diet can contribute to such epigenetic drifts.

In animal models, restraint stress, acute forced swim stress, social isolation stress, and many other types of stress can induce epigenetic modifications, such as on the loci of the glucocorticosteroid receptor (GR) or brain-derived neurotropic factor (BDNF) (Fuchikami et al., 2010; Stankiewicz et al., 2013). There are many studies showing that experimentally induced behavioral changes are linked to these epigenetic modifications; this was also observed in people suffering from depression or anxiety (Bagot et al., 2014).

To date, no published studies have focused on possible epigenetic aspects of tinnitus onset or progression. However, some studies have indirectly approached this topic in the context of hearing loss (Provenzano and Domann, 2007; Wolber et al., 2014). For instance, the pattern of gene methylation in a group of patients with age-related hearing impairment was found to differ from that found in well-hearing subjects (Wolber et al., 2014). Because the incidence of hearing loss in tinnitus patients is high, it would be tempting to speculate that at least some of the epigenetic targets may overlap between the two conditions (Goldman and Holme, 2010; Mazurek et al., 2010). In addition, the comorbidity of psychological conditions such as anxiety or stress (Hébert and Lupien, 2007; Hébert et al., 2012) may possibly create a disease-specific pattern of epigenetic modifications.

The epigenetic modifications often affect specific tissues, but not the entire organism, which renders the study of human auditory tissues challenging (e.g., inner ear and central auditory pathway) due to their limited access. However, epigenetic modifications could occur during fetal development (e.g., maternal stress during gestation)—a phenomenon called "fetal reprogramming" (Moisiadis and Matthews, 2014). Then, peripheral tissues might be used as a proxy for brain-specific alterations (Stenz et al., 2015). Finally, if they occur during adulthood, the consequences of the insults can also be found

across generations both at the level of the phenotype and in the male germline epigenome (Anway et al., 2005; Franklin et al., 2010). These possibilities offer new routes for investigating the relationship between tinnitus and epigenetic changes related to comorbid conditions such as stress, anxiety, and depression.

CONCLUSIONS

Human genetic studies in tinnitus are at the very beginning. Accordingly, concordance studies in twins are an essential first step in defining the heritability of tinnitus. In a second step, the precise selection of subjects based on careful phenotyping will facilitate the identification of genes involved in the resilience or susceptibility to developing tinnitus or tinnitus-related comorbidities. The molecular characterization of tinnitus will not only lead to a better understanding of the pathways and networks regulating the onset of disease, but also shed light on the physiological processes involved, leading to the development of new pharmacological treatments.

AUTHOR CONTRIBUTIONS

JL, CC, TB, RC, PV, MK, BM, AS, contributed to the manuscript. CC, JL, and CC coordinated the writing, designed the tables and figures, and edited the manuscript.

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REFERENCES

Andersson, G. (2003). Tinnitus loudness matchings in relation to annoyance and grading of severity. *Auris Nasus Larynx* 30, 129–133. doi: 10.1016/S0385-8146(03)00008-7

Anway, M. D., Cupp, A. S., Uzumcu, M., and Skinner, M. K. (2005). Epigenetic transgenerational actions of endocrine disruptors and male fertility. *Science* 308, 1466–1469. doi: 10.1126/science.1108190

Ayache, D., Earally, F., and Elbaz, P. (2003). Characteristics and postoperative course of tinnitus in otosclerosis. *Otol. Neurotol.* 24, 48–51. doi: 10.1097/00129492-200301000-00011

Bagot, R. C., Labonté, B., Peña, C. J., and Nestler, E. J. (2014). Epigenetic signaling in psychiatric disorders: stress and depression. *Dialogues Clin. Neurosci.* 16, 281–295.

Baguley, D. M. (2003). Hyperacusis. *J. R. Soc. Med.* 96, 582–585. doi: 10.1258/jrsm.96.12.582

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Biesecker, L. G., and Green, R. C. (2014). Diagnostic clinical genome and exome sequencing. *N. Engl. J. Med.* 371, 1170. doi: 10.1056/NEJMra1312543

Bigal, M. E., Kolodner, K. B., Lafata, J. E., Leotta, C., and Lipton, R. B. (2006). Patterns of medical diagnosis and treatment of migraine and probable migraine in a health plan. *Cephalalgia* 26, 43–49. doi: 10.1111/j.1468-2982.2005.00988.x

Bitsch, A., and Brück, W. (2002). Differentiation of multiple sclerosis subtypes: implications for treatment. *CNS Drugs* 16, 405–418. doi: 10.2165/00023210-200216060-00004

Bohacek, J., and Mansuy, I. M. (2015). Molecular insights into transgenerational non-genetic inheritance of acquired behaviours. *Nat. Rev. Genet.* 16, 641–652. doi: 10.1038/nrg3964

Bozdagi, O., Tavassoli, T., and Buxbaum, J. D. (2013). Insulin-like growth factor-1 rescues synaptic and motor deficits in a mouse model of autism and developmental delay. *Mol. Autism* 4:9. doi: 10.1186/2040-2392-4-9

Bredesen, D. E. (2015). Metabolic profiling distinguishes three subtypes of Alzheimers disease. *Aging* 7, 595–600. doi: 10.18632/aging.100801

Cederroth, C. R., Canlon, B., and Langguth, B. (2013). Hearing loss and tinnitus–are funders and industry listening? *Nat. Biotechnol.* 31, 972–974. doi: 10.1038/nbt.2736

Cederroth, C. R., Vassalli, J. D., and Nef, S. (2007). [Of epigenetics and development]. *Rev. Med. Suisse* 3, 528, 530–522.

Cheung, S. W., and Larson, P. S. (2010). Tinnitus modulation by deep brain stimulation in locus of caudate neurons (area LC). *Neuroscience* 169, 1768–1778. doi: 10.1016/j.neuroscience.2010.06.007

Cianfrone, G., Mazzei, F., Salviati, M., Turchetta, R., Orlando, M. P., Testugini, V., et al. (2015). Tinnitus Holistic Simplified Classification (THoSC): a new assessment for subjective tinnitus, with diagnostic and therapeutic implications. *Ann. Otol. Rhinol. Laryngol.* 124, 550–560. doi: 10.1177/0003489415570931

Cima, R. F., Maes, I. H., Joore, M. A., Scheyen, D. J., El Refaie, A., Baguley, D. M., et al. (2012). Specialised treatment based on cognitive behaviour therapy versus usual care for tinnitus: a randomised controlled trial. *Lancet* 379, 1951–1959. doi: 10.1016/S0140-6736(12)60469-3

CONVERGE, C. (2015). Sparse whole-genome sequencing identifies two loci for major depressive disorder. *Nature* 523, 588–591. doi: 10.1038/nature14659

Cooper, G. M., and Shendure, J. (2011). Needles in stacks of needles: finding disease-causal variants in a wealth of genomic data. *Nat. Rev. Genet.* 12, 628–640. doi: 10.1038/nrg3046

Cooper, J. C. Jr. (1994). Health and nutrition examination survey of 1971–1975: part II. tinnitus, subjective hearing loss, and well-being. *J. Am. Acad. Audiol.* 5, 37–43.

Davis, A., and Refaie, A. E. (2000). "Epidemiology of tinnitus," in *The Handbook of Tinnitus,* ed R. S. Tyler (San Diego, CA: Singular Thompson Learning), 1–23.

Deniz, M., Bayazit, Y. A., Celenk, F., Karabulut, H., Yilmaz, A., Gunduz, B., et al. (2010). Significance of serotonin transporter gene polymorphism in tinnitus. *Otol. Neurotol.* 31, 19–24. doi: 10.1097/MAO.0b013e3181c2dcbc

Don, M., and Eggermont, J. J. (1978). Analysis of the click-evoked brainstem potentials in man unsing high-pass noise masking. *J. Acoust. Soc. Am.* 63, 1084–1092. doi: 10.1121/1.381816

Drevets, W. C., Price, J. L., and Furey, M. L. (2008). Brain structural and functional abnormalities in mood disorders: implications for neurocircuitry models of depression. *Brain Struct. Funct.* 213, 93–118. doi: 10.1007/s00429-008-0189-x

Eggermont, J. J., and Don, M. (1980). Analysis of the click-evoked brainstem potentials in humans using high-pass noise masking. II. Effect of click intensity. *J. Acoust. Soc. Am.* 68, 1671–1675. doi: 10.1121/1.385199

Elgoyhen, A. B., Langguth, B., De Ridder, D., and Vanneste, S. (2015). Tinnitus: perspectives from human neuroimaging. *Nat. Rev. Neurosci.* 16, 632–642. doi: 10.1038/nrn4003

Engdahl, B., Krog, N. H., Kvestad, E., Hoffman, H. J., and Tambs, K. (2012). Occupation and the risk of bothersome tinnitus: results from a prospective cohort study (HUNT). *BMJ Open* 2:e000512. doi: 10.1136/bmjopen-2011-000512

Erlandsson, S. I., and Hallberg, L. R. (2000). Prediction of quality of life in patients with tinnitus. *Br. J. Audiol.* 34, 11–20. doi: 10.3109/03005364000000114

Franklin, T. B., Russig, H., Weiss, I. C., Gräff, J., Linder, N., Michalon, A., et al. (2010). Epigenetic transmission of the impact of early stress

across generations. *Biol. Psychiatry* 68, 408–415. doi: 10.1016/j.biopsych.2010.05.036

Franklin, T. B., Saab, B. J., and Mansuy, I. M. (2012). Neural mechanisms of stress resilience and vulnerability. *Neuron* 75, 747–761. doi: 10.1016/j.neuron.2012.08.016

Fransen, E., Bonneux, S., Corneveaux, J. J., Schrauwen, I., Di Berardino, F., White, C. H., et al. (2015). Genome-wide association analysis demonstrates the highly polygenic character of age-related hearing impairment. *Eur. J. Hum. Genet.* 23, 110–115. doi: 10.1038/ejhg.2014.56

Friberg, E., Jansson, C., Mittendorfer-Rutz, E., Rosenhall, U., and Alexanderson, K. (2012). Sickness absence due to otoaudiological diagnoses and risk of disability pension: a nationwide Swedish prospective cohort study. *PLoS ONE* 7:e29966. doi: 10.1371/journal.pone.0029966

Fuchikami, M., Yamamoto, S., Morinobu, S., Takei, S., and Yamawaki, S. (2010). Epigenetic regulation of BDNF gene in response to stress. *Psychiatry Investig.* 7, 251–256. doi: 10.4306/pi.2010.7.4.251

Gallant, E., Francey, L., Fetting, H., Kaur, M., Hakonarson, H., Clark, D., et al. (2013). Novel COCH mutation in a family with autosomal dominant late onset sensorineural hearing impairment and tinnitus. *Am. J. Otolaryngol.* 34, 230–235. doi: 10.1016/j.amjoto.2012.11.002

Gallus, S., Lugo, A., Garavello, W., Bosetti, C., Santoro, E., Colombo, P., et al. (2015). Prevalence and determinants of tinnitus in the italian adult population. *Neuroepidemiology* 45, 12–19. doi: 10.1159/000431376

Gelernter, J., Panhuysen, C., Wilcox, M., Hesselbrock, V., Rounsaville, B., Poling, J., et al. (2006). Genomewide linkage scan for opioid dependence and related traits. *Am. J. Hum. Genet.* 78, 759–769. doi: 10.1086/503631

Gersdorff, M., Nouwen, J., Gilain, C., Decat, M., and Betsch, C. (2000). Tinnitus and otosclerosis. *Eur. Arch. Otorhinolaryngol.* 257, 314–316. doi: 10.1007/s004059900138

Goldman, D. R., and Holme, R. (2010). Hearing loss and tinnitus–the hidden healthcare time bomb. *Drug Discov. Today* 15, 253–255. doi: 10.1016/j.drudis.2010.01.010

Gopinath, B., McMahon, C. M., Rochtchina, E., Karpa, M. J., and Mitchell, P. (2010). Incidence, persistence, and progression of tinnitus symptoms in older adults: the Blue Mountains Hearing Study. *Ear Hear.* 31, 407–412. doi: 10.1097/AUD.0b013e3181cdb2a2

Hall, D. A., Haider, H., Kikidis, D., Mielczarek, M., Mazurek, B., Szczepek, A. J., et al. (2015). Toward a global consensus on outcome measures for clinical trials in tinnitus: report from the first international meeting of the COMiT Initiative, November 14, 2014, Amsterdam, The Netherlands. *Trends Hear* 19:2331216515580272. doi: 10.1177/2331216515580272

Hasson, D., Theorell, T., Westerlund, H., and Canlon, B. (2010). Prevalence and characteristics of hearing problems in a working and non-working Swedish population. *J. Epidemiol. Community Health* 64, 453–460. doi: 10.1136/jech.2009.095430

Hébert, S., Canlon, B., Hasson, D., Magnusson Hanson, L. L., Westerlund, H., and Theorell, T. (2012). Tinnitus severity is reduced with reduction of depressive mood–a prospective population study in Sweden. *PLoS ONE* 7:e37733. doi: 10.1371/journal.pone.0037733

Hébert, S., and Lupien, S. J. (2007). The sound of stress: blunted cortisol reactivity to psychosocial stress in tinnitus sufferers. *Neurosci. Lett.* 411, 138–142. doi: 10.1016/j.neulet.2006.10.028

Heinonen-Guzejev, M., Vuorinen, H. S., Mussalo-Rauhamaa, H., Heikkilä, K., Koskenvuo, M., and Kaprio, J. (2005). Genetic component of noise sensitivity. *Twin Res. Hum. Genet.* 8, 245–249. doi: 10.1375/twin.8.3.245

Hendrickx, J. J., Huyghe, J. R., Demeester, K., Topsakal, V., Van Eyken, E., Fransen, E., et al. (2007). Familial aggregation of tinnitus: a European multicentre study. *B-ENT* 3(Suppl. 7), 51–60.

Henry, J. A., and Meikle, M. B. (2000). Psychoacoustic measures of tinnitus. *J. Am. Acad. Audiol.* 11, 138–155.

Hiller, W., and Goebel, G. (2007). When tinnitus loudness and annoyance are discrepant: audiological characteristics and psychological profile. *Audiol. Neurootol.* 12, 391–400. doi: 10.1159/000106482

International HapMap, C. (2003). The international hapmap project. *Nature* 426, 789–796. doi: 10.1038/nature02168

Jaenisch, R. (1997). DNA methylation and imprinting: why bother? *Trends Genet.* 13, 323–329. doi: 10.1016/S0168-9525(97)01180-3

Jastreboff, P. J. (1990). Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci. Res.* 8, 221–254. doi: 10.1016/0168-0102(90)90031-9

Jastreboff, P. J., and Hazell, J. W. (1993). A neurophysiological approach to tinnitus: clinical implications. *Br. J. Audiol.* 27, 7–17. doi: 10.3109/03005369309077884

Khedr, E. M., Ahmed, M. A., Shawky, O. A., Mohamed, E. S., El Attar, G. S., and Mohammad, K. A. (2010). Epidemiological study of chronic tinnitus in Assiut, Egypt. *Neuroepidemiology* 35, 45–52. doi: 10.1159/000306630

Knipper, M., Van Dijk, P., Nunes, I., Rüttiger, L., and Zimmermann, U. (2013). Advances in the neurobiology of hearing disorders: recent developments regarding the basis of tinnitus and hyperacusis. *Prog. Neurobiol.* 111, 17–33. doi: 10.1016/j.pneurobio.2013.08.002

Kolevzon, A., Bush, L., Wang, A. T., Halpern, D., Frank, Y., Grodberg, D., et al. (2014). A pilot controlled trial of insulin-like growth factor-1 in children with Phelan-McDermid syndrome. *Mol. Autism* 5:54. doi: 10.1186/2040-2392-5-54

Kolodner, K., Lipton, R. B., Lafata, J. E., Leotta, C., Liberman, J. N., Chee, E., et al. (2004). Pharmacy and medical claims data identified migraine sufferers with high specificity but modest sensitivity. *J. Clin. Epidemiol.* 57, 962–972. doi: 10.1016/j.jclinepi.2004.01.014

Krog, N. H., Engdahl, B., and Tambs, K. (2010). The association between tinnitus and mental health in a general population sample: results from the HUNT Study. *J. Psychosom. Res.* 69, 289–298. doi: 10.1016/j.jpsychores.2010.03.008

Kröner-Herwig, B., Frenzel, A., Fritsche, G., Schilkowsky, G., and Esser, G. (2003). The management of chronic tinnitus: comparison of an outpatient cognitive-behavioral group training to minimal-contact interventions. *J. Psychosom. Res.* 54, 381–389. doi: 10.1016/S0022-3999(02)00400-2

Kvestad, E., Czajkowski, N., Engdahl, B., Hoffman, H. J., and Tambs, K. (2010). Low heritability of tinnitus: results from the second Nord-Trondelag health study. *Arch. Otolaryngol. Head Neck Surg.* 136, 178–182. doi: 10.1001/archoto.2009.220

Landgrebe, M., Langguth, B., Rosengarth, K., Braun, S., Koch, A., Kleinjung, T., et al. (2009). Structural brain changes in tinnitus: grey matter decrease in auditory and non-auditory brain areas. *Neuroimage* 46, 213–218. doi: 10.1016/j.neuroimage.2009.01.069

Landgrebe, M., Zeman, F., Koller, M., Eberl, Y., Mohr, M., Reiter, J., et al. (2010). The Tinnitus Research Initiative (TRI) database: a new approach for delineation of tinnitus subtypes and generation of predictors for treatment outcome. *BMC Med. Inform. Decis. Mak.* 10:42. doi: 10.1186/1472-6947-10-42

Langguth, B., Goodey, R., Azevedo, A., Bjorne, A., Cacace, A., Crocetti, A., et al. (2007). Consensus for tinnitus patient assessment and treatment outcome measurement: tinnitus research initiative meeting, Regensburg, July 2006. *Prog. Brain Res.* 166, 525–536. doi: 10.1016/S0079-6123(07)66050-6

Langguth, B., Kreuzer, P. M., Kleinjung, T., and De Ridder, D. (2013). Tinnitus: causes and clinical management. *Lancet Neurol.* 12, 920–930. doi: 10.1016/S1474-4422(13)70160-1

Lasisi, A. O., Abiona, T., and Gureje, O. (2010). Tinnitus in the elderly: profile, correlates, and impact in the Nigerian Study of Ageing. *Otolaryngol. Head Neck Surg.* 143, 510–515. doi: 10.1016/j.otohns.2010.06.817

Leaver, A. M., Renier, L., Chevillet, M. A., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2011). Dysregulation of limbic and auditory networks in tinnitus. *Neuron* 69, 33–43. doi: 10.1016/j.neuron.2010.12.002

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 274, 1527–1531. doi: 10.1126/science.274.5292.1527

Levine, R. A., and Oron, Y. (2015). Tinnitus. *Handb. Clin. Neurol.* 129, 409–431. doi: 10.1016/B978-0-444-62630-1.00023-8

Lipton, R. B., Scher, A. I., Steiner, T. J., Bigal, M. E., Kolodner, K., Liberman, J. N., et al. (2003). Patterns of health care utilization for migraine in England and in the United States. *Neurology* 60, 441–448. doi: 10.1212/WNL.60.3.441

Lockwood, A. H., Salvi, R. J., Coad, M. L., Towsley, M. L., Wack, D. S., and Murphy, B. W. (1998). The functional neuroanatomy of tinnitus: evidence for limbic system links and neural plasticity. *Neurology* 50, 114–120. doi: 10.1212/WNL.50.1.114

Lowry, L. D., Eisenman, L. M., and Saunders, J. C. (2004). An absence of tinnitus. *Otol. Neurotol.* 25, 474–478. doi: 10.1097/00129492-200407000-00013

Mardis, E. R. (2008). Next-generation DNA sequencing methods. *Annu. Rev. Genomics Hum. Genet.* 9, 387–402. doi: 10.1146/annurev.genom.9.081307.164359

Martín-Sierra, C., Requena, T., Frejo, L., Price, S. D., Gallego-Martinez, A., Batuecas-Caletrio, A., et al. (2016). A novel missense variant in PRKCB segregates low-frequency hearing loss in an autosomal dominant family with Meniere's disease. *Hum. Mol. Genet.* doi: 10.1093/hmg/ddw183. [Epub ahead of print].

Mayer, W., Niveleau, A., Walter, J., Fundele, R., and Haaf, T. (2000). Demethylation of the zygotic paternal genome. *Nature* 403, 501–502. doi: 10.1038/35000656

Mazurek, B., Olze, H., Haupt, H., and Szczepek, A. J. (2010). The more the worse: the grade of noise-induced hearing loss associates with the severity of tinnitus. *Int. J. Environ. Res. Public Health* 7, 3071–3079. doi: 10.3390/ijerph7083071

McCormack, A., Edmondson-Jones, M., Fortnum, H., Dawes, P., Middleton, H., Munro, K. J., et al. (2014). The prevalence of tinnitus and the relationship with neuroticism in a middle-aged UK population. *J. Psychosom. Res.* 76, 56–60. doi: 10.1016/j.jpsychores.2013.08.018

Mertens, G., De Bodt, M., and Van de Heyning, P. (2016). Cochlear implantation as a long-term treatment for ipsilateral incapacitating tinnitus in subjects with unilateral hearing loss up to 10 years. *Hear. Res.* 331, 1–6. doi: 10.1016/j.heares.2015.09.016

Michikawa, T., Nishiwaki, Y., Kikuchi, Y., Saito, H., Mizutari, K., Okamoto, M., et al. (2010). Prevalence and factors associated with tinnitus: a community-based study of Japanese elders. *J. Epidemiol.* 20, 271–276. doi: 10.2188/jea.JE20090121

Mirz, F., Gjedde, A., Sødkilde-Jrgensen, H., and Pedersen, C. B. (2000). Functional brain imaging of tinnitus-like perception induced by aversive auditory stimuli. *Neuroreport* 11, 633–637. doi: 10.1097/00001756-200002280-00039

Moffat, G., Adjout, K., Gallego, S., Thai-Van, H., Collet, L., and Noreña, A. J. (2009). Effects of hearing aid fitting on the perceptual characteristics of tinnitus. *Hear. Res.* 254, 82–91. doi: 10.1016/j.heares.2009.04.016

Moisiadis, V. G., and Matthews, S. G. (2014). Glucocorticoids and fetal programming part 2: mechanisms. *Nat. Rev. Endocrinol.* 10, 403–411. doi: 10.1038/nrendo.2014.74

Mühlau, M., Rauschecker, J. P., Oestreicher, E., Gaser, C., Röttinger, M., Wohlschläger, A. M., et al. (2006). Structural brain changes in tinnitus. *Cereb. Cortex* 16, 1283–1288. doi: 10.1093/cercor/bhj070

Nicolas-Puel, C., Akbaraly, T., Lloyd, R., Berr, C., Uziel, A., Rebillard, G., et al. (2006). Characteristics of tinnitus in a population of 555 patients: specificities of tinnitus induced by noise trauma. *Int. Tinnitus J.* 12, 64–70.

Nondahl, D. M., Cruickshanks, K. J., Wiley, T. L., Klein, B. E., Klein, R., Chappell, R., et al. (2010). The ten-year incidence of tinnitus among older adults. *Int. J. Audiol.* 49, 580–585. doi: 10.3109/14992021003753508

Olze, H., Gräbel, S., Haupt, H., Forster, U., and Mazurek, B. (2012). Extra benefit of a second cochlear implant with respect to health-related quality of life and tinnitus. *Otol. Neurotol.* 33, 1169–1175. doi: 10.1097/MAO.0b013e31825e799f

Park, B., Choi, H. G., Lee, H. J., An, S. Y., Kim, S. W., Lee, J. S., et al. (2014). Analysis of the prevalence of and risk factors for tinnitus in a young population. *Otol. Neurotol.* 35, 1218–1222. doi: 10.1097/mao.0000000000000472

Pawelczyk, M., Rajkowska, E., Kotylo, P., Dudarewicz, A., Van Camp, G., and Sliwinska-Kowalska, M. (2012). Analysis of inner ear potassium recycling genes as potential factors associated with tinnitus. *Int. J. Occup. Med. Environ. Health* 25, 356–364. doi: 10.2478/s13382-012-0061-3

Pierzycki, R. H., McNamara, A. J., Hoare, D. J., and Hall, D. A. (2016). Whole scalp resting state EEG of oscillatory brain activity shows no parametric relationship with psychoacoustic and psychosocial assessment of tinnitus: a repeated measures study. *Hear. Res.* 331, 101–108. doi: 10.1016/j.heares.2015.11.003

Price, A. L., Zaitlen, N. A., Reich, D., and Patterson, N. (2010). New approaches to population stratification in genome-wide association studies. *Nat. Rev. Genet.* 11, 459–463. doi: 10.1038/nrg2813

Provenzano, M. J., and Domann, F. E. (2007). A role for epigenetics in hearing: establishment and maintenance of auditory specific gene expression patterns. *Hear. Res.* 233, 1–13. doi: 10.1016/j.heares.2007.07.002

Rauschecker, J. P., Leaver, A. M., and Mühlau, M. (2010). Tuning out the noise: limbic-auditory interactions in tinnitus. *Neuron* 66, 819–826. doi: 10.1016/j.neuron.2010.04.032

Requena, T., Cabrera, S., Martín-Sierra, C., Price, S. D., Lysakowski, A., and Lopez-Escamez, J. A. (2015). Identification of two novel mutations in FAM136A and DTNA genes in autosomal-dominant familial Meniere's disease. *Hum. Mol. Genet.* 24, 1119–1126. doi: 10.1093/hmg/ddu524

Requena, T., Espinosa-Sanchez, J. M., Cabrera, S., Trinidad, G., Soto-Varela, A., Santos-Perez, S., et al. (2014). Familial clustering and genetic heterogeneity in Meniere's disease. *Clin. Genet.* 85, 245–252. doi: 10.1111/cge.12150

Robinson, P. N., Krawitz, P., and Mundlos, S. (2011). Strategies for exome and genome sequence data analysis in disease-gene discovery projects. *Clin. Genet.* 80, 127–132. doi: 10.1111/j.1399-0004.2011.01713.x

Samuels, D. C., Han, L., Li, J., Quanghu, S., Clark, T. A., Shyr, Y., et al. (2013). Finding the lost treasures in exome sequencing data. *Trends Genet.* 29, 593–599. doi: 10.1016/j.tig.2013.07.006

Sand, P. G., Langguth, B., Itzhacki, J., Bauer, A., Geis, S., Cárdenas-Conejo, Z. E., et al. (2012a). Resequencing of the auxiliary GABA(B) receptor subunit gene KCTD12 in chronic tinnitus. *Front. Syst. Neurosci.* 6:41. doi: 10.3389/fnsys.2012.00041

Sand, P. G., Langguth, B., and Kleinjung, T. (2011). Deep resequencing of the voltage-gated potassium channel subunit KCNE3 gene in chronic tinnitus. *Behav. Brain Funct.* 7:39. doi: 10.1186/1744-9081-7-39

Sand, P. G., Langguth, B., Kleinjung, T., and Eichhammer, P. (2007). Genetics of chronic tinnitus. *Prog. Brain Res.* 166, 159–168. doi: 10.1016/S0079-6123(07)66014-2

Sand, P. G., Langguth, B., Schecklmann, M., and Kleinjung, T. (2012b). GDNF and BDNF gene interplay in chronic tinnitus. *Int. J. Mol. Epidemiol. Genet.* 3, 245–251. doi: 10.1093/hmg/ddu524

Sand, P. G., Luettich, A., Kleinjung, T., Hajak, G., and Langguth, B. (2010). An examination of KCNE1 mutations and common variants in chronic tinnitus. *Genes* 1, 23–37. doi: 10.3390/genes1010023

Sanger, F., Nicklen, S., and Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. U.S.A.* 74, 5463–5467. doi: 10.1073/pnas.74.12.5463

Schaette, R. (2014). Tinnitus in men, mice (as well as other rodents), and machines. *Hear. Res.* 311, 63–71. doi: 10.1016/j.heares.2013.12.004

Schaette, R., and McAlpine, D. (2011). Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J. Neurosci.* 31, 13452–13457. doi: 10.1523/JNEUROSCI.2156-11.2011

Schaette, R., Turtle, C., and Munro, K. J. (2012). Reversible induction of phantom auditory sensations through simulated unilateral hearing loss. *PLoS ONE* 7:e35238. doi: 10.1371/journal.pone.0035238

Schecklmann, M., Landgrebe, M., Langguth, B., and Group, T. R. I. D. S. (2014). Phenotypic characteristics of hyperacusis in tinnitus. *PLoS ONE* 9:e86944. doi: 10.1371/journal.pone.0086944

Schwartz, B., Wetzler, S., Swanson, A., and Sung, S. C. (2010). Subtyping of substance use disorders in a high-risk welfare-to-work sample: a latent class analysis. *J. Subst. Abuse Treat.* 38, 366–374. doi: 10.1016/j.jsat.2010.03.001

Sellgren, C. M., Kegel, M. E., Bergen, S. E., Ekman, C. J., Olsson, S., Larsson, M., et al. (2015). A genome-wide association study of kynurenic acid in cerebrospinal fluid: implications for psychosis and cognitive impairment in bipolar disorder. *Mol. Psychiatry*. doi: 10.1038/mp.2015.186. [Epub ahead of print].

Shargorodsky, J., Curhan, G. C., and Farwell, W. R. (2010). Prevalence and characteristics of tinnitus among US adults. *Am. J. Med.* 123, 711–718. doi: 10.1016/j.amjmed.2010.02.015

Shulman, A. (1995). A final common pathway for tinnitus - the medial temporal lobe system. *Int. Tinnitus J.* 1, 115–126.

Sindhusake, D., Mitchell, P., Newall, P., Golding, M., Rochtchina, E., and Rubin, G. (2003). Prevalence and characteristics of tinnitus in older adults: the blue mountains hearing Study. *Int. J. Audiol.* 42, 289–294. doi: 10.3109/14992020309078348

Sobrinho, P. G., Oliveira, C. A., and Venosa, A. R. (2004). Long-term follow-up of tinnitus in patients with otosclerosis after stapes surgery. *Int. Tinnitus J.* 10, 197–201.

Stankiewicz, A. M., Swiergiel, A. H., and Lisowski, P. (2013). Epigenetics of stress adaptations in the brain. *Brain Res. Bull.* 98, 76–92. doi: 10.1016/j.brainresbull.2013.07.003

Stenz, L., Zewdie, S., Laforge-Escarra, T., Prados, J., La Harpe, R., Dayer, A., et al. (2015). BDNF promoter I methylation correlates between post-mortem human peripheral and brain tissues. *Neurosci. Res.* 91, 1–7. doi: 10.1016/j.neures.2014.10.003

Sztuka, A., Pospiech, L., Gawron, W., and Dudek, K. (2010). DPOAE in estimation of the function of the cochlea in tinnitus patients with normal hearing. *Auris Nasus Larynx* 37, 55–60. doi: 10.1016/j.anl.2009.05.001

Tyler, R., Coelho, C., Tao, P., Ji, H., Noble, W., Gehringer, A., et al. (2008). Identifying tinnitus subgroups with cluster analysis. *Am. J. Audiol.* 17, S176–S184. doi: 10.1044/1059-0889(2008/07-0044)

Vaiserman, A. M. (2015). Epigenetic programming by early-life stress: evidence from human populations. *Dev. Dyn.* 244, 254–265. doi: 10.1002/dvdy.24211

van Gendt, M. J., Boyen, K., de Kleine, E., Langers, D. R., and van Dijk, P. (2012). The relation between perception and brain activity in gaze-evoked tinnitus. *J. Neurosci.* 32, 17528–17539. doi: 10.1523/JNEUROSCI.2791-12.2012

Veltman, J. A., and Brunner, H. G. (2012). De novo mutations in human genetic disease. *Nat. Rev. Genet.* 13, 565–575. doi: 10.1038/nrg3241

Ward, J., Vella, C., Hoare, D. J., and Hall, D. A. (2015). Subtyping Somatic Tinnitus: a cross-sectional UK cohort study of demographic, clinical and audiological characteristics. *PLoS ONE* 10:e0126254. doi: 10.1371/journal.pone.0126254

Wolber, L. E., Steves, C. J., Tsai, P. C., Deloukas, P., Spector, T. D., Bell, J. T., et al. (2014). Epigenome-wide DNA methylation in hearing ability: new mechanisms for an old problem. *PLoS ONE* 9:e105729. doi: 10.1371/journal.pone.0105729

Yoshimura, H., Takumi, Y., Nishio, S. Y., Suzuki, N., Iwasa, Y., and Usami, S. (2014). Deafness gene expression patterns in the mouse cochlea found by microarray analysis. *PLoS ONE* 9:e92547. doi: 10.1371/journal.pone.0092547

Zachriat, C., and Kröner-Herwig, B. (2004). Treating chronic tinnitus: comparison of cognitive-behavioural and habituation-based treatments. *Cogn. Behav. Ther.* 33, 187–198. doi: 10.1080/16506070410029568

Zhang, X. (2014). Exome sequencing greatly expedites the progressive research of Mendelian diseases. *Front. Med.* 8:42–57. doi: 10.1007/s11684-014-0303-9

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MINI REVIEW
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Genetics of Tinnitus: Still in its Infancy

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Tinnitus is the perception of a phantom sound that affects between 10 and 15% of the general population. Despite this considerable prevalence, treatments for tinnitus are presently lacking. Tinnitus exhibits a diverse array of recognized risk factors and extreme clinical heterogeneity. Furthermore, it can involve an unknown number of auditory and non-auditory networks and molecular pathways. This complex combination has hampered advancements in the field. The identification of specific genetic factors has been at the forefront of several research investigations in the past decade. Nine studies have examined genes in a case-control association approach. Recently, a genome-wide association study has highlighted several potentially significant pathways that are implicated in tinnitus. Two twin studies have calculated a moderate heritability for tinnitus and disclosed a greater concordance rate in monozygotic twins compared to dizygotic twins. Despite the more recent data alluding to genetic factors in tinnitus, a strong association with any specific genetic locus is lacking and a genetic study with sufficient statistical power has yet to be designed. Future research endeavors must overcome the many inherent limitations in previous study designs. This review summarizes the previously embarked upon tinnitus genetic investigations and summarizes the hurdles that have been encountered. The identification of candidate genes responsible for tinnitus may afford gene based diagnostic approaches, effective therapy development, and personalized therapeutic intervention.

Keywords: complex disorders, genetics, genetic heterogeneity, genome-wide association study (GWAS), hearing loss, tinnitus, twin study

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INTRODUCTION

Tinnitus is described as a scientific and clinical enigma that affects 10–15% of the general population. Furthermore, ∼1–3% of the population can be diagnosed with debilitating tinnitus connected to sleep disturbances, psychiatric distress, and quality of life consequences (Deniz et al., 2010; Shargorodsky et al., 2010; Baguley et al., 2013). Without question, the personal and societal strain from debilitating tinnitus can be enormous. The American Tinnitus Society describes the annual personal financial burden of tinnitus to be as high as $30,000 from compounded healthcare costs, lost income, and reduced productivity (https://www.ata.org/understanding-facts/impact-tinnitus).

Tinnitus is perceived as ringing, buzzing, beeping, or hissing and is characterized according to various clinical criteria. It can be subjective (perceived by the affected individual) or objective (heard by an observer), continuous or episodic, unilateral or bilateral, or pulsatile (synchronous or

asynchronous). It can range from low- to high-intensity sound and can manifest any frequency. Tinnitus can be acute (<3 months), sub-acute (3–6 months), or chronic (>12 months) with a gradual or sudden onset or be associated with other triggers or comorbidities (Baguley et al., 2013). In combination, these features complicate precise tinnitus phenotyping and have hampered research aiming to uncover a genetic basis for tinnitus.

Risk factors of tinnitus include hearing loss, sound exposure, stress, anxiety, depression, ototoxic drugs, hypertension, and aging. While the association between individual risk factors and tinnitus is not straightforward, tinnitus seems to be correlated with advancing age and hearing loss (Baguley et al., 2013). Interestingly, only about half of patients with tinnitus have recognized risk factors, which is a reason it has been hypothesized that predisposition to tinnitus is linked with genetic background (Shargorodsky et al., 2010).

Secondary tinnitus has been conventionally recognized as a symptom of a variety of monogenic disorders for which many genes or loci have already been identified (**Table 1**). In contrast, recognition of chronic primary tinnitus may be obscured by non-Mendelian inheritance patterns, which contribute to a lack of awareness and underreporting of tinnitus within families and among relatives (Sand et al., 2007). The association between genetic factors and primary tinnitus has historically lacked consensus and replication. Tinnitus could result from a number of pathological processes involving peripheral (cochlear) and/or central auditory abnormalities. The lack of consensus concerning these mechanisms asserts that further research is required.

Identification of genetic factors would provide important insights into the pathogenesis of tinnitus, facilitate understanding of the course and severity of tinnitus burden on patients, and permit novel diagnostic strategies. The majority of research investigations dissecting the genetics of tinnitus have taken the form of association studies that have revealed few borderline-significant results (**Table 2**). Recently, a genome-wide association study (GWAS) has identified potential metabolic pathways meriting further investigation (Gilles et al., 2017) and two twin-study cohorts have uncovered heritability estimates that provide pioneering insight into moderate genetic influences for tinnitus (Bogo et al., 2016; Maas et al., 2017). Although an excellent review discussing the genetics of tinnitus that touches upon phenotyping strategies and proposed pathophysiological mechanisms has been recently published (Lopez-Escamez et al., 2016), the present review exclusively emphasizes the genetic studies that have been published to date, discusses emerging data that suggests a complex or multifactorial genetic etiology, and presents an outlook for future research.

COMPLEX GENETICS APPROACHES

Heritability of tinnitus is defined as tinnitus variance explained by additive genetic factors. The earliest attempts of estimating the heritability of tinnitus stemmed from large family-based questionnaire studies. One of these studies assessed familial aggregation in seven European countries that proposed a sibling-sibling tinnitus correlation of 0.16 in 981 siblings and an increased 1.7-fold likelihood of developing tinnitus with an affected sibling. However, the authors rationalized that this could be due to increased tinnitus awareness within families (Hendrickx et al., 2007). Another study analyzed questionnaire data in Norwegian nuclear families and considered genetic and environmental effects in subjects reporting tinnitus (Kvestad et al., 2010). Heritability estimates returned an upper limit value of 0.11. Criticisms of this study remarked on a lack of attention to questionnaire design and phrasing. Additionally, although the study was conducted as a family-based approach, tinnitus sub-typing of the 28,066 participating individuals was not undertaken and similar replication studies have not followed (Sand, 2011).

Despite the considerable prevalence of tinnitus, the lack of Mendelian inheritance and genetic factors implicated from these early studies support that tinnitus is a complex trait. The identification of complex disease alleles is very challenging in the presence of potential genotype-by-environment interaction, incomplete penetrance, environmental phenocopies, genetic heterogeneity, or polygenic inheritance (Lander and Schork, 1994; Silverman and Palmer, 2000). Complex genetic disorders result from relatively common variants in multiple genes that each contribute effects of varying magnitude and are connected to variants that predispose an individual to a disorder rather than directly causing it (Zondervan and Cardon, 2007). Genetic dissection of tinnitus has followed several different paths that include candidate gene association, twin, and GWAS that are summarized below and in **Figure 1**.

Association Studies

Association studies can take the form of hypothesis-driven candidate gene or hypothesis-free GWAS, with the latter described in a subsequent section in this review. Case-control association testing compares genotype frequencies between unaffected and affected individuals and takes considerable differences between these two groups as evidence for or against disease susceptibility. Association studies in complex disorders can yield useful information if findings are replicated, or alternatively, if associations are confirmed with linkage analyses studying large families. Examples of replicated association findings are the discovery of the genes *ANXA11* and *BTNL2* in sarcoidosis and *DTNBP1* and *NRG1* in schizophrenia (Riley and Kendler, 2006; Spagnolo and du Bois, 2007). However, such findings are rather rare and many studies run the risk of artefactual positive association due to case-control selection bias, population admixture, or alleles residing in linkage disequilibrium (LD) with an allele directly affecting phenotype expression. Furthermore, in candidate gene association studies, the actual gene(s) of interest must already be identified for sequencing or genotyping. Late-onset disorders make selecting control groups challenging and can present a problem in young asymptomatic or undiagnosed individuals with risk alleles (Silverman and Palmer, 2000).

Case-control association testing has been a relatively widely employed approach and has comprised the majority of genetics research conducted in tinnitus patients to date (**Table 2**, **Figure 1**). Tinnitus candidate gene selection has included genes enriched in cardiovascular function (*ACE*, *ADD1*), neurotrophic

TABLE 1 | Monogenic disorders associated with secondary tinnitus with variable onset and severity.

Gene	DFN Locus	MIM	Gene function	Disorder	References
ACTG1	DFNA20/26	102560	Actin gamma 1	Autosomal dominant non-syndromic hearing loss	de Heer et al., 2009
AIFM1	AUNX1	300169	Apoptosis inducing factor, mitochondria associated 1	X-linked non-syndromic hearing loss	Wang et al., 2006; Zong et al., 2015
ANKH	–	605145	ANKH inorganic pyrophosphate transport regulator	Craniometaphyseal dysplasia	Kornak et al., 2010
ATP1A2	–	182340	ATPase Na^+/K^+ transporting subunit alpha 2	Familiar basilar migraine	Ambrosini et al., 2005
CACNA1A	–	601011	Calcium channel, voltage-dependent, P/Q type, alpha-1A subunit	Episodic ataxia type II	Wan et al., 2011
CEACAM16	DFNA4B	614591	Carcinoembryonic antigen related cell adhesion molecule 16	Autosomal dominant non-syndromic hearing loss	Wang et al., 2015
COCH	DFNA9	603196	Cochlin	Autosomal dominant non-syndromic hearing loss	Gallant et al., 2013
COL1A1	–	120150	Collagen, type I, alpha-1	Osteogenesis imperfecta type I	Kuurila et al., 2003
COL1A2	–	120160	Collagen, type I, alpha-2	Osteogenesis imperfecta	Kuurila et al., 2003
DIABLO	DFNA64	605219	Diablo IAP-binding mitochondrial protein	Autosomal dominant non-syndromic hearing loss	Cheng et al., 2011
DSPP	–	125485	Dentin sialophosphoprotein	Dentinogenesis imperfecta with or without progressive hearing loss	Xiao et al., 2001
DTNA	–	601239	Dystrobrevin alpha	Autosomal dominant familial Ménière disease	Requena et al., 2015
FAM136A	–	616275	Family with sequence similarity 136 member A	Autosomal dominant familial Ménière disease	Requena et al., 2015
GJB2	DFNA3A	121011	Gap junction protein beta-2	Autosomal dominant non-syndromic hearing loss	Wang et al., 2017
GJB2	DFNB1	121011	Gap junction protein beta-2	Autosomal recessive non-syndromic hearing loss	Dodson et al., 2011
GJB3	DFNA2A	603324	Gap junction protein beta-3	Autosomal dominant non-syndromic hearing loss	Coucke et al., 1994; Xia et al., 1998
GLA	–	300644	Galactosidase, alpha	Fabry disease	Germain et al., 2002; Conti and Sergi, 2003
JAK2	–	147796	Janus kinase 2	Polycythemia vera	Mihalj et al., 2013
KCNQ4	DFNA2A	603537	Potassium channel, voltage-gated channel subfamily member 4	Autosomal dominant non-syndromic hearing loss	Kubisch et al., 1999
MFN2	–	608507	Mitofusin 2	Hereditary motor and sensory neuropathy VI	Voo et al., 2003
MIR96	DFNA50	611606	MicroRNA 96	Autosomal dominant non-syndromic hearing loss	Modamio-Høybjør et al., 2004; Mencía et al., 2009
MT-TS1	–	590080	Mitochondrially encoded tRNA serine 1	Mitochondrial non-syndromic hearing loss	Chapiro et al., 2002
MT-RNR1	–	561000	Mitochondrially encoded 12S RNA	Mitochondrial non-syndromic hearing loss	Matsunaga et al., 2004; Bravo et al., 2006
MYO7A	DFNA11	276903	Myosin VIIA	Autosomal dominant non-syndromic hearing loss	Sun et al., 2011
NAGA	–	104170	Alpha-N-acetylgalactosamineidase	Kanzaki disease	Kodama et al., 2001
NF2	–	607379	Neurofibromin 2	Neurofibromatosis type 2	Evans et al., 1992
OSBPL2	DFNA67	606731	Oxysterol-binding protein-like protein 2	Autosomal dominant non-syndromic hearing loss	Xing et al., 2015
P2RX2	DFNA41	600844	Purinergic receptor P2X 2	Autosomal dominant non-syndromic hearing loss	Yan et al., 2013
PRKCB	–	176970	Protein kinase C beta	Autosomal dominant familial Ménière disease	Martín-Sierra et al., 2016
PRPS1	DFNX1	311850	Phosphoribosyl pyrophosphate synthetase 1	X-linked non-syndromic hearing loss	Liu et al., 2010
*SDHB**	–	185470	Succinate dehydrogenase complex, subunit B, iron sulfur protein	Paragangliomas 4	Bayley et al., 2006; Sagong et al., 2016
*SDHC**	–	602413	Succinate dehydrogenase complex, subunit C, integral membrane protein, 15-KD	Paragangliomas 3	Bickmann et al., 2014
*SDHD**	–	602690	Succinate dehydrogenase complex, subunit D, integral membrane protein	Paragangliomas 1	Badenhop et al., 2001; Tan et al., 2009
TMC1	DFNA36	606706	Transmembrane cochlear expressed gene 1	Autosomal dominant non-syndromic hearing loss	Zhao et al., 2014

(Continued)

TABLE 1 | Continued

Gene	DFN Locus	MIM	Gene function	Disorder	References
VHL	–	608537	von Hippel-Lindau tumor suppressor	von Hippel-Lindau syndrome	Butman et al., 2007
WFS1	DFNA6/14/38	606201	Wolframin ER transmembrame glycoprotein	Autosomal dominant non-syndromic hearing loss, low-frequency hearing loss	Lesperance et al., 1995
Unknown	DFNA16	603964	–	Autosomal dominant non-syndromic hearing loss	Fukushima et al., 1999
Unknown	DFNA33	614211	–	Autosomal dominant non-syndromic hearing loss	Bönsch et al., 2009
Unknown	DFNA43	608394	–	Autosomal dominant non-syndromic hearing loss	Flex et al., 2003
Unknown	DFNA57	–	–	Autosomal dominant non-syndromic hearing loss	Bönsch et al., 2008
Unknown	DFNA58	615654	–	Autosomal dominant non-syndromic hearing loss	Lezirovitz et al., 2009
Unknown	DFNY1	400043	–	Y-linked hearing loss	Wang et al., 2013

*Pulsatile tinnitus (tympanic paraganglioma) associated.

factors (*BDNF*, *GDNF*), ion recycling pathways (*KCNE1*, *KCNE3*, *SLC12A2*), $GABA_B$ receptor subunit (*KCTD12*), and serotonin receptor/transporter (*HTR1A*, *SLC6A4*) function. Presently, nine case-control studies have examined a combined total of 18 genes that are summarized in **Table 2** (Kleinjung et al., 2006; Deniz et al., 2010; Sand et al., 2010, 2011, 2012a,b; Pawełczyk et al., 2012; Orenay-Boyacioglu et al., 2016; Yüce et al., 2016). For brevity, we describe selected case-control association studies with potentially significant results.

Cardiovascular-Associated Gene

ADD1 encodes ubiquitously expressed alpha-adducin. A well-studied polymorphism (p.G460W) has been linked to cardiovascular disease and hypertension (Staessen and Bianchi, 2005). Hypertension-associated auditory primary lesion sites are the organ of Corti and stria vascularis (Gates et al., 1993). An association study investigated the relationship between severe chronic tinnitus and the p.G460W polymorphism in 89 patients with severe chronic tinnitus and 104 age-matched Turkish-Caucasian controls (Yüce et al., 2016). Clinical tinnitus evaluation and severity assessment were performed by the Structured Tinnitus Interview and the Tinnitus Handicap Inventory, respectively. PCR-based restriction fragment length polymorphism (RFLP) analysis of the *ADD1* GW genotype ($p = 0.009$, $\chi^2 = 9.4$) and the W allele ($p = 0.021$, $\chi^2 = 5.3$) revealed significantly increased allele frequencies in the patient group (Yüce et al., 2016). This study asserted the potential involvement of the p.G460W genotype and W allele in *ADD1* in tinnitus pathophysiology.

Neurotrophic Factors

Tinnitus is thought to stem from central nervous system hyperexcitability and auditory cortical neuronal plasticity. Accumulating evidence indicates that tinnitus adaptation is dependent on cortical tonotopic map remodeling (Eggermont, 2016). An understanding of neurotrophins as important drivers of neural circuit remodeling in the auditory pathway have rationalized their relevance as candidate genes for tinnitus (Tan et al., 2007; Sand et al., 2012b).

PCR-based RFLP analysis in 240 German patients with Tinnitus Questionnaire (TQ)-scored subjective chronic primary tinnitus (Goebel and Hiller, 1994) has been performed for the genes *BDNF* and *GDNF*, encoding brain and glial cell-derived neurotrophic factors, respectively (Sand et al., 2012b). Both genes are essential in early central auditory pathway development. Two and three markers were investigated in *BDNF* (rs2049046 and rs6265) and *GDNF* (rs1110149, rs884344, and rs3812047), respectively. Comparison with reference data did not show significance after multiple testing correction; however, the authors could not exclude a weak modulatory effect. Furthermore, questionnaire intensity scores did not correlate with genetic variants, although notably, age-corrected multiple regression models with joint *BDNF* and *GDNF* genotypes indicated tinnitus severity could be predicted in women ($p = 0.04$, uncorrected; Sand et al., 2012b).

These three *GDNF* markers were similarly screened in a replication study including 52 Turkish patients with chronic tinnitus and 42 controls aged between 18 and 55 years (Orenay-Boyacioglu et al., 2016). No statistically significant distribution was detected in allele frequencies for all three markers between tinnitus and control groups. The only parameter reaching significance was heterozygosity (C:G) in the SNP rs1110149 ($p = 0.02$, χ^2), that was found to have a lower frequency in tinnitus patients compared to controls (Orenay-Boyacioglu et al., 2016).

Potassium Recycling Pathway Genes

Pharmacological research has highlighted ion regulation and transport as potential therapeutic targets (Sand et al., 2011). Voltage-gated ion channels that are involved in auditory neural transmission by regulating endocochlear potentials are intriguing for exploration of tinnitus pathophysiology (Sand et al., 2010). The genes *KCNE1* and *SLC12A2* each encode a homologous β-potassium channel subunit and an inner ear $Na^+/2Cl^-/K^+$ co-transporter, respectively, which have been screened in case-control association studies (Sand et al., 2010; Pawełczyk et al., 2012).

KCNE1 screening in 201 Caucasian chronic TQ-scored tinnitus patients detected four coding and three non-coding variants, including one novel p.Val47Ile substitution and another novel 3′ UTR variant that were concluded as having a non-significant ($p = 0.05$, Fisher's exact test) dominant genotype or compound genotype effect without correction for multiple

TABLE 2 | Genes screened in tinnitus candidate gene studies.

Gene	MIM	Gene function	Number of subjects	Phenotype	Method	Outcome	Stastical power	Multiple testing correction	References
CARDIOVASCULAR ASSOCIATED GENES									
ACE	106180	Angiotensin I converting enzyme	89	Severe chronic tinnitus	PCR-RFLP genotyping	-No significance	Not described	Not described	Yüce et al., 2016
ADD1	102680	Adducin 1	89	Severe chronic tinnitus	PCR-RFLP genotyping	-The p.G460W heterozygous genotype ($p = 0.009$) and W allele ($p = 0.021$) are statistically significantly higher in patients than controls	Not described	Not described	Yüce et al., 2016
NEUROTROPHIC FACTORS									
BDNF	113505	Brain derived neurotrophic factor	240	Chronic tinnitus	PCR-RFLP genotyping	-No correlation between tinnitus and rs2049046 and rs6265 polymorphisms	Underpowered, nearly 15,000 patients and >680,000 controls required for exclusion of a modifying risk from rs6265	Yes	Sand et al., 2012b
GDNF	600837	Glial cell derived neurotrophic factor	52	Chronic tinnitus	PCR-RFLP genotyping	-No correlation between tinnitus and rs884344, rs3812047 and rs1110149 polymorphisms -Heterozygosity was significantly lower ($p = 0.02$) for rs1110149 between patients and controls	Not described	Not described	Orenay-Boyacioglu et al., 2016
			240	Chronic tinnitus	PCR-RFLP genotyping	-No correlation between tinnitus and rs1110149, rs884344 and rs3812047 polymorphisms	Underpowered	Yes	Sand et al., 2012b
POTASSIUM RECYCLING PATHWAY GENES									
KCNE1	176261	Potassium voltage-gated channel subfamily E regulatory subunit 1	201	Chronic tinnitus	Sanger sequencing	-No correlation between tinnitus severity and 46 polymorphic variants -V47I novel variant detected	Underpowered, >12,500 patients required to exclude a modifying allele risk	No	Sand et al., 2010
			128	Noise exposed males with tinnitus	SNP genotyping	-Significance was detected in rs915539 ($p = 0.005$) in noise-resistant subjects and when comparing tinnitus patients vs. controls in noise-resistant and susceptible groups ($p = 0.018$)	Not described	No	Pawełczyk et al., 2012
KCNE3	604433	Potassium voltage-gated channel subfamily E regulatory subunit 3	288	Chronic tinnitus	Sanger sequencing	-No association between tinnitus and 11 polymorphic variants	Underpowered, 2,707 patients and 65,083 controls required	No	Sand et al., 2011
SLC12A2	600840	Solute carrier family 12 member 2	128	Noise exposed males with tinnitus	SNP genotyping	-Significance was detected in rs10089 ($p = 0.016$) in noise susceptible subjects and when comparing tinnitus patients vs. controls in noise-resistant and susceptible groups ($p = 0.026$)	Not described	No	Pawełczyk et al., 2012
$GABA_B$ RECEPTOR SUBUNIT									
KCTD12	610521	Potassium channel tetramerization domain containing 12	95	Chronic tinnitus	Sanger sequencing	-rs34544607 was associated with tinnitus ($p = 0.04$) but weakened after screening 50 additional cases ($p = 0.07$) -Gene did not predict tinnitus severity	Underpowered, 363 tinnitus cases required	No	Sand et al., 2012a

(Continued)

TABLE 2 | Continued

Gene	MIM	Gene function	Number of subjects	Phenotype	Method	Outcome	Stastical power	Multiple testing correction	References
SEROTONIN RECEPTOR/TRANSPORTER									
HTR1A	109760	5-hydroxytrypthamine receptor 1A	88	Chronic tinnitus	Sanger sequencing	-No correlation between tinnitus and rs1800043 polymorphism	Not described	Not described	Kleinjung et al., 2006
SLC6A4	182138	Solute carrier family 6 member 4	54	Chronic tinnitus	PCR and VNTR analysis	-Association between quality of life scores (severity, $p = 0.004$; tinnitus discomfort level, $p = 0.002$; attention deficit, $p = 0.04$; sleep disorder, $p = 0.04$) and patients with the 5-HTTLPR polymorphism	Not described	Not described	Deniz et al., 2010

PCR-RFLP: polymerase chain reaction-restriction fragment length polymorphism; VNTR: variable number tandem repeat
The study from Pawelczyk et al. also included the analysis of eight further genes involved in the potassium recycling pathway (GJB1, GJB2, GJB3, GJB4, GJB6, KCNJ10, KCNQ1, KCNQ4) but results were not significant (Pawelczyk et al., 2012).

testing (Sand et al., 2010). These variants were not found to be causal by themselves or in compound heterozygosity for chronic TQ-scored tinnitus.

In a Polish genotyping study that included 10 potassium recycling genes, 128 noise-exposed subjects with tinnitus, and 498 noise-exposed controls responded to a questionnaire and underwent analysis. Case and control groups were divided into noise-resistant (normal audiograms) and noise-susceptible (abnormal audiograms) groups and individuals with a family history of hearing loss and other clinical indications or medication exposures were excluded. *KCNE1* and *SLC12A2* were associated with tinnitus based on significance in only one genetic marker per gene (rs915539 in *KCNE1*, $p = 0.018$; rs10089 in *SLC12A2*, $p = 0.026$). *p*-values were not subjected to multiple testing correction and, therefore, suggested as nominally significant results (Pawełczyk et al., 2012).

$GABA_B$ Receptor Subunit

Abundant data support chronic tinnitus with neuronal hyperactivity at different levels of the central auditory pathway, making drugs that increase inhibitory neurotransmission or block excitatory neurotransmission candidates for the treatment of tinnitus, and genes encoding these respective receptor complexes of potential interest (Eggermont and Roberts, 2004; Wang et al., 2011; Smith et al., 2012; Sand et al., 2012a). The gene *KCTD12* encodes a potassium channel tetramerization domain-containing protein that is tightly associated with the $GABA_{B2}$ receptor carboxy-terminus (Sand et al., 2012a) and was subsequently subjected to association testing. The genomic DNAs from 95 German chronic TQ-scored tinnitus patients were obtained and the *KCTD12* open reading frame and adjacent 3′ untranslated regions were sequenced. Two rare synonymous and non-coding heterozygous variants were detected. Further, analysis disclosed one significant tinnitus-associated variant (rs34544607; $p = 0.04$, Fisher's exact test), but this significance weakened after screening 50 additional cases ($p = 0.07$, Fisher's exact test). No novel variants were detected and no variants were correlated with or predicted intensity of tinnitus; however, the authors acknowledge the study was underpowered.

Serotonin Transporter

There is considerable overlap in patients reporting disabling tinnitus in conjunction with other comorbidities and a particularly strong association among patients with comorbid depressive disorder that affects ∼5–10% of the general population. There is an estimated 30% concordant overlap between comorbid depressive disorder and tinnitus that implies common molecular mechanisms and, therefore, overlapping genes attributing to both phenotypes (Tyler et al., 2006). As such, genes involved in serotonin regulation, a critical process associated with depressive psychiatric disorders, have been proposed as tinnitus candidate genes. Serotonin is present in hair cells, eighth nerve fibers, brainstem auditory nuclei and nuclei of the lateral lemniscus and superior olivary complex (Tyler et al., 2006).

The gene *SLC6A4* regulates serotonin neurotransmission and has been evaluated for tinnitus-association (Tyler et al.,

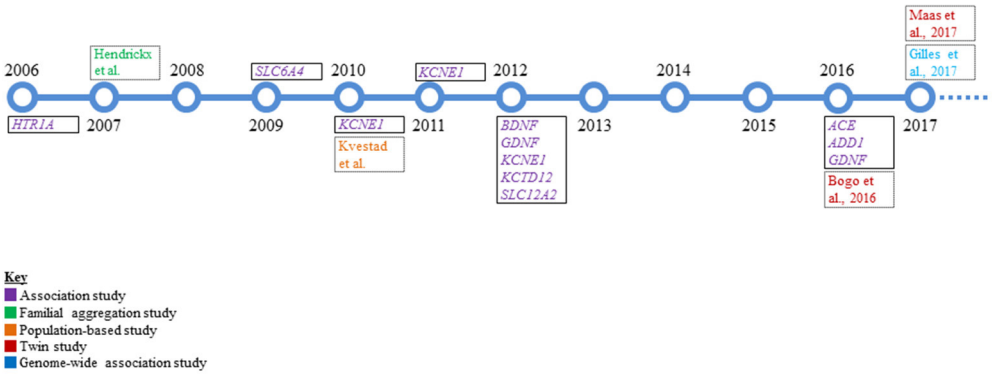

FIGURE 1 | A timeline overview of the genetic research in tinnitus that has been conducted to date. Single genes that were studied via candidate gene association analysis are boxed with a solid black line and represent the majority of work performed in the genetics of tinnitus. All other studies are boxed with a dotted black line.

2006; Deniz et al., 2010). A functional 5′-HTTLPR 44 base pair insertion-deletion polymorphism in the promoter region has been implicated in major depressive disorder (Hoefgen et al., 2005). This polymorphism and a 17 base pair variable number tandem repeat region in intron 2 were screened in 54 patients with subjective tinnitus and 174 population-matched controls. Tinnitus severity and psychoacoustic characteristics were assessed using the Beck Depression Inventory and visual analog scale, respectively. A significant association was detected between the 5′-HTTLPR polymorphism and visual analog scores that measured tinnitus quality of life impact using χ^2 tests (severity, $p = 0.004$; tinnitus discomfort level, $p = 0.002$; attention deficit, $p = 0.04$; sleep disorder, $p = 0.04$). This study linked a polymorphism in the *SLC6A4* promoter with neurophysiological symptoms in tinnitus patients (Deniz et al., 2010).

GWAS

GWAS can be powerful for the association of common variants and genetic loci in complex disorders and are appropriate for dissection of the "common disease-common variant" hypothesis. This hypothesis assumes a significant proportion of phenotypic divergence arises from common variants, typically with a minor allele frequency >5%, and that these variants are important for disease susceptibility (Sharma et al., 2014).

GWAS utilizes up to several million SNP genotypes most commonly generated from genotyping arrays to tag haplotype blocks, occasionally spanning more than 100 kb, on which functional variants reside. These studies utilize non-random co-inheritance of variants in linkage disequilibrium (LD) to test for case-control trait association (Edwards et al., 2013). *p*-value thresholds for statistical significance are very rigorous, typically below 10^{-9}, to reduce the likelihood of false positive results, accommodate multiple testing burden, and provide enough stringency in studies that include lower frequency variants (minor allele frequency >5%; LaFramboise, 2009; Fadista et al., 2016). The conclusion after a successful GWAS is that one or more tag SNPs co-reside on haplotype blocks with variants having a biological function related to the phenotype. Interestingly, over 90% of disease-associated variants from GWAS reside in non-coding regions associated with transcriptional regulatory mechanisms involving promoter and enhancer element modulation (Maurano et al., 2012; Edwards et al., 2013). Following detection of statistically significant association signals, replication and functional experiments are required. A deeper understanding of these variants in a biological context requires experiments analyzing pathogenicity mechanisms such as transcriptional regulation, non-coding RNA function, and epigenetic regulation (Edwards et al., 2013). Functional assessment includes expression quantitative trait loci testing, *in vitro* protein and chromatin-structure assay analysis, as well as model organism experiments (Lee et al., 2014).

The first cross-sectional pilot tinnitus GWAS in ethnically homogeneous individuals between 55 and 65 years old was performed using 167 individuals with tinnitus and 749 non-tinnitus controls from Belgium (Gilles et al., 2017). These patients were previously included in a GWAS for age-related hearing loss in which a polygenic architecture was detected (Fransen et al., 2015). The association between tinnitus phenotype and 4,000,000 SNPs was tested and a gene-set enrichment analysis followed. 3.2% of the phenotypic variance was due to additive genetic effects. Although none of the SNPs reached genome-wide significance, potentially attributed to the limited sample size, the most interesting associations were revealed in the gene-set enrichment analysis that showed significance in seven metabolic pathways. The three most prominent pathways detected were the nuclear factor erythroid 2 like 2 (*NRF2*)-mediated oxidative stress response, endoplasmic reticulum (ER) stress response and serotonin reception mediated signaling pathways with low FDR-corrected *p*-values ranging from 0.004 to 0.02. NRF2-mediated oxidative stress plays a role in noise-induced hearing loss and tinnitus. Interestingly, tinnitus patients have been identified with substantially increased oxidative index levels compared to controls (Delmaghani et al., 2015; Koç et al., 2016). Moreover, ER stress has been associated with

hearing loss via apoptosis (Van Rossom et al., 2015; Xue et al., 2016). Delayed hearing loss progression has been shown in transgenic mice with ER stress inhibitor treatment (Hu et al., 2016). Evidence of serotonin receptor mediated pathway involvement has been proposed from an apparent beneficial outcome of antidepressant usage among tinnitus patients (Baldo et al., 2012). Other pathways reaching significance include RAS, vascular smooth muscle contraction, coenzyme A biosynthesis, and NDK dynamin pathways (Gilles et al., 2017). Several limitations described by the authors included limited power to detect significant individual tinnitus-associated SNPs, the sample set was not selectively enriched for tinnitus patients and comprehensive controlling for risk factors was not undertaken. However, insight into seven potentially implicated pathways in tinnitus was highlighted from gene-set enrichment analysis.

Twin Studies

Twin-based epidemiological studies serve as a means to estimate heritability by comparing disease concordance in monozygotic (MZ) vs. dizygotic (DZ) twins. Increased concordance in genetically identical MZ vs. DZ twins, who share on average half of their alleles, suggests a role for genetic factors. It is assumed both MZ and DZ twins share the same family environment, thus yielding important information about the contribution of genetic factors to disease etiology. Two recent twin studies have been published. While there are differences in the experimental approaches that are detailed below, they independently concluded that genetic factors contribute to tinnitus.

A twin study by Bogo and colleagues evaluated the genetic effects of self-reported tinnitus in male twins aged 52–96 years who were included in a previous longitudinal study (Bogo et al., 2015) that analyzed genetic influence of age-related hearing loss. Male MZ and DZ twins who were born between 1914 and 1958 were included in baseline ($n = 1084$ individuals) and follow-up ($n = 576$ individuals) assessments 18 years apart that included audiometry and self-reported answers to questions about tinnitus status and severity. The hypothesis was that individuals with faster hearing deterioration had the greatest tinnitus risk and that genetic factors influenced tinnitus (Bogo et al., 2016).

No difference in tinnitus prevalence between MZ and DZ twins at either time point was detected and those who reported tinnitus disclosed a mild severity. Individuals ($n = 576$) were placed in one of four categories (never reported tinnitus, $n = 361$; tinnitus only at baseline, $n = 24$; tinnitus only at follow-up, $n = 139$; and tinnitus at both time points, $n = 52$). Those who reported tinnitus at baseline and both baseline and follow-up assessments showed remarkably poorer hearing at follow-up across all frequencies compared to the reference group (those never reporting tinnitus). Those with tinnitus only at follow-up did not have significantly different hearing thresholds compared to the reference group. MZ twin concordant rates were much higher than for DZ twins at both time points (baseline: MZ 0.46; DZ 0.07; follow-up: MZ 0.51; DZ 0.32), proposing that genetic factors were important. A genetic correlation measuring the extent of genetic influences correlated in tinnitus and hearing thresholds ranged from 0.33 to 0.49, and suggested a partial overlap of genes associated with tinnitus and hearing loss; however, the authors also concluded that most of the genetic variation in tinnitus was unique to tinnitus and not associated with co-occurring hearing loss. There was a greater hearing threshold difference between discordant DZ twin-pairs compared to MZ twin-pairs in cases and controls. Interestingly, the hearing thresholds among MZ twins discordant for tinnitus were more similar than for discordant DZ twins, which may be due to genetic background. Compared to controls, individuals with tinnitus have statistically significant hearing threshold shifts. An overall heritability of 0.4 was calculated, which demonstrates a moderate genetic influence on tinnitus. The authors disclosed that their study was underpowered, and that noise exposure and other risk factors were not assessed (Bogo et al., 2016).

Another twin study by Maas and colleagues took a slightly different approach and controlled for tinnitus laterality but did not assess hearing thresholds (Maas et al., 2017). Cross-sectional data from the Swedish Twin Registry, that includes participants from the "Screening Across the Lifespan Twin study" and the "Study of Twin Adults: Genes and Environment" (Magnusson et al., 2013) who were born between 1900 and 1985. Concordance rates between MZ and DZ twin-pairs ($n = 10{,}464$ twin pairs) were assessed. As opposed to the previously described study that enrolled only male twins, this study also included opposite-sexed DZ twins to assess differences due to sex and shared environments. A higher concordance of tinnitus was observed in MZ twins (0.32) vs. DZ twins (0.20). Concordance between DZ same-sex (0.20) and opposite-sex (0.19) twins, same-sex male DZ (0.11) vs. same-sex female DZ (0.13), as well as male MZ (0.25) vs. female MZ (0.23) were all similar. When comparing bilateral tinnitus concordance in both twin groups, a higher concordance rate was detected in MZ (0.49) vs. DZ (0.30) twins that was observed in both sexes. Younger MZ females (0.39) had a greater concordance than DZ females (0.20), but this was not observed in males. Heritability was greater in men (0.68) than in women (0.41), except when considering female twins younger than 40 years of age, where a heritability of 0.62 was determined, although it was noted by the authors that this was a highly variable group. Overall, this study concluded that while tinnitus may be environmentally driven, bilateral tinnitus may have a genetic etiology (Maas et al., 2017).

APPROACHES FOR FUTURE GENETIC STUDIES

Recently published data have begun to dissect a complex genetic basis for tinnitus. However, the general lack of consistent results is not surprising considering that the type of studies presently conducted have not been optimized through streamlined clinical patient classification criteria or by employing enhanced testing strategies of sufficient statistical power for tinnitus studies. There are many lessons that can be learned from previous analyses that include stratified patient selection and careful design of human genetic studies.

Addressing Phenotypic Heterogeneity in Tinnitus Genetics Studies

Identifying the most homogeneous tinnitus patients in terms of etiology, age, sex, severity, onset, and audiometric profile will increase study robustness by limiting genetic variance that would be expected if tinnitus is associated with these factors (Lopez-Escamez et al., 2016). Furthermore, consideration for co-occurring psychiatric disorders and quality of life evaluations are important to recognize as potentially contributing determinants (Langguth et al., 2013). The present collection of case-control association studies has grouped patients into unspecific "chronic tinnitus" cohorts from questionnaire data and only occasionally accounted for concomitant hearing loss, impeding the selection of stratified patient groups. The clinically heterogeneous nature of tinnitus makes assigning patients to one of many clinical sub-categories difficult. Current tinnitus assessments are comprised of self-report questionnaires and psychoacoustic measures (Langguth et al., 2013). There have been several classification systems proposed that consider the origin (i.e., auditory system or head and neck) or classify tinnitus according to auditory, somatosensory or psychopathology alterations (Levine and Oron, 2015). Homogeneous patient selection relies on accurate patient sub-typing; however, the present definition of tinnitus sub-types lacks consensus and is complicated by the many recognized etiologies and risk factors (Lopez-Escamez et al., 2016). The application of universal assessment protocols by clinicians would potentially benefit genetics studies in that once genetic datasets are obtained, the merging of multiple datasets by collaborating working groups can be streamlined to enhance recruitment of the several thousand patients and controls required for adequate statistical power. Furthermore, there are over 100 instruments available for clinical trial primary outcome measures, which further asserts there is a lack of tinnitus assessment consensus (Hall et al., 2016; Müller et al., 2016). Determining the standardized instruments used by practitioners would consolidate some of the assessment practices for accurate phenotyping.

Genetic Investigations in Tinnitus

The unraveling of the human genome has delivered basic knowledge of a reference genome, advanced fundamental knowledge of disease architecture, and catalyzed technological advancements that fuel complex trait research. Recent developments in genetics technology and increased affordability and availability of genotyping array and sequencing data will undoubtedly propel research in the field forward. The present body of research has begun to disclose a genetic architecture for tinnitus but there is still much work ahead for the identification of specific variants influencing critical gene expression and gene products in tinnitus pathophysiology. As previously discussed, study design and patient inclusion are particularly important for a clinically heterogeneous phenotype such as tinnitus. Equally important is the method selection to support appropriate scale and resolution of data for analysis. The next section will discuss the feasibility of case-control association testing, GWAS, and twin and familial approaches in the context of tinnitus research.

Case-Control and Genome-Wide Association Testing in Tinnitus

Population based association studies have long been a popular strategy to identify polymorphisms correlated with complex traits and have thus far been the most widely employed genetic study in tinnitus genetics research (**Table 2**). Historically, case-control candidate gene studies have not yielded abundant success and independent replications are often not possible. The same problem has also been encountered in tinnitus, which makes a reevaluation of the current and future study designs essential.

Future tinnitus association studies should overcome several problematic design setbacks. One key aspect is to exclude controls with unassessed or unrecognized tinnitus burden and select stratified patient cohorts with sufficient statistical power. Furthermore, control individuals should ideally be matched for age, sex, population background, stress/anxiety traits, and other recognized co-morbidities. Studying already characterized tinnitus patients with homogeneous tinnitus phenotypes, families with transgenerational tinnitus aggregation, or cohorts from previously performed epidemiological health studies where individuals experiencing chronic tinnitus can be re-contacted for in-depth tinnitus scoring and auditory assessment would be beneficial (Lopez-Escamez et al., 2016). Finally, drawing upon knowledge from current GWAS for complex traits such as body weight and neuropsychiatric disorders, tens to hundreds of thousands of cases and controls are required to pinpoint significant loci (Ripke et al., 2014; Locke et al., 2015). However, such studies also underscore the power of a GWAS approach for the association of common variants and genetic loci (LD blocks) with specific diseases or traits. Learning from the scale of studies that are required for other complex traits and considering the clinical complexity of tinnitus, it is likely that future studies in tinnitus need to be increased by several orders of magnitude.

GWAS

GWAS approaches have successfully dissected the genomic architecture of complex diseases and remain a robust approach for future tinnitus research. Looking more specifically at the collection of studies presently published, it is reasonable to assume several technological advances will transform study designs. The past decade has endowed affordable sequencing technologies that have revolutionized novel gene discovery by providing an avenue from which to effectively approach complex and Mendelian disorders (Koboldt et al., 2013). Next generation sequencing (NGS), also termed high-throughput sequencing, allows parallel amplification and sequencing of the entire protein coding region of the genome (whole exome sequencing) or the entire sequence of an individual's genome (whole genome sequencing). As the majority of significant findings from GWAS are tag SNP haplotype blocks in non-coding regions and NGS provides nucleotide-resolution of scalable coding and non-coding sequence (i.e., whole exome or whole genome sequencing), NGS can be regarded as a complementary methodology to GWAS and both methods have the potential

to contribute important findings to the field. This would be especially promising if a combination of causal rare coding and common non-coding regulatory variants underlie tinnitus pathology. Examples from Alzheimer and Parkinson disease research underscore this parallel approach from the implication of both common and rare variants in these diseases that were detected from GWAS and NGS approaches, respectively (Sharma et al., 2012, 2014; Guerreiro et al., 2013).

The present collection of case-control studies for tinnitus has emphasized the need for several design considerations. There has yet to be a tinnitus case-control study with adequate statistical power. Complex disorders are more challenging to detect common susceptibility alleles and require larger sample numbers to separate signal from noise and to detect frequent alleles of modest effect (Risch and Merikangas, 1996). Furthermore, population differences can have drastically differing allelic architecture that can complicate the mapping of putative risk factors when attempting association replication in patients of differing ethnicities (Sharma et al., 2014). Although underpowered, even if significance were achieved in the initial association study analyzing *GDNF* markers in German tinnitus patients, the replication study that followed studied the same three markers in a Turkish tinnitus group (Sand et al., 2012b; Orenay-Boyacioglu et al., 2016).

The only pilot tinnitus GWAS published to date in a Belgian tinnitus cohort has highlighted the worthwhile investment of this approach in tinnitus patients (Gilles et al., 2017). In light of these results, it would be worth repeating a GWAS with a stratified patient cohort of extremely severe tinnitus patients or patients who are young and therefore have a greater chance of having tinnitus due to a genetic etiology. The patients included would need to have the same tinnitus sub-type and controls should be specifically assessed for tinnitus. Replication using a tinnitus cohort would be important to highlight the same pathways and potentially achieve genome-wide significance.

Familial Aggregation and Twin Studies

Early studies utilizing questionnaire data from families with tinnitus returned low heritability estimates. Although both of the twin studies that have been performed each had their limitations and utilized different inclusion criteria, these recently published studies served as groundbreaking evidence suggesting tinnitus is multifactorial with both genetic and non-genetic factors contributing to its etiology (Bogo et al., 2016; Maas et al., 2017). Another central nervous system disorder, Parkinson disease, has witnessed a similar period of debate about genetic factors contributing to the disease. Early evidence disclosed low heritability estimates from twin studies (Wirdefeldt et al., 2004) and lack of familial aggregation (Levy et al., 2004), while concurrent evidence also uncovered familial aggregation (Maher et al., 2002; Payami et al., 2002; Marder et al., 2003) and heritability estimates were later calculated at 0.34 (Wirdefeldt et al., 2011). Continued research has indeed uncovered the genetic complexity of Parkinson disease. It remains to be seen whether continued research into the genetic underpinnings of tinnitus will uncover similar observations. With respect to twin studies, future studies would have to take into account the same clinical phenotyping and study design details that other genetic studies must also address.

FUTURE OUTLOOK

Understanding the genetic basis for tinnitus is of great public health significance that is clearly in its infancy. Many of the attempts dissecting the genetic contribution have likely been clouded by tinnitus heterogeneity that emphasizes the need for a consensus for tinnitus measures and increased sample size by several orders of magnitude. Recent heritability estimates from twin studies assert genetic factors are important in tinnitus etiology, which allows a potential understanding of basic molecular mechanisms of tinnitus and eventual diagnostic and therapeutic options. An improvement in study design will further clarify results emerging from genetics studies.

Although gene identification in tinnitus will itself represent a major advancement to the field, it will trigger several new lines of research. (1) Knowledge of candidate genes will permit basic research into the specific pathophysiological mechanisms involving animal models. A recent example is shown in a mouse knockout model with a glutamate aspartate transporter (GLAST) that leaves mice genetically susceptible to tinnitus-inducing agents such as salicylate. Such studies could contribute an understanding to how tinnitus is triggered and maintained (Yu et al., 2016). (2) Streamline effective research into drug development that profits from knowledge of specific mechanisms. (3) Clinical research into genotype-phenotype correlations can be based on knowledge of alleles involved. Furthermore, this may also enhance not only diagnostic development to support informed healthcare decisions, but also the identification of high-risk individuals to direct preventative care.

The impact from research breakthroughs analyzing the genetics of tinnitus would be enormous for tinnitus sufferers and would allow personalized and optimized therapies to be possible for tinnitus patients. The most promising data are yet to emerge and will provide much needed insights into the role of genetics in primary chronic tinnitus.

AUTHOR CONTRIBUTIONS

BV, IN, WS, and TH participated in manuscript preparation and editing. All authors have read and approved the final manuscript.

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REFERENCES

Ambrosini, A., D'Onofrio, M., Grieco, G. S., Di Mambro, A., Montagna, G., Fortini, D., et al. (2005). Familial basilar migraine associated with a new mutation in the ATP1A2 gene. *Neurology* 65, 1826–1828. doi: 10.1212/01.wnl.0000187072.71931.c0

Badenhop, R. F., Cherian, S., Lord, R. S., Baysal, B. E., Taschner, P. E., and Schofield, P. R. (2001). Novel mutations in the SDHD gene in pedigrees with familial carotid body paraganglioma and sensorineural hearing loss. *Genes Chromosomes Cancer* 31, 255–263. doi: 10.1002/gcc.1142

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Baldo, P., Doree, C., Molin, P., McFerran, D., and Cecco, S. (2012). Antidepressants for patients with tinnitus. *Cochrane Database Syst. Rev.* 12:Cd003853. doi: 10.1002/14651858.CD003853.pub3

Bayley, J. P., van Minderhout, I., Weiss, M. M., Jansen, J. C., Oomen, P. H., Menko, F. H., et al. (2006). Mutation analysis of SDHB and SDHC: novel germline mutations in sporadic head and neck paraganglioma and familial paraganglioma and/or pheochromocytoma. *BMC Med. Genet.* 7:1. doi: 10.1186/1471-2350-7-1

Bickmann, J. K., Sollfrank, S., Schad, A., Musholt, T. J., Springer, E., Miederer, M., et al. (2014). Phenotypic variability and risk of malignancy in SDHC-linked paragangliomas: lessons from three unrelated cases with an identical germline mutation (p.Arg133*). *J. Clin. Endocrinol. Metab.* 99, E489–E496. doi: 10.1210/jc.2013-3486

Bogo, R., Farah, A., Johnson, A. C., Karlsson, K. K., Pedersen, N. L., Svartengren, M., et al. (2015). The role of genetic factors for hearing deterioration across 20 years: a twin study. *J. Gerontol. A Biol. Sci. Med. Sci.* 70, 647–653. doi: 10.1093/gerona/glu245

Bogo, R., Farah, A., Karlsson, K. K., Pedersen, N. L., Svartengren, M., and Skjönsberg, Å. (2016). Prevalence, Incidence Proportion, and Heritability for Tinnitus: A Longitudinal Twin Study. *Ear Hear.* doi: 10.1097/AUD.0000000000000397. [Epub ahead of print].

Bönsch, D., Schmidt, C. M., Scheer, P., Bohlender, J., Neumann, C., Am Zehnhoff-Dinnesen, A., et al. (2009). [A new gene locus for an autosomal-dominant non-syndromic hearing impairment (DFNA 33) is situated on chromosome 13q34-qter]. *HNO* 57, 371–376. doi: 10.1007/s00106-008-1832-9

Bönsch, D., Schmidt, C. M., Scheer, P., Bohlender, J., Neumann, C., Am Zehnhoff-Dinnesen, A., et al. (2008). [A new locus for an autosomal dominant, non-syndromic hearing impairment (DFNA57) located on chromosome 19p13.2 and overlapping with DFNB15]. *HNO* 56, 177–182. doi: 10.1007/s00106-007-1633-6

Bravo, O., Ballana, E., and Estivill, X. (2006). Cochlear alterations in deaf and unaffected subjects carrying the deafness-associated A1555G mutation in the mitochondrial 12S rRNA gene. *Biochem. Biophys. Res. Commun.* 344, 511–516. doi: 10.1016/j.bbrc.2006.03.143

Butman, J. A., Kim, H. J., Baggenstos, M., Ammerman, J. M., Dambrosia, J., Patsalides, A., et al. (2007). Mechanisms of morbid hearing loss associated with tumors of the endolymphatic sac in von Hippel-Lindau disease. *JAMA* 298, 41–48. doi: 10.1001/jama.298.1.41

Chapiro, E., Feldmann, D., Denoyelle, F., Sternberg, D., Jardel, C., Eliot, M. M., et al. (2002). Two large French pedigrees with non syndromic sensorineural deafness and the mitochondrial DNA T7511C mutation: evidence for a modulatory factor. *Eur. J. Hum. Genet.* 10, 851–856. doi: 10.1038/sj.ejhg.5200894

Cheng, J., Zhu, Y., He, S., Lu, Y., Chen, J., Han, B., et al. (2011). Functional mutation of SMAC/DIABLO, encoding a mitochondrial proapoptotic protein, causes human progressive hearing loss DFNA64. *Am. J. Hum. Genet.* 89, 56–66. doi: 10.1016/j.ajhg.2011.05.027

Conti, G., and Sergi, B. (2003). Auditory and vestibular findings in Fabry disease: a study of hemizygous males and heterozygous females. *Acta Paediatr. Suppl.* 92, 33–37. discussion: 27. doi: 10.1111/j.1651-2227.2003.tb00219.x

Coucke, P., Van Camp, G., Djoyodiharjo, B., Smith, S. D., Frants, R. R., Padberg, G. W., et al. (1994). Linkage of autosomal dominant hearing loss to the short arm of chromosome 1 in two families. *N. Engl. J. Med.* 331, 425–431. doi: 10.1056/NEJM199408183310702

de Heer, A. M., Huygen, P. L., Collin, R. W., Oostrik, J., Kremer, H., and Cremers, C. W. (2009). Audiometric and vestibular features in a second Dutch DFNA20/26 family with a novel mutation in ACTG1. *Ann. Otol. Rhinol. Laryngol.* 118, 382–390. doi: 10.1177/000348940911800511

Delmaghani, S., Defourny, J., Aghaie, A., Beurg, M., Dulon, D., Thelen, N., et al. (2015). Hypervulnerability to sound exposure through impaired adaptive proliferation of peroxisomes. *Cell* 163, 894–906. doi: 10.1016/j.cell.2015.10.023

Deniz, M., Bayazit, Y. A., Celenk, F., Karabulut, H., Yilmaz, A., Gunduz, B., et al. (2010). Significance of serotonin transporter gene polymorphism in tinnitus. *Otol. Neurotol.* 31, 19–24. doi: 10.1097/MAO.0b013e3181c2dcbc

Dodson, K. M., Blanton, S. H., Welch, K. O., Norris, V. W., Nuzzo, R. L., Wegelin, J. A., et al. (2011). Vestibular dysfunction in DFNB1 deafness. *Am. J. Med. Genet. A* 155a, 993–1000. doi: 10.1002/ajmg.a.33828

Edwards, S. L., Beesley, J., French, J. D., and Dunning, A. M. (2013). Beyond GWASs: illuminating the dark road from association to function. *Am. J. Hum. Genet.* 93, 779–797. doi: 10.1016/j.ajhg.2013.10.012

Eggermont, J. J. (2016). Effects of long-term non-traumatic noise exposure on the adult central auditory system. Hearing problems without hearing loss. *Hear. Res.* S0378–5955(16)30442-7. doi: 10.1016/j.heares.2016.10.015

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Evans, D. G., Huson, S. M., Donnai, D., Neary, W., Blair, V., Newton, V., et al. (1992). A clinical study of type 2 neurofibromatosis. *Q. J. Med.* 84, 603–618.

Fadista, J., Manning, A. K., Florez, J. C., and Groop, L. (2016). The (in)famous GWAS *P*-value threshold revisited and updated for low-frequency variants. *Eur. J. Hum. Genet.* 24, 1202–1205. doi: 10.1038/ejhg.2015.269

Flex, E., Mangino, M., Mazzoli, M., Martini, A., Migliosi, V., Colosimo, A., et al. (2003). Mapping of a new autosomal dominant non-syndromic hearing loss locus (DFNA43) to chromosome 2p12. *J. Med. Genet.* 40, 278–281. doi: 10.1136/jmg.40.4.278

Fransen, E., Bonneux, S., Corneveaux, J. J., Schrauwen, I., Di Berardino, F., White, C. H., et al. (2015). Genome-wide association analysis demonstrates the highly polygenic character of age-related hearing impairment. *Eur. J. Hum. Genet.* 23, 110–115. doi: 10.1038/ejhg.2014.56

Fukushima, K., Kasai, N., Ueki, Y., Nishizaki, K., Sugata, K., Hirakawa, S., et al. (1999). A gene for fluctuating, progressive autosomal dominant nonsyndromic hearing loss, DFNA16, maps to chromosome 2q23–24.3. *Am. J. Hum. Genet.* 65, 141–150. doi: 10.1086/302461

Gallant, E., Francey, L., Fetting, H., Kaur, M., Hakonarson, H., Clark, D., et al. (2013). Novel COCH mutation in a family with autosomal dominant late onset sensorineural hearing impairment and tinnitus. *Am. J. Otolaryngol.* 34, 230–235. doi: 10.1016/j.amjoto.2012.11.002

Gates, G. A., Cobb, J. L., D'Agostino, R. B., and Wolf, P. A. (1993). The relation of hearing in the elderly to the presence of cardiovascular disease and cardiovascular risk factors. *Arch. Otolaryngol. Head Neck Surg.* 119, 156–161. doi: 10.1001/archotol.1993.01880140038006

Germain, D. P., Avan, P., Chassaing, A., and Bonfils, P. (2002). Patients affected with Fabry disease have an increased incidence of progressive hearing loss and sudden deafness: an investigation of twenty-two hemizygous male patients. *BMC Med. Genet.* 3:10. doi: 10.1186/1471-2350-3-10

Gilles, A., Van Camp, G., Van de Heyning, P., and Fransen, E. (2017). A pilot genome-wide association study identifies potential metabolic pathways involved in tinnitus. *Front. Psychol.* 8:71. doi: 10.3389/fnins.2017.00071

Goebel, G., and Hiller, W. (1994). The tinnitus questionnaire. A standard instrument for grading the degree of tinnitus. Results of a multicenter study with the tinnitus questionnaire. *HNO* 42, 166–172.

Guerreiro, R., Wojtas, A., Bras, J., Carrasquillo, M., Rogaeva, E., Majounie, E., et al. (2013). TREM2 variants in Alzheimer's disease. *N. Engl. J. Med.* 368, 117–127. doi: 10.1056/NEJMoa1211851

Hall, D. A., Haider, H., Szczepek, A. J., Lau, P., Rabau, S., Jones-Diette, J., et al. (2016). Systematic review of outcome domains and instruments used in clinical trials of tinnitus treatments in adults. *Trials* 17, 270. doi: 10.1186/s13063-016-1399-9

Hendrickx, J. J., Huyghe, J. R., Demeester, K., Topsakal, V., Van Eyken, E., Fransen, E., et al. (2007). Familial aggregation of tinnitus: a European multicentre study. *B-ENT* 3(Suppl. 7), 51–60.

Hoefgen, B., Schulze, T. G., Ohlraun, S., von Widdern, O., Höfels, S., Gross, M., et al. (2005). The power of sample size and homogenous sampling: association between the 5-HTTLPR serotonin transporter

polymorphism and major depressive disorder. *Biol. Psychiatry* 57, 247–251. doi: 10.1016/j.biopsych.2004.11.027

Hu, J., Li, B., Apisa, L., Yu, H., Entenman, S., Xu, M., et al. (2016). ER stress inhibitor attenuates hearing loss and hair cell death in Cdh23erl/erl mutant mice. *Cell Death Dis.* 7:e2485. doi: 10.1038/cddis.2016.386

Kleinjung, T., Langguth, B., Fischer, B., Hajak, G., Eichhammer, P., and Sand, P. G. (2006). Systematic screening of the seratonin receptor 1A (5-HT1A) gene in chronic tinnitus. *J. Otol.* 1, 83–85. doi: 10.1016/S1672-2930(06)50018-2

Koboldt, D. C., Steinberg, K. M., Larson, D. E., Wilson, R. K., and Mardis, E. R. (2013). The next-generation sequencing revolution and its impact on genomics. *Cell* 155, 27–38. doi: 10.1016/j.cell.2013.09.006

Koç, S., Akyüz, S., Somuk, B. T., Soyalic, H., Yilmaz, B., Taskin, A., et al. (2016). Paraoxonase activity and oxidative status in patients with tinnitus. *J. Audiol. Otol.* 20, 17–21. doi: 10.7874/jao.2016.20.1.17

Kodama, K., Kobayashi, H., Abe, R., Ohkawara, A., Yoshii, N., Yotsumoto, S., et al. (2001). A new case of alpha-N-acetylgalactosaminidase deficiency with angiokeratoma corporis diffusum, with Meniere's syndrome and without mental retardation. *Br. J. Dermatol.* 144, 363–368. doi: 10.1046/j.1365-2133.2001.04028.x

Kornak, U., Brancati, F., Le Merrer, M., Lichtenbelt, K., Höhne, W., Tinschert, S., et al. (2010). Three novel mutations in the ANK membrane protein cause craniometaphyseal dysplasia with variable conductive hearing loss. *Am. J. Med. Genet. A* 152a, 870–874. doi: 10.1002/ajmg.a.33301

Kubisch, C., Schroeder, B. C., Friedrich, T., Lütjohann, B., El-Amraoui, A., Marlin, S., et al. (1999). KCNQ4, a novel potassium channel expressed in sensory outer hair cells, is mutated in dominant deafness. *Cell* 96, 437–446. doi: 10.1016/S0092-8674(00)80556-5

Kuurila, K., Kentala, E., Karjalainen, S., Pynnönen, S., Kovero, O., Kaitila, I., et al. (2003). Vestibular dysfunction in adult patients with osteogenesis imperfecta. *Am. J. Med. Genet. A* 120a, 350–358. doi: 10.1002/ajmg.a.20088

Kvestad, E., Czajkowski, N., Engdahl, B., Hoffman, H. J., and Tambs, K. (2010). Low heritability of tinnitus: results from the second Nord-Trøndelag health study. *Arch. Otolaryngol. Head Neck Surg.* 136, 178–182. doi: 10.1001/archoto.2009.220

LaFramboise, T. (2009). Single nucleotide polymorphism arrays: a decade of biological, computational and technological advances. *Nucleic Acids Res.* 37, 4181–4193. doi: 10.1093/nar/gkp552

Lander, E. S., and Schork, N. J. (1994). Genetic dissection of complex traits. *Science* 265, 2037–2048. doi: 10.1126/science.8091226

Langguth, B., Kreuzer, P. M., Kleinjung, T., and De Ridder, D. (2013). Tinnitus: causes and clinical management. *Lancet Neurol.* 12, 920–930. doi: 10.1016/S1474-4422(13)70160-1

Lee, S., Abecasis, G. R., Boehnke, M., and Lin, X. (2014). Rare-variant association analysis: study designs and statistical tests. *Am. J. Hum. Genet.* 95, 5–23. doi: 10.1016/j.ajhg.2014.06.009

Lesperance, M. M., Hall, J. W. III, Bess, F. H., Fukushima, K., Jain, P. K., Ploplis, B., et al. (1995). A gene for autosomal dominant nonsyndromic hereditary hearing impairment maps to 4p16.3. *Hum. Mol. Genet.* 4, 1967–1972. doi: 10.1093/hmg/4.10.1967

Levine, R. A., and Oron, Y. (2015). Tinnitus. *Handb. Clin. Neurol.* 129, 409–431. doi: 10.1016/B978-0-444-62630-1.00023-8

Levy, G., Louis, E. D., Mejia-Santana, H., Côté, L., Andrews, H., Harris, J., et al. (2004). Lack of familial aggregation of Parkinson disease and Alzheimer disease. *Arch. Neurol.* 61, 1033–1039. doi: 10.1001/archneur.61.7.1033

Lezirovitz, K., Braga, M. C., Thiele-Aguiar, R. S., Auricchio, M. T., Pearson, P. L., Otto, P. A., et al. (2009). A novel autosomal dominant deafness locus (DFNA58) maps to 2p12-p21. *Clin. Genet.* 75, 490–493. doi: 10.1111/j.1399-0004.2008.01130.x

Liu, X., Han, D., Li, J., Han, B., Ouyang, X., Cheng, J., et al. (2010). Loss-of-function mutations in the PRPS1 gene cause a type of nonsyndromic X-linked sensorineural deafness, DFN2. *Am. J. Hum. Genet.* 86, 65–71. doi: 10.1016/j.ajhg.2009.11.015

Locke, A. E., Kahali, B., Berndt, S. I., Justice, A. E., Pers, T. H., Day, F. R., et al. (2015). Genetic studies of body mass index yield new insights for obesity biology. *Nature* 518, 197–206. doi: 10.1038/nature14177

Lopez-Escamez, J. A., Bibas, T., Cima, R. F., Van de Heyning, P., Knipper, M., Mazurek, B., et al. (2016). Genetics of tinnitus: an emerging area for molecular diagnosis and drug development. *Front. Neurosci.* 10:377. doi: 10.3389/fnins.2016.00377

Maas, I. L., Brüggemann, P., Requena, T., Bulla, J., Edvall, N. K., Hjelmborg, J. v. B., et al. (2017). Genetic susceptibility to bilateral tinnitus in a Swedish twin cohort. *Genet. Med.* doi: 10.1038/gim.2017.4. [Epub ahead of print].

Magnusson, P. K., Almqvist, C., Rahman, I., Ganna, A., Viktorin, A., Walum, H., et al. (2013). The Swedish Twin Registry: establishment of a biobank and other recent developments. *Twin Res. Hum. Genet.* 16, 317–329. doi: 10.1017/thg.2012.104

Maher, N. E., Golbe, L. I., Lazzarini, A. M., Mark, M. H., Currie, L. J., Wooten, G. F., et al. (2002). Epidemiologic study of 203 sibling pairs with Parkinson's disease: the GenePD study. *Neurology* 58, 79–84. doi: 10.1212/WNL.58.1.79

Marder, K., Levy, G., Louis, E. D., Mejia-Santana, H., Cote, L., Andrews, H., et al. (2003). Familial aggregation of early- and late-onset Parkinson's disease. *Ann. Neurol.* 54, 507–513. doi: 10.1002/ana.10711

Martín-Sierra, C., Requena, T., Frejo, L., Price, S. D., Gallego-Martinez, A., Batuecas-Caletrio, A., et al. (2016). A novel missense variant in PRKCB segregates low-frequency hearing loss in an autosomal dominant family with Meniere's disease. *Hum. Mol. Genet.* 25, 3407–3415. doi: 10.1093/hmg/ddw183

Matsunaga, T., Kumanomido, H., Shiroma, M., Ohtsuka, A., Asamura, K., and Usami, S. (2004). Deafness due to A1555G mitochondrial mutation without use of aminoglycoside. *Laryngoscope* 114, 1085–1091. doi: 10.1097/00005537-200406000-00024

Maurano, M. T., Humbert, R., Rynes, E., Thurman, R. E., Haugen, E., Wang, H., et al. (2012). Systematic localization of common disease-associated variation in regulatory DNA. *Science* 337, 1190–1195. doi: 10.1126/science.1222794

Mencía, A., Modamio-Høybjør, S., Redshaw, N., Morin, M., Mayo-Merino, F., Olavarrieta, L., et al. (2009). Mutations in the seed region of human miR-96 are responsible for nonsyndromic progressive hearing loss. *Nat. Genet.* 41, 609–613. doi: 10.1038/ng.355

Mihalj, M., Titlic, M., Bonacin, D., and Dogaš, Z. (2013). Sensomotor axonal peripheral neuropathy as a first complication of polycythemia rubra vera: a report of 3 cases. *Am. J. Case Rep.* 14, 385–387. doi: 10.12659/AJCR.884016

Modamio-Høybjør, S., Moreno-Pelayo, M. A., Mencía, A., del Castillo, I., Chardenoux, S., Morais, D., et al. (2004). A novel locus for autosomal dominant nonsyndromic hearing loss, DFNA50, maps to chromosome 7q32 between the DFNB17 and DFNB13 deafness loci. *J. Med. Genet.* 41:e14. doi: 10.1136/jmg.2003.012500

Müller, K., Edvall, N. K., Idrizbegovic, E., Huhn, R., Cima, R., Persson, V., et al. (2016). Validation of Online Versions of Tinnitus Questionnaires Translated into Swedish. *Front. Aging Neurosci.* 8:272. doi: 10.3389/fnagi.2016.00272

Orenay-Boyacioglu, S., Coskunoglu, A., Caki, Z., and Cam, F. S. (2016). Relationship between chronic tinnitus and glial cell line-derived neurotrophic factor gene rs3812047, rs1110149, and rs884344 polymorphisms in a Turkish Population. *Biochem. Genet.* 54, 552–563. doi: 10.1007/s10528-016-9741-1

Pawełczyk, M., Rajkowska, E., Kotyło, P., Dudarewicz, A., Van Camp, G., and Śliwińska-Kowalska, M. (2012). Analysis of inner ear potassium recycling genes as potential factors associated with tinnitus. *Int. J. Occup. Med. Environ. Health* 25, 356–364. doi: 10.2478/s13382-012-0061-3

Payami, H., Zareparsi, S., James, D., and Nutt, J. (2002). Familial aggregation of Parkinson disease: a comparative study of early-onset and late-onset disease. *Arch. Neurol.* 59, 848–850. doi: 10.1001/archneur.59.5.848

Requena, T., Cabrera, S., Martín-Sierra, C., Price, S. D., Lysakowski, A., and Lopez-Escamez, J. A. (2015). Identification of two novel mutations in FAM136A and DTNA genes in autosomal-dominant familial Meniere's disease. *Hum. Mol. Genet.* 24, 1119–1126. doi: 10.1093/hmg/ddu524

Riley, B., and Kendler, K. S. (2006). Molecular genetic studies of schizophrenia. *Eur. J. Hum. Genet.* 14, 669–680. doi: 10.1038/sj.ejhg.5201571

Ripke, S., Neale, B. M., Corvin, A., Walters, J. T. R., Farh, K.-H., Holmans, P. A., et al. (2014). Biological insights from 108 schizophrenia-associated genetic loci. *Nature* 511, 421–427. doi: 10.1038/nature13595

Risch, N., and Merikangas, K. (1996). The future of genetic studies of complex human diseases. *Science* 273, 1516–1517. doi: 10.1126/science.273.5281.1516

Sagong, B., Seo, Y. J., Lee, H. J., Kim, M. J., Kim, U. K., and Moon, I. S. (2016). A mutation of the succinate dehydrogenase B gene in a Korean family with paraganglioma. *Fam. Cancer* 15, 601–606. doi: 10.1007/s10689-016-9874-8

Sand, P. G. (2011). *Genetic Risk Factors in Chronic Tinnitus*. New York, NY: Springer.

Sand, P. G., Langguth, B., Itzhacki, J., Bauer, A., Geis, S., Cárdenas-Conejo, Z. E., et al. (2012a). Resequencing of the auxiliary GABA(B) receptor subunit gene KCTD12 in chronic tinnitus. *Front. Syst. Neurosci.* 6:41. doi: 10.3389/fnsys.2012.00041

Sand, P. G., Langguth, B., and Kleinjung, T. (2011). Deep resequencing of the voltage-gated potassium channel subunit KCNE3 gene in chronic tinnitus. *Behav. Brain Funct.* 7:39. doi: 10.1186/1744-9081-7-39

Sand, P. G., Langguth, B., Kleinjung, T., and Eichhammer, P. (2007). Genetics of chronic tinnitus. *Prog. Brain Res.* 166, 159–168. doi: 10.1016/s0079-6123(07)66014-2

Sand, P. G., Langguth, B., Schecklmann, M., and Klienjung, T. (2012b). GDNF and BDNF gene interplay in chronic tinnitus. *Int. J. Mol. Epidemiol. Genet.* 3, 245–251.

Sand, P. G., Luettich, A., Kleinjung, T., Hajak, G., and Langguth, B. (2010). An Examination of KCNE1 mutations and common variants in chronic tinnitus. *Genes (Basel)* 1, 23–37. doi: 10.3390/genes1010023

Shargorodsky, J., Curhan, G. C., and Farwell, W. R. (2010). Prevalence and characteristics of tinnitus among US adults. *Am. J. Med.* 123, 711–718. doi: 10.1016/j.amjmed.2010.02.015

Sharma, M., Krüger, R., and Gasser, T. (2012). LRRK2: understanding the role of common and rare variants in Parkinson's disease. *Mov. Disord.* 27:475. doi: 10.1002/mds.24937

Sharma, M., Krüger, R., and Gasser, T. (2014). From genome-wide association studies to next-generation sequencing: lessons from the past and planning for the future. *JAMA Neurol.* 71, 5–6. doi: 10.1001/jamaneurol.2013.3682

Silverman, E. K., and Palmer, L. J. (2000). Case-control association studies for the genetics of complex respiratory diseases. *Am. J. Respir. Cell Mol. Biol.* 22, 645–648. doi: 10.1165/ajrcmb.22.6.f191

Smith, P. F., Zheng, Y., and Darlington, C. L. (2012). Revisiting baclofen for the treatment of severe chronic tinnitus. *Front. Neurol.* 3:34. doi: 10.3389/fneur.2012.00034

Spagnolo, P., and du Bois, R. M. (2007). Genetics of sarcoidosis. *Clin. Dermatol.* 25, 242–249. doi: 10.1016/j.clindermatol.2007.03.001

Staessen, J. A., and Bianchi, G. (2005). Adducin and hypertension. *Pharmacogenomics* 6, 665–669. doi: 10.2217/14622416.6.7.665

Sun, Y., Chen, J., Sun, H., Cheng, J., Li, J., Lu, Y., et al. (2011). Novel missense mutations in MYO7A underlying postlingual high- or low-frequency non-syndromic hearing impairment in two large families from China. *J. Hum. Genet.* 56, 64–70. doi: 10.1038/jhg.2010.147

Tan, J., Rüttiger, L., Panford-Walsh, R., Singer, W., Schulze, H., Kilian, S. B., et al. (2007). Tinnitus behavior and hearing function correlate with the reciprocal expression patterns of BDNF and Arg3.1/arc in auditory neurons following acoustic trauma. *Neuroscience* 145, 715–726. doi: 10.1016/j.neuroscience.2006.11.067

Tan, T. M., Hatfield, E. C., Thakker, R. V., Maher, E. R., Meeran, K., Martin, N. M., et al. (2009). A legacy of tinnitus: multiple head and neck paragangliomas. *Rare Tumors* 1:e29. doi: 10.4081/rt.2009.e29

Tyler, R. S., Coelho, C., and Noble, W. (2006). Tinnitus: standard of care, personality differences, genetic factors. *ORL J. Otorhinolaryngol. Relat. Spec.* 68, 14–19. discussion: 20–12. doi: 10.1159/000090486

Van Rossom, S., Op de Beeck, K., Hristovska, V., Winderickx, J., and Van Camp, G. (2015). The deafness gene DFNA5 induces programmed cell death through mitochondria and MAPK-related pathways. *Front. Cell. Neurosci.* 9:231. doi: 10.3389/fncel.2015.00231

Voo, I., Allf, B. E., Udar, N., Silva-Garcia, R., Vance, J., and Small, K. W. (2003). Hereditary motor and sensory neuropathy type VI with optic atrophy. *Am. J. Ophthalmol.* 136, 670–677. doi: 10.1016/S0002-9394(03)00390-8

Wan, J., Mamsa, H., Johnston, J. L., Spriggs, E. L., Singer, H. S., Zee, D. S., et al. (2011). Large genomic deletions in CACNA1A Cause Episodic Ataxia Type 2. *Front. Neurol.* 2:51. doi: 10.3389/fneur.2011.00051

Wang, H., Brozoski, T. J., and Caspary, D. M. (2011). Inhibitory neurotransmission in animal models of tinnitus: maladaptive plasticity. *Hear. Res.* 279, 111–117. doi: 10.1016/j.heares.2011.04.004

Wang, H., Wang, X., He, C., Li, H., Qing, J., Grati, M., et al. (2015). Exome sequencing identifies a novel CEACAM16 mutation associated with autosomal dominant nonsyndromic hearing loss DFNA4B in a Chinese family. *J. Hum. Genet.* 60, 119–126. doi: 10.1038/jhg.2014.114

Wang, H., Wu, K., Yu, L., Xie, L., Xiong, W., Wang, D., et al. (2017). A novel dominant GJB2 (DFNA3) mutation in a Chinese family. *Sci. Rep.* 7:34425. doi: 10.1038/srep34425

Wang, Q. J., Li, Q. Z., Rao, S. Q., Lee, K., Huang, X. S., Yang, W. Y., et al. (2006). AUNX1, a novel locus responsible for X linked recessive auditory and peripheral neuropathy, maps to Xq23-27.3. *J. Med. Genet.* 43:e33. doi: 10.1136/jmg.2005.037929

Wang, Q., Xue, Y., Zhang, Y., Long, Q., and Asan, Y. F. (2013). Genetic basis of Y-linked hearing impairment. *Am. J. Hum. Genet.* 92, 301–306. doi: 10.1016/j.ajhg.2012.12.015

Wirdefeldt, K., Gatz, M., Reynolds, C. A., Prescott, C. A., and Pedersen, N. L. (2011). Heritability of Parkinson disease in Swedish twins: a longitudinal study. *Neurobiol. Aging* 32, e1921–e1928. doi: 10.1016/j.neurobiolaging.2011.02.017

Wirdefeldt, K., Gatz, M., Schalling, M., and Pedersen, N. L. (2004). No evidence for heritability of Parkinson disease in Swedish twins. *Neurology* 63, 305–311. doi: 10.1212/01.WNL.0000129841.30587.9D

Xia, J. H., Liu, C. Y., Tang, B. S., Pan, Q., Huang, L., Dai, H. P., et al. (1998). Mutations in the gene encoding gap junction protein beta-3 associated with autosomal dominant hearing impairment. *Nat. Genet.* 20, 370–373. doi: 10.1038/3845

Xiao, S., Yu, C., Chou, X., Yuan, W., Wang, Y., Bu, L., et al. (2001). Dentinogenesis imperfecta 1 with or without progressive hearing loss is associated with distinct mutations in DSPP. *Nat. Genet.* 27, 201–204. doi: 10.1038/84848

Xing, G., Yao, J., Wu, B., Liu, T., Wei, Q., Liu, C., et al. (2015). Identification of OSBPL2 as a novel candidate gene for progressive nonsyndromic hearing loss by whole-exome sequencing. *Genet. Med.* 17, 210–218. doi: 10.1038/gim.2014.90

Xue, Q., Li, C., Chen, J., Guo, H., Li, D., and Wu, X. (2016). The Protective effect of the endoplasmic reticulum stress-related factors BiP/GRP78 and CHOP/Gadd153 on noise-induced hearing loss in guinea pigs. *Noise Health* 18, 247–255. doi: 10.4103/1463-1741.192481

Yan, D., Zhu, Y., Walsh, T., Xie, D., Yuan, H., Sirmaci, A., et al. (2013). Mutation of the ATP-gated P2X receptor leads to progressive hearing loss and increased susceptibility to noise. *Proc. Natl. Acad. Sci. U.S.A.* 110, 2228–2233. doi: 10.1073/pnas.1222285110

Yüce, S., Sancakdar, E., Bağci, G., Koç, S., Kurtulgan, H. K., Bağci, B., et al. (2016). Angiotensin-Converting Enzyme (ACE) I/D and Alpha-Adducin (ADD1) G460W gene polymorphisms in Turkish Patients with severe chronic tinnitus. *J. Int. Adv. Otol.* 12, 77–81. doi: 10.5152/iao.2016.1732

Yu, H., Vikhe Patil, K., Han, C., Fabella, B., Canlon, B., Someya, S., et al. (2016). GLAST Deficiency in mice exacerbates gap detection deficits in a model of salicylate-induced tinnitus. *Front. Behav. Neurosci.* 10:158. doi: 10.3389/fnbeh.2016.00158

Zhao, Y., Wang, D., Zong, L., Zhao, F., Guan, L., Zhang, P., et al. (2014). A novel DFNA36 mutation in TMC1 orthologous to the Beethoven (Bth) mouse associated with autosomal dominant hearing loss in a Chinese family. *PLoS ONE* 9:e97064. doi: 10.1371/journal.pone.0097064

Zondervan, K. T., and Cardon, L. R. (2007). Designing candidate gene and genome-wide case-control association studies. *Nat. Protoc.* 2, 2492–2501. doi: 10.1038/nprot.2007.366

Zong, L., Guan, J., Ealy, M., Zhang, Q., Wang, D., Wang, H., et al. (2015). Mutations in apoptosis-inducing factor cause X-linked recessive auditory neuropathy spectrum disorder. *J. Med. Genet.* 52, 523–531. doi: 10.1136/jmedgenet-2014-102961

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A Pilot Genome-Wide Association Study Identifies Potential Metabolic Pathways Involved in Tinnitus

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Tinnitus, the perception of an auditory phantom sound in the form of ringing, buzzing, roaring, or hissing in the absence of an external sound source, is perceived by ∼15% of the population and 2.5% experiences a severely bothersome tinnitus. The contribution of genes on the development of tinnitus is still under debate. The current manuscript reports a pilot Genome Wide Association Study (GWAS) into tinnitus, in a small cohort of 167 independent tinnitus subjects, and 749 non-tinnitus controls, who were collected as part of a cross-sectional study. After genotyping, imputation, and quality checking, the association between the tinnitus phenotype and 4,000,000 single-nucleotide polymorphisms (SNPs) was tested followed by gene set enrichment analysis. None of the SNPs reached the threshold for genome-wide significance ($p < 5.0e–8$), with the most significant SNPs, situated outside coding genes, reaching a p-value of 3.4e–7. By using the Genetic Analysis of Complex Traits (GACT) software, the percentage of the variance explained by all SNPs in the GWAS was estimated to be 3.2%, indicating that additive genetic effects explain only a small fraction of the tinnitus phenotype. Despite the lack of genome-wide significant SNPs, which is, at least in part, due to the limited sample size of the current study, evidence was found for a genetic involvement in tinnitus. Gene set enrichment analysis showed several metabolic pathways to be significantly enriched with SNPs having a low p-value in the GWAS. These pathways are involved in oxidative stress, endoplasmatic reticulum (ER) stress, and serotonin reception mediated signaling. These results are a promising basis for further research into the genetic basis of tinnitus, including GWAS with larger sample sizes and considering tinnitus subtypes for which a greater genetic contribution is more likely.

Keywords: genome-wide association study, tinnitus, heritability, GWAS, phenotype, oxidative stress, serotonin, gene set enrichment analysis

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INTRODUCTION

Tinnitus is the perception of a sound which can be perceived as hissing, ringing, buzzing, or roaring in the absence of an external sound source. It is therefore often referred to as an auditory phantom sound (Eggermont and Roberts, 2004). The prevalence of tinnitus in the adult population is ∼15% (Seidman and Jacobson, 1996; Gilles et al., 2012, 2013), and in 2.5% of the population, the tinnitus

causes significant burden (Axelsson and Ringdahl, 1989). The evolvement of incapacitating tinnitus may lead to social isolation, anxiety, depression, sleep disorders, and concentration issues severely affecting ones quality of life (Henry et al., 2005).

The causes of tinnitus have been extensively investigated over the years. Tinnitus appears as a symptom with increasing prevalence with age. Tinnitus appears to emerge from a complex interaction between aging, diseases, auditory malfunctions, and environmental stressors. In 50% of the cases, tinnitus is attributable to otologic malfunctions (hearing loss, noise trauma, Ménière disease, vestibular schwannoma, temporomandibular junction disorder, ototoxic medications, or substances etc.) (Gilles et al., 2014). In others, the tinnitus is a result of neurologic, metabolic, and/or psychogenic disorders (Crummer and Hassan, 2004). Environmental factors increasing the chances to develop tinnitus are recreational and work-related noise exposure (Sindhusake et al., 2003; Kim et al., 2015), smoking, sleep deprivation (Park et al., 2014; Schecklmann et al., 2015), stress (Nondahl et al., 2011), smaller households (Kim et al., 2015), and obesity (Gallus et al., 2015; Martines et al., 2015) whereas high household income and moderate alcohol consumption are considered to be inversely related to tinnitus occurrence (Nondahl et al., 2011; Gallus et al., 2015).

In contrast to the largely known environmental risk factors for tinnitus, less is known about the genes involved. Many genes have been put forward for possibly underlying tinnitus susceptibility but no consensus has been reached so far. An important complicating factor is the heterogeneity in tinnitus causes and types (Sand et al., 2010; Pawelczyk et al., 2012; Elgoyhen et al., 2014; Lopez-Escamez et al., 2016). In several monogenic disorders associated with secondary chronic tinnitus, such as neurofibromatosis type II, episodic ataxia type II, osteogenesis imperfecta type I and Fabry disease, causative genes have been identified. For an overview of monogenic diseases associated with secondary tinnitus and its associated mutated genes, we refer the reader to Sand et al. (2007). The role of genetics in the more common types of primary, chronic tinnitus in a healthy population, is under debate as data on primary chronic tinnitus heritability is very limited and family and/or twin studies are mostly lacking. The study of Kvestad et al. (2010) was the first large population-based family study to provide an estimation of the relative contribution of genetic effects on the susceptibility to tinnitus. The data were collected by use of a self-report questionnaire including questions concerning tinnitus characteristics (duration, distress, frequency) and first-degree family relationships. In total, a sample of 12,294 spouses, 27,607 parent-offspring, and 11,498 siblings was used for structural equation modeling. A heritability of 11% was obtained suggesting that the involvement of genetic factors in tinnitus is rather low (Kvestad et al., 2010).

It seems unlikely that families with hereditary tinnitus are under-reported in the literature given the large number of families reported with hereditary hearing loss. Therefore, the lack of reported families showing a Mendelian pattern of inheritance, probably indicates that primary chronic tinnitus is not a single-gene disorder but rather a complex trait, controlled by complex interactions between multiple genes and environmental risk factors. In recent years, much effort has been devoted to the genetic elucidation of the genes involved in complex traits. This showed that most complex traits and diseases are highly polygenic, involving the combined action of a multitude of genetic variants, each of which having small effect sizes. Odds ratios are typically smaller than 1.1, although variants with a larger effect size (odds ratios above 1.25) have been reported (Michailidou et al., 2015). For tinnitus, the previously estimated heritability of 11% suggests that only a small fraction of the variance is due to genetic variants. However, since the heritability represents the variance accounted for by the combined effect of several genes, it cannot be excluded that the number of involved genes is limited, and that one or more major genes account for most of the heritability. A recent study provides novel insights into the genetic contribution to tinnitus. This longitudinal study in a male twin cohort investigated the genetic contribution to tinnitus revealing a ratio of 40/60% for, respectively, genetic and environmental influences. These genetic influences for tinnitus were independent of those for hearing loss (Bogo et al., 2016). Genome-wide association studies (GWAS) are the method of choice to identify the genes involved in complex diseases. In a GWAS, genotypes from many single nucleotide polymorphisms (SNPs) throughout the entire genome (often 1 million or more) are determined and tested for association with the disease status. A strong association between a particular SNP can indicate a possible role of the variant (or the gene in which it resides) in the pathophysiology of the disease. To date, more than 2000 different disease-associated variants have been identified through GWAS analysis, providing many new insights into the pathophysiology of several diseases and traits (Welter et al., 2014).

The authors of the present manuscript previously carried out a GWAS on age-related hearing impairment (ARHI) for which a highly polygenic character of ARHI was shown, with presumably no major genes involved (Fransen et al., 2015). In the ARHI dataset, 18% of the participants reported to perceive tinnitus. The aim of the current study was to gain an insight into the genetic influence of SNPs on the tinnitus phenotype using the genome-wide SNP data from the previously collected ARHI dataset.

METHODS

Ethics Approval

The collection of the original ARHI dataset was approved by the ethics committee of the University Hospital Antwerp on October 22, 2002 (file #A02-055).

Sample Collection

The local city councils in southern Antwerp (Belgium) made population registries available by which a population-based sample could be obtained. It was requested that at least three out of the four grandparents originated from Belgium which made the population ethnically homogenous. All responding subjects underwent clinical examination, otoscopy, and completed a detailed questionnaire on medical history and exposure to environmental risk factors. A list of all questions and answers used in this study is available on request.

Strict exclusion criteria were applied to exclude persons having or having had a condition that possibly leads to hearing impairment. No phenotypic inclusion criteria were used for the sample collection. Subjects with ear diseases, possible monogenic forms of hearing impairment or other major pathologies with a possible influence on hearing, were excluded. The main goal was to study healthy subjects and therefore persons with multiple hospitalizations were excluded. The extensive, complete list of exclusion criteria was previously reported (Van Eyken et al., 2006) and is provided as Supplementary Information (Supplement 1).

Pure tone audiometry was measured in all participating subjects according to the current clinical standards (ISO 8253-1, 2010) using a two-channel Interacoustics AC-40 audiometer in a soundproof booth. Air conduction thresholds were measured at 125, 250, 500 Hz, 1, 2, 4, and 8 kHz. Bone conduction thresholds were measured within the range of 250 Hz and 4 kHz.

The phenotype of tinnitus was scored in a very basic fashion using the question "Nowadays, do you ever hear noises in your head or ear(s) (tinnitus) which usually last longer than 5 min?" No further phenotyping of tinnitus was performed as this was a retrospective study to initially identify genes involved in age-related hearing impairment (Fransen et al., 2015). Noise exposure was addressed through the question "Have you ever worked for more than a year in an environment where you had to shout to an individual standing at less than a meter from you."

Collection of the study subjects was cross-sectional with no enrichment for any phenotype. However, the selection of the genotyped samples was based upon audiometric criteria as discussed in Fransen et al. (2015). In brief, the pure-tone thresholds from 250, 500, 1000, 2000, 4000, and 8000 Hz on age, age^2, and age^3 were regressed, fitting separate models for males and females. Subsequently, principal component analysis was performed on the residuals. Principal component (PC) scores for PC1, PC2, and PC3 were calculated. Since the aim was to obtain a maximal statistical power to detect genes involved in hearing loss, the phenotypically most extreme individuals for the three PC scores were selected for genotyping.

Genotyping

DNA was extracted from blood samples using standard procedures. Genomic DNA concentrations were determined with PicoGreen (Invitrogen, Carlsbad, CA, USA). Additionally, the quality of the genomic DNA was assessed for each sample by gel electrophoresis. Five-hundred DNA samples were genotyped with the Illumina CNV370 quad chip, 1060 DNA samples using the Illumina HumanOmniExpress BeadChip (Illumina, Inc., San Diego, CA, USA).

Imputation and Filtering

Pre-imputation filtering was carried out to exclude SNPs with a minor allele frequency (MAF) below 1%, a *p*-value for Hardy-Weinberg equilibrium below 1.0e–6, a call rate below 95% across all samples and across all SNPs. Duplicate samples were identified using pi-hat analysis (pi-hat > 0.99). For each of these duplicates, the sample with the lowest genotyping call rate was removed.

Imputation was performed using the program impute2, version 2.1.2 (Howie et al., 2009) with the 1000 Genomes Phase 1 Interim panel (June 2011) as reference panel. Imputation resulted in a total of 11 626 570 SNPs. An additional post-imputation filtering was carried out using the same exclusion criteria as in the pre-imputation stage, and removing imputed SNPs with an Impute2 info metric below 0.5. Gene boundaries used for mapping SNPs onto genes are: 110 kb upstream to the most extreme gene transcript start position, and 40 kb downstream to the most extreme gene transcript end position, taking gene orientation into account.

Association Analysis

Association between the tinnitus status (affected or not affected) and the common variant genotypes (MAF > 0.01) was tested using the logistic regression option in the software package PLINK v1.07. An additive model was fitted taking the affection status as a dependent variable. This analysis tests for the main effect of a SNP on the tinnitus phenotype.

The effect of SNP genotype to noise sensitivity was tested using logistic regression by fitting a model with SNP genotype, noise exposure, and their interaction. Two significance tests were carried out on this model: first the significance on the interaction term (1df-test) was tested as well as the joint significance of the SNP genotype and the interaction term (2df-test).

Heritability Estimates

The cumulative effect of all genotyped SNPs on the phenotypic variance was estimated through variance component analysis using the genomic-relatedness-based restricted maximum-likelihood (GREML) method implemented in the Genetic Analysis of Complex Traits (GACT) software (Yang et al., 2011). All SNPs that passed the quality control were included. The Genetic Relationship Matrix was estimated correcting for incomplete Linkage Disequilibrium (LD) between genotyped and causative variants. The percentage of variance explained by the SNPs is obtained as the ratio of the genetic variance to the total variance.

The presence of genome-wide gender-specific effects was tested using a likelihood ratio test, comparing the models with and without a variance component for gene-sex interaction, as implemented in GACT.

Gene Set Enrichment Analysis

Gene set enrichment analysis was performed using the software package MAGENTA (Meta-Analysis Gene-set Enrichment of varianT Associations; Segre et al., 2010). This program first expresses the significance of a whole gene as a gene score, based upon the individual *p*-values of the SNPs within the gene, adjusting for confounders (gene size, number of SNPs within the gene, amount of LD, and number of recombination hotspots within the gene). Subsequently, gene scores are combined at the level of gene sets, whereby the *p*-values of a given gene set are generated by counting the fraction of the genes within that gene set that have a gene score above a predefined enrichment cut-off. As recommended by the authors, the 75th percentile of all observed gene scores was used as enrichment cut-offs to generate the *p*-values for the individual pathways. Correction for multiple

testing was performed using the False Discovery Rate (FDR) method.

Analysis was performed using the *p*-values of the GWAS, using the three tests (main effects, interaction test, and joint test) as discussed earlier. SNPs were mapped to genes using Genome build36 (hg18), using gene sets from the following databases: Gene Ontology (April 2010), PANTHER (January 2010), Ingenuity (June 2008), KEGG (2010), Reactome and BioCarta.

RESULTS

Sample Collection

All individuals in this study were recruited through population registries in a residential suburb of Antwerp, Belgium. No enrichment for any phenotype was carried out, but strict exclusion criteria were applied to exclude individuals having or having had a disease with a possible influence on hearing. A detailed list with the exclusion criteria is provided as Supplementary Data (Supplement 1). All individuals were of Belgian ancestry. In total, 2161 individuals were randomly collected in a cross-sectional way. Of these, 1560 were selected for genotyping based upon audiometric data described in Fransen et al. (2015). Of these genotyped individuals, tinnitus information was available for 916 persons (456 females and 460 males), of which 167 (18%) were reporting episodes of tinnitus lasting longer than 5 min and the remaining 749 did not. The fraction of individuals with tinnitus was significantly different between males and females. Twenty-two percent of the males in the dataset reported tinnitus, against only 15% of the females ($p = 0.006$, chi-square test; odds ratio = 1.61 with 95% confidence interval 1.15–2.27). This difference may, at least in part, be due to a difference in noise exposure: 94 of the 167 tinnitus patients reported having worked in a noisy environment for more than a year. Only 13 of them were female, vs. 81 males ($p < 0.001$, chi-square test). **Table 1** provides an overview of the characteristics of the affected and non-affected individuals in this study.

TABLE 1 | Social-demographic factors of the study population.

	Affected by tinnitus	Control	*N*
Total population	167	749	916
Belgian ethnic origin	167 (100%)	749 (100%)	
Age range	55–65	55–65	
Mean age (st. deviation)	61.2 (3.1)	61.4 (3.1)	
Number of males (%)	100 (60%)	360 (48%)	460
Noise exposure data available	164 (98%)	733 (98%)	897
Noise exposure (%)	23 (14%)	71 (10%)	897

Association Testing

All association tests were carried out on the 916 individuals with a known tinnitus status, with the 167 tinnitus patients as cases and the 749 non-tinnitus individuals as controls.

Three types of statistical tests were performed to test the effect of the SNP genotypes on tinnitus. First, the main effect of the SNPs on the phenotype was tested, via the association between tinnitus, and the genotype without accounting for noise exposure. This highlights the SNPs that lead to an increased risk for tinnitus across both noise-exposed and non-exposed individuals. Results of this analysis are shown in the table "MainEffect" (Supplement 2). None of the SNPs reached the threshold for genome-wide significance ($p < 5.0e{-}8$). The most significant SNPs reach a *p*-value of 3.4e–7. Two genes contain SNPs with a *p*-value below 1.0e–5 (VDAC1 and NKTR). The significance of the SNPs is graphically shown in a Manhattan plot in **Figure 1**, where the *p*-value is plotted vs. the chromosomal location. The SNPs within a coding gene, with a *p*-value below 1.0e–5, are shown in green.

Since it is known that noise exposure is a risk factor for tinnitus, the interaction between the SNPs and noise exposure was analyzed. This analysis would identify SNPs having a different effect on the phenotype depending on noise exposure. For instance, it could identify SNPs that render an individual susceptible to tinnitus, following noise. These SNPs would not be identified in the first analysis, as their effect on the phenotype depends on noise exposure. *P*-values for this interaction were calculated in two ways: the significance of the interaction term and the joint test for genotype and interaction with noise In general, *p*-values for the joint test are more significant than those for the interaction test, but not as significant as the *p*-values for the main effects. Genic SNPs in three genes (VDAC1, NKTR, and COG3) reach a *p*-value below 1.0e–5 for the joint test, as highlighted in the Manhattan plots (**Figures 2**, **3**).

Tables with results are provided as Supplementary Materials (Supplement 2). These tables have been sorted by *p*-value (most significant value on top), and show the SNPs with a *p*-value below 0.0001. The far right column ("ANNOT") shows the gene in which the SNP is located. If the SNP is further than 110 kb upstream the most extreme gene transcript start site of a coding gene, or more than 40 kb downstream to the most extreme gene transcript end position of a coding gene, this column is empty. For each of the three significance tests (main effects, interaction test and joint test), we present two tables. The first table shows all SNPs with a *p*-value below 1e–4, whereas the second one (extension "Genic") shows the SNPs within or close to a coding gene.

Pathway Analysis

To investigate if genes, belonging to certain metabolic pathways or biological processes, were enriched in SNPs with low *p*-values, gene set enrichment analysis (GSEA) was carried out using the MAGENTA package. The analysis was performed with the results from both the main effects test, the interaction test and the joint test. Upon FDR correction for multiple testing, a total of seven pathways were significantly enriched in low *p*-values. An overview of the significant pathways is provided in **Table 2**. A complete overview is provided in the Supplementary Materials (Supplement 3).

Using GACT software, the fraction of the phenotypic variance attributable to additive genetic effects was estimated. All genotyped SNPs passing quality control had a cumulative effect

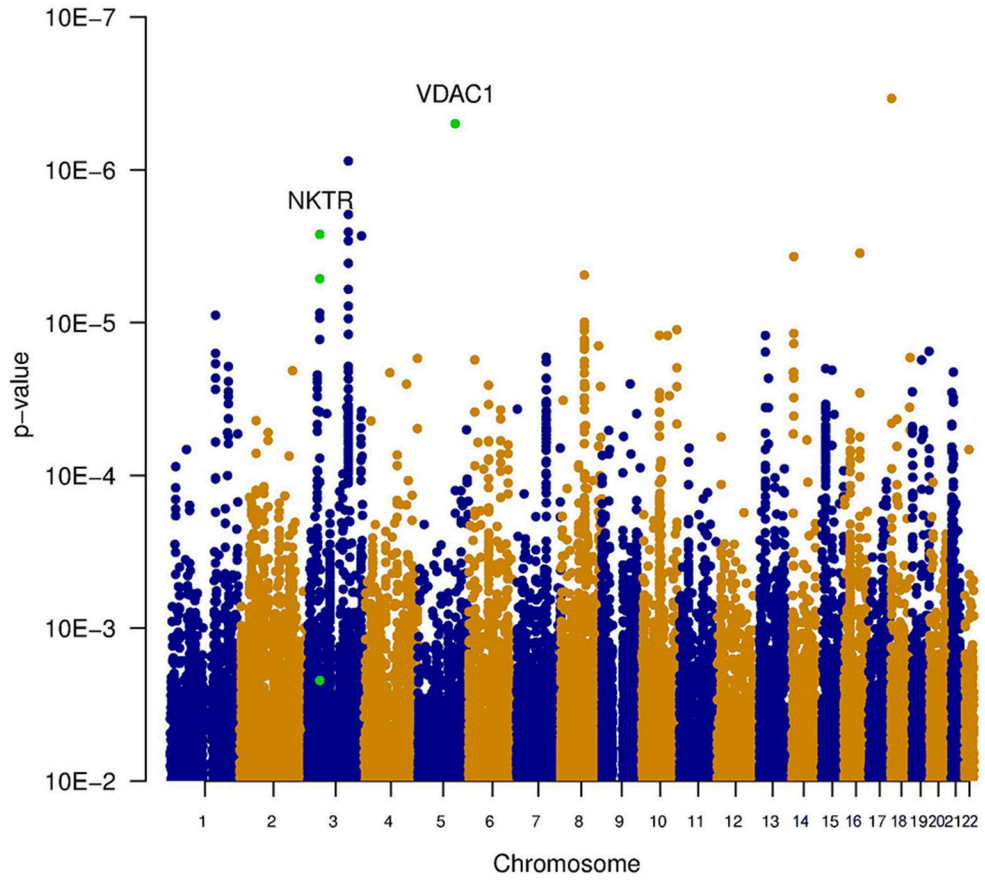

FIGURE 1 | Manhattan plot illustrating the main effect of the SNPs on the phenotype, tested via the association between tinnitus and the genotype without accounting for noise exposure. This highlights the SNPs that lead to an increased risk for tinnitus across both noise-exposed and non-exposed individuals. None of the SNPs reached the threshold for genome-wide significance ($p < 5e^{-8}$). The most significant SNPs reach a p-value of $3.4e^{-7}$. Two genes contain SNPs with a p-value below $1e^{-5}$ (VDAC1 and NKTR). These SNPs are indicated in green.

accounting for 3.2% of the phenotypic variance in the trait, with a 95% confidence interval ranging from 0 to 21%.

DISCUSSION AND CONCLUSIONS

To our knowledge, this is the first genome-wide association study on tinnitus phenotype ever performed, including 916 individuals aged between 55 and 65, consisting of 167 tinnitus cases and 749 controls. Although none of the SNPs reached the conventional threshold for genome-wide significance ($p < 5.0e{-}8$), and the proportion of the variance accounted for by all SNPs together is small, evidence was found for a genetic involvement in tinnitus through gene set enrichment analysis (GSEA). Throughout the three methods with which the SNPs were tested for association, seven pathways with a significant enrichment in significant SNPs emerged. On the basis of a literature search, we could not find an obvious link between tinnitus and the RAS pathway, vascular smooth muscle contraction, NDK Dynamin, and coenzyme A biosynthesis.

On the other hand, a beneficial effect of antidepressants on tinnitus has been suggested several times (Baldo et al., 2012; Beebe Palumbo et al., 2015), and this possibly involves serotonin (5HT) receptor mediated signaling pathways. Deniz et al. reported that one allelic variant of the serotonin transporter gene SLC6A4 was associated with the emotional distress associated with tinnitus (Deniz et al., 2010). Because the studies are small and often of limited quality, the results remain inconclusive at this time (Baldo et al., 2012). Moreover, since anxiety is associated with both serotonin and tinnitus (Lesch et al., 1996), it is unclear whether serotonin and tinnitus are directly associated or whether the apparent link between tinnitus and serotonin is attributable to anxiety as a confounder. The involvement of genetic variants in the serotonin mediated signaling pathways would provide a biological link between the action of antidepressants and the improvement of tinnitus (Deniz et al., 2010).

The most remarkable associations are those with endoplasmatic reticulum (ER) stress, and with the NRF2-mediated oxidative stress response. Oxidative stress plays an important role in the pathogenesis of noise-induced hearing loss and noise-induced tinnitus. Delmaghani et al. showed a direct molecular link between noise-induced hearing loss and an impaired oxidative stress defense, in a study on mice with

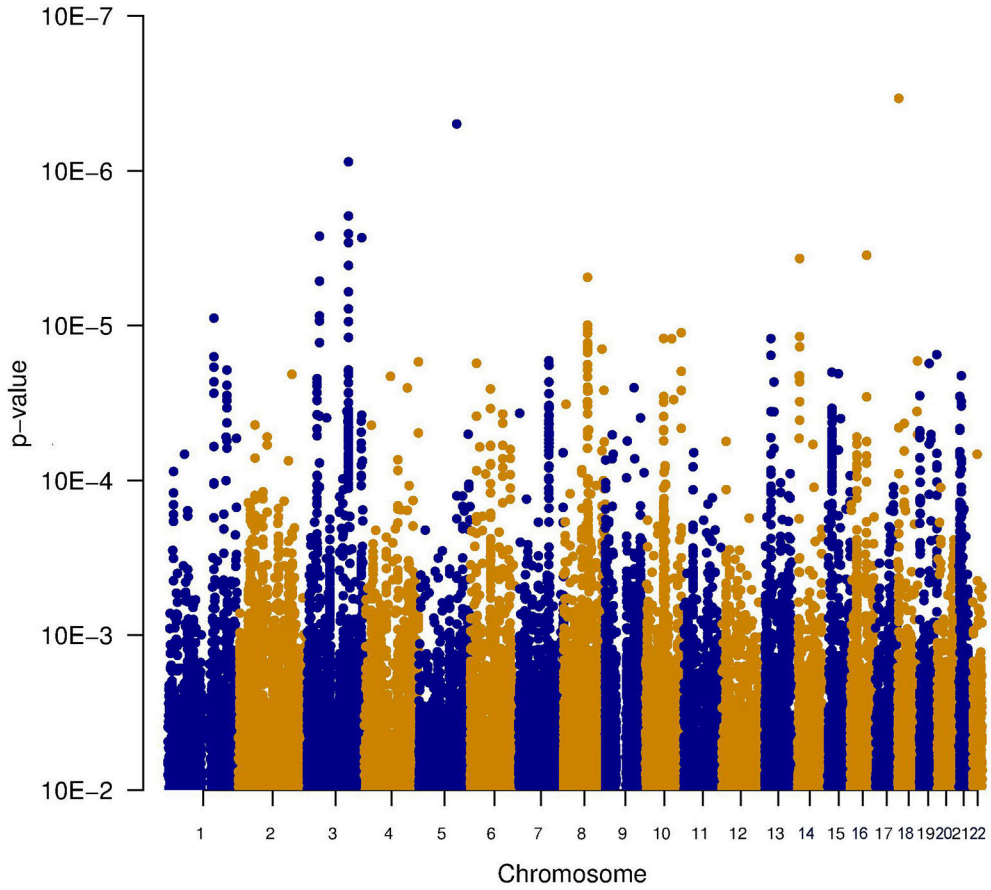

FIGURE 2 | Manhattan plot illustrating the interaction test between genotype and noise exposure. The *p*-values test if the effect of the SNP genotype on the phenotype is altered by noise exposure. This test could highlight SNPs making an individual susceptible to tinnitus, following noise exposure.

an inactivation of the Pejvakin (*Pjvk*) gene (Delmaghani et al., 2015). They showed that in regular animals, sensory cells in the inner hair respond to noise-induced oxidative stress by upregulating *Pjvk* expression. This in turns upregulates the number of peroxysomes, which defend the cell against reactive oxygen species by restoring the normal redox balance. In mice with an inactivated *Pjvk* gene, this defense mechanism against noise-induced oxidative stress was totally absent. Following noise exposure, levels of oxidative stress were significantly increased compared to normal animals, and hearing function sharply declined. A recent study showed that tinnitus patients express significantly higher total oxidant status and oxidative index levels compared to control subjects (Koç et al., 2016) which can possibly cause dysfunctions in the microcirculation of the inner ear (Neri et al., 2002, 2006). The exact role of oxidative stress in tinnitus is still under debate. However, Honkura et al. showed that NRF2 is an undeniably crucial player in the defense mechanism against noise-induced oxidative stress (Honkura et al., 2016). A significant association between an NRF2 SNP influencing expression and susceptibility for noise-induced hearing damage was shown in noise-exposed subjects. In particular a noise notch at 4 kHz was more prevalent in subjects with a low NRF2 gene expression resulting into lower antioxidant capacity. In the current study all subjects had age-related hearing impairment, which is the most common form of sensorineural hearing loss. Oxidative stress is known to play a vital role in the development of ARHI. Along the aging process progressive decline of mitochondrial function emerges resulting into increased ROS production which in turn elicits oxidative damage and dysfunction in various tissues (Wallace, 2005). With ARHI being the most frequent cause of tinnitus development, it can be argued that besides the increase in susceptibility to hearing loss, a low NRF2 expressor allele also contributes to tinnitus development.

The endoplasmatic reticulum (ER) overload response, also referred to as ER stress or the unfolding protein response (UPR), is an evolutionary conserved response of the ER to the accumulation of unfolded proteins in the ER lumen. Although the response is primarily aimed at re-establishing the normal ER function, prolonged ER stress results in cell death through apoptosis (Xu et al., 2005). Kalinec et al. showed that the previously reported ototoxicity of N-acetyl-para-aminophenol (APAP) acts through oxidative stress and the ER overload pathway in cells derived from mouse organ of Corti (Kalinec et al., 2014). ER stress has also been linked to hearing loss in cellular and animal models for noise induced and age related

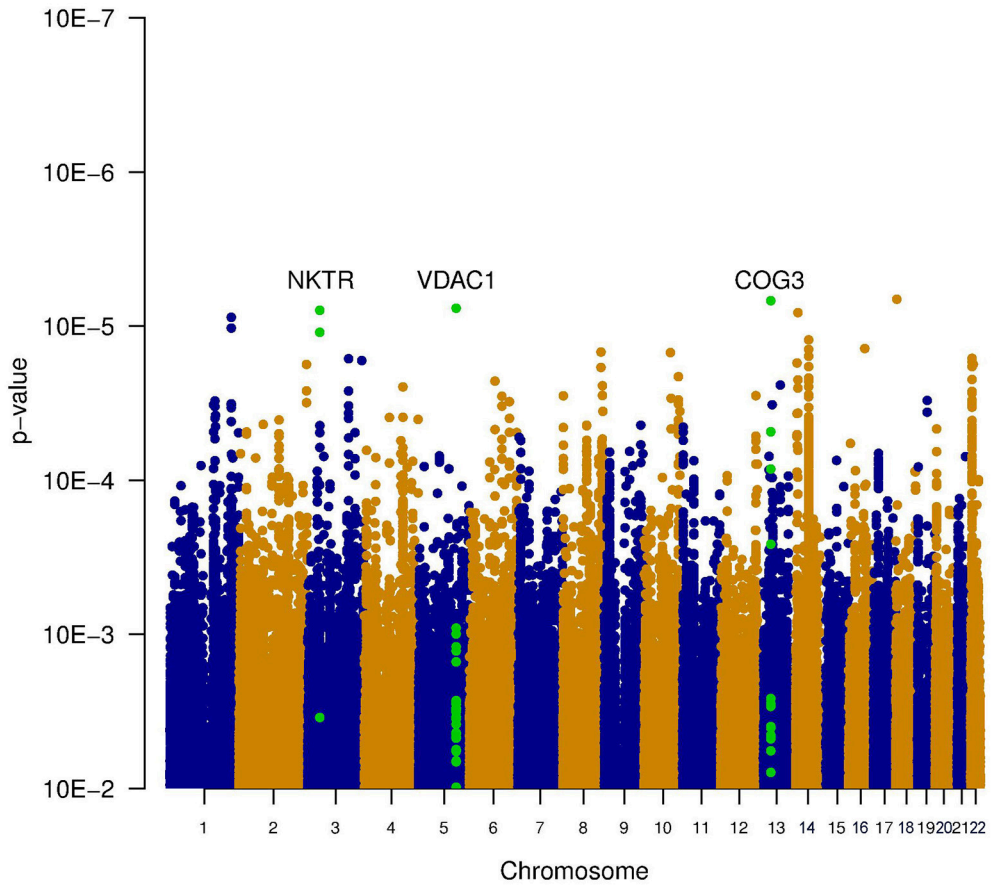

FIGURE 3 | Manhattan plot illustrating the joint test for genotype and interaction with noise. This test combines the significance of the main effect test (**Figure 1**) and the interaction test (**Figure 2**). Two genes contain SNPs with a p-value below 1e-5 (VDAC1 and NKTR). The SNPs within these genes are shown in green.

TABLE 2 | Results of Gene set enrichment analysis.

Pathway	Database	No. of genes above 75th percentile		*p*-value	
		Expected	Observed no.	Nominal	FDR-corrected
MAIN EFFECTS					
RAS	Panther	3	9	8.0 e–4	0.02
Vascular Smooth Muscle contraction	KEGG	26	43	1.0 e–4	0.03
INTERACTION TEST					
Coenzyme A biosynthesis	Panther	2	6	1.6 e–3	0.02
5HT2-type receptor mediated signaling pathway		2	6	1.4 e–3	0.03
NDK Dynamin	Biocarta	4	11	5.0 e–4	0.05
JOINT TEST					
NRF2-mediated oxidative stress response	Ingenuity	12	25	4.7 e–5	0.002
ER overload response	GOTERM	2	8	1.7 e–5	0.004

hearing loss (Van Rossom et al., 2015; Hu et al., 2016; Xue et al., 2016). ER stress was suggested to be the earliest molecular event leading to hearing loss (Hu et al., 2016), and progression could be delayed with an ER stress inhibitor.

The identification of pathways involved in tinnitus is a useful first step into a better understanding of the pathophysiology and the identification of possible drug targets. A further step would be the identification of individual SNPs associated with tinnitus, which would deepen our understanding how the symptom arises on a molecular basis. A potential follow-up study could therefore focus on genotyping SNPs in genes belonging to the pathways identified in the current study.

It is not surprising that the current study has not been able to find individual SNPs associated with tinnitus. First of all, this sample set was initially not optimized to study the genetic causes of tinnitus. It was collected as a cross-sectional study to investigate age-related hearing impairment, and no selective enrichment for tinnitus patients was carried out. The observed prevalence of 18% is in line with previously reported epidemiological studies (Axelsson and Ringdahl, 1989; Gilles et al., 2013). There was no matching for other tinnitus risk factors, although noise exposure was accounted for in the analysis stage. Hence, the current dataset had limited power to detect individual SNPs associated with tinnitus. A sensitivity analysis using the Genetic Power calculator (Purcell et al., 2003) showed that the current dataset holds 80% power to detect an allele with a genotype relative risk of 2, under an additive model, at a genome-wide significance level ($p < 5e{-}8$). Since no genome-wide significant SNPs were found in the current GWAS, it can be concluded that presumably, no major genes with a genotype relative risk of 2 or larger in tinnitus exist.

The finding of several pathways significantly enriched in significant SNPs, seems in contradiction with the lack of SNPs reaching genome-wide significance and the low heritability. The genetic signal, picked up using the GSEA, must therefore reside in the SNPs reaching a nominal, but not a genome-wide, significance level. For instance, for both the main effect test and the joint test, there are about 700 SNPs (both genic and non-genic) reaching a *p*-value below 1.0e–4. Due to the multitude of hypotheses tested in a GWAS, it is quite likely that a large part of these association signals represent false positives, but a fraction of the SNPs with low *p*-values will represent a genuine association signal. This is in line with previous studies into, amongst others, psychiatric diseases and age-related hearing loss, where the SNPs with a low *p*-value not reaching genome-wide significance, still hold substantial genetic information on the phenotype (International Schizophrenia Consortium et al., 2009; Fransen et al., 2015).

A very low heritability estimate was obtained through variance components analysis as implemented in GACT. This showed that the cumulative effect of all SNPs in the GWAS on the phenotype only explains 3.2% of the phenotypic variance in tinnitus. Several explanations are possible to reconcile this weak effect of genes with the presence of genetic signals through the GSEA. A first explanation is the inaccuracy of the current estimate due to the small sample size. The standard error of the estimate was 9.2%, which leads to a confidence interval for the heritability ranging from 0 to 21%. The previously reported heritability of 11% by Kvestad et al. (2010), falls within this 95% confidence interval. Moreover, the GCTA-GREML method implemented in GCTA only accounts for the additive genetic effects of the SNPs in the GWAS. The actual genetic effect may be larger due to, amongst other, rare SNPs that are not analyzed in a GWAS or SNP-SNP interactions. The combination of this very weak effect of all individual SNPs combined, with the significant results of the GSEA, seems to indicate that only a very limited number of SNPs are involved in tinnitus, but these SNPs seem to be confined in a small number of disease-associated pathways.

Although GWASs have been very successful in the identification of disease genes, the technique has its limitations. Most SNP variants genotyped in the current GWAS are common, typically with a minor allele frequency of at least 5%. The effects of low-frequency and rare SNPs are not captured. Novel techniques, such as next-generation sequencing, now allow to more thoroughly study the role of rare variants in complex diseases, and traits (Rivas et al., 2011; Gudmundsson et al., 2012). Moreover, intense research is being carried out on the role of non-coding variation in the genome. Since most SNPs identified in GWAS are located outside coding genes, and probably reside within regulatory regions, subtle alterations in gene expression level may be a major player in complex traits and diseases. However, there are very few non-coding SNPs for which the exact role in gene expression has already been elucidated.

The ideal study design, for a genetic study on tinnitus, would be a case-control dataset with an equal number of tinnitus cases and matched controls taking into account potential confounders such as anxiety, age, hearing loss, occupational/recreational noise exposure, and distress. Since most genetic effect sizes for complex traits are small, with the variance explained by a single SNP in the order of 0.1% of the phenotypic variance and genotype relative risks around 1.1, detecting such risk alleles is only feasible using massive sample sizes of 10,000 individuals, collected through large, international consortia. In addition, these studies should more carefully study the tinnitus phenotype and take into account the clinical heterogeneity of the trait. In the current study, the anamnesis was based upon one simple question in a questionnaire, without any distinction between the various subgroups of tinnitus. Tinnitus phenotype has proven to be very heterogeneous and, as such, the tinnitus phenotype in the present study was inevitably heterogeneous as well. Further improvement can be reached by construction of a standardized operation protocol to collect and phenotype tinnitus patients and controls. Limiting the tinnitus patients to certain subtypes, excluding for example somatic tinnitus for which a non-genetic etiology is likely (Lopez-Escamez et al., 2016), would increase the power of genetic studies into tinnitus.

So far no large GWAS into tinnitus has been carried out. The lack of large international studies into tinnitus genetics is possibly attributable to the general assumption that tinnitus is a symptom rather than a trait with few genetic factors involved. Interestingly, a recent longitudinal twin study (Bogo et al., 2016) estimated the proportion of additive genetic factors in tinnitus at 40%, which is substantially higher than the 11% previously estimated by Kvestad (Kvestad et al., 2010).Together with the results from the current study, the idea seems to emerge that the role of genetic factors in tinnitus is larger than initially assumed, and that the identification of SNPs involved in tinnitus is feasible if sufficiently large sample sets of well-characterized tinnitus and control subjects are collected.

AUTHOR CONTRIBUTIONS

AG wrote the manuscript, co-operated in the statistical analyses, and is the corresponding author. GV and PV were involved in

the design of the study, the collecting of the original data, and reviewed the draft manuscript. EF performed statistical analyses and co-operated in writing the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnins.2017.00071/full#supplementary-material

Public Data Access

Data from this study are publicly available through the following link: http://bit.ly/2jYmRii.

REFERENCES

Axelsson, A., and Ringdahl, A. (1989). Tinnitus–a study of its prevalence and characteristics. *Br. J. Audiol.* 23, 53–62. doi: 10.3109/03005368909077819

Baldo, P., Doree, C., Molin, P., McFerran, D., and Cecco, S. (2012). Antidepressants for patients with tinnitus. *Cochrane Database Syst. Rev.* 12:CD003853. doi: 10.1002/14651858.CD003853.pub3

Beebe Palumbo, D., Joos, K., De Ridder, D., and Vanneste, S. (2015). The management and outcomes of pharmacological treatments for tinnitus. *Curr. Neuropharmacol.* 13, 692–700. doi: 10.2174/1570159X13666150415002743

Bogo, R., Farah, A., Karlsson, K. K., Pedersen, N. L., Svartengren, M., and Skjonsberg, A. (2016). Prevalence, incidence proportion, and heritability for tinnitus: a longitudinal twin study. *Ear Hear.* doi: 10.1097/AUD.0000000000000397. [Epub ahead of print].

Crummer, R. W., and Hassan, G. A. (2004). Diagnostic approach to tinnitus. *Am. Fam. Physician* 69, 120–126.

Delmaghani, S., Defourny, J., Aghaie, A., Beurg, M., Dulon, D., Thelen, N., et al. (2015). Hypervulnerability to sound exposure through impaired adaptive proliferation of peroxisomes. *Cell* 163, 894–906. doi: 10.1016/j.cell.2015.10.023

Deniz, M., Bayazit, Y. A., Celenk, F., Karabulut, H., Yilmaz, A., Gunduz, B., et al. (2010). Significance of serotonin transporter gene polymorphism in tinnitus. *Otol. Neurotol.* 31, 19–24. doi: 10.1097/MAO.0b013e3181c2dcbc

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Elgoyhen, A. B., Langguth, B., Nowak, W., Schecklmann, M., De Ridder, D., and Vanneste, S. (2014). Identifying tinnitus-related genes based on a side-effect network analysis. *CPT Pharmacometrics Syst. Pharmacol.* 3, e97. doi: 10.1038/psp.2013.75

Fransen, E., Bonneux, S., Corneveaux, J. J., Schrauwen, I., Di, B. F., White, C. H., et al. (2015). Genome-wide association analysis demonstrates the highly polygenic character of age-related hearing impairment. *Eur. J. Hum. Genet.* 23, 110–115. doi: 10.1038/ejhg.2014.56

Gallus, S., Lugo, A., Garavello, W., Bosetti, C., Santoro, E., Colombo, P., et al. (2015). Prevalence and determinants of tinnitus in the italian adult population. *Neuroepidemiology* 45, 12–19. doi: 10.1159/000431376

Gilles, A., De Ridder, D., Van Hal, G., Wouters, K., Kleine Punte, A., and Van de Heyning, P. (2012). Prevalence of leisure noise-induced tinnitus and the attitude toward noise in university students. *Otol. Neurotol.* 33, 899–906. doi: 10.1097/mao.0b013e31825d640a

Gilles, A., Goelen, S., and Van de Heyning, P. (2014). Tinnitus: a cross-sectional study on the audiologic characteristics. *Otol. Neurotol.* 35, 401–406. doi: 10.1097/MAO.0000000000000248

Gilles, A., Van Hal, G., De Ridder, D., Wouters, K., and Van de Heyning, P. (2013). Epidemiology of noise-induced tinnitus and the attitudes and beliefs towards noise and hearing protection in adolescents. *PLoS ONE* 8:e70297. doi: 10.1371/journal.pone.0070297

Gudmundsson, J., Sulem, P., Gudbjartsson, D. F., Masson, G., Agnarsson, B. A., Benediktsdottir, K. R., et al. (2012). A study based on whole-genome sequencing yields a rare variant at 8q24 associated with prostate cancer. *Nat. Genet.* 44, 1326–1329. doi: 10.1038/ng.2437

Henry, J. A., Dennis, K. C., and Schechter, M. A. (2005). General review of tinnitus: prevalence, mechanisms, effects, and management. *J. Speech Lang. Hear. Res.* 48, 1204–1235. doi: 10.1044/1092-4388(2005/084)

Honkura, Y., Matsuo, H., Murakami, S., Sakiyama, M., Mizutari, K., Shiotani, A., et al. (2016). NRF2 is a key target for prevention of noise-induced hearing loss by reducing oxidative damage of cochlea. *Sci. Rep.* 6:19329. doi: 10.1038/srep19329

Howie, B. N., Donnelly, P., and Marchini, J. (2009). A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet.* 5:e1000529. doi: 10.1371/journal.pgen.1000529

Hu, J., Li, B., Apisa, L., Yu, H., Entenman, S., Xu, M., et al. (2016). ER stress inhibitor attenuates hearing loss and hair cell death in Cdh23erl/erl mutant mice. *Cell Death Dis.* 7, e2485. doi: 10.1038/cddis.2016.386

International Schizophrenia Consortium, Purcell, S. M., Wray, N. R., Stone, J. L., Visscher, P. M., O'Donovan, M. C., et al. (2009). Common polygenic variation contributes to risk of schizophrenia and bipolar disorder. *Nature* 460, 748–752. doi: 10.1038/nature08185

Kalinec, G. M., Thein, P., Parsa, A., Yorgason, J., Luxford, W., Urrutia, R., et al. (2014). Acetaminophen and NAPQI are toxic to auditory cells via oxidative and endoplasmic reticulum stress-dependent pathways. *Hear. Res.* 313, 26–37. doi: 10.1016/j.heares.2014.04.007

Kim, H. J., Lee, H. J., An, S. Y., Sim, S., Park, B., Kim, S. W., et al. (2015). Analysis of the prevalence and associated risk factors of tinnitus in adults. *PLoS ONE* 10:e0127578. doi: 10.1371/journal.pone.0127578

Koç, S., Akyuz, S., Somuk, B. T., Soyalic, H., Yilmaz, B., Taskin, A., et al. (2016). Paraoxonase activity and oxidative status in patients with tinnitus. *J. Audiol. Otol.* 20, 17–21. doi: 10.7874/jao.2016.20.1.17

Kvestad, E., Czajkowski, N., Engdahl, B., Hoffman, H. J., and Tambs, K. (2010). Low heritability of tinnitus: results from the second Nord-Trondelag health study. *Arch. Otolaryngol. Head Neck Surg.* 136, 178–182. doi: 10.1001/archoto.2009.220

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., et al. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 274, 1527–1531. doi: 10.1126/science.274.5292.1527

Lopez-Escamez, J. A., Bibas, T., Cima, R. F., Van de Heyning, P., Knipper, M., Mazurek, B., et al. (2016). Genetics of tinnitus: an emerging area for molecular diagnosis and drug development. *Front. Neurosci.* 10:377. doi: 10.3389/fnins.2016.00377

Martines, F., Sireci, F., Cannizzaro, E., Costanzo, R., Martines, E., Mucia, M., et al. (2015). Clinical observations and risk factors for tinnitus in a Sicilian cohort. *Eur. Arch. Otorhinolaryngol.* 272, 2719–2729. doi: 10.1007/s00405-014-3275-0

Michailidou, K., Beesley, J., Lindstrom, S., Canisius, S., Dennis, J., Lush, M. J., et al. (2015). Genome-wide association analysis of more than 120,000 individuals identifies 15 new susceptibility loci for breast cancer. *Nat. Genet.* 47, 373–380. doi: 10.1038/ng.3242

Neri, S., Mauceri, B., Cilio, D., Bordonaro, F., Messina, A., Malaguarnera, M., et al. (2002). Tinnitus and oxidative stress in a selected series of elderly patients. *Arch. Gerontol. Geriatr. Suppl.* 8, 219–223. doi: 10.1016/S0167-4943(02)00137-1

Neri, S., Signorelli, S., Pulvirenti, D., Mauceri, B., Cilio, D., Bordonaro, F., et al. (2006). Oxidative stress, nitric oxide, endothelial dysfunction and tinnitus. *Free Radic. Res.* 40, 615–618. doi: 10.1080/10715760600623825

Nondahl, D. M., Cruickshanks, K. J., Huang, G. H., Klein, B. E., Klein, R., Nieto, F. J., et al. (2011). Tinnitus and its risk factors in the Beaver Dam offspring study. *Int. J. Audiol.* 50, 313–320. doi: 10.3109/14992027.2010.551220

Park, B., Choi, H. G., Lee, H. J., An, S. Y., Kim, S. W., Lee, J. S., et al. (2014). Analysis of the prevalence of and risk factors for tinnitus in a young population. *Otol. Neurotol.* 35, 1218–1222. doi: 10.1097/mao.0000000000000472

Pawelczyk, M., Rajkowska, E., Kotylo, P., Dudarewicz, A., Van Camp, G., and Sliwinska-Kowalska, M. (2012). Analysis of inner ear potassium recycling genes as potential factors associated with tinnitus. *Int. J. Occup. Med. Environ. Health* 25, 356–364. doi: 10.2478/s13382-012-0061-3

Purcell, S., Cherny, S. S., and Sham, P. C. (2003). Genetic power calculator: design of linkage and association genetic mapping studies of complex traits. *Bioinformatics* 19, 149–150. doi: 10.1093/bioinformatics/19.1.149

Rivas, M. A., Beaudoin, M., Gardet, A., Stevens, C., Sharma, Y., Zhang, C. K., et al. (2011). Deep resequencing of GWAS loci identifies independent rare variants associated with inflammatory bowel disease. *Nat. Genet.* 43, 1066–1073. doi: 10.1038/ng.952

Sand, P. G., Langguth, B., Kleinjung, T., and Eichhammer, P. (2007). Genetics of chronic tinnitus. *Prog. Brain Res.* 166, 159–168. doi: 10.1016/S0079-6123(07)66014-2

Sand, P. G., Luettich, A., Kleinjung, T., Hajak, G., and Langguth, B. (2010). An examination of KCNE1 mutations and common variants in chronic tinnitus. *Genes* 1, 23–37. doi: 10.3390/genes1010023

Schecklmann, M., Pregler, M., Kreuzer, P. M., Poeppl, T. B., Lehner, A., Cronlein, T., et al. (2015). Psychophysiological associations between chronic tinnitus and sleep: a cross validation of tinnitus and insomnia questionnaires. *Biomed Res. Int.* 2015:461090. doi: 10.1155/2015/461090

Segre, A. V., DIAGRAM Consortium, MAGIC Investigators, Groop, L., Mootha, V. K., Daly, M. J., et al. (2010). Common inherited variation in mitochondrial genes is not enriched for associations with type 2 diabetes or related glycemic traits. *PLoS Genet.* 6:e1001058. doi: 10.1371/journal.pgen.1001058

Seidman, M. D., and Jacobson, G. P. (1996). Update on tinnitus. *Otolaryngol. Clin. North Am.* 29, 455–465.

Sindhusake, D., Golding, M., Newall, P., Rubin, G., Jakobsen, K., and Mitchell, P. (2003). Risk factors for tinnitus in a population of older adults: the blue mountains hearing study. *Ear Hear.* 24, 501–507. doi: 10.1097/01.AUD.0000100204.08771.3D

Van Eyken, E., Van Laer, L., Fransen, E., Topsakal, V., Lemkens, N., Laureys, W., et al. (2006). KCNQ4: a gene for age-related hearing impairment? *Hum. Mutat.* 27, 1007–1016. doi: 10.1002/humu.20375

Van Rossom, S., Op de Beeck, K., Hristovska, V., Winderickx, J., and Van Camp, G. (2015). The deafness gene DFNA5 induces programmed cell death through mitochondria and MAPK-related pathways. *Front. Cell. Neurosci.* 9:231. doi: 10.3389/fncel.2015.00231

Wallace, D. C. (2005). A mitochondrial paradigm of metabolic and degenerative diseases, aging, and cancer: a dawn for evolutionary medicine. *Annu. Rev. Genet.* 39, 359–407. doi: 10.1146/annurev.genet.39.110304.095751

Welter, D., MacArthur, J., Morales, J., Burdett, T., Hall, P., Junkins, H., et al. (2014). The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res.* 42, D1001–D1006. doi: 10.1093/nar/gkt1229

Xu, C., Bailly-Maitre, B., and Reed, J. C. (2005). Endoplasmic reticulum stress: cell life and death decisions. *J. Clin. Invest.* 115, 2656–2664. doi: 10.1172/JCI26373

Xue, Q., Li, C., Chen, J., Guo, H., Li, D., and Wu, X. (2016). The Protective effect of the endoplasmic reticulum stress-related factors BiP/GRP78 and CHOP/Gadd153 on noise-induced hearing loss in guinea pigs. *Noise Health* 18, 247–255. doi: 10.4103/1463-1741.192481

Yang, J., Lee, S. H., Goddard, M. E., and Visscher, P. M. (2011). GCTA: a tool for genome-wide complex trait analysis. *Am. J. Hum. Genet.* 88, 76–82. doi: 10.1016/j.ajhg.2010.11.011

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Biomarkers of Presbycusis and Tinnitus in a Portuguese Older Population

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Introduction: Presbycusis or age-related hearing loss (ARHL) is a ubiquitous health problem. It is estimated that it will affect up to 1.5 billion people by 2025. In addition, tinnitus occurs in a large majority of cases with presbycusis. Glutamate metabotropic receptor 7 (*GRM7*) and *N*-acetyltransferase 2 (*NAT2*) are some of the genetic markers for presbycusis.

Objectives: To explore patterns of hearing loss and the role of *GRM7* and *NAT2* as possible markers of presbycusis and tinnitus in a Portuguese population sample.

Materials and Methods: Tonal and speech audiometry, tinnitus assessment, clinical interview, and DNA samples were obtained from patients aged from 55 to 75 with or without tinnitus. *GRM7* analysis was performed by qPCR. Genotyping of single nucleotide polymorphisms (SNPs) in *NAT2* was performed by PCR amplification followed by Sanger sequencing or by qPCR.

Results: We screened samples from 78 individuals (33 men and 45 women). T allele at *GRM7* gene was the most observed (60.3% T/T and 33.3% A/T). Individuals with a T/T genotype have a higher risk for ARHL and 33% lower risk for tinnitus, compared to individuals with A/A and A/T genotype, respectively. Being a slow acetylator (53%) was the most common *NAT2* phenotype, more common in men (55.8%). Intermediate acetylator was the second most common phenotype (35.9%) also more frequent in men (82.6%). Noise exposed individuals and individuals with 'high frequency' hearing loss seem to have a higher risk for tinnitus. Our data suggests that allele AT of *GRM7* can have a statistically significant influence toward the *severity* of tinnitus.

Conclusion: For each increasing year of age the chance of HL increases by 9%. The risk for ARHL was not significantly associated with *GRM7* neither *NAT2*. However, we cannot conclude from our data whether the presence of T allele at *GRM7* increases the odds for ARHL or whether the A allele has a protective effect. Genotype A/T at *GRM7* could potentially be considered a biomarker of tinnitus severity. This is the first study evaluating the effect of *GRM7* and *NAT2* gene in tinnitus.

Keywords: presbycusis, *GRM7*, *NAT2*, tinnitus, markers, comorbidities

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INTRODUCTION

Presbycusis [age-related hearing loss (ARHL)] is a universal feature of mammalian aging in which the auditory function is compromised, hearing thresholds increase, and frequency resolution gets poorer. As a result, in noisy environments speech-understanding deteriorates and temporal processing deficits in gap detection measures increase (Lee, 2013). In humans, this condition affects tens of millions of people world-wide (Yamasoba et al., 2013). Many people with hearing loss also experience tinnitus, which is the perception of a sound in one or both ears or in the head in the absence of an external sound source (Jastreboff and Hazell, 1993).

Presbycusis is complex in that it has repercussions at a physical, cognitive, emotional, and social level; quality of life can deteriorate, and for some people presbycusis could lead to depression, social isolation and lower self-esteem (Lee, 2013; Ciorba et al., 2015). Environmental factors such as diet, physical exercise, smoking, and intake of medications are some of the extrinsic factors predisposing to presbycusis. There are several auditory structures affected by presbycusis, such as hair cells, *stria vascularis,* afferent spiral ganglion neurons and the central auditory pathways (Fuentes-Santamaría et al., 2013). Based on results of audiometric tests and temporal bone pathology, Schuknecht and Gacek (1993) and later modified by Nelson and Hinojosa (2003), classified presbycusis as either sensory (downslope audiometry and cochlear degeneration), neural (downslope audiometry and very poor speech discrimination, spiral ganglion and nerve fibers degeneration), metabolic (audiometry in a platform and strial atrophy), cochlear conductive (downslope audiometry and thickening and stiffening of basilar membrane), mixed (mixture of the above), or undetermined (none of the above) types.

Depending on the type and severity of the hearing loss, several options are available to reduce the hearing difficulties and consequently improve quality of life. When patients are appropriately fitted and motivated, hearing aids and cochlear implants (CIs) are the most commonly used devices for treating mild-severe presbycusis. Electric-acoustic stimulation and active middle ear implants may also be suitable solutions for treating presbycusis (Sprinzl and Riechelmann, 2010).

Biological markers are widely used in oncology, hematology and in other medical disciplines to diagnose or to monitor various diseases. In otology, biological markers are not yet widely used, but once identified, they could provide a means of determining the time-course or most effective treatment for an individual with presbycusis or tinnitus. Potential biomarkers include mutations in mitochondrial DNA, chromosomal mutations, state of chronic inflammation, presence of certain diseases associated with earlier onset or progression of presbycusis (e.g., diabetes, hypertension) and metabolic diseases (Van Eyken et al., 2007; Verschuur et al., 2014). It was recently estimated that 35–55% of auditory aging could have a genetic background (Ruan et al., 2014). Of interest are genes coding for glutamate receptors as glutamate is the main excitatory neurotransmitter in the peripheral and central auditory pathways. It has been suggested that increased release of glutamate may be involved in the auditory aging and the generation and maintenance of tinnitus by causing "excitotoxicity." There are many types of glutamate receptors, such as *N*-methyl-D-aspartate (NMDA) and alfa-amine propionic acid (AMPA), the latter being the most relevant receptor in physiological neurotransmission at auditory pathways. NMDA receptors are not essential for the auditory transmission, but they have been shown to be expressed in the cochlea after induction of tinnitus. Moreover, it has been demonstrated that the application of NMDA antagonists directly into the cochlear fluid can block salicylate-induced tinnitus in animals (Figueiredo et al., 2008).

GRM7 encodes a metabotropic glutamate receptor subtype 7 (mGluR7), a G protein-coupled receptor regulating auditory nerve excitability. When bound by L-glutamate, mGluR7changes the configuration of adenylyl cyclase, which has implications in the metabolism of AMPc, control of cellular cycle, and normal functioning of central nervous pathways. mGluR7 plays a general role in glutamate synaptic transmission (Voytenko and Galazyuk, 2011). In the auditory periphery, mGluR7 is thought to mediate glutamate excitotoxicity (Pujol et al., 1993) and in the cochlea mGluR7 maintains the glutamate-dependent equilibrium between the inner hair cells and the spiral ganglion neurons (Newman et al., 2012). Its role in the higher auditory pathways remains unclear (Lu, 2014). Single nucleotide polymorphisms (SNPs) of *GRM7* have been demonstrated to be associated with auditory aging in European (Friedman et al., 2009) and American populations (Newman et al., 2012) but not of a Chinese population (Luo et al., 2013). Interestingly, Newman et al. (2012) have reported that certain SNP variants of *GRM7* associate with poorer speech recognition in the elderly. The importance of *GRM7* in the auditory system is supported by the detection of mGluR7 in the inner and outer hair cells and in the spiral ganglion nerve (Friedman et al., 2009).

Highly concentrated glutamate may affect membrane permeability in the hair cells, causing an increase in Cl^- influx, and consequently an osmotic imbalance and membrane disruption (Puel et al., 1998). In addition, glutamate excitotoxicity induces apoptotic cell death and inflammation (Sahley et al., 2013). This was demonstrated in an animal model to be directly responsible for the loss of inner hair cells in a time-, dose- and tonotopy-dependent manner (Hu et al., 2015). Interestingly, neonatal exposure to monosodium glutamate has been shown to induce neuronal atrophy and dysmorphia in the cochlear nucleus and in the superior olivary complex (Foran et al., 2017). The physiological effects of glutamate excitotoxicity therefore are concluded to include ARHL (Pujol et al., 1993) and tinnitus (Brozoski et al., 2012; Sahley et al., 2013; Yu et al., 2016).

Oxidative stress represents an imbalance between the production of reactive oxygen species (ROS) and their detoxification and has been postulated to play a major role in the overall aging process and to significantly contribute to the ARHL. Oxidative stress in the inner ear, secondary to impairments in defense mechanisms caused by certain polymorphisms related to a battery of antioxidant systems, could make individuals more susceptible to ARHL (Seidman et al., 2002; Fujimoto and Yamasoba, 2014).

In the adult inner ear, presence of several detoxification and antioxidant enzymes including catalase, superoxide dismutase,

glutathione peroxidase, and glutathione *S*-transferases (GST) has been demonstrated.

One of the sources leading to accumulation of ROS are insufficiently acetylated drugs which accumulate and may be converted into reactive drug metabolites by oxidative enzymes. *N*-acetyltransferase (*NAT*) are enzymes responsible for the detoxification of exogenic substrates via *N*-acetylation or *O*-acetylation. In humans, the catalytic activity by *NAT* isoenzymes *NAT1* and *NAT2* may be regulate by these substrate concentration. Both isoenzymes are highly polymorphic and catalyze many aromatic amines and hydrazine substances important for the balance of the oxidative status. In addition, *NATs* are known to be involved in the detoxification of harmful xenobiotics (Vatsis and Weber, 1993; Hein, 2002; Ünal et al., 2005b).

Variation in *NAT2* alleles or haplotypes resulting from combination of SNPs is responsible for the *N*-acetylation polymorphism. Regarding the latter, rapid, intermediate, and slow acetylator phenotypes have been demonstrated. These phenotypes are associated with the rate of catalytic activity and accordingly predispose toward drug toxicity (Rajasekaran et al., 2011).

Because the individuals with the null genotype for *NAT2* may be more susceptible to effects of environmental toxins and oxidative free radical cellular damage, the presbycusis becomes an ideal model for evaluation of gene-environmental interaction (Ünal et al., 2005a,b). Although many individuals have been exposed to several environmental risk factors, the ARHL develops to a different degree in various age groups. This suggests genetic host factor(s) contributing to the degenerative mechanisms (Ünal et al., 2005b).

Previous studies demonstrated the association between the common human *NAT2* alleles and ARHL. Independent studies have showed a significant association between *NAT2* polymorphisms and presbycusis, namely *NAT2***6A* in the Turkish population (Ünal et al., 2005b) and in the European population (Van Eyken et al., 2007) with Caucasian subjects carrying a *NAT***6A* mutant allele having an increased risk to Presbycusis (Bared et al., 2010). Other studies considering different *NAT2* alleles reported negative associations with ARHL (Dawes et al., 2015) and with the shape of the audiograms (Angeli et al., 2012), when considering audiometric patterns of presbycusis in older individuals. However, most authors suggested that *NAT2* gene is a susceptibility factor for development of hearing impairment (Ünal et al., 2005b; Dawes et al., 2015).

Here we explore the relationships between presbycusis, tinnitus, co-morbidities, and the genotypes of *GRM7* and *NAT2*, in a sample of older Portuguese adults.

PATIENTS AND METHODS

Subjects

Inclusion criteria was the presence of sensory presbycusis, with or without tinnitus, in adults of any gender, aged between 55 and 75 years, from the Portuguese population.

Our sample included 78 older individuals ($n = 45$ women, $n = 33$ men).

For the purposes of inclusion presbycusis was defined as bilateral sensorineural deafness in downslope audiometric pattern, above 1000 Hz with poor speech discrimination (discrimination threshold > 40 dB SPL and 100% discrimination to 60 dB or worse). Although all included participants have presbycusis we will consider a subgroup with normal hearing because the adopted classification uses conversational frequencies.

Exclusion criteria were considered: inability to understand and sign the informed consent due to a significant cognitive impairment, an uncompensated medical disorder that requires urgent evaluation or if the individual has a serious psychiatric disorder. Also individuals over 55 years who presented possible factors that may overlap the variables under study were excluded [e.g., Ménière's disease, chronic otitis media, otosclerosis, tinnitus from disease of the outer ear (occlusive exostosis, outer otitis)], history of ototoxic drugs use, massive noise exposure, a history of previous malignancy with chemotherapy, history of autoimmune disorders and neurodegenerative and demyelinating diseases.

This study had the approval of the Ethical Committees from Hospital Cuf Infante Santo (November 26th, 2014), Nova Medical School (n°65/2014/CEFCM) and the National Department of Personal Data Protection (authorization number:1637/2016).

Accordingly we obtained the Institutional Scientific Review Board approval of the process for taking informed consent and overall study design. The study was conducted in accordance with the Declaration of Helsinki.

Clinical Evaluation

Written informed consent, clinical and familial history, audiological evaluation and a blood sample, using Whatman® FTA® card technology, was obtained from every subject.

A questionnaire concerning epidemiologic data (demographic, previous and present diseases, toxicological habits and noise exposure) was completed by the researcher through participant interview.

Audiological Assessment

Hearing thresholds were determined by pure tone audiometry (air and bone) according to ISO 8253 and 389. The exam was performed in a soundproof booth using an Interacoustics®, Assens, Denmark audiometer (Model: AC40, Serial No.: 98 019 046) and TDH39 headphones fitted with noise-excluding headset ME70 and bone conductor B-71. Audiometry was performed at frequencies from 0.25 to 16 kHz (standard tonal audiometry and extended high frequency). The category of Hearing Loss (HL) was defined according to the average threshold across 500, 1000, 2000, and 4000 Hz in the better ear as mild (21–40 dB), moderate (41–70 dB), severe (71–95 dB) or profound (> 95 dB), from an average of thresholds at 500, 1000, 2000, and 4000 Hz in the better ear, according to the European Working Group Genetics of Hearing Impairment (After Liu and Xu, 1994; Parving and Newton, 1995).

Speech audiometry evaluation was obtained with headphones (using mp3 player), or in open field, where the evaluator was

hiding his lips to prevent lip-reading. The number of disyllables that patient repeats correctly was recorded. This intelligibility threshold for two-syllable words intends to measure hearing sensitivity threshold through the intensity level identification in which the patient can correctly identify 50% or more of a disyllables list. On the other hand, the speech discrimination evaluates the lowest intensity level at which a listener can understand speech.

Tinnitus Assessment

Psychoacoustic assessment consisted of loudness match, pitch match, minimum masking level (MML) or Feldmann masking curves, residual inhibition, and loudness discomfort levels (LDL). The severity of tinnitus was evaluated using the Tinnitus Handicap Inventory (THI; Newman et al., 1996). THI comprises 25 questions concerning tinnitus, and the response options are "Yes," "Sometimes," and "No," respectively, corresponds to 4, 2, and 0, accounting for a total score that may vary between 0 and 100. The questionnaire comprehends three sub-scales or dimensions: Functional (11 items – contributing 0–44 for the final score), Emotional (9 items – contributing 0–36 for the final score) and Catastrophic (5 items – contributing 0–20 for the final score). This allow to verify which are the most affected aspects and accordingly choose the therapeutic interventions. The total score of the responses allows tinnitus classification according to its severity or impact in daily life – 0–16: Slight or no handicap (Grade 1), 18–36: Mild handicap (Grade 2), 38–56: Moderate handicap (Grade 3), 58–76: Severe handicap (Grade 4), 78–100: Catastrophic handicap (Grade 5).

Additionally THI is a self-administered instrument, easy to quote, to interpret and has good psychometrics properties (McCombe et al., 2001).

Genetic Analysis

Total genomic DNA was extracted from a blood sample on FTA cards using a commercial NZY Tissue gDNA Isolation Kit (NZYTech, Lisbon, Portugal), strictly according to the manufacturer's instructions. Molecular analysis of *GRM7* gene was assessed by qPCR for A/A, A/T, and T/T genotypes, at rs11928865 SNP. Concerning *NAT2* gene, rs1041983, rs1801280, rs1799929, rs1799930, rs108 and s1799931 were assessed by qPCR or by bidirectional sequencing of the target region in order to identify all the SNPs.

Statistical Analysis

We conducted a descriptive analysis for variables such as gender and age. The audiograms were analyzed considering the best ear (estimated based on the lowest average of frequencies of 0.5–4 kHz). We also evaluated the "high frequency" pure-tone average (PTA) at 2, 4, and 8 kHz (Newman et al., 2012). Chi-square Test or Fisher Exact Test for general association between two variables were used. Mann–Whitney or Kruskal–Wallis (for more than two groups) tests were employed to compare hearing thresholds. A Dunn's test with a Bonferroni correction was applied for multiple pairwise comparisons. The level of significance considered was $p = 0.05$.

All the results were analyzed through logistic regression model, where age and gender where considered as control for all other variables.

RESULTS

Participants in our study were 78 older adults aged 64.6 ± 5.58 years old (range = 55–75 years old). Most participants were female ($n = 45$, 57.7%), presenting an average age of 64.1 ± 5.35 years old. For men ($n = 33$, 42.3%), the mean age was 65.3 ± 5.89 years old (**Table 1**).

Hearing Thresholds

The average hearing threshold values (by gender and age) are shown in **Figure 1**. There were significant differences between the gender groups regarding average and median values of hearing thresholds at the frequencies of 4 kHz (p-value = 0.007) and 8 kHz ($p = 0.031$) when comparing male and female (**Figure 1**). There were significant differences between age groups regarding the average and median values of hearing thresholds at frequencies of 4 kHz ($p = 0.003$), 8 kHz ($p < 0.001$), 10 kHz ($p < 0.001$) and 12 kHz ($p < 0.001$) when comparing the different age groups (**Figure 1**).

When comparing hearing thresholds between the different age groups, we found significant differences in females at 4 kHz ($p = 0.009$), 8 kHz ($p = 0.011$), 10 kHz ($p = 0.018$) and 12 kHz ($p = 0.002$) (**Figure 2**). For males statistically significant differences were observed between age groups at 8 kHz ($p = 0.009$), 10 kHz ($p = 0.003$) and 12 kHz ($p = 0.004$) (**Figure 2**).

According to age and gender grouping and comparing males to females we found significant differences for hearing thresholds for the age group 55–60 years old for 1 kHz frequency ($p = 0.022$) and 4 kHz frequency ($p = 0.028$) (**Figure 2**).

Distribution of the individuals according to the hearing loss and tinnitus presence (**Table 1**) shows that in subgroup 1, 18 (23.1%) individuals who had normal hearing thresholds at speech frequencies (0.5–4 kHz) but not tinnitus; subgroup 2, 23 (29.5%) individuals who had normal hearing thresholds at speech frequencies and tinnitus; subgroup 3, 10 (12.8%) individuals who had hearing loss but not tinnitus; and subgroup 4, 27 (34.6%) individuals who had hearing loss and tinnitus (see also **Figure 3**). There are no statistical differences in age or gender between those four subgroups.

TABLE 1 | Distribution of the individuals by subgroups according to hearing loss, tinnitus presence (PTA = Pure Tone Average) and gender.

Subgroup	Audiological characteristic	Gender		*n*
		Male	Female	
1	PTA ≤ 20 without Tinnitus	5	13	18 (28%)
2	PTA ≤ 20 with Tinnitus	8	15	23 (29.5%)
3	PTA ≥ 20 without Tinnitus	6	4	10 (12.8%)
4	PTA ≥ 20 with Tinnitus	14	13	27 (34.6%)
Total		33	45	**78**

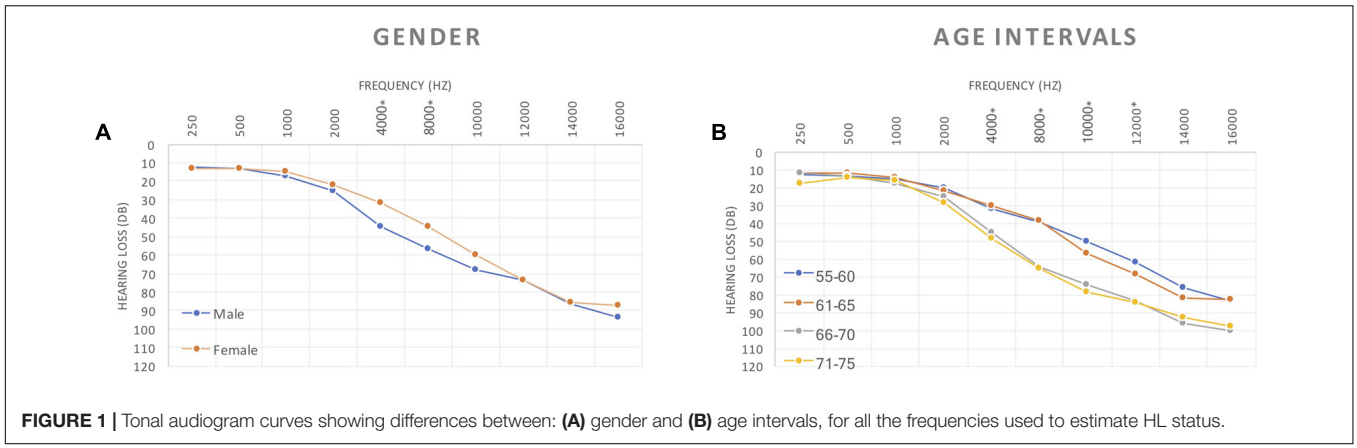

FIGURE 1 | Tonal audiogram curves showing differences between: **(A)** gender and **(B)** age intervals, for all the frequencies used to estimate HL status.

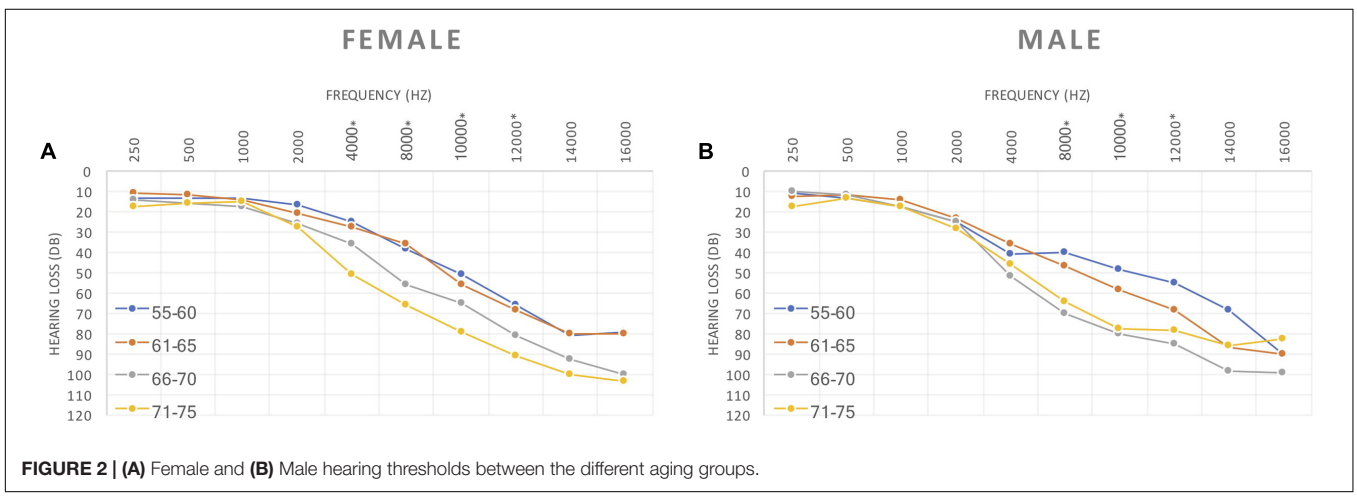

FIGURE 2 | (A) Female and **(B)** Male hearing thresholds between the different aging groups.

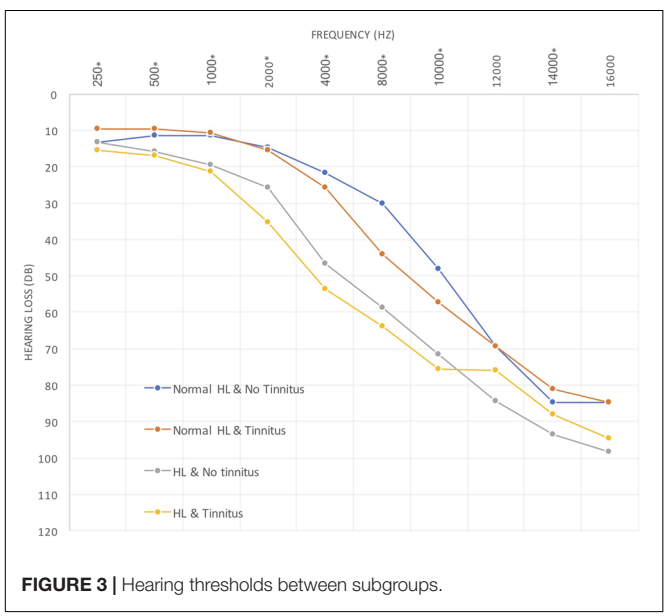

FIGURE 3 | Hearing thresholds between subgroups.

We found statistically relevant differences between the four described groups which corroborates the logical of having chosen this subdivision of our study population. ($p < 0.001$ at the majority of frequencies).

There were significant differences in speech audiograms (PTA, speech recognition threshold [SRT], 100%; p-value = <0.001; <0.001; <0.001, respectively) between subgroups, either for the right ear or for the left ear. The differences were found between subgroups 4 or 3 and the subgroups 1 and 2 for PTA (0%), SRT (50%) and (100%) (**Figure 4**).

Because our study population represents older adult individuals with sensory presbycusis we evaluated the "high frequency" pure-tone average (PTA) at 2, 4, and 8 kHz. We compared the groups of individuals with and without tinnitus and the four subgroups (**Table 1**). In respect to having or not tinnitus we found statistical differences between those groups ($p = 0.003$) (for more details see Appendix 1). We found statistically significant differences ($p < 0.001$) when comparing the four subgroups described in **Table 1** (for more details see Appendix 2).

Characterization of the considered comorbidities in our sample are presented in **Table 2**. Concerning hearing thresholds according to presence or not of the studied comorbidities, we found the following relevant significant differences: from 0.5 to 4 kHz for cholesterol; at 4 kHz for measles.

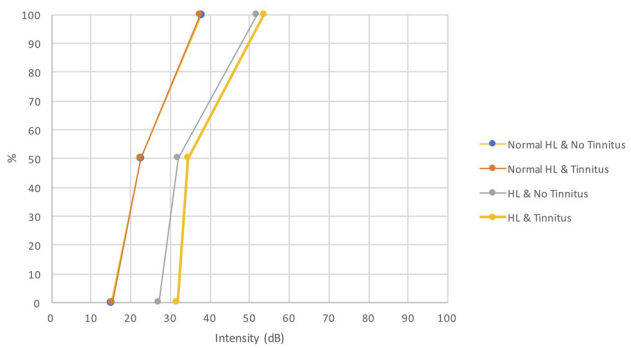

FIGURE 4 | Speech audiograms between subgroups.

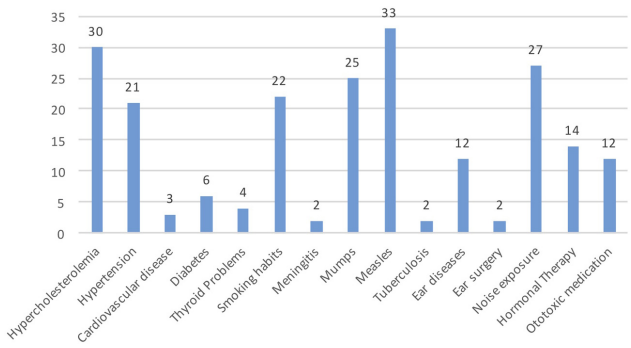

FIGURE 5 | Distribution of the most common comorbidities in the individuals with tinnitus.

When comparing the group of participants with and without tinnitus the most statistical relevant results were concerning 'high frequency' hearing loss and noise exposure. In our study population, 50 individuals (64.1%) had tinnitus.

We have determined the distribution of studied comorbidities in our tinnitus population (**Figure 5**). The most prevalent were measles, hypercholesterolemia, noise exposure, mumps, smoking and hypertension, in a descendent order of frequency.

In our sample, 49 participants (62.8%) reported to have high blood values of cholesterol. Of those, 27 individuals (55.1%) were taking medication (statins) (Appendix 3). There was a significant association between tinnitus and statins intake in those individuals reporting hypercholesterolemia ($\widehat{OR} = 0.28$, $p = 0.045$, $CI = 0.08 - 0.99$) (**Table 3**). We found no relevant association between statins intake and hearing loss.

TABLE 2 | Distribution of the most common comorbidities in the individual of the sample.

Comorbidities	*n*	
	Absent	**Present**
Cholesterol	29 (37.2%)	49 (62.8%)
Hypertension	43 (55.1%)	35 (44.9%)
Cardiovascular disease	73 (93.6%)	5 (6.4%)
Tinnitus	28 (35.8%)	50 (64.1%)
Diabetes	65 (83.3%)	13 (16.7%)
Thyroid problems	70 (89.7%)	8 (10.3%)
Smoking habits	44 (56.4%)	34 (43.6%)
Meningitis	77 (98.7%)	1 (1.3%)
Mumps	44 (56.4%)	34 (43.6%)
Measles	21 (26.9%)	57 (73.1%)
Tuberculosis	75 (96.2%)	3 (3.8%)
Ear diseases	62 (79.5%)	16 (20.5%)
Ear surgery	76 (97.4%)	2 (2.6%)
Noise exposure	51 (65.4%)	27 (34.6%)
Hormonal therapy	55 (70.5%)	23 (29.5%)
Ototoxic medication	58 (74.4%)	20 (25.6%)

Tinnitus Evaluation

Subgroups 2 and 4 included participants with tinnitus. Concerning tinnitus laterality, 33 of them reported to have a unilateral tinnitus (12 on the right ear and 21 on the left ear) and 17 participants have a bilateral tinnitus. According to THI score (**Figure 6**) for most participants tinnitus was bothersome, only 10 subjects had a slight handicap.

Modeling the data

All the results were analyzed through a logistic regression model age and gender were considered in all the models with the objective of controlling eventual confounding since these two factors are known to be related to hearing loss.

The regression logistic model was applied to HL considering female as reference (for more details see Appendix 4). The odds of developing presbycusis was significantly higher for males than for females ($\widehat{OR} = 2.9, p = 0.032$). When considering age as a covariate, the effect was slight but significant, being the odds of having hearing loss 9% higher for each increasing year ($\widehat{OR} = 1.09, p = 0.03$).

Using this statistical model for all the comorbidities considered and controlled for age the odds of having hearing loss was significantly lower for subjects with high cholesterol ($\widehat{OR} = 0.33, p = 0.034$).

We found no association between HL and high blood pressure or noise exposure.

In addition, using the regression logistic model for tinnitus considering men and the absence of tinnitus as reference, we found that noise exposure seems to influence the occurrence of tinnitus ($\widehat{OR} = 3.65, p = 0.026, CI = 1.2 - 11.4$), when considered isolated. This result is statistically very relevant.

There were no other statistically significant results concerning other comorbidities in this study (for more details see Appendix 5).

GRM7 and NAT2 genes

Results for *GRM7* gene at rs11928865 SNP refers to A or T alleles and contribute for three possible genotypes: A/A, A/T, or T/T. *GRM7* data are presented comparatively (**Table 4**) with data for Iberian Peninsula and Europe in order to compare our population with others.

TABLE 3 | Association between tinnitus and statins intake in individuals reporting hypercholesterolemia.

	Statins intake	Without Statins	$\widehat{OR}$	*p*-value (fisher test)	95% IC
Tinnitus	13	17	0.28	$p = 0.045$	[0.08 – 0.99]
Without tinnitus	14	5			

*p-value < 0.05.

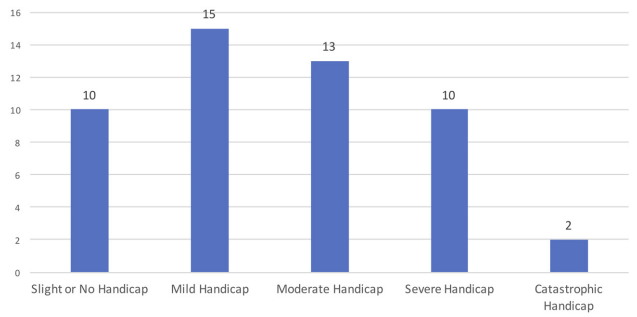

FIGURE 6 | Distribution of the individuals according to THI.

Some genetic specificity has been reported for different populations regarding deafness genes, and, interestingly, genotypes representativeness for the individuals of our sample were in accordance with values described in the European population as well as in the Iberian population.

Analyzing these results and considering the hearing thresholds, no significant differences were found in males or females when the three genotypes were compared, however, some differences in the pattern of the curves on the audiogram can be seen.

Considering the Tinnitus Handicap Inventory scores (please see **Figure 6**) the variable severe tinnitus ($n = 12$) joins the severe and catastrophic grades. We found relevant statistical association between the presence of *GRM7* and severe tinnitus (individuals having scored severe or catastrophic grade in THI). The results are present in the **Table 5**.

We found no relevant statistical differences considering *GRM7* when comparing the four sub groups already described in **Table 1**, evidencing no relation with this SNP and the presence of presbycusis with or without tinnitus.

When considering genetic data, *GRM7* genotype was found not to be associated with the risk of developing presbycusis ($p = 0.889$). However, the odds of HL is higher in individuals presenting A/T (29%) or T/T (2%) genotype, than in A/A genotype. The same results were observed when controlling for age and gender, however, in this case the odds of HL in A/T genotype individuals was nearly 39% higher than for A/A genotype individuals. The odds of HL in T/T genotype was 15% higher than in A/A genotype.

TABLE 4 | Comparative results for rs11928865 SNP on *GRM7* gene and comparison with other populations.

Genotypes	*N*	Frequency	Europe	United Kingdom	Iberian peninsula
A/A	5	0.064	0.087	0.055	0.065
A/T	26	0.333	0.382	0.473	0.393
T/T	47	0.603	0.531	0.473	0.542
Total	**78**	1	1	1	1

TABLE 5 | Association between THI score (tinnitus severity) and *GRM7* gene.

GRM7	Severe tinnitus	*p*-value	Fisher test
A/A	1		
A/T	7	**0.0233***	**0.0175**
T/T	4		

*p-value < 0.05.

The relation between tinnitus and GRM7 gene was evaluated considering two groups, one defined as "having an A allele" (AA + AT) other defined as "not having an A allele" (TT). Results were not significant ($\widehat{OR} = 0.96$) however, since the estimated $\widehat{OR} < 1$, a decrease in the risk for tinnitus could be thought. The GRM7 genotype was not identified as a risk factor for tinnitus, neither when controlling for age ($\widehat{OR} = 0.94$) (OR = 0.94) for gender ($\widehat{OR} = 0.93$) or both simultaneously ($\widehat{OR} = 0.93$). Similar analysis was performed considering also two groups but defined as "having a T allele" (TT and AT genotypes). However, no significant association with tinnitus was found.

Genetic analysis of *NAT2* gene was performed in 65 individuals, 39 females (60%), and 26 males (40%). Rapid (R) phenotype was least common (12.3%, $n = 8$), followed by Intermediate (I) phenotype (35.4%, $n = 23$) and Slow (S) phenotype (52.3%, $n = 34$) (please see **Table 6**).

The genotype 4/4 (considered as wild type) was observed in 9.1% ($n = 6$) of the individuals being the allele 4 present in 56.9% ($n = 37$) of the genotypes. The genotype 6A/6A previously associated with presbycusis was found in 6.2% ($n = 4$) of the individuals being the allele 6A present in 23.1% ($n = 15$) of the individuals. The most common genotype is 5B/5B accounting for 50% ($n = 11$) of all the homozygous genotypes (33.9%, $n = 22$) the sample (Kuznetsov et al., 2009).

We found no statistical differences in *NAT2* gene expression across our four subgroups described in **Table 1**, evidencing no relation with the presence of presbycusis with or

TABLE 6 | Genotypes observed in the sample and their corresponding phenotypes.

Genotype	Phenotype
*NAT2*4/NAT2*5U; NAT2*6A/NAT2*6A; NAT2*5B/NAT2*5D; NAT2*6J/NAT2*13A; NAT2*5A/NAT2*5B; NAT2*6N/NAT2*6N; NAT2*6A/NAT2*6B; NAT2*5D/NAT2*5G; NAT2*5B/NAT2*5B; NAT2*5R/NAT2*12A; NAT2*4/NAT2*5J*	S
*NAT2*4/NAT2*4; NAT2*12A/NAT2*12C; NAT2*4/NAT2*12C*	R
*NAT2*4/NAT2*5B; NAT2*4/NAT2*6A; NAT2*4/NAT2*5B; NAT2*4/NAT2*5A; NAT2*5B/NAT2*12A; NAT2*4/NAT2*13A; NAT2*4/NAT2*5V; NAT2*6A/NAT2*13A; NAT2*5B/NAT2*13A*	I

without tinnitus. No significant association with ARHL was found, for the in the right and left ear or best or worst ear.

Considering the Tinnitus Handicap Inventory scores, we found significant association between severity of tinnitus (grades severe and catastrophic from THI) and the presence of *NAT2* gene (please see more details in the next sub-heading).

Modeling the Data – *GRM7* and *NAT2*

All the results were analyzed through logistic regression model (**Tables 7**, **8**) where age, gender and noise exposure were considered in the models with the purpose of controlling for confounding. The independent variable in the model was severe tinnitus ($n = 12$), (the sum of severe and catastrophic grades from THI) (**Figure 6**).

We have considered the genotype T/T because after crossing the *GRM7* gene with the tinnitus population, we found that the T/T genotype is more frequent and it is the most representative so it was chosen as the reference category.

The odds of developing severe tinnitus was significantly higher in the presence of genotype A/T when compared to genotype T/T ($\widehat{OR} = 14.2$, $p = 0.009$, $CI = 2.0 - 97.8$). When considering the genotype A/A, no statistically significant difference was found ($\widehat{OR} = 2.9$, $p = 0.443$, $CI = 0.2 - 42.2$). The probability of severe tinnitus among individuals with genotype A/T is significantly higher when compared with individuals with the genotype T/T (for more details see Appendix 6).

When analyzing the presence of severe tinnitus through a logistic regression model considering *NAT2* as the independent variable and controlling for age, gender and noise exposure, the odds of developing severe tinnitus was significantly higher in the presence of slow acetylator phenotype when compared to intermediate acetylator ($\widehat{OR} = 5.7$, $p = 0.095$, $CI = 1.5 - 21.9$). No statistically significant difference was found with respect to rapid acetylator ($\widehat{OR} = 2.8$, $p = 0.504$, $CI = 0.4 - 20.8$) (for more details see Appendix 7).

TABLE 7 | Logistic regression model in the *GRM7* applied to severe tinnitus considering the genotype T/T as reference.

Variable*	$\widehat{OR}$	*p*-value (Wald test)	(95% IC)
GRM7			
A/A	2.9	0.443	(0.2, 42.2)
A/T	14.2	**0.009****	(2.0, 97.8)

***p-value < 0.05.*

TABLE 8 | Logistic regression model in the *NAT2* applied to severe tinnitus considering intermediate acetylator as reference.

Variable*	$\widehat{OR}$	*p*-value (Wald test)	(90% IC)
NAT2			
Rapid acetylator	2.8	0.504	(0.4, 20.8)
Slow acetylator	5.7	0.095	(1.5, 21.9)

DISCUSSION

In the present research, we conducted a case history questionnaire, hearing evaluation and gene screening analysis for *GRM7* and *NAT2* in a sample of patients aged between 55 and 75 years, in an attempt to find factors that might contribute to the diagnosis of presbycusis and tinnitus, which could be useful for diagnosis and future therapeutic interventions.

Comorbidities Effect

Although in previous literature was described that individuals with thyroid problems present increased hearing thresholds, suggesting that thyroid hormones may act as regulators of the auditory system (Forrest et al., 1996) our results do not show any statistical relevance concerning this, one possible explanation is the sample size. Only 10% of our participants report thyroid problems which precludes statistical analysis.

Possibly for a similar reason our data doesn't show that individuals with high blood pressure may be at greater risk of presbycusis than the normotensive. Hypertension has previously been associated with increasing of the hearing threshold (Agarwal et al., 2013, p. 614). Since both presbycusis and hypertension are common and widespread disorders, the fact that hypertension may influence presbycusis strongly suggests adding cardiologists to the multidisciplinary team of professionals screening for presbycusis and improving the quality of life of positively identified individuals (Agarwal et al., 2013).

Our results found that hypercholesterolemic individuals had a lower risk of HL, probably this is due to the fact that the majority of them (67%) were having medication (statins) to control cholesterol levels. These results are in accordance with previous publications (Gopinath et al., 2011). In individuals with hypercholesterolemia the chance of occurring tinnitus is 72% lower in those who have statins intake. According to our results It seems like the statins have a protector effect.

Noise exposure and "high frequency" hearing loss seems to influence the occurrence of tinnitus, those were two of the most statistical relevant findings in our study population, which is in accordance with previous literature (Hoffman and Reed, 2004).

Gender and Age Effect

Significant differences on the HL degree were observed in different frequencies for different age groups (**Figure 2**). Our results show a significant age-dependent increase of hearing loss in about 13% for both genders, although the risk of developing presbycusis is about three times higher for men. This finding is consistent with a previous reports (Pearson et al., 1995) but contradicts another (Homans et al., 2016) where women were found to have more hearing loss.

According to our data, the risk of presbycusis increases 9% per year of life. Considering the increase in life expectancy of the population in industrialized countries, our result presents obvious consequences and must be considered for future clinical management guidelines.

In our sample tinnitus was present in 60.7% of the participants and men showed 53% more likelihood of developing tinnitus than women. This contradicts other results (Vielsmeier et al.,

2012) who reported higher tinnitus prevalence in women but in a much younger population.

According to our data, and in agreement with previous literature (Hoffman and Reed, 2004; Shargorodsky et al., 2010) age is not associated with the risk of developing tinnitus.

GRM7 and *NAT2* Effect

We did not find a significant relationship between *GRM7* genotype and either presbycusis or tinnitus. Especially for men, some differences concerning the pattern in the audiogram curves were observed in relation to *GRM7* phenotypes. For both genders, the T allele in *GRM7* gene is the most common allele in our sample of older adults with presbycusis and tinnitus, where genotypes A/T and T/T present higher level of hearing loss compared to A/A genotype. Perhaps in a larger population it could be demonstrated that the allele A of *GRM7* plays a protective role in presbycusis.

Hence, according to our results, *GRM7* genotype does not seem to be predictive of presbycusis since the odds to have ARHL is not significant ($p = 0.78$). Corroborating our results, Luo et al. (2013) studying an all-male population found that the T-allele frequency was significantly different from the genotype A/A+A/T comparing ARHL patients and healthy controls and that the *GRM7* SNP A > T was significantly different between the two groups (Luo et al., 2013). On the other hand, our findings differ from Friedman et al. (2009) most likely due to sample size (Luo et al., 2013). Moreover, the impact of the other variables – environmental, lifestyle, noise exposure, cholesterol levels and stochastic element – perhaps has prevailed over the genetic factor, declining the importance between *GRM7* gene and ARHL. Certainly multicenter studies with higher sample sizes would overcome these aspects.

Concerning *NAT2* gene, Rapid (R) phenotype was the least common, followed by the Intermediate (I) and Slow (S) phenotypes.

We found relevant statistical association between the presence of the allele A/T of *GRM7* and severe tinnitus. The chance for having a severe grade of tinnitus (severe or catastrophic grades in THI) is 14,2 higher for those carrying the allele A/T compared to T/T. Probably in larger scale studies could be demonstrated the role of allele A/A that is the less frequent in our sample.

The odds of developing severe tinnitus was relatively higher in the presence of slow acetylator phenotype of *NAT2* when compared to intermediate acetylator.

Our data suggests that allele A/T of *GRM7* can have a statistically significant influence toward the *severity* of tinnitus. As well slow acetylator phenotype of *NAT2* seems to have a similar influence (not statistically relevant in our results). Nevertheless, those results should be interpreted with caution and future studies in larger scale are necessary to confirm this correlation.

However, present data shows that genotype A/T and T/T present, respectively, a 70 and 33.3% lower risk of developing tinnitus, when compared to A/A genotype. No other studies were found relating *GRM7, NAT2* and tinnitus.

CONCLUSION

To the best of our knowledge, this is the first study on the association between *GRM7* and *NAT2* gene and the presbycusis and tinnitus in a population of Portuguese older adults.

Tinnitus was present in the majority of the presbycusis individuals.

Age and gender significantly influence the risk for presbycusis but not for tinnitus. Overall hearing thresholds rates increase exponentially with age (9% per year), and the increment rate and speed were gender-specific, but this increasing rate and velocity are different for women and men.

High blood pressure, thyroid diseases and hypercholesterolemia seem to have an effect on the hearing thresholds but no significant associations were found.

Our findings agree with previously observed correlations between tinnitus, noise exposure, and "high frequency" hearing loss.

No significant associations between presbycusis, tinnitus, and *GRM7* or *NAT2* were found in our sample. Our results precludes a definitive clarification about the role of *GRM7* as a possible genetic biomarkers for ARHL, although since the genotypes A/T and T/T have higher odds for HL than A/A genotypes, thus A allele could be pointed as protective biomarker for HL Nevertheless, the current state of knowledge regarding *GRM7* impact in presbycusis is insufficient to make conclusions, and so, further large-scale studies are necessary to clarify this relation.

Considering tinnitus severity (according to THI), our results bring-up very innovative conclusions.

Our data suggests the tracks that can lead to the pathway of a tinnitus severity biomarker. Potentially individuals carrying the allele A/T of *GRM7* and slow acetylator phenotype of *NAT2* (the later one with smaller statistic relevance) are prone to develop a more severe form of tinnitus, that requires specific therapeutic interventions and ideally personally tailored.

The occurrence of presbycusis is thought to be determined by genetic factors but can also be influenced by environmental or comorbidities effects, with a huge impact on quality of life and general health (Huang and Tang, 2010; Ciorba et al., 2015). However, there is still much research to explore and elucidate which risk factors contribute more to presbycusis and tinnitus, so this could help on therapeutic or preventive interventions (Huang and Tang, 2010).

Information on family history and clinical epidemiological data may help the design and development of future clinical management plans for an increasing presbycusis population.

AUTHOR CONTRIBUTIONS

HH conceived and designed this study and had contributions to all its stages. MAp and MA performed the statistical analysis. HH, MF, and HC contributed equally to all other stages of the manuscript development, drafted and revised the manuscript. DR worked with HH on interpretation of results and created appendices. DR created all audiometric figures. JP, MA, AS, DH, and GF provided consultative advice and revised the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnagi.2017.00346/full#supplementary-material

REFERENCES

Agarwal, S., Mishra, A., Jagade, M., Kasbekar, V., and Nagle, S. K. (2013). Effects of hypertension on hearing. *Indian J. Otolaryngol. Head Neck Surg.* 65, 614–618.

Angeli, S., Lin, X., and Liu, X. Z. (2012). Genetics of hearing and deafness. *Anat. Rec.* 295, 1812–1829.

Bared, A., Ouyang, X., Angeli, S., Du, L. L., Hoang, K., Yan, D., et al. (2010). Antioxidant enzymes, presbycusis, and ethnic variability. *Otolaryngol. Head Neck Surg.* 143, 263–268. doi: 10.1016/j.otohns.2010.03.024

Brozoski, T., Odintsov, B., and Bauer, C. (2012). Gamma-aminobutyric acid and glutamic acid levels in the auditory pathway of rats with chronic tinnitus: a direct determination using high resolution point-resolved proton magnetic resonance spectroscopy (H-MRS). *Front. Syst. Neurosci.* 6:9. doi: 10.3389/fnsys.2012.00009

Ciorba, A., Hatzopoulos, S., Bianchini, C., Aimoni, C., Skarzynski, H., and Skarzynski, P. H. (2015). Genetics of presbycusis and presbystasis. *Int. J. Immunopathol. Pharmacol.* 28, 29–35. doi: 10.1177/0394632015570819

Dawes, P., Emsley, R., Cruickshanks, K. J., Moore, D. R., Fortnum, H., Edmondson-Jones, M., et al. (2015). Hearing loss and cognition: the role of hearing AIDS, social isolation and depression. *PLOS ONE* 10:e0119616. doi: 10.1371/journal.pone.0119616

Figueiredo, R. R., Langguth, B., de Oliveira, P. M., and de Azevedo, A. A. (2008). Tinnitus treatment with memantine. *Otolaryngol. Head Neck Surg.* 138, 492–496. doi: 10.1016/j.otohns.2007.11.027

Foran, L., Blackburn, K., and Kulesza, R. J. (2017). Auditory hindbrain atrophy and anomalous calcium binding protein expression after neonatal exposure to monosodium glutamate. *Neuroscience* 344, 406–417. doi: 10.1016/j.neuroscience.2017.01.004

Forrest, D., Erway, L. C., Ng, L., Altschuler, R., and Curran, T. (1996). Thyroid hormone receptor b is essential for development of auditory function. *Nat. Genet.* 13, 354–357.

Friedman, R. A., Van Laer, L., Huentelman, M. J., Sheth, S. S., Van Eyken, E., Corneveaux, J. J., et al. (2009). *GRM7* variants confer susceptibility to age-related hearing impairment. *Hum. Mol. Genet.* 18, 785–796. doi: 10.1093/hmg/ddn402

Fuentes-Santamaría, V., Alvarado, J. C., Gabaldón-Ull, M. C., and Manuel Juiz, J. (2013). Upregulation of insulin like growth factor and interleukin 1β occurs in neurons but not in glial cells in the cochlear nucleus following cochlear ablation. *J. Comp. Neurol.* 521, 3478–3499.

Fujimoto, C., and Yamasoba, T. (2014). Oxidative stresses and mitochondrial dysfunction in age-related hearing loss. *Oxid. Med. Cell. Longev.* 2014:582849.

Gopinath, B., Flood, V. M., Teber, E., McMahon, C. M., and Mitchell, P. (2011). Dietary intake of cholesterol is positively associated and use of cholesterol-lowering medication is negatively associated with prevalent age-related hearing loss. *J. Nutr.* 141, 1355–1361. doi: 10.3945/jn.111.138610

Hein, D. W. (2002). Molecular genetics and function of *NAT1* and *NAT2*: role in aromatic amine metabolism and carcinogenesis. *Mutat. Res.* 506, 65–77.

Hoffman, H. J., and Reed, G. W. (2004). "Epidemiology of tinnitus," in *Tinnitus: Theory and Management*, ed. J. B. Snow (Lewiston, NY: BC Decker Inc.), 16–41.

Homans, N. C., Metselaar, R. M., Dingemanse, J. G., van der Schroeff, M. P., Brocaar, M. P., Wieringa, M. H., et al. (2016). Prevalence of age-related hearing loss, including sex differences, in older adults in a large cohort study. *Laryngoscope* 127, 725–730. doi: 10.1002/lary.26150

Hu, Y., Zhou, L. Q., Lu, H. T., Yuan, K., and Gong, S. S. (2015). Excitotoxic effects of glutamate on cochlear organotypic cultures. *J. Huazhong Univ. Sci. Technol.* 35, 117–121. doi: 10.1007/s11596-015-1399-0

Huang, Q., and Tang, J. (2010). Age-related hearing loss or presbycusis. *Eur. Arch. Otorhinolaryngol.* 267, 1179–1191. doi: 10.1007/s00405-010-1270-7

Jastreboff, P. J., and Hazell, J. W. (1993). A neurophysiological approach to tinnitus: clinical implications. *Br. J. Audiol.* 27, 7–17.

Kuznetsov, I. B., McDuffie, M., and Moslehi, R. (2009). A web-server for inferring the human N-acetyltransferase-2 (*NAT2*) enzymatic phenotype from *NAT2* genotype. *Bioinformatics* 25, 1185–1186. doi: 10.1093/bioinformatics/btp121

Lee, K. Y. (2013). Pathophysiology of age-related hearing loss (peripheral and central). *Korean J. Audiol.* 17, 45–49. doi: 10.7874/kja.2013.17.2.45

Liu, X., and Xu, L. (1994). Nonsyndromic hearing loss: an analysis of audiograms. *Ann. Otol. Rhinol. Laryngol.* 103, 428–433. doi: 10.1177/000348949410300602

Lu, Y. (2014). Metabotropic glutamate receptors in auditory processing. *Neuroscience* 274, 429–445. doi: 10.1016/j.neuroscience.2014.05.057

Luo, H., Yang, T., Jin, X., Pang, X., Li, J., Chai, Y., et al. (2013). Association of *GRM7* variants with different phenotype patterns of age-related hearing impairment in an elderly male Han Chinese population. *PLOS ONE* 8:e77153. doi: 10.1371/journal.pone.0077153

McCombe, A., Baguley, D., Coles, R., McKenna, L., McKinney, C., and Windle-Taylor, P. (2001). Guidelines for the grading of tinnitus severity: the results of a working group commissioned by the British Association of Otolaryngologists, Head and Neck Surgeons. *Clin. Otolaryngol.* 26, 388–393.

Nelson, E. G., and Hinojosa, R. (2003). Presbycusis: a human temporal bone study of individuals with flat audiometric patterns of hearing loss using a new method to quantify stria vascularis volume. *Laryngoscope* 113, 1672–1686.

Newman, D. L., Fisher, L. M., Ohmen, J., Parody, R., Fong, C. T., Frisina, S. T., et al. (2012). *GRM7* variants associated with age-related hearing loss based on auditory perception. *Hear. Res.* 294, 125–132. doi: 10.1016/j.heares.2012.08.016

Newman, C. W., Jacobson, G. P., and Spitzer, J. B. (1996). Development of the tinnitus handicap inventory. *Arch. Otolaryngol. Head Neck Surg.* 122, 143–148.

Parving, A., and Newton, V. (1995). Guidelines for description of inherited hearing loss. *J. Audiol. Med.* 4, 2–5.

Pearson, J. D., Morrell, C. H., Gordon Salant, S., Brant, L. J., Metter, E. J., Klein, L. L., et al. (1995). Gender differences in a longitudinal study of age-associated hearing loss. *J. Acoust. Soc. Am.* 97, 1196–1205.

Puel, J. L., Ruel, J., d'Aldin, C. G., and Pujol, R. (1998). Excitotoxicity and repair of cochlear synapses after noise trauma induced hearing loss. *Neuroreport* 9, 2109–2114.

Pujol, R., Puel, J. L., D'aldin, C. G., and Eybalin, M. (1993). Pathophysiology of the glutamatergic synapses in the cochlea. *Acta Otolaryngol.* 113, 330–334.

Rajasekaran, M., Abirami, S., and Chen, C. (2011). Effects of single nucleotide polymorphisms on human N-acetyltransferase 2 structure and dynamics by molecular dynamics simulation. *PLOS ONE* 6:e25801. doi: 10.1371/journal.pone.0025801

Ruan, Q., Ma, C., Zhang, R., and Yu, Z. (2014). Current status of auditory aging and anti-aging research. *Geriatr. Gerontol. Int.* 14, 40–53. doi: 10.1111/ggi.12124

Sahley, T. L., Hammonds, M. D., and Musiek, F. E. (2013). Endogenous dynorphins, glutamate and N-methyl-d-aspartate (NMDA) receptors may participate in a stress-mediated Type-I auditory neural exacerbation of tinnitus. *Brain Res.* 1499, 80–108. doi: 10.1016/j.brainres.2013.01.006

Schuknecht, H. F., and Gacek, M. R. (1993). Cochlear pathology in presbycusis. *Ann. Otol. Rhinol. Laryngol.* 102(1 Pt 2), 1–16.

Seidman, M. D., Khan, M. J., Tan, W. X., and Quirk, W. S. (2002). Influence of lecithin on mitochondrial DNA and age-related hearing loss. *Otolaryngol. Head Neck Surg.* 127, 138–144.

Shargorodsky, J., Curhan, G. C., and Farwell, W. R. (2010). Prevalence and characteristics of tinnitus among US adults. *Am. J. Med.* 123, 711–718. doi: 10.1016/j.amjmed.2010.02.015

Sprinzl, G. M., and Riechelmann, H. (2010). Current trends in treating hearing loss in elderly people: a review of the technology and treatment options–a mini-review. *Gerontology* 56, 351–358. doi: 10.1159/000275062

Ünal, M., Tamer, L., Akbaş, Y., Pata, Y. S., Vayisoğlu, Y., Değirmenci, U., et al. (2005a). Genetic polymorphism of N-acetyltransferase 2 in the susceptibility to laryngeal squamous cell carcinoma. *Head Neck* 27, 1056–1060.

Ünal, M., Tamer, L., Doğruer, Z. N., Yildirim, H., Vayisoğlu, Y., and Çamdeviren, H. (2005b). N-acetyltransferase 2 gene polymorphism and presbycusis. *Laryngoscope* 115, 2238–2241.

Van Eyken, E., Van Camp, G., and Van Laer, L. (2007). The complexity of age-related hearing impairment: contributing environmental and genetic factors. *Audiol. Neurotol.* 12, 345–358.

Vatsis, K. P., and Weber, W. W. (1993). Structural heterogeneity of Caucasian N-acetyltransferase at the *NAT1* gene locus. *Arch. Biochem. Biophys.* 301, 71–76.

Verschuur, C., Agyemang-Prempeh, A., and Newman, T. A. (2014). Inflammation is associated with a worsening of presbycusis: evidence from the MRC national study of hearing. *Int. J. Audiol.* 53, 469–475. doi: 10.3109/14992027.2014.891057

Vielsmeier, V., Strutz, J., Kleinjung, T., Schecklmann, M., Kreuzer, P. M., Landgrebe, M., et al. (2012). Temporomandibular joint disorder complaints in tinnitus: further hints for a putative tinnitus subtype. *PLOS ONE* 7:e38887. doi: 10.1371/journal.pone.0038887

Voytenko, S. V., and Galazyuk, A. V. (2011). mGluRs modulate neuronal firing in the auditory midbrain. *Neurosci. Lett.* 492, 145–149. doi: 10.1016/j.neulet.2011.01.075

Yamasoba, T., Lin, F. R., Someya, S., Kashio, A., Sakamoto, T., and Kondo, K. (2013). Current concepts in age-related hearing loss: epidemiology and mechanistic pathways. *Hear. Res.* 303, 30–38. doi: 10.1016/j.heares.2013.01.021

Yu, H., Patil, K. V., Han, C., Fabella, B., Canlon, B., Someya, S., et al. (2016). GLAST deficiency in mice exacerbates gap detection deficits in a model of salicylate-induced tinnitus. *Front. Behav. Neurosci.* 10:158. doi: 10.3389/fnbeh.2016.00158

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Genetics of Tinnitus: Time to Biobank Phantom Sounds

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Tinnitus is a common phantom sensation resulting most often from sensory deprivation, and for which little knowledge on the molecular mechanisms exists. While the existing evidence for a genetic influence on the condition has been until now sparse and underpowered, recent data suggest that specific forms of tinnitus have a strong genetic component revealing that not all tinnitus percepts are alike, at least in how they are genetically driven. These new findings pave the way for a better understanding on how phantom sensations are molecularly driven and call for international biobanking efforts.

Keywords: tinnitus, genetics, heritability, subtype, neuropsychiatry, GWAS (genome-wide association study), whole exome sequencing

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PERSPECTIVE

For decades, tinnitus was considered a consequence of environmental factors, with low genetic contribution. The numerous etiologies, such as aging (presbycusis), noise exposure, stress, hypertension, diabetes, ototoxic medications, temporomandibular joint disorders, traumatic or ischemic damage, vascular problems, middle-ear problems, and the complex pathophysiology involving peripheral and central auditory and non-auditory structures, have led to the belief that tinnitus is a consequence of some other disease.

The knowledge on the genetic basis of tinnitus was recently reviewed (Vona et al., 2017) and phenotyping strategies have been proposed based on the assumption that tinnitus should be considered as an ensemble of sub-entities called subtypes (Lopez-Escamez et al., 2016). A small familial aggregation study ($n = 198$ families) found no obvious heritability (Hendrickx et al., 2007), and the first large population-based family study ($n = 52{,}045$) made an estimate of heritability of 0.11 (Kvestad et al., 2010). But a recent twin study revealed a higher heritability of 0.4, indicating that a larger fraction of the variance can be due to genetic variants than previously reported (Bogo et al., 2016). Such discrepancies may originate from differences in the design and formulation of the questionnaires, which have been found to vary greatly in prevalence studies on tinnitus (McCormack et al., 2016).

The tinnitus phenotype could also be grounds for diverging heritability values, but what defines a tinnitus phenotype is highly debated. We indeed consider that a more precise definition of a homogeneous phenotype will be essential in the design of genetic studies. A larger twin study performed by members of the TINNET[1] consortium recently

[1] http://tinnet.tinnitusresearch.net/

considered the laterality of tinnitus as a potential genetic subtype (Maas et al., 2017). A key finding was that bilateral tinnitus had higher heritability than unilateral tinnitus. The study was based on self-reported data from the Swedish Twin Registry, one of the largest twin registries in the world (Lichtenstein et al., 2002, 2006; Pedersen et al., 2002; van Dongen et al., 2012). Of a total of 70,186 twins that answered questions related to tinnitus, 15% of them experienced tinnitus. 10,464 concordant or discordant pairs for tinnitus were identified, in which 6,990 subjects had tinnitus. When considering tinnitus as a whole, a moderate genetic contribution (near 40%) was found (Bogo et al., 2016). However, when twins were stratified – based on tinnitus experienced in one ear (unilateral) or in both ears (bilateral) as well as on gender – bilateral tinnitus reached a heritability of 0.68 in men (Maas et al., 2017). Such values are close to the levels of heritability for schizophrenia and attention deficit hyperactive disorder (ADHD), two well known heritable conditions (**Table 1**). Although more work is required for establishing the contribution of hearing loss in such high heritability values (e.g., by including exhaustive auditory data), these findings open the possibility of specific forms of tinnitus being more genetically driven than others and pave the way for future genetic studies considering subtypes. These findings, however, need to be replicated in other twin cohorts as well as familial studies.

In line with genetic association studies of other complex traits, published studies to find genetic markers for chronic tinnitus patients in candidate genes have been underpowered (n = 95–288) and failed to identify robustly associated genetic variants (Sand et al., 2010, 2011, 2012a,b; Gallant et al., 2013). In spite of a lowly powered tinnitus group (N = 167) and no significant associations found, a recent genome-wide association study (GWAS) identified some pathways (e.g., oxidative stress, endoplasmatic reticulum stress, and serotonin reception mediated signaling) potentially involved in tinnitus (Gilles et al., 2017). Supporting the need of better characterizing the tinnitus cases, Pawelczyk and colleagues investigated 99 single nucleotide polymorphisms targeting 10 genes involved in the potassium recycling pathway in the inner ear (128 tinnitus cases and 498 controls both exposed to occupational noise) (Pawelczyk et al., 2012). However, two of the identified SNPs were not subjected to multiple testing and were thus considered nominally significant. An important lesson from GWAS on other complex traits, such as schizophrenia and major depressive disorder (Sullivan et al., in press), is that far larger sample sizes are needed in order to identify genome-wide significant genetic variants. Therefore, an important next step in the search for genetic variants associated with tinnitus will be to perform joint GWAS analysis of thousands of tinnitus patients and healthy controls.

Since familial tinnitus is a rare condition, the selection of multiplex tinnitus families, in addition to unrelated cases and controls, for exome sequencing, is another potential strategy to be used for the discovery of genes involved in tinnitus. This strategy has been successful in the identification of *DTNA*, *PRKCB*, *SEMA3D* and *DPT* in autosomal dominant Meniere disease (Requena et al., 2015; Martin-Sierra et al., 2016, 2017).

With tinnitus being a condition with highly unmet clinical needs (Cederroth et al., 2013), the recent identification of a high heritability opens door to exciting research. Since it is more than likely that tinnitus is a polygenic trait and it will require the study of several thousand samples, audiologists and ENT doctors should optimize their phenotyping strategies for instance by using high frequency audiometry and multivariate questionnaire data (Muller et al., 2016; Schlee et al., 2017), initiate incentives to allocate a specific ICD-code for bilateral tinnitus, and start biobanking samples (Lopez-Escamez et al., 2016). Regarding the latter, since it is not custom for an ENT clinic to collect samples for DNA biobanking, guidelines should emerge to promote good practice (Fuller et al., 2017) and enable the creation of a large consortium to join efforts to decipher the genetic basis of tinnitus.

TABLE 1 | Classification of tinnitus heritability against other disorders.

Trait	Heritability	Number of Twin pairs
Diabetes, type 1	0.88	22 650
Schizophrenia	0.81	Meta-analysis
ADHD	0.76	Review
Autism	0.71	11 535
Bilateral tinnitus (men)	0.68	10 464
Diabetes, type 2	0.64	13 888
Coronary heart disease	0.57	10 483
Alzheimer's disease	0.48	662
Any tinnitus	0.43	10 464
Prostate cancer	0.42	21 000
Systolic blood pressure	0.42	1 617
Bilateral tinnitus (women)	0.41	10 464
Colorectal cancer	0.35	44 788
Parkinson's disease	0.34	46 436
Breast cancer	0.27	23 788
Unilateral tinnitus	0.27	10 464
MS	0.25	Review

Modified from van Dongen et al. (2012) with permission from the Nature Publishing Group. Tinnitus values are marked in red.

AUTHOR CONTRIBUTIONS

CC conceived the paper and prepared the table. CC co-wrote the paper with JL-E. AK and PS helped to develop the scientific arguments. All authors played a role in writing the manuscript and approved the final version.

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REFERENCES

Bogo, R., Farah, A., Karlsson, K. K., Pedersen, N. L., Svartengren, M., and Skjonsberg, A. (2016). Prevalence, incidence proportion, and heritability for tinnitus: a longitudinal twin study. *Ear Hear.* 38, 292–300. doi: 10.1097/AUD.0000000000000397

Cederroth, C. R., Canlon, B., and Langguth, B. (2013). Hearing loss and tinnitus–are funders and industry listening? *Nat. Biotechnol.* 31, 972–974. doi: 10.1038/nbt.2736

Fuller, T. E., Haider, H. F., Kikidis, D., Lapira, A., Mazurek, B., Norena, A., et al. (2017). Different teams, same conclusions? A systematic review of existing clinical guidelines for the assessment and treatment of tinnitus in adults. *Front. Psychol.* 8:206. doi: 10.3389/fpsyg.2017.00206

Gallant, E., Francey, L., Fetting, H., Kaur, M., Hakonarson, H., Clark, D., et al. (2013). Novel COCH mutation in a family with autosomal dominant late onset sensorineural hearing impairment and tinnitus. *Am. J. Otolaryngol.* 34, 230–235. doi: 10.1016/j.amjoto.2012.11.002

Gilles, A., Van Camp, G., Van de Heyning, P., and Fransen, E. (2017). A pilot genome-wide association study identifies potential metabolic pathways involved in tinnitus. *Front. Neurosci.* 11:71. doi: 10.3389/fnins.2017.00071

Hendrickx, J. J., Huyghe, J. R., Demeester, K., Topsakal, V., Van Eyken, E., Fransen, E., et al. (2007). Familial aggregation of tinnitus: a European multicentre study. *B-ENT* 3(Suppl. 7), 51–60.

Kvestad, E., Czajkowski, N., Engdahl, B., Hoffman, H. J., and Tambs, K. (2010). Low heritability of tinnitus: results from the second Nord-Trondelag health study. *Arch. Otolaryngol. Head Neck Surg.* 136, 178–182. doi: 10.1001/archoto.2009.220

Lichtenstein, P., De Faire, U., Floderus, B., Svartengren, M., Svedberg, P., and Pedersen, N. L. (2002). The Swedish Twin Registry: a unique resource for clinical, epidemiological and genetic studies. *J. Intern. Med.* 252, 184–205. doi: 10.1046/j.1365-2796.2002.01032.x

Lichtenstein, P., Sullivan, P. F., Cnattingius, S., Gatz, M., Johansson, S., Carlstrom, E., et al. (2006). The Swedish Twin Registry in the third millennium: an update. *Twin Res. Hum. Genet.* 9, 875–882. doi: 10.1375/183242706779462444

Lopez-Escamez, J. A., Bibas, T., Cima, R. F., Van de Heyning, P., Knipper, M., Mazurek, B., et al. (2016). Genetics of tinnitus: an emerging area for molecular diagnosis and drug development. *Front. Neurosci.* 10:377. doi: 10.3389/fnins.2016.00377

Maas, I. L., Bruggemann, P., Requena, T., Bulla, J., Edvall, N. K., Hjelmborg, J. V., et al. (2017). Genetic susceptibility to bilateral tinnitus in a Swedish twin cohort. *Genet Med.* doi: 10.1038/gim.2017.4 [Epub ahead of print].

Martin-Sierra, C., Gallego-Martinez, A., Requena, T., Frejo, L., Batuecas-Caletrio, A., and Lopez-Escamez, J. A. (2017). Variable expressivity and genetic heterogeneity involving DPT and SEMA3D genes in autosomal dominant familial Meniere's disease. *Eur. J. Hum. Genet.* 25, 200–207. doi: 10.1038/ejhg.2016.154

Martin-Sierra, C., Requena, T., Frejo, L., Price, S. D., Gallego-Martinez, A., Batuecas-Caletrio, A., et al. (2016). A novel missense variant in PRKCB segregates low-frequency hearing loss in an autosomal dominant family with Meniere's disease. *Hum. Mol. Genet.* 25, 3407–3415. doi: 10.1093/hmg/ddw183

McCormack, A., Edmondson-Jones, M., Somerset, S., and Hall, D. (2016). A systematic review of the reporting of tinnitus prevalence and severity. *Hear. Res.* 337, 70–79. doi: 10.1016/j.heares.2016.05.009

Muller, K., Edvall, N. K., Idrizbegovic, E., Huhn, R., Cima, R., Persson, V., et al. (2016). Validation of online versions of tinnitus questionnaires translated into Swedish. *Front. Aging Neurosci.* 8:272. doi: 10.3389/fnagi.2016.00272

Pawelczyk, M., Rajkowska, E., Kotylo, P., Dudarewicz, A., Van Camp, G., and Sliwinska-Kowalska, M. (2012). Analysis of inner ear potassium recycling genes as potential factors associated with tinnitus. *Int. J. Occup. Med. Environ. Health* 25, 356–364. doi: 10.2478/S13382-012-0061-3

Pedersen, N. L., Lichtenstein, P., and Svedberg, P. (2002). The Swedish Twin Registry in the third millennium. *Twin Res.* 5, 427–432. doi: 10.1375/136905202320906219

Requena, T., Cabrera, S., Martin-Sierra, C., Price, S. D., Lysakowski, A., and Lopez-Escamez, J. A. (2015). Identification of two novel mutations in FAM136A and DTNA genes in autosomal-dominant familial Meniere's disease. *Hum. Mol. Genet.* 24, 1119–1126. doi: 10.1093/hmg/ddu524

Sand, P. G., Langguth, B., Itzhacki, J., Bauer, A., Geis, S., Cardenas-Conejo, Z. E., et al. (2012a). Resequencing of the auxiliary GABA(B) receptor subunit gene KCTD12 in chronic tinnitus. *Front. Syst. Neurosci.* 6:41. doi: 10.3389/fnsys.2012.00041

Sand, P. G., Langguth, B., and Kleinjung, T. (2011). Deep resequencing of the voltage-gated potassium channel subunit KCNE3 gene in chronic tinnitus. *Behav. Brain Funct.* 7:39. doi: 10.1186/1744-9081-7-39

Sand, P. G., Langguth, B., Schecklmann, M., and Kleinjung, T. (2012b). GDNF and BDNF gene interplay in chronic tinnitus. *Int. J. Mol. Epidemiol. Genet.* 3, 245–251.

Sand, P. G., Luettich, A., Kleinjung, T., Hajak, G., and Langguth, B. (2010). An examination of KCNE1 mutations and common variants in chronic tinnitus. *Genes* 1, 23–37. doi: 10.3390/genes1010023

Schlee, W., Hall, D., Edvall, N. K., Langguth, B., Canlon, B., and Cederroth, C. R. (2017). Visualization of global disease burden for the optimization of patient management and treatment. *Front. Med.* 4:86. doi: 10.3389/fmed.2017.00086

Sullivan, P. F., Agrawal, A., Bulik, C. M., Andreassen, O. A., Børglum, A. D., Breen, G., et al. (in press). Psychiatric genomics: an update and an agenda. *Am. J. Psychiatry*.

van Dongen, J., Slagboom, P. E., Draisma, H. H., Martin, N. G., and Boomsma, D. I. (2012). The continuing value of twin studies in the omics era. *Nat. Rev. Genet.* 13, 640–653. doi: 10.1038/nrg3243

Vona, B., Nanda, I., Shehata-Dieler, W., and Haaf, T. (2017). Genetics of tinnitus: still in its infancy. *Front. Neurosci.* 11:236. doi: 10.3389/fnins.2017.00236

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REVIEW
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Auditory Brainstem Responses in Tinnitus: A Review of Who, How, and What?

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The auditory brainstem response (ABR) in tinnitus subjects has been extensively investigated over the last decade with the hopes of finding possible abnormalities related to the pathology. Despite this effort, the use of the ABR for tinnitus diagnosis or as an outcome measure is under debate. The present study reviewed published literature on ABR and tinnitus. The authors searched PubMed, MedLine, Embase, PsycINFO, and CINAHL, and identified additional records through manually searching reference lists and gray literature. There were 4,566 articles identified through database searching and 151 additional studies through the manual search (4,717 total): 2,128 articles were removed as duplicates, and 2,567 records did not meet eligibility criteria. From the final 22 articles that were included, ABR results from 1,240 tinnitus subjects and 664 control subjects were compiled and summarized with a focus on three main areas: the participant characteristics, the methodology used, and the outcome measures of amplitude and/or latency of waves I, III, and V. The results indicate a high level of heterogeneity between the studies for all the assessed areas. Amplitude and latency differences between tinnitus and controls were not consistent between studies. Nevertheless, the longer latency and reduced amplitude of wave I for the tinnitus group with normal hearing compared to matched controls was the most consistent finding across studies. These results support the need for greater stratification of the tinnitus population and the importance of a standardized ABR method to make comparisons between studies possible.

Keywords: tinnitus, ABR, review, brainstem, synaptopathy, meta-analysis, hearing loss

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INTRODUCTION

Tinnitus is known as a phantom sound that is perceived in the absence of an acoustic stimulation. It is described by patients in a variety of ways that can be as simple as a single pure tone and as complex as a combination of different sounds (Stouffer and Tyler, 1990). It can also be perceived differently in one ear, both ears or in the head, and can be modulated in some individuals by orofacial movements (Levine, 1999), touch, background noise, stress, anxiety, depression, and attention (Tyler et al., 2008).

Although, the pathophysiology of tinnitus is still not clear, various origins, and mechanisms have been described in the literature (Henry et al., 2014). The fact that tinnitus is not always suppressed when the cochlear nerve is sectioned suggests there are, at least, two distinct tinnitus sub-types:

cochlear tinnitus and central tinnitus (House and Brackmann, 1981; Berliner et al., 1992). Cochlear tinnitus can be defined as a tinnitus subtype that results from aberrant activity in the cochlear nerve (Noreña, 2011). Central tinnitus can be defined as a tinnitus subtype that does not result from an increase of activity (or synchrony) in the cochlear nerve but rather at cortical levels within the central auditory pathways (Noreña, 2011). In this latter subtype, the tinnitus perception may result from cortical changes associated with the reduction of sensory inputs due to hearing loss.

One technique used to assess the activation along the neural pathways between the eighth peripheral nerve of the cochlear nucleus up to the inferior colliculus is the Auditory Brainstem Response (ABR) (Melcher and Kiang, 1996). Auditory Brainstem Responses (ABRs) are acoustically stimulated signals that represent the synchronized neural activation along the neural pathways. A study investigating the generation sites of ABRs in cats revealed the first wave (I) of the ABR reflects activity of the spiral ganglion cells at the distal part of the eighth auditory nerve, wave II is predominantly from the globular cells in the cochlear nucleus, wave III is generated by the cochlear nucleus spherical cells and globular cells, and waves IV and V generate from the medial superior olive and its projections to the nuclei in the lateral lemniscus and the inferior colliculus (Melcher and Kiang, 1996). These electrophysiological responses are typically less than a microvolt in amplitude (Burkard and Secor, 2002; Chalak et al., 2013). The success of revealing true and reliable responses relies heavily on averaging techniques employed to reduce noise contamination thereby improving the signal to noise ratio (Burkard and Secor, 2002). ABRs have been used clinically for two main purposes: hearing threshold estimations and neurodiagnostics. Indeed, the ABR is a well known cost-effective test that is routinely used in clinical practice as an objective diagnostic measure for determining the presence of hearing loss in infants, young children and patients that are difficult to test behaviorally. More so, the ABR is an important clinical tool for identifying the presence of retrocochlear lesions, acoustic neuromas, and vestibular schwannomas (Kotlarz et al., 1992; Rupa et al., 2003). This is achieved by identifying waves I, III, and V peaks and comparing the absolute latency values to normative ranges for each wave. For example, the presence of an acoustic neuroma at the level of the auditory nerve could significantly delay neural conduction. As a result, the latency between waves I and V is usually extended from the normative value by more than 0.2 ms (Wilson et al., 1992). Normative ABR latency values, for clicks at 70 dB nHL, collected on the most reliable waves I, III, and V are, respectively, 1.66, 3.68, and 5.64 ms for the left ear, and 1.66, 3.65, and 5.59 ms for the right ear (Chalak et al., 2013). When comparing between genders of the same age, the latencies are shorter and the amplitudes are larger in women compared to men (Hultcrantz et al., 2006). Hearing loss of different configurations affect the ABR: high frequency hearing losses show a delayed wave V at low intensities and a greater degree of wave I delay at all intensities, low frequency hearing losses show an earlier wave V at low intensities (Keith and Greville, 1987; Watson, 1996). Furthermore, elevated hearing thresholds also reduce the amplitude of waves I and V using the click-ABR (Sand and Saunte, 1994) and wave V using tone-burst ABR when the tone-burst characteristic frequency falls within the frequency region of the hearing loss (Lewis et al., 2015). The ABR sensitivity and specificity for both hearing threshold estimations and neurodiagnostics, have been shown to be very high with values of 100 and 91% for the former (Hyde et al., 1990) and 88 and 92% for the latter (Bauch et al., 1996). ABR assessment is also used for the diagnosis of auditory neuropathy (Starr et al., 1996). In such a case, the function of the outer hair cell of the cochlea is mostly normal, irrespective of hearing thresholds, even though the ABR waves are absent due to a lack of synchronized neural activity or excessive auditory fatigue (see Giraudet and Avan, 2012). The ABR technique thus provides information about the integrity of the central auditory system and can be a valuable diagnostic tool. Moreover, ABRs are relatively easy to obtain from only a few electrodes and are mostly insensitive to cognitive states (e.g., attention or arousal) or even consciousness (Burkard and Secor, 2002).

In tinnitus research, ABRs have been used in a variety of ways in humans. ABRs have been used to differentiate peripheral from central lesion sites in patients (Kehrle et al., 2008), and to investigate tinnitus treatment efficacy following drug administration (Shulman and Seitz, 1981; Milicic and Alcada, 1999; Bayar et al., 2001; Gopal et al., 2015). ABRs have also been used to identify noise-induced hidden hearing loss. In brief, Kujawa and Liberman found that the ABR wave I amplitude of mice significantly decreased at moderately-high levels (above 70 dB) up to 2 months following noise exposure even when the auditory thresholds had recovered to normal values (Kujawa and Liberman, 2006, 2009). In addition to the amplitude reduction, damage to the synaptic ribbons of the inner hair cells and spiral ganglion cells were revealed, suggesting that reduced wave I amplitude may be indicative of auditory nerve deafferentation. The term "cochlear synaptopathy" was further proposed to describe damage at the cochlear synapse without loss of hair cells resulting in "hidden hearing loss," a functional hearing deficit without an elevation of audiometric thresholds (Liberman and Kujawa, 2017). In tinnitus patients with normal hearing ($\leq$20 dB HL, Freq: 0.25–8 kHz), Schaette and McAlpine (2011) and Gu et al. (2012) showed similar reduced wave I amplitudes at high levels (80–90 dB SPL) compared to non-tinnitus matched controls, which were both interpreted as diminished activity of the low spontaneous rate auditory nerve fibers (LSR). Interestingly, the amplitude of wave V (measured baseline to peak) was reported to be significantly higher in only Gu et al. (2012). Schaette and McAlpine (2011) suggest the normal wave V amplitude, despite a reduction in wave I, is due to the central auditory system increasing its neural responsiveness to compensate for the reduced activity of the auditory nerve. Conversely, Gu et al. (2012) suggest that the higher amplitude of wave V is an artifact from the use of a lower frequency filter cutoff. Based on these findings, people suffering from tinnitus with normal audiometric thresholds show ABR amplitude changes that may be indicative of cochlear synaptopathy (reduced wave I) and the compensated responses of central/cortical regions (normal or elevated wave V). The increased responsiveness of central regions would generate

increased spontaneous activity leading to tinnitus generation. Hickox and Liberman (2014) attempted to link synaptopathy to the generation of tinnitus in noise-exposed mice. The mice exposed to loud noise displayed the typical auditory nerve degeneration (determined by ribbon counts), reduced wave I amplitude/enhanced wave V ABR responses, and subtle changes in the behavioral response of tinnitus that did not reached significance (using the gap prepulse inhibition acoustic startle reflex or GPIAS). Low efficacy of this particular behavioral technique (GPIAS) in CBA-mice might explain the failure of significant results (Yu et al., 2016). Using another strain with better GPIAS could maximize these effects and link wave I reduction to a behavioral measure of tinnitus in animals. Another animal study (Rüttiger et al., 2013), exposed animals to loud noise and separated them based on tinnitus behavior. They found that although the ABR waveform was generally reduced after the trauma for both groups, wave I did not significantly change amplitude after recovery. Interestingly, the tinnitus group showed reduced wave IV and V amplitude after recovery, which the authors proposed to arise from a failure to compensate for the cochlear loss at the central levels of the auditory system.

It is noteworthy that ABR wave amplitude may be altered by the number of neural components activated by the stimulation and/or the level of synchronization between them. As amplitude of wave I is mostly due to tightly synchronized activity at the level of the cochlear nerve, the reduction in amplitude noted previously at high intensities might indicate not only a loss of neural fibers but also a decrease of synchronization. Conversely, increased neural synchrony has been proposed as a potential mechanism of tinnitus generation (Eggermont, 1984; Moeller, 1984). It was postulated that increased synchrony of the spontaneous firing rate even at the peripheral level of the auditory nervous system could be sufficient to produce a perception of a sound in the absence of external stimulation. The higher wave V amplitude reported in tinnitus subjects might reflect increased neural synchronization at higher levels of the auditory system. In brief, changes in wave I might reflect damage to the periphery and the following wave modifications might reflect compensation mechanisms such as higher increased neural synchrony in tinnitus. Still, modifications of wave III and wave V amplitude might occur without being related to wave I alterations. In a recent study, decreases in the amplitude of waves III and V were not adequately explained by changes in wave I in older participants compared to younger ones (Konrad-Martin et al., 2012). In this study, the reduction of the peak amplitudes of waves III and V, seemed to be linked to the effects of aging, instead of wave I amplitude reduction (and latency shift), which is believed to be the consequence of reduced auditory nerve inputs.

The current purpose of the study was to review ABR findings on tinnitus to assess any consistencies across studies in terms of absolute wave amplitudes and latencies. As ABR waves are affected by hearing loss (Don et al., 1998), and tinnitus mechanisms may differ between normal hearing and hearing loss participants (Henry et al., 2014), studies were separated based on this variable. A potential decrease in wave I amplitude in tinnitus subjects with normal hearing is expected to be one of the most consistent findings across studies (Schaette and McAlpine, 2011; Gu et al., 2012). The current review might also bring insight on possible modifications of the other waves such as wave III and wave V, in populations reporting tinnitus. A careful analysis of studies on tinnitus and ABR from 1980 to 2016 was made and convergent evidence was extracted based on the population/sample (who?), the methodology (how?), and the outcome (what?). The investigated outcomes were related to the latency and amplitude of waves I, III, and V.

METHODS

Database Search

A scoping review of the literature was conducted using the method described by Arksey and O'Malley (2005). This approach uses a five stage framework that includes (1) identifying the research question, (2) identifying relevant studies, (3) selecting the study, (4) charting the data, and (5) collating, summarizing, and reporting the results. Statistics for each level of data collection are tabulated in the PRISMA schema (Moher et al., 2009; **Figure 1**).

A team approach to the process of the review was used to eliminate the level of error produced by a single individual and a second reviewer was used to independently analyze all the abstracts for inclusion (Levac et al., 2010). Given the high volume of articles yielded from the comprehensive search strategy, a "liberal accelerated" approach was used: the second reviewer analyzed only articles excluded by the first reviewer instead of the entire yield (Khangura et al., 2012).

The primary outcome of interest is measurement of the absolute peak amplitudes (peak to following trough) and latencies of the ABR waveform in tinnitus patients with and without hearing loss. Searches were conducted, between April 2015 and August 2016, by the principal investigator (V.M.) using the strategies detailed in Supplementary Table 1. In brief, the search terms (and their variations) used alone or in combination referring to tinnitus were: tinnitus, ear, buzz, ring, roar, click, pulsate, or pulse, and referring to the auditory brainstem measurements were: brainstem, brain, stem, auditory, response, potential, ABR, BAER, BSER, and evoked. The databases PubMed, CINAHL, Medline, PsycInfo, and Embase were searched separately and results were compiled in a Microsoft Excel (2011) spreadsheet where the search strategy yields were dated and organized (Supplementary Table 1). Gray literature which includes conference papers, master dissertations, and doctoral theses was also searched (October 2016) using ProQuest Dissertations and Theses Global and Conference Papers Index and added to the compiled list of articles. No conference papers, master dissertations, or doctoral theses were included in the final compilation as all those found were later published. Articles were limited to those published after 1980 as the waveforms were only just described in the 1970s by Jewett and colleagues and not yet applied to subjects with tinnitus (Jewett et al., 1970; Jewett and Williston, 1971). Any articles assessing populations with tinnitus due to underlying medical conditions were excluded for the purposes of this review (i.e., acoustic neuroma, otitis media, otitis externa, etc.). Excluding various comorbidities ensured the ABR outcomes

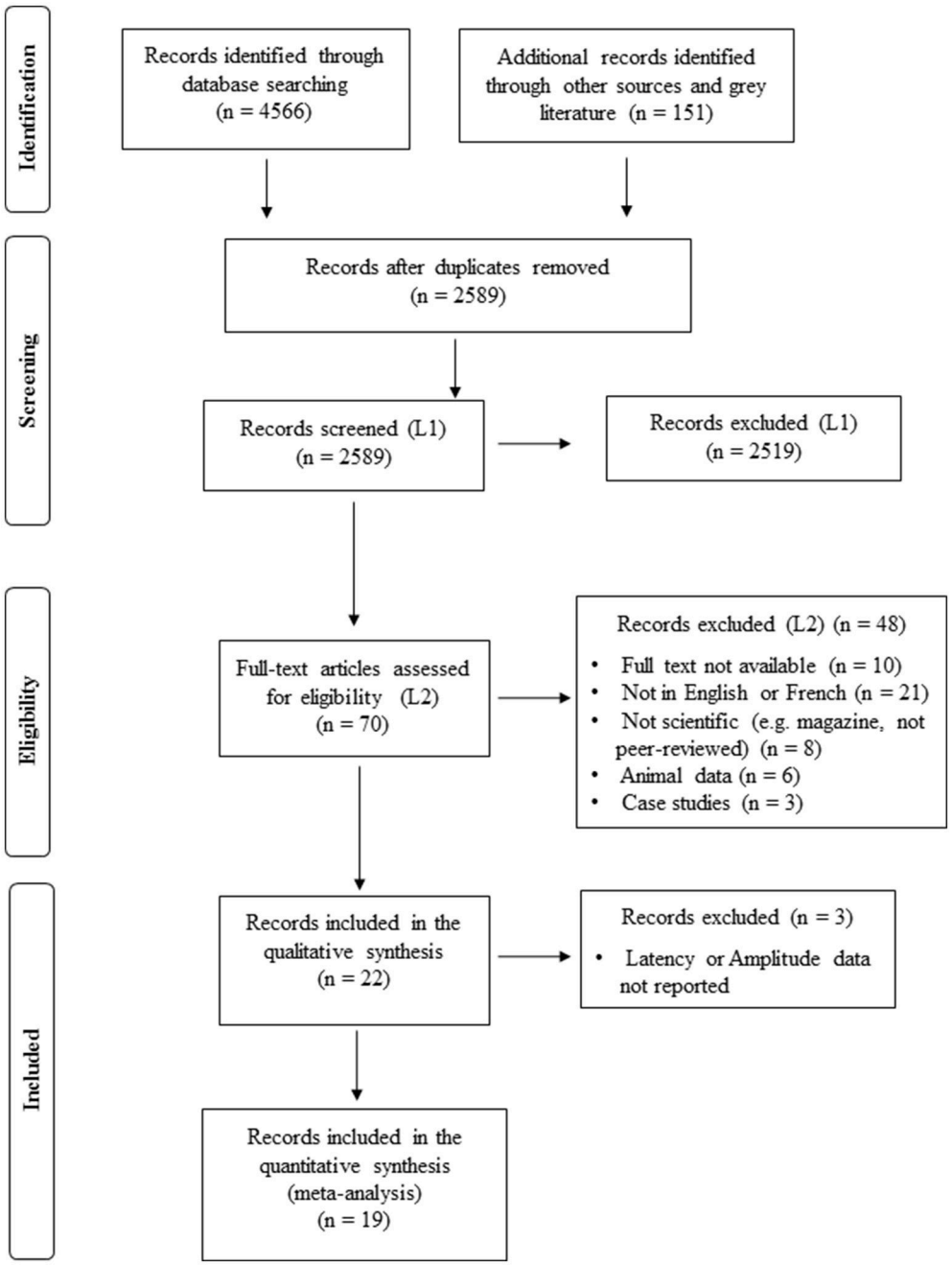

FIGURE 1 | Flowchart of study selection (PRISMA diagram).

reported were not due to known covariables. Any articles discussing ABRs for the purpose of measuring hearing loss such as threshold searching and newborn hearing screenings were also excluded. Preoperative and intraoperative ABRs were not included in this review as clinical populations with other underlying conditions, such as acoustic neuromas, or vascular abnormalities, are typically involved in these studies and are known covariables of ABR (Berliner et al., 1992; De Ridder et al., 2015).

The first screening (L1) consisted of excluding the articles that did not meet the criteria described in **Table 1** based on the analysis of title and abstract of the article. The second reviewer screened the articles rejected by the first reviewer. Both reviewers completed the second screening (L2) where eligibility was based on the analysis of the full text. The use of a language translator was not feasible for this study, therefore all texts written in languages other than French or English were eliminated. Three case-report studies, where a single participant was reported

TABLE 1 | Inclusion and exclusion criteria applied to the scoping review search.

	Inclusion criteria	Exclusion criteria
Population	Subjective tinnitus with or without hearing loss. This includes individuals with noise-induced hearing loss.	Subjective tinnitus with a history of ontological conditions (i.e., hypertension, tumors, demylenation, multiple sclerosis, Meniere's disease, auditory neuropathy, otitis media, otitis externa, middle ear pathologies etc.).
		Individuals with cochlear implants, CAPD, head trauma, psychological disorders, sudden hearing loss.
		Individuals with objective tinnitus or pulsatile tinnitus.
		Studies with a sample size of 1.
Evaluation	Auditory evoked potentials of the brainstem.	Long Latency Auditory Evoked Potentials (P1, N1, P2, N2); Event Related Potentials (MMN, P300, N400, P600 etc.). Auditory Steady State Responses, intraoperative ABR, preoperative monitoring, newborn hearing screening.
Publication type	Peer-reviewed journals only with articles published after 1980 in English or French.	Any unscientific papers: Magazine articles, conference proceedings, editorials, and manuals.
Outcomes	Measured peak amplitudes and latencies of the ABR wave.	

(Shulman and Seitz, 1981; Milicic and Alcada, 1999; Gopal et al., 2015), were removed from the remaining yield. The sample size was too small to significantly contribute to the analysis, and the purpose of these studies was mostly to measure the responsiveness to treatment.

After the two levels of title and abstract screening (L1) and full-text screening (L2) were completed, a narrative synthesis was used to organize key points of the data into two charts. Key points of interest included population (i.e., age, tinnitus etiology, tinnitus localization, and hearing status), technical information (i.e., transducer used, ABR system used, stimulus type, presentation levels, tinnitus characterization, and recording filters), and results.

Meta-Analysis of the Compiled Data

The results were compiled in Microsoft Excel (2011) for a meta-analysis of the data using two different methodologies. The first method (meta-analysis 1) consisted of compiling the latency and amplitude values from Waves I, III, and V for all the subjects, with or without tinnitus, from all the studies reporting these values. The mean values for absolute latency and amplitude, standard deviations, and sample size for each study were organized in a table as a function of the ABR waves. The results were also separated based on hearing status and reported tinnitus. For example, the results of the four groups were determined based on the participants having (a) normal hearing without tinnitus, (b) normal hearing with tinnitus, (c) hearing loss without tinnitus, and (d) hearing loss with tinnitus. In these cases, we used the normal hearing and hearing loss definitions established within each article. These definitions varied from one study to the other (see Section Results and Discussion). Meta-analysis calculations were carried out on these data to determine (a) the total number of subjects for all the studies separated by hearing status; (b) the total mean latency/amplitudes weighted according to sample size of each study; (c) the composite standard deviation calculated as a combination of all the groups from all the studies; (d) the 95% confidence interval (CI) determined based on the composite standard deviation; (e) the mean difference latency/amplitude (i.e., the mean latency/amplitude of the tinnitus group subtracted by the mean latency/amplitude of the non-tinnitus group) again weighted according to the sample size. The confidence interval was calculated using Microsoft Excel software and consisted of adding or subtracting the confidence value from the weighted mean. The confidence value was calculated based on the composite standard deviation and the total number of observations of the pooled data grouped in one of the categories: normal hearing, normal hearing and tinnitus, hearing loss, or hearing loss and tinnitus. Confidence intervals were chosen because the interval estimate obtained from this method is more informative for data comparison of future studies than a sample mean and *T*-test. The confidence level of the confidence intervals was set at 95%, which is the equivalent of $p < 0.05$.

The second method (meta-analysis 2) consisted of calculating the difference of the mean amplitude and latency for waves I, III, and V (and the 95% CI) between the tinnitus and control groups, only for studies with at least matched age and hearing status. This method was added to minimize the risk of identifying differences in population variables, assessment techniques or methodologies between tinnitus and control groups. The differences found with the second methodology are thus presumed to be the result of group differences as both groups were tested with very similar protocols.

RESULTS

Study Selection

A total of 4,566 articles were retrieved from the databases PubMed, MedLine, Embase, PsycINFO, and CINAHL. An additional 133 articles were found by manually searching citations from the reference lists of articles that met the eligibility criteria and another 18 from gray literature (i.e., doctoral theses and conference papers). After the duplicates were removed, 2,589 articles were screened by title and abstract and 70 of those articles were analyzed by reading the full text. Of the remaining articles, 22 were included in the qualitative narrative synthesis and 19 in the meta-analysis (see **Figure 1**). The most common objective of the studies is the assessment of possible changes to the ABR of tinnitus patients compared to those without tinnitus ($n = 19$)

TABLE 2 | Demographics include the number of subjects, the mean age, the tinnitus etiology, and localization and the hearing status criteria.

Study	Subjects	Mean age in years (Range)	Tinnitus characterization	Tinnitus localization	Hearing status criteria	Results: latency	Results: amplitude
NOISE-INDUCED ETIOLOGY							
Attias et al., 1993	Tinnitus ($n = 12$) Controls ($n = 12$) Matched: Age, HL severity and configuration	Not mentioned (26-45) Not mentioned (26-45)	Pitch Matching Loudness Matching	Bilateral ($n = 12$)	Audiometrically matched, No definition Noise induced hearing loss	No differences	No differences
Attias et al., 1996	Tinnitus ($n = 13$) Controls ($n = 11$) Matched: Age and Hearing	35 (21-45) Not mentioned (age and hearing matched)	Pitch Matching Loudness Matching Tinnitus severity profile	Unilateral ($n = 8$) Bilateral ($n = 5$)	Normal hearing: ≤20 dB HL, Freq: 0.25–2 kHz Hearing loss: 20–45 dB HL, Freq: 2–8 kHz	No differences	Enhanced Wave III amplitude
Gilles et al., 2016	Tinnitus ($n = 19$) Controls ($n = 23$) Matched: Age, Sex, and Hearing	∼23 (SD: 2.4)	VAS–Loudness TQ	Head ($n = 1$) Unilateral ($n = 2$) Bilateral ($n = 16$)	Normal hearing (<25 dB HL, Freq: 0.25–16 kHz?) Hearing loss (>25 dB HL, Freq: 0.25–16 kHz?) HF tested (up to 16 kHz)	No differences	No differences
Santos-Filha et al., 2014	Tinnitus ($n = 30$) Controls ($n = 30$) Matched: Age, Sex, Hearing	41 (27-50) 41.6 (27-50)	VAS—Severity	Bilateral (67%) Unilateral (33%)	Normal hearing (<25 dB HL, Freq: 0.25–8 kHz)	No differences	Not reported
IDIOPATHIC ETIOLOGY							
Cartocci et al., 2012	Tinnitus ($n = 10$) Controls ($n = 14$) Matched: Age, Sex, Hearing	43.9 (SD: 11.0) 45.1 (SD: 11.9)	Not reported	Unilateral ($n = 5$) Bilateral ($n = 5$)	Normal hearing (≤20 dB HL, Freq: 0.125–8 kHz)	Longer Wave V and III–V	Not reported
Mahmoudian et al., 2013	Tinnitus ($n = 44$) No Controls	43.45 (18-65)	Not reported	Unilateral ($n = 19$) Bilateral ($n = 25$)	Included Hearing levels (≤30 dB HL, Freq: 0.5–2 kHz and <60 dB HL, Freq: 4–8 kHz)	No latency changes	III/V and I/V ratio modifications following electrical RI
Maurizi et al., 1985	Tinnitus ($n = 54$) No Controls	Not mentioned (23-78)	Residual Inhibition	Unilateral ($n = 54$)	Classification of hearing loss: 1) ≤20 dB HL, Freq: 0.5–4 kz 2) 21–49 dB HL, Freq: 0.5–4 kHz and >50 dB HL, Freq: 0.5–4 kHz	Prolonged Wave V in tinnitus ears vs. control ears	Not reported
McKee and Stephens, 1992	Tinnitus ($n = 18$) Controls ($n = 19$) Matched: Age and Hearing	26 (18-37) 27.5 (17-38)	Not reported	Head ($n = 2$) Unilateral ($n = 2$) Bilateral ($n = 14$)	Normal hearing (≤20 dB HL, Freq: 0.25–8 kHz) HF tested (up to 18 kHz)	No differences	Not reported
Nemati et al., 2014	Tinnitus ($n = 25$) Controls ($n = 16$) Matched: Age, Sex, Hearing	34.4 (20-57) Not mentioned (matched)	Not reported	Unilateral ($n = 19$) Bilateral ($n = 6$)	Normal hearing (<25 dB HL, Freq: 0.25–8 kHz)	No differences	Amplitude ratio V/I larger
Singh et al., 2011	Tinnitus ($n = 25$) Controls ($n = 20$) Matched: Age, Sex, Hearing	32 (18-45) Not mentioned (matched)	Not reported	Unilateral ($n = 19$) Bilateral ($n = 6$)	Normal hearing (<25 dB HL, Freq: 0.25–8 kHz)	Longer Wave I Shorter Wave V Shorter I–II and I–V	Not reported

(Continued)

TABLE 2 | Continued

Study	Subjects	Mean age in years (Range)	Tinnitus characterization	Tinnitus localization	Hearing status criteria	Results: latency	Results: amplitude
HETEROGENEOUS ETIOLOGY							
Kim et al., 2016	Tinnitus ($n = 123$) No Controls	53.5 (SD: 13.4)	VAS—Discomfort Minimum masking level Residual inhibition THI Pitch matching	Unilateral ($n = 79$) Bilateral ($n = 44$)	Audiometric configurations: 1) Flat 2) High frequency gently sloping 3) High frequency steeply sloping	Prolonged latencies I, III and V for steeply high frequency hearing loss group	Not reported
ETIOLOGY NOT MENTIONED							
Barnea et al., 1990	Tinnitus ($n = 12$) Controls ($n = ??$) Matched: Age, Sex, Hearing	35 (21-45) Not mentioned (matched)	Pitch matching Loudness matching	Unilateral (60%) Bilateral (40%)	Normal hearing (≤20 dB HL, Freq: 0.25–8 kHz) HF tested (up to 20 kHz)	No difference	No differences
De Lavernhe-Lemaire and Beutter, 1989	Tinnitus ($n = 164$) Controls ($n = 57$) Not Matched	Not mentioned	Not reported	Unilateral ($n = 112$) Bilateral ($n = 52$)	Not mentioned	Longer Wave I, but decreased inter-peak I–V	Not reported
De Lavernhe-Lemaire and Beutter, 1990	Tinnitus ($n = 139$) Controls ($n = 20$) Not Matched	27-74	Not reported	Unilateral ($n = 93$) Bilateral ($n = 46$)	Not mentioned	Not reported	Decrease wave I and III amplitude
Gerken et al., 2001	Tinnitus ($n = 9$) Controls with normal hearing ($n = 11$) Controls with hearing loss ($n = 8$) Controls-elderly ($n = 7$) Not Matched	45.7 (26-68) 28 (22-37) 40.9 (23-53) 63.6 (60-68)	Pitch matching Loudness matching Minimum masking level	Not mentioned	Normal hearing (≤15 dB HL, Freq: 0.5–8 kHz) Hearing loss (>15 dB HL, Freq: 0.5–8 kHz)	Problem tinnitus group longer wave VII	No differences
Gu et al., 2012	Tinnitus ($n = 15$) Controls ($n = 21$) Matched: Age, Sex, Hearing	42 (SD: 6) 43 (SD: 7)	Pitch matching Loudness matching Minimum masking level Residual inhibition	Head ($n = 4$) Unilateral ($n = 2$) Bilateral ($n = 9$)	Normal hearing (≤20 dB HL, Freq: 0.25–8 kHz) HF tested (up to 16 kHz)	No latency differences	Reduced wave I and enhanced wave V
Ikner and Hassen, 1990	Tinnitus ($n = 35$) Controls ($n = 35$) Matched: Age, Sex, Hearing	40 36	Not reported	Not mentioned	Normal hearing (<20 dB HL, Freq: 1–4 kHz)	Longer wave I, III, V, and III–V interval	Not reported
Kehrle et al., 2008	Tinnitus ($n = 37$) Controls ($n = 38$) Matched: Age, Sex, Hearing	36 (SD: 7.2) Not mentioned (matched)	Not reported	Unilateral ($n = 13$) Bilateral ($n = 24$)	Normal hearing (<25 dB HL, Freq: 0.5–8 kHz)	Longer Wave I, III, V, and III–V	Ratio V/I
Kehrle et al., 2016	Tinnitus ($n = 84$) Controls ($n = 47$) Matched: Age, Sex, Hearing	37.2 (18-48) 35.7 (18-48)	VAS— severity Pitch matching Loudness matching THI	Unilateral ($n = 26$) Bilateral ($n = 58$)	Normal hearing (≤25 dB HL, Freq: 0.25–8 kHz)	Abnormal for wave I, wave III, wave V, inter-peak I–III, inter-peak III–V, Inter-peak I–V	Not reported

(Continued)

TABLE 2 | Continued

Study	Subjects	Mean age in years (Range)	Tinnitus characterization	Tinnitus localization	Hearing status criteria	Results: latency	Results: amplitude
Lemaire and Beutter, 1995	Tinnitus (n = 355) Controls (n = 129) Not matched	52.1 (SD: 16.4) <25	Pitch matching Loudness matching	Unilateral (n = 220) Bilateral (n = 135)	Normal hearing (<20 dB HL, Freq: ??)	Longer for 0–I and I–V on the tinnitus affected side	Reduced Wave I, III, and sometimes V
Rosenhall and Axelsson, 1994	Tinnitus with hearing loss (n = 57) Tinnitus with normal hearing (n = 56) Controls with hearing loss (n = 166) Controls with normal hearing (n = 54) Matched: Age, Sex, Hearing	57.2 (SD: 10.6) 42.1 (SD: 13.8) Not mentioned (matched) Not mentioned (matched)	Not reported	Unilateral (n = 30) Bilateral (n = 83)	Normal hearing (<20 dB HL, Freq: 0.125–2 kHz and <35 dB HL, Freq: 4–8 kHz) Hearing loss (45-60 dB HL, Freq: 4 kHz)	Longer Wave I, III, and V	Not reported
Schaette and McAlpine, 2011	Tinnitus (n = 15) Controls (n = 18) Matched: Age, Sex, Hearing	36.3 (SD: 2.6) 33.2 (SD: 1.9)	Modified tinnitus spectrum	Not mentioned	Normal hearing (≤20 dB HL, Freq: 0.25–8 kHz) HF tested (up to 16 kHz)	Not reported	Reduced Wave I, No change Wave V

SD, Standard deviation; dB, decibels; HL, hearing loss; Freq, frequency; n, number of subjects; VAS, visual analog scale; TQ, Tinnitus Questionnaire; THI, Tinnitus Handicap Inventory; HF, high frequency thresholds above 8 kHz.

for the purpose of distinguishing between peripheral and central tinnitus or identifying lesions or deafferentation of the auditory nerve. Other objectives were to compare the ABR to tinnitus perception ($n = 1$), emotions ($n = 1$), and the behavioral effects of residual inhibition ($n = 1$).

Population Characteristics

Characteristics of the populations tested in the 22 studies are shown in **Table 2**. Nineteen out of the 22 studies had a control group. The matching procedures varied between the studies, if mentioned at all. In 12 studies subjects were matched by sex, age, and hearing status, in three studies by age and hearing only and in four studies not matched (see **Table 2**). The mean age of the tinnitus and control groups was 40.1 and 38.0 years old, respectively, but varied widely between the studies ranging from 18 to 78-year-old participants. Since the data were not reported for smaller age groups, this data could not be separated into more narrowly defined age divisions. The tinnitus etiology was characterized as noise-induced for five of the studies, idiopathic for six studies, and not mentioned for the remaining 11 studies. Seventeen studies assessed patients with both bilateral or unilateral tinnitus, two studies used either bilateral tinnitus (Attias et al., 1993) or unilateral tinnitus exclusively (Maurizi et al., 1985) and three studies did not mention the lateralization of the tinnitus. Gender of the population was reported in 19 of the 22 studies of which five separated ABR data for males and/or females.

Hearing status was reported in all except two studies (De Lavernhe-Lemaire and Beutter, 1989, 1990). Among the studies that evaluated hearing, 17 studies used hearing status as a way to match controls to the tinnitus group. Three articles (Maurizi et al., 1985; Mahmoudian et al., 2013; Kim et al., 2016) did not have a control group and focused their comparisons on subgroups of tinnitus patients based on the configuration of the audiogram. These 17 studies either ensured that they used only a normal hearing population for both the tinnitus and control groups ($n = 12$), or a mixture of normal hearing and hearing loss for the control and tinnitus groups, matched based on the degree of hearing loss ($n = 5$). These studies used average audiometric thresholds of ≤15 dB HL ($n = 1$), <20 dB HL ($n = 3$), ≤20 dB HL ($n = 6$), <25 dB HL ($n = 5$), ≤25 dB HL ($n = 1$) as the criteria for normal hearing for the standard clinical frequencies. One study did not mention the criteria used to define normal hearing (Attias et al., 1993). Still, the frequency by which the audiometric criteria for normal hearing were applied varied from one study to the other. Indeed, there were typically defined from 0.25 to 8 kHz ($n = 9$), 0.125 to 8 kHz ($n = 1$), or 0.5 to 8 kHz ($n = 2$). Some studies limited the frequencies to a narrower range ($n = 5$). Of the five studies that used hearing loss populations, hearing loss was either undefined (Ikner and Hassen, 1990; Attias et al., 1993), defined within a limited range (i.e., 20-45, 21-49, 31-60, or 45-60 dB HL), or an unlimited range (i.e., above 15, 25 or 50 dB HL). A small number of studies ($n = 5$) tested frequencies above 8 kHz (Barnea et al., 1990; McKee and Stephens, 1992; Schaette and McAlpine, 2011; Gu et al., 2012; Gilles et al., 2016) (see also **Table 2**).

Characteristics of the Assessment and ABR Technique

Tinnitus was characterized in only 12 of the studies (see **Table 2**). Of these studies, four used a visual analog scale to determine either loudness ($n = 1$), severity ($n = 2$), or discomfort ($n = 1$), and eight used matching psychoacoustic procedures for loudness and pitch or a variation called the modified tinnitus spectrum procedure where pitch and loudness are rated (see **Table 2**). Residual inhibition was measured in three studies. The characteristics of the assessment and the ABR technique can be found in **Table 3**. The most commonly reported systems used to acquire the ABR were the Nicolet CA-1000 ($n = 4$) and the Bio-Logic NavPro or Traveler Express ($n = 5$). Transducers used were typically the supra-auricular headphones TDH-39(P) ($n = 6$) and TDH-49 ($n = 4$), or the insert headphones ER-3(A) ($n = 3$) with the exception of one study that used high frequency Sennheiser HDA-200 circumauricular headphones (Gu et al., 2012). The stimulus type was largely broadband clicks ($n = 19$) with a typical duration of 0.1 ms ($n = 16$) presented at a rate of 10–31 clicks per second. Exceptionally, one study used 0.05 ms clicks (Schaette and McAlpine, 2011) and another study used 3 ms tone bursts (Gerken et al., 2001). Presentation levels were generally high (>80 dB) and were either expressed in HL ($n = 8$), nHL ($n = 6$), or SPL ($n = 7$). Of the six studies reporting stimulus level in dB nHL, three reported using their own subjects to determine the minimum click intensity in dB SPL that elicited a behavioral response (Maurizi et al., 1985; Gu et al., 2012; Mahmoudian et al., 2013). When filter characteristics were reported ($n = 16$), the cutoff frequency of the high-pass filters ranged from 5 to 200 Hz and from the 1,500 to 5,000 Hz for the low-pass filters. Contralateral masking was used in 5 studies, all of which used a white noise at an intensity of 55 dB nHL (Gilles et al., 2016) or 50 dB HL (De Lavernhe-Lemaire and Beutter, 1989, 1990; Lemaire and Beutter, 1995; Kehrle et al., 2008).

Latency was reported in all studies except for De Lavernhe-Lemaire and Beutter (1990) and Schaette and McAlpine (2011). The most common outcome was no change in latency ($n = 9$) or an increase in the latency of waves I ($n = 8$), III ($n = 5$), and V ($n = 7$) for the tinnitus group (See **Table 2**). Only one study reported a decrease in wave V latency. Other latency changes for the tinnitus group varied considerably from increased interlatencies between waves III–V ($n = 4$), I–V ($n = 1$), and I–III ($n = 1$) to decreased interlatencies between waves I–II ($n = 1$) and I–V ($n = 3$). Out of the 12 studies that reported amplitude, four did not report any changes. The others reported the tinnitus group amplitudes either increased for waves III ($n = 1$), or decreased for waves I ($n = 4$), III ($n = 2$) and V ($n = 2$). Amplitude ratios were reported in four studies: V/III ($n = 1$) and V/I ($n = 3$). Gilles et al. (2016) was the sole study that reported the latency and amplitude of waves II and IV.

Meta-Analysis 1: Quantitative Analysis of ABR Latency and Amplitude Changes Separated by Hearing Status

The data of the 19 studies were compiled: a total of 1,240 subjects included in the tinnitus population and 664 control subjects were found. Three studies were not included because they have not reported any amplitude or latency data in format suitable for the analysis (see **Figure 1**). A summary of the mean latency and amplitude pooled from all studies is presented in **Table 4**. Each ear was treated as a separate data point when available in the literature. The raw latency and amplitude values for all the studies are available in Supplementary Table 2. **Table 4** shows that for the normal hearing populations, there is no significant difference between the tinnitus and non-tinnitus groups. The difference in mean latency for the normal hearing tinnitus group was 0, 0.01, and 0.03 ms higher than the control group for waves I, III, and V, respectively. For the hearing loss populations, the tinnitus group lower limit (95% CI) values of 1.75 (I), 3.83 (III), and 5.80 (V) ms were significantly higher than the upper limit (95% CI) values of 1.62 (I), 3.76 (III), and 5.68 (V) ms for the non-tinnitus groups. When comparing hearing loss groups, the tinnitus groups were 0.21, 0.15, and 0.22 ms delayed for waves I, III, and V compared to the group without tinnitus. Amplitudes for the normal hearing population were 0.04 μV lower for the wave I, and 0.02 and 0.01 μV higher for waves III, and V, respectively, for the tinnitus group. The hearing loss population showed 0.1, 0.09, and 0.06 μV lower wave I, III, and V amplitudes for the tinnitus group. Amplitudes were significantly different for the hearing loss population but not significantly different for normal hearing populations. Nevertheless, the wave I amplitude reduction in the tinnitus with normal hearing compared to the normal hearing controls was close to significance with a higher limit of the 95% CI of 0.24 μV compared to 0.23 μV 95% CI lower limit for the controls.

Meta-Analysis 2: Quantitative Analysis of ABR Latency and Amplitude Mean Difference between Tinnitus and Matched Control Groups Separated by Hearing Status

The mean differences in latencies and amplitudes between the tinnitus group and the matched controls (age and hearing status) were extracted when possible. For the latencies, the extraction of the mean difference was possible for only 10 out of the 15 studies that minimally matched their controls for age and hearing status. From the five excluded studies, one study did not report latencies (Schaette and McAlpine, 2011) and four studies provided insufficient information for data extraction (i.e., only the tinnitus data were presented, standard deviation was omitted, etc.; Barnea et al., 1990; McKee and Stephens, 1992; Rosenhall and Axelsson, 1994; Nemati et al., 2014). To note, only two studies out of the 10 studies that were kept for the latency meta-analysis did not include gender in their matching procedure (Attias et al., 1993, 1996). Interestingly, they were also the two studies with the highest degree of variability for both latency and amplitude analysis. For amplitudes, the number of included studies is even lower with five studies included in the second meta-analysis. Given that the amplitudes of waves III and V are poorly reported, the analysis was made on wave I only. Overall, similar problems extracting sufficient information were found for both the amplitude and latency data.

TABLE 3 | Characteristics of the Auditory Brainstem Responses methodologies used including the latency and amplitude outcome results.

Study	ABR system	Transducer	Polarity	Stimulus type	Presentation level(s)	Recording filters (Hz)
Attias et al., 1993	Microshev-4000 System	Not reported	Alternating	Broadband clicks, (0.1 ms duration 10 clicks/s)	120 dB SPL	200–3,000
Attias et al., 1996	Not mentioned	TDH-49	Alternating	Broadband clicks, (0.1 ms duration 10.3 clicks/s)	120 dB peSPL	100–2,000
Barnea et al., 1990	Microshev-2000	TDH-49	Alternating	Broadband clicks, (0.1 ms duration, 10 clicks/s)	120 dB SPL	200–2,000
Cartocci et al., 2012	Epic Plus	Not reported	Alternating	Broadband clicks, (0.1 ms duration, 11 clicks/s)	90 dB HL 80 dB HL	150–1,500
De Lavernhe-Lemaire and Beutter, 1989	Nicolet CA-1000	TDH-39P	Alternating	Broadband clicks, (0.1 ms duration, 11.1 clicks/s)	90 dB HL	150–1,500
De Lavernhe-Lemaire and Beutter, 1990	Nicolet CA-1000	TDH-39P	Alternating	Broadband clicks, (0.1 ms duration, 11.1 clicks/s)	90 dB HL	150–1,500
Gerken et al., 2001	Tucker-davis FT5 Grass P511k	ER2 inserts	Not reported	Tone bursts (1–8 kHz) (3 ms duration, 9.7 clicks/s)	112.5 peak dB SPL	1–3,000
Gilles et al., 2016	Bio-Logic with Nav Pro	ER-3A	Alternating	Broadband clicks, (0.1 ms duration, 31 clicks/s)	80 dB nHL	100–3,000
Gu et al., 2012	Tucker-Davis Medusa	HDA-200	Condensation	Broadband clicks, (0.1 ms duration, 11 clicks/s)	30, 50, 70, 80 dB nHL	5–5,000
Ikner and Hassen, 1990	Nicolet CA-1000	TDH-39	Condensation	Broadband clicks, (0.1 ms duration, 21.9 clicks/s)	75 dB nHL	Not reported
Kehrle et al., 2008	Amplaid Mk-15	TDH-50P	Alternating	Broadband clicks, (0.1 ms duration, 12 clicks/s)	80 dB HL	100–2,500
Kehrle et al., 2016	Biologic Navigator Pro AEP	E-A-RTONE 3A	Rarefaction	Broadband clicks, (0.1 ms duration, 21.1 clicks/s)	80 dB HL	100–3,000
Kim et al., 2016	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported
Lemaire and Beutter, 1995	Nicolet CA-1000	TDH-39P	Alternating	Broadband clicks, (0.1 ms duration, 11.1 clicks/s)	90 dB HL	150–1,500
Mahmoudian et al., 2013	Bio-Logic with Nav Pro	ER-3	Alternating	Broadband clicks, (0.1 ms duration, 11.1 clicks/s)	20 dB over the AEP threshold level	30–3,000
Maurizi et al., 1985	Amplaid MK 6	TDH-49	Rarefaction	Broadband clicks, (0.1 ms duration, 21 clicks/s)	60 dB nHL	200–2,000
McKee and Stephens, 1992	Biologic Evoked Potential System	Not reported	Not reported	Broadband clicks, (0.1 ms duration, 19.1 clicks/s)	85 dB HL	Not reported
Nemati et al., 2014	ICS CHARTR	Not reported	Alternating	Broadband clicks, (duration not reported, 11.1 clicks/s)	90 dB SPL	Not reported
Rosenhall and Axelsson, 1994	Madsen 2250 ERA	Not reported	Rarefaction	Broadband clicks, (duration not reported, 20 clicks/s)	80 dB nHL	150–2,000
Santos-Filha et al., 2014	Bio-Logic Traveler Express	TDH-39	Rarefaction	Broadband clicks (0.1 ms duration 19 clicks/s)	80 dB HL	Not mentioned
Schaette and McAlpine, 2011	Medelec Synergy T-EP system	TDH-49	Not reported	Broadband clicks, (0.05 ms duration, 11 clicks/s)	90 and 100 dB SPL	100–1,500
Singh et al., 2011	Not reported	Not reported	Not reported	Not reported	Not reported	Not reported

dB, Decibels; HL, hearing loss; SPL, sound pressure level; TDH, Telephonics supraauricular headphones; ER, Etymonic insert earphones; HAD, Sennheiser circumauricular headphones.

TABLE 4 | Summary table of the meta-analysis (1) of the mean latency and amplitude of waves I, III, and V for tinnitus and non-tinnitus groups separated by hearing status.

	Tinnitus			No tinnitus		
	I	III	V	I	III	V
NORMAL HEARING						
Mean latency (ms)	1.59	3.73	5.61	1.59	3.72	5.58
Standard error	0.02	0.02	0.02	0.01	0.02	0.02
95% CI	1.55–1.62	3.69–3.76	5.56–5.65	1.58–1.61	3.68–3.75	5.53–5.62
N-value	142	142	152	490	118	132
Mean Amplitude (μV)	0.21	0.34	0.43	0.25	0.32	0.42
Standard error	0.01	0.01	0.02	0.008	0.009	0.01
95% CI	0.19–0.24	0.31–0.36	0.39–0.48	0.23–0.26	0.30–0.34	0.40–0.44
N-value	105	75	75	248	212	212
HEARING LOSS						
Mean latency (ms)	1.77	3.86	5.84	1.56	3.71	5.62
Standard error	0.007	0.02	0.02	0.03	0.03	0.03
95% CI	**1.75**–1.78	**3.83**–3.89	**5.80**–5.88	1.5–**1.62**	3.66–**3.76**	5.57–**5.68**
N-value	1,407	369	385	69	69	69
Mean Amplitude (μV)	0.15	0.19	0.33	0.25	0.28	0.39
Standard error	0.004	0.004	0.005	0.04	0.03	0.03
95% CI	0.14–**0.16**	0.18–**0.20**	0.32–**0.34**	**0.17**–0.32	**0.23**–0.34	**0.34**–0.45
N-value	831	919	919	34	34	34

Confidence intervals (CI) and number of observations (n-value) are presented for each group. Bolded values are significant (comparing the limits of the 95% CI of the tinnitus to the no tinnitus group). Number of observations was obtained by adding the reported number of subjects or ears of each study within the group.

For the latency mean differences, the meta-analysis revealed that only three studies out of 10 found a significantly prolonged wave I in the tinnitus group compared to controls and two other studies were close to significance (**Figure 2**). To note, two of the studies that are not close to significance were the only ones that tested participants (tinnitus and controls) with hearing loss (**Figure 2**, white diamonds), all the other studies used normal hearing individuals for both the tinnitus and control groups (**Figure 2**, black diamonds). As previously mentioned, they were also the two only studies that did not match their groups on the basis of gender. For wave III and V latencies, significantly prolonged latencies were found in three studies for the former and four for the latter. Kehrle et al. (2008, 2016) were the only studies that showed all three waves were significantly prolonged although Ikner and Hassen (1990) reported a similar trend. Interestingly, none of the studies with a specific noise induced tinnitus etiology inclusion criteria reported a significant latency effect for any of the waves (**Figure 2**, studies in Bold).

For the mean amplitude differences, the second meta-analysis revealed that only two studies out of five found a significant reduction of wave I (see **Figure 3**). Two of the studies that did not report significant reduction of wave I amplitude tested noise-induced hearing loss participants with and without tinnitus (**Figure 3**, white diamonds). Gilles et al. (2016) reported a tendency of the wave I amplitude to be increased in the tinnitus group, although this did not reach significance (**Figure 3**).

DISCUSSION

The aim of the present scoping review was to investigate whether consistent ABR abnormalities are prevalent in populations with tinnitus. Although there is increasing interest in the use of ABRs for measuring auditory function in tinnitus individuals, the present scoping review found that the evidence of abnormalities within this population is sparse. Only 22 studies corresponding to the broad inclusion and exclusion criteria were found. Of these 22 studies, only 19 used control groups to make their comparisons. The present review unfortunately indicates that the tested tinnitus populations (i.e., *who*) are typically poorly defined across ABR studies as the vast majority did not report tinnitus etiology, assess and/or report the psychoacoustic properties of tinnitus, did not measure high frequency thresholds (above 8 kHz) and used various definitions of normal hearing and/or hearing loss. In regards to the methodology used (i.e., *how*), the ABR system, the type of transducer, the presentation level and the filtering strategies varied significantly across the studies. Still, the results of these studies (i.e., *what*) showed significant changes in amplitude and/or latency for high intensity stimulation levels as the current review did not assess low stimulation levels. In addition to this, longer latency and reduced amplitude of wave I for the normal hearing tinnitus group compared to hearing matched controls was consistently shown across numerous studies. Since high sound levels of stimulation were used in most studies, these results might indicate cochlear nerve fiber degeneration, a loss of neural synchrony, or both. These results will be further discussed by looking at the population characteristics, the various techniques and assessments, and the outcomes of the included studies. Based on these results, recommendations for future studies will be made as well as a description of the future direction of electrophysiology in tinnitus research.

Heterogeneous Population Characteristics

One of the issues found by the current review is the poorly defined and undefined tinnitus population tested. Many of the studies reported the tinnitus from their test groups were either subjective or idiopathic. The vast majority did not report the tinnitus etiology at all. Only four studies chose noise-induced tinnitus as their sample population of which two included sensorineural hearing loss participants. Interestingly, none of the studies on noise-induced tinnitus reported any significant effects on wave latencies and amplitudes with the only exception being Attias et al. (1996) who found higher wave III amplitude in the tinnitus group compared to age and hearing matched controls. These null results contrast the findings by Gu et al. (2012) and Schaette and McAlpine (2011) showing reduced wave I amplitude in human tinnitus subjects, as well as animal studies showing decrease ABR wave I amplitudes at high stimulation levels after noise exposure without significant hearing threshold shifts (Kujawa and Liberman, 2006, 2009). Still, these human studies did not mention the etiology of the tinnitus nor did they classify their subjects as having noise-induced tinnitus. Conversely, the

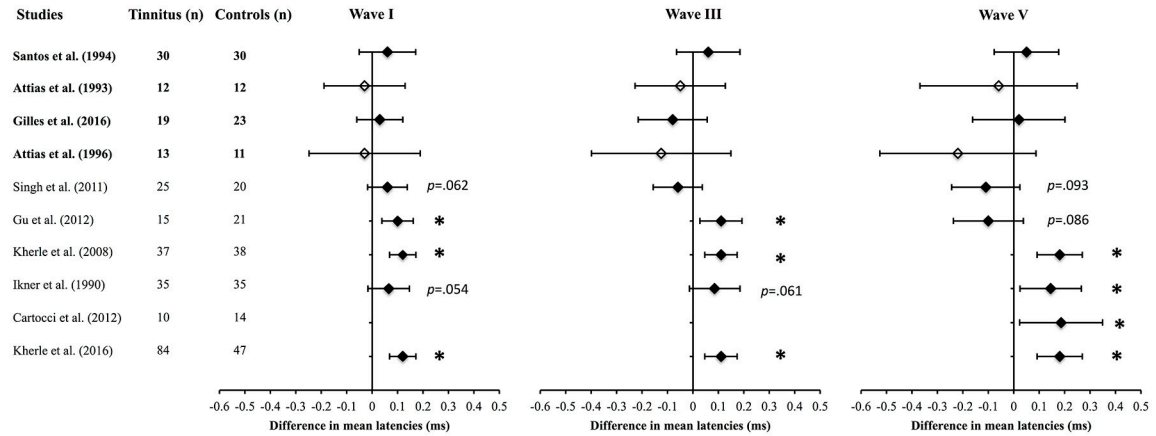

FIGURE 2 | Meta-analysis (2) of the net difference in latencies for Wave I, III, and V (95% CI) between tinnitus and control groups when matched for hearing status. Black diamonds represent studies with normal hearing participants and white diamonds studies with hearing loss participants. The studies in bold included only noise-induced tinnitus. *Asterisks represent significant differences ($p < 0.05$).

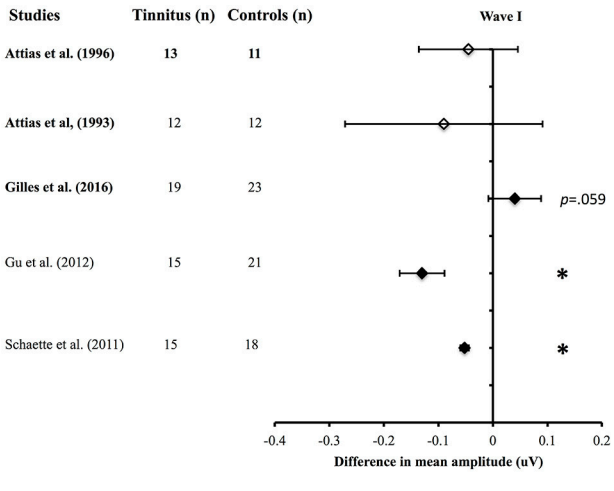

FIGURE 3 | Meta-analysis (2) of the net difference in amplitudes for Wave I (95% CI) between tinnitus and control groups when matched for hearing status. Black diamonds represent studies with normal hearing participants and white diamonds studies with hearing loss participants. The studies in bold included only noise-induced tinnitus. *Asterisks represent significant differences ($p < 0.05$).

Kujawa and Liberman (2006, 2009) animal studies did not assess tinnitus. Thus, the direct link between ABR abnormalities obtained in noise-induced animals and humans is not completely elucidated. Indeed, a recent study investigating ABRs in a young adult cohorts with normal audiograms but exposed to noise, did not find any significant reductions of wave I (Prendergast et al., 2017). More so, a recent study on a young adult sample (early 20's) of noise-induced tinnitus found no differences in the amplitude and latency of any of the ABR waves (Gilles et al., 2016). In that study, the ABR was assessed only on a subgroup of tinnitus and controls subjects. Their participants were matched for age, sex, and hearing thresholds for pure tones of 1–4 kHz only. Since a measure of synaptopathy such as the AP/SP ratio of the electrocochleography and very high frequency thresholds have been shown to be correlated (Liberman et al., 2016), it is thus possible that the tinnitus group had better thresholds at very high frequencies (>8 kHz) than the controls, and less synaptopathy. Still, this would be very unlikely considering that tinnitus subjects usually display more hearing loss than controls for those high frequencies when matched for normal thresholds at conventional frequencies (Fournier and Hébert, 2013). One possible interpretation of these results is that it takes some time, maybe years, for the nerve fibers to degenerate and therefore to effect the ABR responses. Another possibility is that ABRs are not sensitive enough to reveal synaptopathy and/or that synaptopathy loss is not necessary to develop tinnitus. Also, differences across species have been noted in the development of synaptopathy (Prendergast et al., 2017): losses of cochlear synapses have been shown to be irreversible in rodents but not in guinea pigs (although their function remained abnormal) (Liu et al., 2012; Shi et al., 2013; Song et al., 2016). Thus, one has to be cautious when comparing results from different animal species and, even more cautious, when translating such results to human listeners.

Very few studies assessed ABR wave characteristics between tinnitus and controls with hearing loss. Considering that tinnitus is often associated with hearing loss and remains rare in individuals with normal hearing, why have so few studies assessed ABR abnormalities in tinnitus participants with significant hearing loss? It is well known that two of three individuals with hearing loss will go on to develop tinnitus (Hoffman and Reed, 2004). It is possible these studies purposefully avoided recruiting participants with hearing loss in order to prevent known confounding effects of hearing loss on the ABR. However, ABR abnormalities in individuals with hearing loss might nevertheless help reveal certain underlying neural mechanisms responsible for tinnitus generation. To date, the only reported significant effect in this

specific population is higher wave III amplitude (Attias et al., 1996).

We conducted the first meta-analysis to demonstrate the effects of tinnitus within a large clinical population separated based on hearing loss. The advantage of such an approach is that the population is more comparable to what would be seen in a clinical setting, and the higher power, due to the large sample size, increases the chances of revealing tinnitus-related cofactors, such as hearing loss, whilst reducing the effects of random variables not related to tinnitus (i.e., gender, thickness of the scalp, transducer frequency response). This analysis shows increased latencies and reduced amplitudes for all three waves (I, III, and V) for tinnitus subjects compared to controls (**Table 4**) with hearing loss. However, these results must be interpreted cautiously as the number of subjects with tinnitus was five to 20 times higher than the number of controls depending on the wave. This imbalance of the number of subjects is the result of compiling all the data available from the entire yield of studies even though four studies did not report a control group. These ABR effects did not survive the second meta-analysis where only studies with matched control groups were used: the only two studies that used matched hearing loss control groups did not report any significant changes (Attias et al., 1993, 1996). It can be argued that the longer latencies and lower amplitudes found in the first meta-analysis may be the result of the compiled tinnitus group having a greater degree of hearing loss than controls (for amplitude: Sand and Saunte, 1994; for latency: Keith and Greville, 1987). The possibility of a gender and/or an aging bias could also account for the differences obtained in meta-analysis one.

The present review highlighted some variability in the criteria used to define normal hearing and an even larger variability in defining hearing loss. Future studies should define normal hearing as thresholds of less or equal to 15 dB HL minimally at all standard clinical frequencies thus from 250 to 8,000 Hz (Clark, 1981). More so, the measurements of high frequency thresholds (>8 kHz) need to be undertaken as significant threshold elevation for those frequencies (10–16 kHz) have been recently shown and interpreted as an early sign of synaptopathy in humans (Liberman et al., 2016). More so, high frequency hearing loss (>8 kHz) in tinnitus patients with conventional normal hearing (250– 8,000 Hz) have been reported (Fournier and Hébert, 2013; Vielsmeier et al., 2015). It is thus crucial to control for high frequency thresholds, at least up to 16 kHz, when comparing tinnitus subjects to controls in order to distinguish with confidence the presence of synaptopathy. Participants should be separated and grouped based on the presence of hearing loss. In addition to this, the degree (mild, moderate, severe, or profound), the origin (cochlear vs. neural), and the configuration (flat, high, or low frequency slope, notch) of the hearing loss should be clearly defined and reported.

One other recommendation is to recruit tinnitus participants based on tinnitus etiology (or report etiology) and/or psychoacoustic measurements in order to separate the test groups. This in turn might show ABR related patterns within each subgroup that would be otherwise masked by the heterogeneity of the sample. Few studies used characteristics of the tinnitus perception, such as pitch and loudness matching of the tinnitus percept or residual inhibition, as a means to separate various tinnitus subtypes. For instance, Noreña et al. (1999) classified the late auditory evoked potentials in three tinnitus subgroups based on their self-reported changes of tinnitus perception in relation to noise. They were classified as having decreased, increased, or unchanged tinnitus perception in the presence of noise. Based on this classification, they found that patients with decreased tinnitus perception in noise had greater intensity-dependence and longer N1 latency than the subgroup that reported increased tinnitus perception. Within the included ABR articles for this review, Maurizi et al. (1985) used residual inhibition (RI); a known phenomenon where a temporary reduction in the loudness or even disappearance of tinnitus follows the cessation of a masking noise, to stratify unilateral tinnitus into positive or no RI subgroups. They found wave I was prolonged for the positive RI group and wave V was prolonged for the no RI group of the tinnitus ear compared to the contralateral ear. They also performed ABR testing before and after treatment for each group. Interestingly, they found that after masking, the positive RI group's longer wave I latency had disappeared but the no RI group's wave V latency did not change. Stratification of the tinnitus test population based on psychoacoustic methods and added information on the tinnitus etiology would be crucial for future studies on auditory evoked potentials.

To note, only one study reported a potential adverse effect of the ABR on tinnitus subject. Indeed, Gu et al. (2012) reported that they could not complete the ABR assessment in 10 participants because they did not tolerate the stimulus intensity level. The co-occurrence of hyperacusis, which is defined as a hypersensivitity to moderate to loud sounds, and tinnitus have been shown to be very high (Hébert et al., 2004, 2013; Dauman and Bouscau-Faure, 2005). Still, the Gu and colleagues group is the only one to have reported that the hypersensitivity was detrimental for the assessment. From all the studies found in the current review, four measured hyperacusis in different ways within their sample: one used the Khalfa questionnaire (Gilles et al., 2016) and the others used loudness discomfort levels (LDL) (Gerken et al., 2001; Cartocci et al., 2012; Gu et al., 2012). It is not known whether hyperacusis was detrimental for the ABR assessment in those studies, as none reported it. Future studies should address the potential adverse effects of ABR testing such as discomfort or pain on tinnitus patients with and without hyperacusis. A potential cut-off on a hyperacusis questionnaire or on a psychophysical method such as LDL could be used to triage those participants for which the procedure is judged to be safe from those at risk of discomfort.

Various Techniques and Assessments

Several techniques and assessments revealed by the review may have impacted the ABR results. One suspect issue occurs with the type of transducer. Out of the studies that reported the type of transducer used, 11 used various types of supra-auricular headphones while only four used insert earphones. According to Van Campen et al. (1992), insert earphones such as the ER-3A insert earphones can increase interaural attenuation, ambient noise attenuation, patient comfort, and eliminate ear canal collapse. Their study measured the acoustic output of TDH 39P, TDH 49P, and ER-3A inserts earphones on a KEMAR mannequin

and used the same transducers for measuring click ABRs on normal hearing adults. One of the main differences they showed was that both TDH earphones had greater ringing than ER-3As for stimulus intensities down to 15 dB nHL. In addition to this, when tested on normal hearing adults, the insert earphones elicited a wave V that was significantly more delayed by 1.15 and 1 ms when stimulated at 40 dB nHL than the TDH earphones. Additionally, ER-3A earphones produced a significantly smaller wave I but similar wave V amplitude at 80 dB nHL than the TDH earphones, resulting in a greater V/I amplitude ratio. Given these differences, comparing data between insert and TDH earphones may be problematic.

Another potential issue comes from the frequency response of using various transducers with different response bandwidths. For example, the frequency response of an ER-3A earphone to a 500 Hz tone at 118.5 dB SPL is flat up to 4 kHz (E-A-R® Tone™ calibration specification sheet). This contrasts the Sennheiser HDA-200 headphones used by Gu et al. (2012) that was reported to have a bandwidth up to 8 kHz or the TDH 49 headphones that stimulate up to 7.1 kHz (Guest et al., 2016). Derived band measurements of the ABR to click stimuli show that wave I is mostly generated by characteristic frequencies above 2 kHz however wave V can be evoked by lower frequencies (Don and Eggermont, 1978; Abdala and Folsom, 1995). This may mean that the frequency response of the transducers used may influence the intensity of certain frequencies that may differ between studies. This variability may also contribute to the differences in latencies and amplitudes reported.

Heterogeneous Outcomes

The results found from the 22 studies were quite heterogeneous. For latencies, nine studies reported no change for any of the waves compared to nine who reported increased latency for waves I, V, and VII. Still, most well-controlled studies with appropriate matching procedures reported longer latencies for tinnitus compared to controls with wave I being the most consistently affected wave (**Figure 2**). A significant latency shift for all the three waves was found in Kehrle et al. (2008, 2016) and close to significance in Ikner and Hassen (1990) study. In these cases, the latency shift seen for waves III and V are not likely to be the result of the delayed wave I latency (due to neural damage) following through the other waves because the inter-peaks (I–V, III–V) were reported as abnormal in those same studies (see **Table 3**). These results might be related to a lack of central compensation in tinnitus individuals as suggested by Rüttiger et al. (2013).

Similar discordances were found within the amplitude data: four studies did not find any changes in amplitude for tinnitus, four reported decreased wave I, and either a decreased, an increased or even no modifications of the following amplitudes of waves III and V. Overall, only two of the five well-controlled studies reported decreased wave I amplitude. In addition to this, two well-controlled studies reported a higher V/I ratio (Kehrle et al., 2008; Nemati et al., 2014). More well-controlled studies are thus needed to clarify the presence of synaptopathy, as measured by wave I amplitude, in tinnitus patients. The large variability within the two studies with hearing loss tinnitus subjects prevent any conclusions that the trend towards lower wave I amplitude is due to an actual effect in tinnitus.

These mixed amplitude results may be related to the relative contribution of each type of nerve fibers (i.e., low-, medium-, and high-spontaneous rate) on the ABR signal. All the reviewed studies used high intensity stimuli that can be presumed to saturate the HSR fibers revealing potential difference linked only to LSR fibers. However, the specific contribution of each neural population (i.e., low-, medium-, and high- spontaneous rate) on the ABR waveform is not known. Substantial damage, for example to LSR fibers, may contribute to changes in the ABR amplitude in addition to the high spontaneous rate fibers. Bourien et al. (2014) have recently demonstrated that LSR fibers might have a negligible contribution to wave I ABR by measuring the compound action potential after selective damage to the LSR auditory nerve fibers of gerbils. They suggested that wave I reduction might be the result of damage to medium spontaneous rate fibers, which are usually mixed with LSR fibers in previous studies. It is also possible that damage to the LSR and MSR fibers varies across the length of the basilar membrane in such a way that the regions corresponding to certain frequencies have less damage than other areas. One way to target regions specifically affected by synaptopathy is to use specific frequencies or tone bursts, however narrowing the stimulus to include fewer frequencies may further reduce the number of responding fibers. For example, Gerken et al. (2001) used 10 tone bursts (1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, and 8 kHz) at a level of 112 dB SPL to elicit the ABR in a tinnitus and non-tinnitus population with and without hearing loss. They found no significant differences for the ABR amplitude and latencies (Wave I, III, V) of the tinnitus group compared to the non-tinnitus subjects. Still, they did not use any matching procedures to compose their groups and included only nine tinnitus subjects with hearing loss. It may be interesting to replicate the Gerken et al. (2001) study on normal hearing populations (for all frequencies up to 16 kHz) with and without tinnitus, and with appropriate matching procedures (gender, age, and hearing status) for different intensity levels, for different frequencies (from low to high, including tinnitus pitch) in order to compare the response of the HSR, MSR, and LSR fibers.

Since the search for this review was conducted, two more articles on ABR and tinnitus populations have been published: Ravikumar and Murthy (2016) and Guest et al. (2016). Both studies compared tinnitus populations with normal hearing to normal hearing matched controls. Normal hearing was not defined in the former, and was defined as pure tone thresholds of $\leq$20 dB HL at 0.25–8 kHz for the latter. Latencies of wave I, III, and V were significantly ($p = 0.05$) prolonged (Ravikumar and Murthy, 2016) and wave V amplitude was higher but not significant (Guest et al., 2016). The latter study also reported no differences in the amplitude of wave I and found there was no correlation between the amplitude of wave I and history of noise exposure.

Recommendations for Future Studies on ABR

Clear and simple recommendations for future ABR investigations on tinnitus can be determined from these

findings with the aim of improving future reviews on the subject, showing more reliable evidence of tinnitus, and making it easier to replicate previous studies. Most imperatively, it is highly encouraged that researchers report all the data collected including latencies and amplitudes of all the waves in a format that is suitable for meta-analysis. The meta-analysis of the current study was difficult particularly when latency or amplitude data was left unreported. From 22 studies found on ABR investigations of tinnitus in humans, only 10 studies could be used for the second meta-analysis to compare the mean difference of latencies between tinnitus and controls. Unfortunately, this represented <50% of all the studies found. For amplitude, even fewer studies were retained (n = 5), which represents <25% of all the studies. The mean and standard deviation of the latencies and amplitudes of the waves found within their paradigm for the tinnitus and control groups should be reported separately. Negative and non-significant results should always be reported in a similar fashion.

Secondly, all future ABR protocols should at least include control groups matched for gender, age and hearing status for sufficient control over these covariables. As mentioned previously, the two studies displaying the greatest variability for latencies and amplitudes in the second meta-analysis (Attias et al., 1993, 1996) did not match for gender between groups. Still, when comparing more recent studies to older ones, there appears to be a clear trend toward the use of more restrictive matching procedures. It is further suggested that hearing be assessed and matched for frequencies up to at least 16 kHz between groups. Studies should recruit participants with similar tinnitus etiologies (e.g., noise trauma) and include psychoacoustic measurements such as pitch and loudness matching, minimum masking level and residual inhibition. Future research should also consider separating participants into narrower age bands or at least separate younger and older subjects into two different groups. The two studies reporting reduced wave I in tinnitus (Schaette and McAlpine, 2011; Gu et al., 2012) tested participants approximately 10 years older on average than the study of Gilles et al. (2016) which included only participants below the age of 30. The absence of synaptopathy using wave I amplitude has also been reported in a study on noise-exposed young adults (mean age of 23, ranging from 18 to 36 years old), however tinnitus was not assessed (Prendergast et al., 2017). It is thus recommended that an age cut-off around 30 years old be used for future work. A sample size of at least 30 subjects per group is also recommended in order to reduce variability of the measures and to increase statistical power. Regarding the technical aspects of ABR measurement, insert headphones are preferred over circumauricular ones in order to optimize interaural attenuation, ambient noise attenuation, and to reduce the risk of ear canal collapsing. The frequency response of the transducer should also include as many frequencies above 2 kHz as possible.

Future Directions

Since the key publication of animal research demonstrating evidence of cochlear synaptopathy after noise exposure (Kujawa and Liberman, 2006, 2009), there has been a growing interest in improving ABR measurements in humans. Reliable ABR waveforms can sometimes be difficult to obtain mostly because of high inter-subject variability due to factors such as small signal to noise ratios, head shape, sex, as well as the various methodological concerns described above. Many research groups have attempted to address these issues by either improving the methodology of the click or tone-burst ABR method or by proposing new methods of assessment. For example, one study used an electrode placed on the tympanic membrane (TM) in order to improve the signal to noise ratio (Stamper and Johnson, 2015). Using a similar electrode tip on the TM, another group used electrocochleography instead of ABR to show significant differences in the SP/AP ratio between high and low noise exposure risk groups of participants (Liberman et al., 2016). This finding still needs to be replicated, but electrocochleography could potentially become a standard measure of synaptopathy instead of the classical ABR. Another group showed delayed wave V ABRs when responding to clicks in background noise as evidence of the presence of synaptopathy in animals and humans (Mehraei et al., 2016). The use of envelope following responses (EFR) with amplitude-modulated tones in notched noise with varying modulation depth have also shown deficits that are consistent with synaptopathy (Bharadwaj et al., 2015). All these new techniques could easily be applied to tinnitus participants. This in turn can bring new insight on a possible role, if any, played by cochlear synaptopathy in the generation of tinnitus. More so, the application of a paradigm to desynchronize neural activity may help reveal potential tinnitus mechanisms. Indeed, when click-rate is increased, the nerve fibers appear to have more difficulty synchronizing their discharge to the stimulus, resulting in smaller ABR amplitudes and prolonged wave V latencies (Konrad-Martin et al., 2012). Higher synchronous activity at higher levels of the auditory system related to tinnitus might thus only be revealed when using high click-rates.

Finally, ABRs have more recently been used not only to understand the pathophysiology of tinnitus but also objectify its presence in individuals. The gap-in-noise ABR (or GIN-ABR) has been used in animal subjects with different background noise frequencies before and after tinnitus induction by salicylate (Lowe and Walton, 2015). Using this method, they found a significant reduction in gap detection after salicylate treatment for only the 16 kHz background noise condition. The authors concluded that since salicylate is known to produce a 16 kHz tinnitus percept that appears to fill the gap, the GIN-ABR may be effective for objectifying the presence of tinnitus in animals. This in turn may be a promising new avenue for future auditory brainstem research applied to humans with tinnitus.

AUTHOR CONTRIBUTIONS

All the authors contributed to this work. VM and AK provided the original conception and design of the study. VM and PF worked on data acquisition, analysis, and interpretation. They both wrote the manuscript. DB, AN, and AK provided

intellectual feedback and revised the content of several previous versions of the manuscript. All authors (VM, PF, DB, AN, and AK) approved the final version of the manuscript.

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REFERENCES

Abdala, C., and Folsom, R. C. (1995). Frequency contribution to the click-evoked auditory-brain-stem response in human adults and infants. *J. Acoust. Soc. Am.* 97, 2394–2404. doi: 10.1121/1.411961

Arksey, H., and O'Malley, L. (2005). Scoping studies: towards a methodological framework. *Int. J. Soc. Res. Methodol.* 8, 19–32. doi: 10.1080/1364557032000119616

Attias, J., Pratt, H., Reshef, I., Bresloff, I., Horowitz, G., Polyakov, A., et al. (1996). Detailed analysis of auditory brainstem responses in patients with noise-induced tinnitus. *Audiology* 35, 259–270. doi: 10.3109/00206099609071946

Attias, J., Urbach, D., Gold, S., and Shemesh, Z. (1993). Auditory event related potentials in chronic tinnitus patients with noise induced hearing loss. *Hear. Res.* 71, 106–113. doi: 10.1016/0378-5955(93)90026-W

Barnea, G., Attias, J., Gold, S., and Shahar, A. (1990). Tinnitus with normal hearing sensitivity: extended high-frequency audiometry and auditory-nerve brain-stem-evoked responses. *Audiology* 29, 36–45. doi: 10.3109/00206099009081644

Bauch, C. D., Olsen, W. O., and Pool, A. F. (1996). ABR indices: sensitivity, specificity, and tumor size. *Am. J. Audiol.* 5, 97–104. doi: 10.1044/1059-0889.0501.97

Bayar, N., Böke, B., Turan, E., and Belgin, E. (2001). Efficacy of amitriptyline in the treatment of subjective tinnitus. *J. Otolaryngol.* 30, 300–303. doi: 10.2310/7070.2001.19597

Berliner, K., Shelton, C., and Hitselberger, W. (1992). Acoustic tumors: effect of surgical removal on tinnitus. *Otol. Neurotol.* 13, 13–17. doi: 10.1097/00129492-199201000-00005

Bharadwaj, H. M., Masud, S., Mehraei, G., Verhulst, S., and Shinn-Cunningham, B. G. (2015). Individual differences reveal correlates of hidden hearing deficits. *J. Neurosci.* 35, 2161–2172. doi: 10.1523/JNEUROSCI.3915-14.2015

Bourien, J., Tang, Y., Batrel, C., Huet, A., Lenoir, M., Ladrech, S., et al. (2014). Contribution of auditory nerve fibers to compound action potential of the auditory nerve. *J. Neurophysiol.* 112, 1025–1039. doi: 10.1152/jn.00738.2013

Burkard, R., and Secor, C. (2002). "Overview of auditory evoked potentials," in *Handbook of Clinical Audiology*, ed T. L. Julet (Baltimore, MD: Lippincott Williams and Wilkins), 233–248.

Cartocci, G., Attanasio, G., Fattapposta, F., Locuratolo, N., Mannarelli, D., and Filipo, R. (2012). An electrophysiological approach to tinnitus interpretation. *Int. Tinnitus J.* 17, 152–157. doi: 10.5935/0946-5448.20120027

Chalak, S., Kale, A., Deshpande, V. K., and Biswas, D. A. (2013). Establishment of normative data for monaural recordings of auditory brainstem response and its application in screening patients with hearing loss: a cohort study. *J. Clin. Diagn. Res.* 7, 2677–2679. doi: 10.7860/jcdr/2013/6768.3730

Clark, J. (1981). Uses and abuses of hearing loss. *ASHA* 23, 493–500.

Dauman, R., and Bouscau-Faure, F. (2005). Assessment and amelioration of hyperacusis in tinnitus patients. *Acta Otolaryngol.* 125, 503–509. doi: 10.1080/00016480510027565

De Lavernhe-Lemaire, M. C., and Beutter, P. (1989). Potentiels évoqués auditifs dans la distinction entre acouphènes périphériques et centraux. *Arch. Int. Physiol. Biochim.* 97, 135–144. doi: 10.3109/13813458909104533

De Lavernhe-Lemaire, M. C., and Beutter, P. (1990). Modifications des amplitudes des potentiels évoques auditifs précoces observées dans les acouphènes. *Arch. Int. Physiol. Biochim.* 98, 403–409. doi: 10.3109/13813459009114002

De Ridder, D., Vanneste, S., Langguth, B., and Llinas, R. (2015). Thalamocortical dysrhythmia: a theoretical update in tinnitus. *Front. Neurol.* 6:124. doi: 10.3389/fneur.2015.00124

Don, M., and Eggermont, J. J. (1978). Analysis of the click-evoked brainstem potentials in man using high-pass noise masking. *J. Acoust. Soc. Am.* 63, 1084–1092. doi: 10.1121/1.381816

Don, M., Ponton, C. W., Eggermont, J. J., and Kwong, B. (1998). The effects of sensory hearing loss on cochlear filter times estimated from auditory brainstem response latencies. *J. Acoust. Soc. Am.* 104, 2280–2289. doi: 10.1121/1.423741

Eggermont, J. J. (1984). Tinnitus: some thoughts about its origin. *J. Laryngol. Otol.* 98, 31–37. doi: 10.1017/S1755146300090089

Fournier, P., and Hébert, S. (2013). Gap detection deficits in humans with tinnitus as assessed with the acoustic startle paradigm: does tinnitus fill in the gap? *Hear. Res.* 295, 16–23. doi: 10.1016/j.heares.2012.05.011

Gerken, G. M., Hesse, P. S., and Wiorkowski, J. J. (2001). Auditory evoked responses in control subjects and in patients with problem-tinnitus. *Hear. Res.* 157, 52–64. doi: 10.1016/S0378-5955(01)00277-5

Gilles, A., Schlee, W., Rabau, S., Wouters, K., Fransen, E., and Van de Heyning, P. (2016). Decreased speech-in-noise understanding in young adults with tinnitus. *Front. Neurosci.* 10:288. doi: 10.3389/fnins.2016.00288

Giraudet, F., and Avan, P. (2012). Auditory neuropathies. *Curr. Opin. Neurol.* 25, 50–56. doi: 10.1097/WCO.0b013e32834f0351

Gopal, K. V., Thomas, B. P., Mao, D., and Lu, H. (2015). Efficacy of carnitine in treatment of tinnitus: evidence from audiological and MRI measures—a case study. *J. Am. Acad. Audiol.* 26, 311–324. doi: 10.3766/jaaa.26.3.10

Gu, J. W., Herrmann, B. S., Levine, R. A., and Melcher, J. R. (2012). Brainstem auditory evoked potentials suggest a role for the ventral cochlear nucleus in tinnitus. *J. Assoc. Res. Otolaryngol.* 13, 819–833. doi: 10.1007/s10162-012-0344-1

Guest, H., Munro, K. J., Prendergast, G., Howe, S., and Plack, C. J. (2016). Tinnitus with a normal audiogram: relation to noise exposure but no evidence for cochlear synaptopathy. *Hear. Res.* 344, 265–274. doi: 10.1016/j.heares.2016.12.002

Hébert, S., Fournier, P., and Noreña, A. (2013). The auditory sensitivity is increased in tinnitus ears. *J. Neurosci.* 33, 2356–2364. doi: 10.1523/JNEUROSCI.3461-12.2013

Hébert, S., Paiement, P., and Lupien, S. J. (2004). A physiological correlate for the intolerance to both internal and external sounds. *Hear. Res.* 190, 1–9. doi: 10.1016/S0378-5955(04)00021-8

Henry, J. A., Roberts, L. E., Caspary, D. M., Theodoroff, S. M., and Salvi, R. J. (2014). Underlying mechanisms of tinnitus: review and clinical implications. *J. Am. Acad. Audiol.* 25, 5–22. doi: 10.3766/jaaa.25.1.2

Hickox, A. E., and Liberman, M. C. (2014). Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus? *J. Neurophysiol.* 111, 552–564. doi: 10.1152/jn.00184.2013

Hoffman, H., and Reed, G. (2004). "Epidemiology of tinnitus," in *Tinnitus: Theory and Management*, ed J. B. Snow (Lewiston, NY: BC Decker), 16–41.

House, J. W., and Brackmann, D. E. (1981). "Tinnitus: surgical treatment," in *Ciba Foundation Symposium 85: Tinnitus*, eds D. Evered and G. Lawrens (London: Pitman), 204–212.

Hultcrantz, M., Simonoska, R., and Stenberg, A. E. (2006). Estrogen and hearing: a summary of recent investigations. *Acta Otolaryngol.* 126, 10–14. doi: 10.1080/00016480510038617

Hyde, M. L., Riko, K., and Malizia, K. (1990). Audiometric accuracy of the click ABR in infants at risk for hearing loss. *J. Am. Acad. Audiol.* 1, 59–66.

Ikner, C. L., and Hassen, A. H. (1990). The effect of tinnitus on ABR latencies. *Ear Hear.* 11, 16–20. doi: 10.1097/00003446-199002000-00005

Jewett, D., Romano, M., and Williston, J. (1970). Human auditory evoked potentials: possible brain stem components detected on the scalp. *Science* 167, 1517–1518. doi: 10.1126/science.167.3924.1517

Jewett, D., and Williston, J. S. (1971). Auditory-evoked far fields averaged from the scalp of humans. *Brain* 94, 681–696. doi: 10.1093/brain/94.4.681

Kehrle, H. M., Granjeiro, R. C., Sampaio, A. L. L., Bezerra, R., Almeida, V. F., and Oliveira, C. A. (2008). Comparison of auditory brainstem response results in normal-hearing patients with and without tinnitus. *Arch. Otolaryngol. Head Neck Surg.* 134, 647–651. doi: 10.1001/archotol.134.6.647

Kehrle, H. M., Sampaio, A. L., Granjeiro, R. C., De Oliveira, T. S., and Oliveira, C. A. (2016). Tinnitus annoyance in normal-hearing individuals: correlation with depression and anxiety. *Ann. Otol. Rhinol. Laryngol.* 125, 185–194. doi: 10.1177/0003489415606445

Keith, W. J., and Greville, K. A. (1987). Effects of audiometric configuration on the auditory brain stem response. *Ear Hear.* 8, 49–55. doi: 10.1097/00003446-198702000-00009

Khangura, S., Konnyu, K., Cushman, R., Grimshaw, J., and Moher, D. (2012). Evidence summaries: the evolution of a rapid review approach. *Syst. Rev.* 1:10. doi: 10.1186/2046-4053-1-10

Kim, S. I., Kim, M. G., Kim, S. S., Byun, J. Y., Park, M. S., and Yeo, S. G. (2016). Evaluation of tinnitus patients by audiometric configuration. *Otolaryngol. Head Neck Surg.* 37, 1–5. doi: 10.1016/j.amjoto.2015.08.009

Konrad-Martin, D., Dille, M. F., McMillan, G., Griest, S., McDermott, D., Fausti, S. A., et al. (2012). Age-related changes in the auditory brainstem response. *J. Am. Acad. Audiol.* 23, 18–35. doi: 10.3766/jaaa.23.1.3

Kotlarz, J. P., Eby, T. L., and Borton, T. E. (1992). Analysis of the efficiency of retrocochlear screening. *Laryngoscope* 102, 1108–1112. doi: 10.1288/00005537-199210000-00004

Kujawa, S. G., and Liberman, M. C. (2006). Acceleration of age-related hearing loss by early noise exposure: evidence of a misspent youth. *J. Neurosci.* 26, 2115–2123. doi: 10.1523/JNEUROSCI.4985-05.2006

Kujawa, S. G., and Liberman, M. C. (2009). Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. *J. Neurosci.* 29, 14077–14085. doi: 10.1523/JNEUROSCI.2845-09.2009

Lemaire, M. C., and Beutter, P. (1995). Brainstem auditory evoked responses in patients with tinnitus. *Audiology* 34, 287–300. doi: 10.3109/00206099509071919

Levac, D., Colquhoun, H., and O'Brien, K. K. (2010). Scoping studies: advancing the methodology. *Implement. Sci.* 5:69. doi: 10.1186/1748-5908-5-69

Levine, R. A. (1999). Somatic (craniocervical) tinnitus and the dorsal cochlear nucleus hypothesis. *Am. J. Otolaryngol.* 6, 351–362. doi: 10.1016/S0196-0709(99)90074-1

Lewis, J. D., Kopun, J., Neely, S. T., Schmid, K. K., and Gorga, M. P. (2015). Tone-burst auditory brainstem response wave V latencies in normal-hearing and hearing-impaired ears. *J. Acoust. Soc. Am.* 138, 3210–3219. doi: 10.1121/1.4935516

Liberman, M. C., Epstein, M. J., Cleveland, S. S., Wang, H., and Maison, S. F. (2016). Toward a differential diagnosis of hidden hearing loss in humans. *PLoS ONE* 11:e0162726. doi: 10.1371/journal.pone.0162726

Liberman, M. C., and Kujawa, S. G. (2017). Cochlear synaptopathy in acquired sensorineural hearing loss: manifestations and mechanisms. *Hear. Res.* 349, 138–147. doi: 10.1016/j.heares.2017.01.003

Liu, L., Wang, H., Shi, L., Almuklass, A., He, T., Aiken, S., et al. (2012). Silent damage of noise on cochlear afferent innervation in guinea pigs and the impact on temporal processing. *PLoS ONE* 7:e49550. doi: 10.1371/journal.pone.0049550

Lowe, A. S., and Walton, J. P. (2015). Alterations in peripheral and central components of the auditory brainstem response: a neural assay of tinnitus. *PLoS ONE* 10:e0117228. doi: 10.1371/journal.pone.0117228

Mahmoudian, S., Lenarz, M., Esser, K.-H., Salamat, B., Alaeddini, F., Dengler, R., et al. (2013). Alterations in early auditory evoked potentials and brainstem transmission time associated with tinnitus residual inhibition induced by auditory electrical stimulation. *Int. Tinnitus J.* 18, 63–74. doi: 10.5935/0946-5448.20130009

Maurizi, M., Ottaviani, F., Paludetti, G., Almadori, G., and Tassoni, A. (1985). Contribution to the differentiation of peripheral versus central tinnitus via auditory brain stem response evaluation. *Audiology* 24, 207–216. doi: 10.3109/00206098509070104

McKee, G. J., and Stephens, S. D. G. (1992). An investigation of normally hearing subjects with tinnitus. *Audiology* 31, 313–317. doi: 10.3109/00206099209072919

Mehraei, G., Hickox, A. E., Bharadwaj, H. M., Goldberg, H., Verhulst, S., Liberman, M. C., et al. (2016). Auditory brainstem response latency in noise as a marker of cochlear synaptopathy. *J. Neurosci.* 36, 3755–3764. doi: 10.1523/JNEUROSCI.4460-15.2016

Melcher, J. R., and Kiang, N. Y. (1996). Generators of the brainstem auditory evoked potential in cat III: identified cell populations. *Hear. Res.* 93, 52–71. doi: 10.1016/0378-5955(95)00200-6

Milicic, D., and Alcada, M. N. (1999). A tinnitus objectivization: how we do it. *Int Tinnitus J.* 5, 5–15.

Moeller, A. R. (1984). Pathophysiology of tinnitus. *Ann. Otol. Rhinol. Laryngol.* 93, 39–44. doi: 10.1177/000348948409300110

Moher, D., Liberati, A., Tetzlaff, J., and Altman, D. G. (2009). Academia and clinic annals of internal medicine preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann. Intern. Med.* 151, 264–269. doi: 10.7326/0003-4819-151-4-200908180-00135

Nemati, S., Habibi, A. F., Panahi, R., and Pastadast, M. (2014). Cochlear and brainstem audiologic findings in normal hearing tinnitus subjects in comparison with non-tinnitus control group. *Acta Med. Iran.* 51, 822–826.

Noreña, A., Cransac, H., and Chéry-Croze, S. (1999). Towards an objectification by classification of tinnitus. *Clin. Neurophysiol.* 110, 666–675.

Noreña, A. J. (2011). An integrative model of tinnitus based on a central gain controlling neural sensitivity. *Neurosci. Biobehav. Rev.* 35, 1089–1109. doi: 10.1016/j.neubiorev.2010.11.003

Prendergast, G., Guest, H., Munro, K. J., Kluk, K., Léger, A., Hall, D. A., et al. (2017). Effects of noise exposure on young adults with normal audiograms I: electrophysiology. *Hear. Res.* 344, 68–81. doi: 10.1016/j.heares.2016.10.028

Ravikumar, G., and Murthy, V. A. (2016). A study of brainstem auditory evoked responses in normal hearing patients with tinnitus. *Indian J. Otolaryngol. Head Neck Surg.* 68, 429–433. doi: 10.1007/s12070-015-0917-5

Rosenhall, U., and Axelsson, A. (1994). Auditory brainstem response latencies in patients with tinnitus. *Scand. Audiol.* 24, 97–100. doi: 10.3109/01050399509047521

Rupa, V., Job, A., George, M., and Rajshekhar, V. (2003). Cost-effective initial screening for vestibular schwannoma: auditory brainstem response or magnetic resonance imaging? *Otolaryngol. Head Neck Surg.* 128, 823–828. doi: 10.1016/S0194-5998(03)00358-9

Rüttiger, L., Singer, W., Panford-Walsh, R., Matsumoto, M., Lee, S. C., Zuccotti, A., et al. (2013). The reduced cochlear output and the failure to adapt the central auditory response causes tinnitus in noise exposed rats. *PLoS ONE* 8:e57247. doi: 10.1371/journal.pone.0057247

Sand, T., and Saunte, C. (1994). ABR amplitude and dispersion variables: relation to audiogram shape and click polarity. *Scand. Audiol.* 23, 7–12. doi: 10.3109/01050399409047482

Santos-Filha, V., Samelli, A., and Matas, C. (2014). Noise-induced tinnitus: auditory evoked potential in symptomatic and asymptomatic patients. *Clinics* 69, 487–490. doi: 10.6061/clinics/2014(07)08

Schaette, R., and McAlpine, D. (2011). Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J. Neurosci.* 31, 13452–13457. doi: 10.1523/JNEUROSCI.2156-11.2011

Shi, L., Liu, L., He, T., Guo, X., Yu, Z., Yin, S., et al. (2013). Ribbon synapse plasticity in the cochleae of Guinea pigs after noise-induced silent damage. *PLoS ONE* 8:e81566. doi: 10.1371/journal.pone.0081566

Shulman, A., and Seitz, M. R. (1981). Central tinnitus- diagnosis and treatment. Observations simultaneous binaural auditory brain responses with monaural stimulation in the tinnitus patient. *Laryngoscope* 91, 2025–2036. doi: 10.1288/00005537-198112000-00005

Singh, S., Munjal, S. K., Panda, N. K., Munjal, K., S., Panda, K., N., Munjal, S. K., et al. (2011). Comparison of auditory electrophysiological responses in normal-hearing patients with and without tinnitus. *J. Laryngol. Otol.* 125, 668–672. doi: 10.1017/S0022215111000569

Song, Q., Shen, P., Li, X., Shi, L., Liu, L., Wang, J., et al. (2016). Coding deficits in hidden hearing loss induced by noise: the nature and impacts. *Sci. Rep.* 6:25200. doi: 10.1038/srep25200

Stamper, G. C., and Johnson, T. A. (2015). Auditory function in normal-hearing, noise-exposed human ears. *Ear Hear.* 36, 172–184. doi: 10.1097/AUD.0000000000000107

Starr, A., Picton, T. W., Sininger, Y., Hood, L. J., and Berlin, C. I. (1996). Auditory neuropathy. *Brain* 119, 741–753. doi: 10.1093/brain/119.3.741

Stouffer, J. L., and Tyler, R. S. (1990). Characterisation of tinnitus by tinnitus patients. *J. Speech Hear. Disord.* 55, 439–453. doi: 10.1044/jshd.5503.439

Tyler, R., Coelho, C., Tao, P., and Ji, H. (2008). Identifying tinnitus subgroups with cluster analysis. *Am. J. Audiol.* 17, S176–S184. doi: 10.1044/1059-0889(2008/07-0044)

Van Campen, L. E., Sammeth, C. A., Hall, J. W., and Peek, B. F. (1992). Comparison of Etymotic insert and TDH supra-aural earphones in auditory brainstem response measurement. *J. Am. Acad. Audiol.* 3, 315–323.

Vielsmeier, V., Lehner, A., Strutz, J., Steffens, T., Kreuzer, P. M., Schecklmann, M., et al. (2015). The relevance of the high frequency audiometry in tinnitus patients with normal hearing in conventional pure-tone audiometry. *Biomed. Res. Int.* 2015:302515. doi: 10.1155/2015/302515

Watson, D. R. (1996). The effects of cochlear hearing loss. *Audiology* 35, 246–258. doi: 10.3109/00206099609071945a

Wilson, D. F., Hodgson, R. S., Gustafson, M. F., Hogue, S., and Mills, L. (1992). The sensitivity of auditory brainstem response testing in small acoustic neuromas. *Laryngoscope* 102, 961–964. doi: 10.1288/00005537-199209000-00001

Yu, H., Patil, K. V., Han, C., Fabella, B., Canlon, B., Someya, S., et al. (2016). GLAST deficiency in mice exacerbates gap detection deficits in a model of salicylate-induced tinnitus. *Front. Behav. Neurosci.* 10:158. doi: 10.3389/fnbeh.2016.00158

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frontiers in Neurology

CASE REPORT
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A Case of Acoustic Shock with Post-trauma Trigeminal-Autonomic Activation

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This study reports the case of an acoustic shock injury (ASI), which did not result in a significant hearing loss, but was followed by manifold chronic symptoms both within (tinnitus, otalgia, tingling in the ear, tension in the ear, and red tympanum) and outside the ears (blocked nose, pain in the neck/temporal region). We suggest that these symptoms may result from a loop involving injury to middle ear muscles, peripheral inflammatory processes, activation and sensitization of the trigeminal nerve, the autonomic nervous system, and central feedbacks. The pathophysiology of this ASI is reminiscent of that observed in post-traumatic trigeminal-autonomic cephalalgia. This framework opens new and promising perspectives on the understanding and medical management of ASI.

Keywords: tinnitus and hyperacousis, otalgia, pain, trigeminal nerve, acoustic shock, inflammation, referred pain

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INTRODUCTION

Acoustic shocks are brief exposure to loud sounds that do not cause substantial hearing loss but can trigger a cluster of debilitating symptoms, i.e., otalgia, ear fullness, ear tension, tinnitus, sound intolerance, dizziness and head, face or neck aches (1, 2). In most cases, these symptoms are temporary and disappear within a few hours or days following the acoustic incident. However, in certain cases, they can become chronic and seriously affect quality of life (1). The pathophysiological mechanisms underlying these symptoms remain unknown, even though some authors have hypothesized a dysfunction in the tensor tympani muscle (TTM) (1, 2). The patient described here was able to precisely report his symptoms, their temporal evolution, and take pictures of his eardrums over time during symptom severity fluctuations. The psychoacoustic characteristics of his tinnitus and the functional integrity of the middle ears were also investigated. This invaluable dataset provides critical insights into the pathophysiology of the acoustic shock injury (ASI) and beyond, i.e., tinnitus, hyperacusis, and otalgia.

METHODS

The patient (NC) was a 27-year-old Caucasian male working as a general medical practitioner at the time of the acoustic shock. Written informed consent was obtained from the participant for the publication of this case report. On November 10th 2013, in a leisure shooting stand, without wearing any hearing protection, he was exposed to a unique and unexpected gunfire at an

approximate 7 m distance from his right side. As bothersome symptoms progressively emerged after this acoustic incident, he attended several ENT clinics. Audiograms at week 3 and 7 after ASI (thresholds <15 dBHL at all tested frequencies from 0.125 to 8 kHz) and all other exams (tympanometry, blood check, and cerebral MRI) were normal. Later, he was asked to assess the severity of his symptoms, using a 0–10 visual analog scale, for each ear several times a day (from May 1st to May 9th 2015). The symptoms were tingling, otalgia, ear tension, tinnitus loudness, neck pain/tension, temporal pain/tension, and pain/blocked nose. He also managed to photograph his eardrums using a video-otoscope (Firefly DE500 v1.1, 4 mm speculum). For analysis purpose, the images were transformed into numeric values estimating the "redness" of the eardrum. The number of red pixels was normalized according to the eardrum surface captured by the video-otoscope. In March 2017, NC visited our laboratory in Marseille to assess the psychoacoustic properties of his tinnitus and explored the middle ear function (Multifrequency Tympanometer Zodiac, Otometrics). Static admittance was obtained at four frequencies (226, 678, 800, and 1,000 Hz). Admittance variation while the patient voluntarily (but not forcefully) eye blinked was investigated over time both at the pressure at which the admittance is maximal and at ±50 daPa from this value. Symptom severity from each ear was analyzed using principal component analysis (PCA). The PCA was performed on all symptoms, excluding neck and temporal pain as these symptoms did not affect the left side.

RESULTS

In regard to the clinical course, immediately after ASI, the patient felt a clicking in the right ear and thereafter reported a subjective perception of ear tension and fullness. At week 2 emerged a bilateral high-pitched fluctuating tinnitus. These auditory symptoms were associated with an erratic acute pain (sting or electrical shock) located deep in either ear. More than 3 weeks after the acoustic shock, the patient reported additional painful sensations starting around the concha and irradiating to the mid-face (constriction, blocked nose, and clear nasal discharge) and to the temporal region or the neck. Interestingly, the laterality and severity of the pain was correlated with the amount of tension perceived in the ipsilateral (right) ear. The psychoacoustic properties of the tinnitus, its amplitude modulation (tremolo), also varied according to the ear tension level. From low to mid tension, the tremolo varied from low frequency ("morse code") to high frequency ("cricket-like sound"). For high tension, tinnitus was described as a high-pitched whistling. The pitch of this tinnitus was measured at 12 kHz and the tremolo, estimated from an amplitude modulated stimulus at the tinnitus frequency, was found at 32 Hz (the severity of ear tension and tinnitus loudness were 4 and 3, respectively). The loudness of this high-pitched tinnitus is reported in **Figure 1**. The patient also reported a low-pitched tinnitus associated with a sensation of fluttering in the ear. The low-pitched tinnitus was enhanced when something (earplug, stethoscope) was inserted in the ear canal. The high- and low-pitched tinnitus could be absent when the ear was completely relaxed. In contrast the tinnitus was not modulated by forceful head and neck contractions (3).

The temporal evolution of symptoms severity (**Figure 1**) was investigated using PCA. The first principal component (48% of the total variance) represents the severity of the symptoms and does not discriminate between them. On the other hand, the second principal component (18% of the total variance) separates two symptom clusters, namely "tension," "blocked nose," and "tympanum redness" for symptom cluster 1 and "tingling," "tinnitus," and "otalgia" for symptom cluster 2 (**Figure 2**, upper panels). The feeling of pain in the temporal region and the neck (plotted on the first two PCA axes) represent a third group of symptoms (symptom cluster 3), different from cluster 1 and 2. The symptom cluster 3 is reported in the right side only, is moderate in severity, and is present only occasionally compared to the other symptoms. The PCA indicates that the correlation between symptom cluster 3 and both symptom cluster 1 and 2 is low. **Figure 2** (middle panels) shows the multi-dimensional data (symptom severity) plotted on the new coordinate system defined by the first two principal components at each time point. The symptoms are usually more severe in the right ear than in the left ear, and the maximum severity is also greater in the right ear. Interestingly, the two symptom clusters are reported in the right ear, while only symptom cluster 1 is reported in the left ear. The temporal dynamics of symptom severity (first principal component) in the left and right ear (**Figure 2**, bottom panel) is also clearly anti-correlated across the two ears.

The sensation of tension in the ear was associated with tympanum hyperemia (**Figure 1**, also see Video S1 in Supplementary Material). This hyperemia was also present during jaw muscle contraction (hypothetically involving the mylohyoid muscle) and was associated with an increase of the high-pitch tinnitus (see Video S2 in Supplementary Material). This phenomenon was reported to be only present when the sensation of tension in the ear was high and coincidentally, the tympanum was always showing dilated vessels.

The tympanometry measurements demonstrated different middle ear function for the two ears (**Figure 3**). Indeed, for the right ear, the static admittance was larger (**Figure 3**, upper panel) and the resonant frequency (estimated from the frequency at which the susceptance is null) was abnormally low (<678 Hz) (**Figure 3**, middle panel). Moreover, the admittance was modulated by eyelid closure, especially in the right ear (**Figure 3**, bottom panel). Finally, the stapedial reflexes were present and clinically normal in both ears at 500 Hz and 4 kHz at 95–100 dB HL.

DISCUSSION

This report completes and adds to previous studies on acoustic shock (1). First, the symptoms reported after the acoustic shock, including their temporal dynamics and characteristics, have been described both qualitatively and quantitatively. This case reports all the symptoms that are commonly described after ASI, except vertigo and/or dizziness (1), and additional symptoms (nasal congestion, rhinorrhea, and tympanum hyperemia). Second, the functional integrity of the middle ears was also investigated

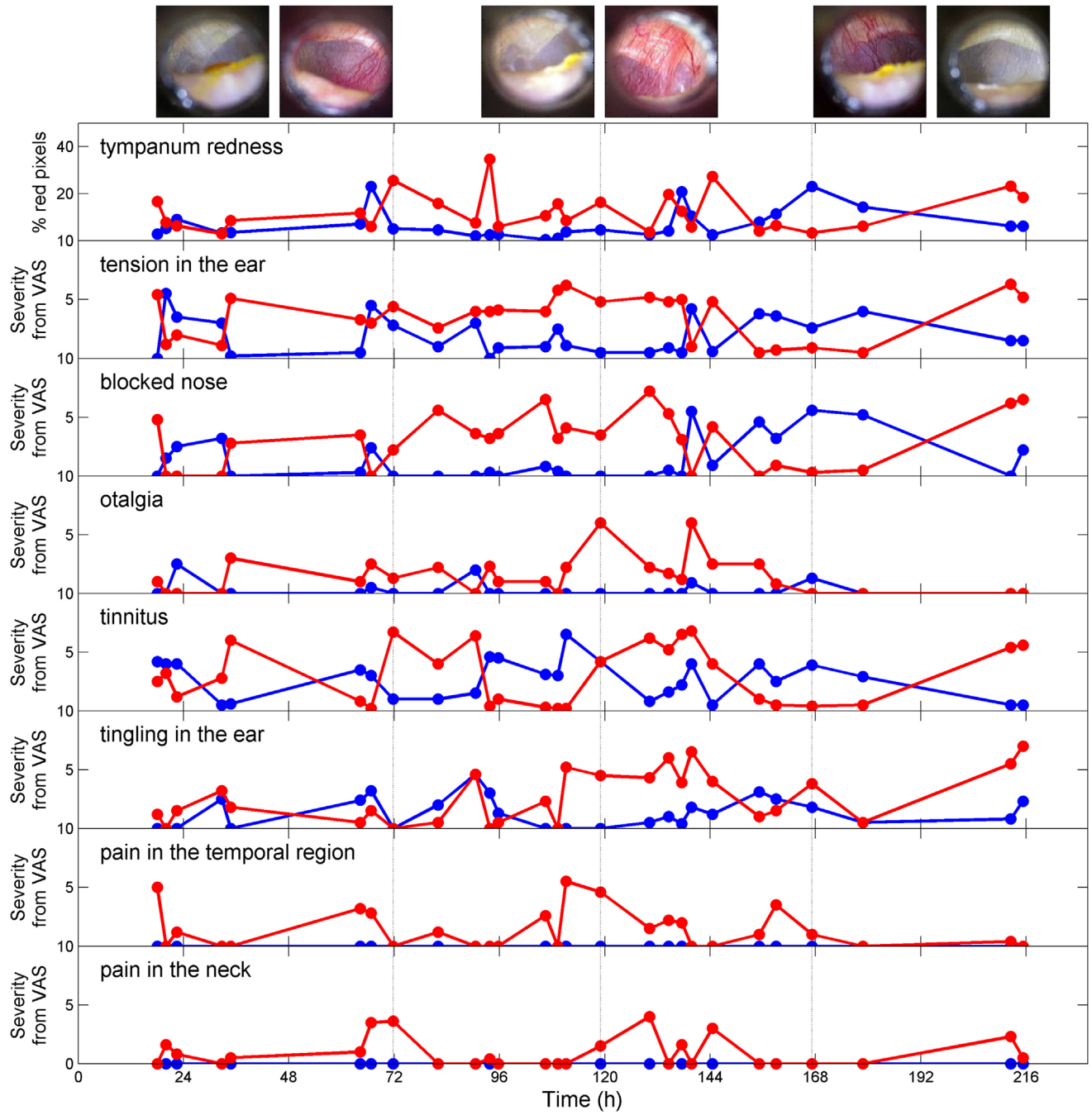

FIGURE 1 | Severity of acoustic shock symptoms (assessed by VAS) as a function of time. The blue line represents the left ear and the red line represents the right ear. Examples of tympanum pictures are shown at the top (left images: left ear; right images: right ear, the vertical lines corresponds to the time at which the pictures were taken).

using multifrequency tympanometry and direct examination of the eardrums. Our findings provide further insights into the mechanisms involved in ASI, and in particular they question and extend the framework previously developed by others (1, 2).

This study is the first to provide experimental support suggesting that middle ear muscles (MEM) can behave abnormally after ASI. Indeed, middle ear admittance is changed by simple eyelid closure, eardrum movements in the right ear can be large enough to be visible by the naked eye and the fluttering sensation (likely accompanying MEM contraction) is enhanced by cutaneous stimulation of the ear canal (4–7). This abnormal behavior of MEM may result from a dysfunction and/or injury of MEM caused by an exaggerated contraction in response to the acoustic incident. While stapedius muscle contraction is activated by loud

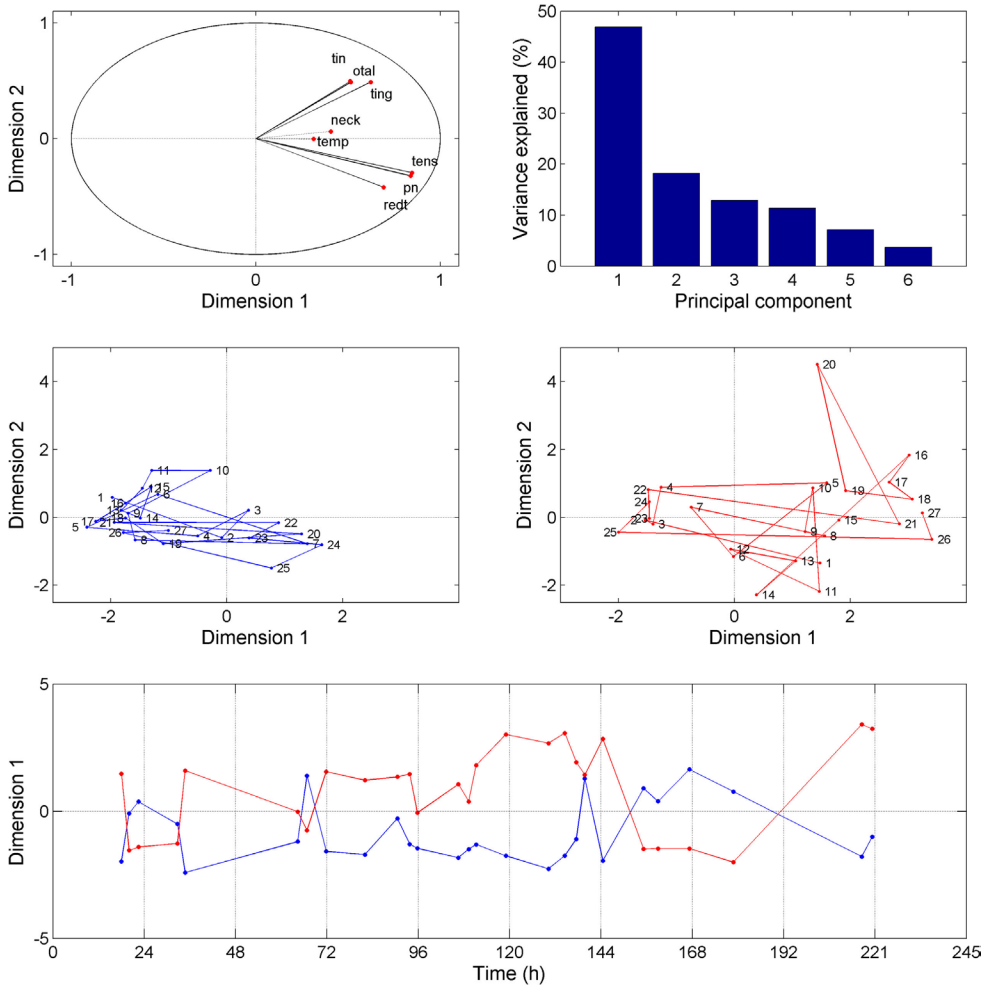

FIGURE 2 | Results obtained from the principal component analysis (PCA). The upper left panel represents each symptom as a function of the first two principal components derived from the PCA. The upper right panel shows the percentage of variance explained by the different principal components. The middle panels (left: left ear, right: right ear) show the symptoms plotted at each time point as a function the first two principal components. The bottom panel shows the first principal component for each ear (blue line: left ear, red line: right ear) as a function of time.

sounds, TTM contraction can be triggered by unexpected sounds through the general startle reflex (8). The low resonant frequency and large admittance in the most symptomatic (right) ear may reflect an abnormal ossicle arrangement, possibly caused by the exaggerated contraction of the MEM. The sensation of tension in the ear reported immediately after the acoustic shock may result from an abnormal contraction of the MEM signaled by the muscle spindles (9) and tympanum mechanoreceptors due to eardrum deformation (10). The traumatic acoustic episode may lead to anxiety and sensory hypersensitivity, which may in turn reduce the startle reflex threshold and/or potentiate the startle reflex (11), possibly through a descending serotoninergic innervation (12). This central feedback may facilitate tonic and phasic TTM contraction.

The putative exaggerated contraction of the MEM, eventually followed by tonic contraction, may be associated with MEM injury and inflammatory processes. In particular, the TTM is innervated by fibers containing substance P and calcitonin gene-related peptide, which may play a key role in the noise-induced inflammation of the middle ear (13–15). Inflammation can then diffuse from the MEM to the tympanum and middle ear mucosa and can even become neurogenic as these tissues are rich in mastocytes (16–18). The diverse pain feelings in the ear (otalgia and tingling) likely result from middle ear inflammation and activation of the trigeminal nerve (TGN), which innervates the middle ear mucosa, the eardrum, and the TTM (15, 19). Intriguingly, tinnitus loudness is correlated to the severity of otalgia and tingling in the ear, suggesting that middle ear inflammation plays a role in tinnitus generation and/or severity. One can propose that antidromic activation of the TGN may change the vascular permeability of the blood vessels in the stria vascularis (20), which may eventually increase the endocochlear potential and produce tinnitus.

The MEM dysfunction/injury and the chronic inflammatory processes following ASI may be associated with neural hyperactivity in the trigeminal pathways. This neural hyperactivity may

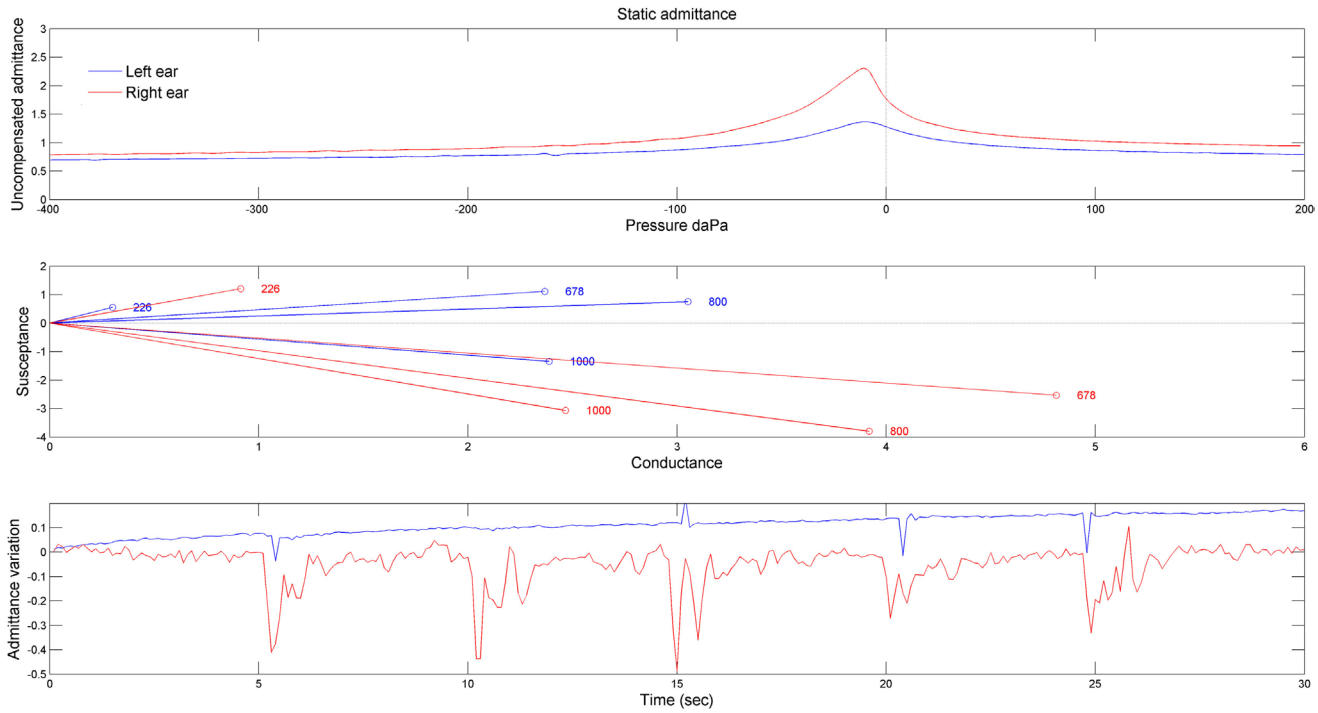

FIGURE 3 | Results obtained by tympanometry measurements. The upper panel shows the static admittance for the two ears obtained for a 226 Hz probe. The middle panel shows the susceptance as a function of the conductance at four frequencies of probe tone stimulation (226, 678, 800, and 1,000 Hz). The bottom panel shows the admittance variation when the patient blinked voluntarily (but not forcefully) his eyes. The patient was asked to do so every 5 s (blue line: left ear, red line: right ear).

in turn lead to plastic changes (sensitization) at several levels of the trigeminal pathways (21). Pain in the neck and the temporal region may result from referred pain as a consequence of central sensitization, possibly at the trigemino-cervical complex (TCC) level where multimodal neurons are present (22). Sensitization may also contribute to shift the pathophysiology from acute to chronic (21).

Finally, the PCA suggests that the symptoms from cluster 1 originate from tightly coupled mechanisms. The trigeminal sensory inputs from regions of the head, including middle ear mucosa, eardrums, and the TTM (14, 15, 19), are collected by the TCC that has a reflex connection with the superior salivatory nucleus (SSN). Interestingly, the SSN provides a parasympathetic innervation to the head region *via* the sphenopalatine ganglion. This reflex loop between the TGN and the parasympathetic pathway of the head has been suggested to account for the autonomic symptoms associated with trigeminal-autonomic cephalalgia (TAC) (23). The activation of the trigeminal-autonomic reflex in this case may account for the nasal congestion, rhinorrhea, and the tympanic hyperemia. The autonomic nervous system, which innervates the middle ear (18, 24–26), may also contribute to enhance the MEM tonus and the feeling of tension and aural fullness (27).

In summary, this case suggests that a complex pathophysiology can be involved in ASI. Initially, the acoustic shock may trigger an exaggerated MEM response causing muscle dysfunction/injury and inflammatory processes, which may later diffuse to the tympanum and middle ear mucosa. The activation of the trigeminal pathways due to inflammation may account for the diverse pain feelings in the ear and tinnitus (symptom cluster 2) and also for referred pain in the neck and the temporal region (symptom cluster 3) after induction of central sensitization. Finally, nasal congestion and tympanum hyperemia, which are tightly coupled to the feeling of ear tension (symptom cluster 1), may result from the activation of a trigeminal-autonomic reflex. Interestingly, the pathophysiology of ASI is strongly reminiscent of that of post-traumatic TAC (28). In this context, the case reported here may define a new clinical entity that we suggest calling post-traumatic trigeminal-autonomic otalgia. The clinical picture presented here, i.e., with numerous, diverse, and severe symptoms, could be an extreme form of this clinical entity and may be relatively rare. However, the prevalence of this clinical entity may be more prevalent than expected at first sight, as less symptomatic forms may also exist. Many patients with tinnitus and/or hyperacusis report one or more additional symptoms such as ear fullness, otalgia, tympanic flutter, and/or pain in the neck (2). This framework opens new and promising perspectives on the understanding and medical management of ASI and beyond, i.e., tinnitus and hyperacusis (23).

ETHICS STATEMENT

The case reported in the study gave written informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

AL, NC, DP, PF, LP, and AN collected data. LP and AN analyzed data and made figures. AN wrote a first version of the manuscript. All authors contributed to the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fneur.2017.00420/full#supplementary-material.

VIDEO S1 | The video shows the right eardrum when the feeling of tension in the ear is high and when it is low.

VIDEO S2 | The video shows the right eardrum, when the tension in the ear is high, before, during, and after the mylohyoid muscle contraction.

REFERENCES

1. Westcott M. Acoustic shock injury (ASI). *Acta Otolaryngol Suppl* (2006) 556:54–8. doi:10.1080/03655230600895531
2. Westcott M, Sanchez TG, Diges I, Saba C, Dineen R, McNeill C, et al. Tonic tensor tympani syndrome in tinnitus and hyperacusis patients: a multi-clinic prevalence study. *Noise Health* (2013) 15:117–28. doi:10.4103/1463-1741.110295
3. Levine RA, Abel M, Cheng H. CNS somatosensory-auditory interactions elicit or modulate tinnitus. *Exp Brain Res* (2003) 153:643–8. doi:10.1007/s00221-003-1747-3
4. Klockhoff IH, Anderson H. Recording of the stapedius reflex elicited by cutaneous stimulation; preliminary report. *Acta Otolaryngol* (1959) 50:451–4. doi:10.3109/00016485909129218
5. Ellenstein A, Yusuf N, Hallett M. Middle ear myoclonus: two informative cases and a systematic discussion of myogenic tinnitus. *Tremor Other Hyperkinet Mov (N Y)* (2013) 3. doi:10.7916/D8RX9BS1
6. Salomon G, Starr A. Electromyography of middle ear muscles in man during motor activities. *Acta Neurol Scand* (1963) 39:161–8. doi:10.1111/j.1600-0404.1963.tb05317.x
7. Watanabe I, Kumagami H, Tsuda Y. Tinnitus due to abnormal contraction of stapedial muscle. An abnormal phenomenon in the course of facial nerve paralysis and its audiological significance. *ORL J Otorhinolaryngol Relat Spec* (1974) 36:217–26. doi:10.1159/000275177
8. Klockhoff I, Anderson H. Reflex activity in the tensor tympani muscle recorded in man; preliminary report. *Acta Otolaryngol* (1960) 51:184–8. doi:10.3109/00016486009124480
9. Kierner AC, Zelenka I, Lukas JR, Aigner M, Mayr R. Observations on the number, distribution and morphological peculiarities of muscle spindles in the tensor tympani and stapedius muscle of man. *Hear Res* (1999) 135:71–7. doi:10.1016/S0378-5955(99)00092-1
10. Nagai T, Tono T. Encapsulated nerve corpuscles in the human tympanic membrane. *Arch Otorhinolaryngol* (1989) 246:169–72. doi:10.1007/BF00456661
11. Grillon C. Models and mechanisms of anxiety: evidence from startle studies. *Psychopharmacology (Berl)* (2008) 199:421–37. doi:10.1007/s00213-007-1019-1
12. Thompson AM, Thompson GC, Britton BH. Serotoninergic innervation of stapedial and tensor tympani motoneurons. *Brain Res* (1998) 787:175–8. doi:10.1016/S0006-8993(97)01020-2
13. Yamazaki M, Sato I. Distribution of substance P and the calcitonin gene-related peptide in the human tensor tympani muscle. *Eur Arch Otorhinolaryngol* (2014) 271:905–11. doi:10.1007/s00405-013-2469-1
14. Kierner AC, Mayer R, Adunka O. Is there a double innervation of the tensor tympani muscle in humans? *Ann Otol Rhinol Laryngol* (2003) 112:1056–8. doi:10.1177/000348940311201211
15. Uddman R, Grunditz T, Larsson A, Sundler F. Sensory innervation of the ear drum and middle-ear mucosa: retrograde tracing and immunocytochemistry. *Cell Tissue Res* (1988) 252:141–6. doi:10.1007/BF00213835
16. Ebmeyer J, Furukawa M, Pak K, Ebmeyer U, Sudhoff H, Broide D, et al. Role of mast cells in otitis media. *J Allergy Clin Immunol* (2005) 116:1129–35. doi:10.1016/j.jaci.2005.07.026
17. Ylikoski J, Panula P. Neuropeptides in the middle ear mucosa. *ORL J Otorhinolaryngol Relat Spec* (1988) 50:176–82. doi:10.1159/000275987
18. Nagaraj BS, Linthicum FH. Autonomic innervation of the human middle ear: an immunohistochemical study. *Am J Otolaryngol* (1998) 19:75–82. doi:10.1016/S0196-0709(98)90099-0
19. Oyagi S, Ito J, Honjo I. The trigeminal sensory innervation to the middle ear, eustachian tube, and pharynx: a study by the horseradish peroxidase tracer method. *Laryngoscope* (1990) 100:873–7. doi:10.1288/00005537-199008000-00014
20. Vass Z, Steyger PS, Hordichok AJ, Trune DR, Jancsó G, Nuttall AL. Capsaicin stimulation of the cochlea and electric stimulation of the trigeminal ganglion mediate vascular permeability in cochlear and vertebro-basilar arteries: a potential cause of inner ear dysfunction in headache. *Neuroscience* (2001) 103:189–201. doi:10.1016/S0306-4522(00)00521-2
21. Bernstein C, Burstein R. Sensitization of the trigeminovascular pathway: perspective and implications to migraine pathophysiology. *J Clin Neurol* (2012) 8:89–99. doi:10.3988/jcn.2012.8.2.89
22. Piovesan EJ, Kowacs PA, Oshinsky ML. Convergence of cervical and trigeminal sensory afferents. *Curr Pain Headache Rep* (2003) 7:377–83. doi:10.1007/s11916-003-0037-x
23. Eller M, Goadsby PJ. Trigeminal autonomic cephalalgias. *Oral Dis* (2016) 22:1–8. doi:10.1111/odi.12263
24. Ogawa T, Rutka J. The presence of ganglion cells in the human middle ear: a histological survey. *Acta Otolaryngol Suppl* (1999) 540:38–41.
25. Oyagi S, Ito J, Honjo I. The origin of autonomic nerves of the middle ear as studied by the horseradish peroxidase tracer method. *Acta Otolaryngol* (1987) 104:463–7. doi:10.3109/00016488709128275
26. Ito J, Oyagi S, Honjo I. Autonomic innervations in the middle ear and pharynx. *Acta Otolaryngol Suppl* (1993) 506:90–3. doi:10.3109/00016489309130249
27. Yuasa R, Kambayashi J, Saijo S, Hozawa K, Iino Y, Kaneko Y. Sensation of aural fullness and its treatment with an autonomic nerve blocking agent. *Acta Otolaryngol Suppl* (1987) 435:122–9. doi:10.3109/00016488709107361
28. Charney L, Rubino A, Cohen JM. A case of barotrauma-induced post-traumatic headache with a cluster headache phenotype. *Headache* (2016) 56:769–72. doi:10.1111/head.12727

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ORIGINAL RESEARCH
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Tinnitus in Normal-Hearing Participants after Exposure to Intense Low-Frequency Sound and in Ménière's Disease Patients

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Tinnitus is one of the three classical symptoms of Ménière's disease (MD), an inner ear disease that is often accompanied by endolymphatic hydrops. Previous studies indicate that tinnitus in MD patients is dominated by low frequencies, whereas tinnitus in non-hydropic pathologies is typically higher in frequency. Tinnitus of rather low-frequency (LF) quality was also reported to occur for about 90 s in normal-hearing participants after presentation of intense, LF sound (120 dB SPL, 30 Hz, 90 s). LF sound has been demonstrated to also cause temporary endolymphatic hydrops in animal models. Here, we quantify tinnitus in two study groups with chronic (MD patients) and presumably transient endolymphatic hydrops (normal-hearing participants after LF exposure) with a psychophysical procedure. Participants matched their tinnitus either with a pure tone of adjustable frequency and level or with a noise of adjustable spectral shape and level. Sensation levels of matching stimuli were lower for MD patients (mean: 8 dB SL) than for normal-hearing participants (mean: 15 dB SL). Transient tinnitus after LF-exposure occurred in all normal-hearing participants (N = 28). About half of the normal-hearing participants matched noise to their tinnitus, the other half chose a pure tone with frequencies below 2 kHz. MD patients matched their tinnitus with either high-frequency pure tones, mainly above 3 kHz, or with a noise. Despite a significant proportion of MD patients matching low-pass (roaring) noises to their tinnitus, the range of matched stimuli was more heterogeneous than previous data suggested. We propose that in those participants with noise-like tinnitus, the percept is probably generated by increased spontaneous activity of auditory nerve fibers with a broad range of characteristic frequencies, due to an impaired ion balance in the cochlea. For tonal tinnitus, additional mechanisms are conceivable: focal hair cell loss can result in decreased auditory nerve firing and a central auditory overcompensation. Also, normal-hearing participants after LF-exposure experience alterations in spontaneous otoacoustic emissions, which may contribute to a transient tonal tinnitus.

Keywords: tinnitus, low-frequency, Ménière's disease, bounce phenomenon, endolymphatic hydrops

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Abbreviations: BP, bounce phenomenon; DPOAE, distortion product otoacoustic emission; ICC, intra-class correlation coefficients; IHCs, inner hair cells; LF, low-frequency; MD, Ménière's disease; NH, normal-hearing; OAE, otoacoustic emission; OHCs, outer hair cells; SOAE, spontaneous otoacoustic emission.

INTRODUCTION

Tinnitus is defined as the perception of sound in the absence of external acoustic stimulation and can take various forms from pure tones to more atonal percepts (1, 2).

Tinnitus can be distinguished into two main classes: objective tinnitus and subjective tinnitus. Objective tinnitus is caused by sounds originating from internal sources, e.g., from the patient's inner ear such as prominent spontaneous otoacoustic emissions (SOAEs) (3, 4). Subjective tinnitus is characterized by an abnormal spontaneous activity within the auditory periphery or the central auditory pathway in the absence of any acoustic stimulation, which is interpreted by the brain as sound (5, 6). A psychophysical characterization of tinnitus serves to quantify loudness and pitch/timbre, which can, in the second step, contribute to the understanding of the underlying pathology (7). For the sake of simplicity, in the following, the term pitch will be applied to pure tones and noises, although the term pitch refers to pure tones only and the equivalent for noises would be timbre. Pitch and loudness of a tinnitus sensation are typically characterized by matching a synthesized sound to the tinnitus percept [see Ref. (8, 9) for an overview]. Pitch matches of tinnitus patients are usually in the rather high-frequency region above 3 kHz and only rarely below 1 kHz (10).

However, patients with Ménière's disease (MD) seem to be an exception regarding tinnitus pitch. MD is classically characterized by a triad of symptoms: fluctuating hearing loss, episodes of vertigo, and tinnitus (11). In early stages of the disease, tinnitus seems to be related to episodes of vertigo, being more intense before and during attacks. With progression of the disease, which is typically associated with increasing hearing loss and ceasing of vertigo, tinnitus stays and its intensity increases (12–14). Descriptions of tinnitus in MD patients range from roaring over buzzing to ringing (13, 15); except for few MD patients presumably in later stages of the disease, who describe their tinnitus as tonal and containing high-pitched components (12, 13). Overall, studies concluded that tinnitus in the majority of MD patients was confined to lower-frequency ranges below 1 kHz and in particular between 125 and 250 Hz (16–21).

An excess of endolymph volume, termed endolymphatic hydrops, is often suggested to cause MD and specifically symptoms such as tinnitus. A transient endolymphatic hydrops was experimentally induced in rodents by an intense low-frequency (LF) sound (22).

In human participants, transient endolymphatic hydrops induced by an intense LF sound has not been shown directly. However, a post-LF-exposure phenomenon called "Bounce Phenomenon" (BP), lasting only a few minutes, was found in humans. The BP includes a transient tinnitus percept, fluctuating hearing thresholds with transient improvement and subsequent worsening (23–26), and biphasic amplitude level changes in otoacoustic emissions (OAEs) (27–31). As OAEs are sounds emitted by the cochlea due to active amplification processes by outer hair cells (OHCs) (32), the origin of the BP is thought to be cochlear (33). Increased endocochlear potentials in the cochlea have been recorded after presentation of LF sound (22) and were suggested to lead to an increase in the spontaneous firing rate of auditory nerve fibers, resulting in a "rate tinnitus" (34), presumably in the absence of any structural cochlear impairment.

In BP studies, human participants reported a transient "roaring" tinnitus immediately after LF sound exposure (23, 26, 27). The time course and relative loudness of the transient tinnitus percept were characterized with psychophysical studies (26, 30), but tinnitus pitch has not been systematically described with psychophysical measures yet. This study employed a tinnitus-matching procedure to quantify and compare tinnitus percepts in MD patients and normal-hearing (NH) participants after exposure to intense LF sound. Both study groups presumably presented with impaired cochlear ion balance that might manifest in endolymphatic hydrops in MD patients and shows indirectly as BP after LF sound in NH participants.

MATERIALS AND METHODS

Subjects

The study population consisted of two groups: a group of NH participants and a group of patients with MD. In the following, members of both groups will be referred to as participants. NH participants comprised 18 females and 10 males (age range 20–29, mean age 23.7) with no reported hearing problems, no ear surgery, no recent ear infections, and no tinnitus. All NH participants had hearing thresholds of less than 25 dB HL between 0.25 and 8 kHz, tested with a Matlab-based automated procedure [Automatic Pure Tone Audiometry APTA-HF 2012 V2.28 (HörTech, Oldenburg, Germany)]. One ear was pseudo-randomly chosen (14 left ears, 14 right ears) and exposed to a LF sound. The transient tinnitus was lateralized and perceived on the exposed side only.

The group of MD patients consisted of nine females and nine males (age range 26–74, mean age 53.1) diagnosed with definite MD according to the criteria recently formulated by the Bárány Society joint with several national and international organizations (14). Additionally, endolymphatic hydrops had been detected with magnetic resonance imaging after intra-tympanic gadolinium injection (35) before study participation in 15 of 18 patients.

Ménière's disease patients were included when they were unilaterally affected only (10 left ears, 8 right ears) and when they reported tinnitus localized to the affected ear.

Patients with middle ear disorders, pathologies of the auditory nerve or recent ear infections were excluded from the study. Patients with obvious noise-related hearing damage (i.e., notched audiograms) as well as patients with a history of noise exposure were not eligible either. All MD patients were required to be in a stage of the disease with fluctuating symptoms, with at least one vertigo attack during the 6 months preceding the experiment. In the contralateral, non-affected ear patients were required to have hearing thresholds better than 40 dB HL below 2 kHz, and better than 70 dB HL at higher frequencies.

This study was approved by the ethics committee of the University Hospital of the Ludwig-Maximilians-Universität Munich, Germany, in agreement with the Code of Ethics of the World Medical Association for experiments involving humans

(Declaration of Helsinki) and all participants (MD and NH) gave their written informed consent.

Experiments with NH participants were conducted in a double-walled, sound-attenuated booth at the Department of Biology, Ludwig-Maximilians-Universität Munich, Martinsried. Experiments with MD patients were carried out in a sound-attenuated booth at the ENT Department at the University Hospital of the Ludwig-Maximilians-Universität Munich, Germany.

Signal Generation and Data Acquisition

Signal generation and data acquisition was implemented with scripts written in MATLAB 7.5 (MathWorks, Natick, MA, USA). Sound generation and acquisition was done with an RME Fireface UC 24-bit external sound card (RME, Audio AG, Haimhausen, Germany). The sampling rate was 44.1 kHz. For sound stimulation, SoundMexPro (HörTech GmbH, Oldenburg, Germany) was employed, which enables low-latency multi-channel Audio Stream Input/Output and interactive changes of stimuli properties within the MATLAB environment.

SOAEs Recording

In NH participants, SOAEs were recorded with the ER-10C distortion product otoacoustic emission (DPOAE) probe microphone (Etymotic Research Inc., Elk Grove Village, IL, USA). The recorded signal was amplified 30 dB by the preamplifier of the external sound card. Level and frequency of SOAEs were recorded in the control trial for 120 s (measured in 21 of 28 NH participants) and after LF stimulation for 240 s (measured in 15 of 28 NH participants). In an artificial ear (B&K 4157, Brüel & Kjaer Sound and Vibration Measurement A/S, Denmark), no artifacts exceeding the noise floor of the system could be detected during recording. A probe-fit-check procedure preceded and concluded each measurement by presenting a band-stop noise consisting of a low- and a high-frequency band and analyzing the ear response using a Fourier transform analysis. If the probe-fit-check procedure at the end of a trial indicated that the probe position had changed, the trial was rejected and repeated.

Tinnitus-Matching Procedure

In experiments with MD patients, the output of the sound card was sent to HDA 200 headphones (Sennheiser, Wedemark-Wennebostel, Germany). In NH participants, two different sound systems were used for the two ears: an ER4 insert ear phone (Etymotic Research Inc., Elk Grove Village, IL, USA) was used to present the matching stimuli to one ear. An ER-10C DPOAE probe system with an additional tube coupled to an external transducer was used to present LF sounds (30 Hz sine wave, 120 dB SPL, 90 s, including 0.1 s raised-cosine ramps) to the other ear in order to elicit a tinnitus sensation.

The external transducer was a small broadband unit (NSW1-205-8A, Aura Sound Inc., Santa Fe Springs, CA, USA) driven by a RB-960BX power amplifier (Rotel, Worthing, UK). This transducer was connected to a 50-cm long polyethylene tube (inner diameter 1 mm), the tip of which was fed through the foam ear tip of the ER-10C DPOAE probe. The ER-10C includes a microphone for recording sound pressure in the ear canal enabling the examiners to calibrate the LF sound. The amplitude response of the DPOAE probe microphone was compared with the amplitude response in an artificial ear (B&K 4157, Brüel & Kjaer Sound and Vibration Measurement A/S, Denmark) and corrected for deviations. The level of the first harmonic of the LF sound was at least 50 dB lower than the level of the desired LF frequency.

Participants (NH and MD) were given standardized, written and illustrated instructions for the tinnitus-matching procedure. In NH participants, each trial was started with LF-sound exposure to one ear, thereby inducing a transient tinnitus percept in the exposed ear (see **Figure 1A**). Since the transient tinnitus lasted only for about 90 s after LF exposure (30), NH participants were allowed to restart the LF stimulus playback if necessary. As tinnitus was a unilateral symptom in both groups, matching stimuli were presented to the contralateral, unaffected ear (see **Figures 1A,B**). Participants were able to interactively adjust matching stimuli regarding loudness and pitch with a gamepad (Bigben Interactive GmbH, Bergheim, Germany). Matching stimuli were continuous and generated in real time by a SoundMexPro plug-in.

The matching procedure was split into two stages. The first stage served to determine whether the participants' tinnitus was matched best either with a pure tone or with a noise. Therefore, 10 different synthesized stimuli were presented. The stimuli comprised pure tones of five different frequencies, equally spaced on a logarithmic frequency axis between 0.25 and 8 kHz (0.25, 0.595, 1.414, 3.364, and 8.0 kHz) and five noises derived from Gaussian noise with different filter slopes (see **Figure 1C**). In the following, those slopes are referred to as spectral tilts with units of decibel per octave. They ranged from negative values with dominant LF components (−12 and −6 dB/octave) over white Gaussian noise (0 dB/octave) to positive values with dominant high-frequency components (+6 and +12 dB/octave). Stimuli were filtered such that linear phase and frequency distortions of the transducers (NH participants: ER4, Etymotic Research; MD patients: HDA 200, Sennheiser) were exactly compensated for. Participants started the playback of the individual stimuli successively by choosing from a graphical representation of the stimuli on a user interface. No information regarding the physical properties of the stimuli was available from the graphical representation. The 10 stimuli were identical but reordered on the user interface for each trial.

After having listened to all stimuli, participants were asked to select the stimulus best matching their tinnitus. After stimulus selection, participants had to adjust the stimulus level, such that the selected tone matched their tinnitus as well as possible. Selecting 1 out of the 10 stimuli and adjusting its loudness was counted as one trial in the first stage of the matching procedure. In NH participants, trials were repeated until the same stimulus class was selected in two successive trials. In MD patients, due to time constraints, only two trials were carried out regardless of whether participants selected the same stimulus or different stimuli in those two trials.

In the second stage, participants carried out fine adjustments of level and pitch of either a pure tone or a noise. Matching stimuli were presented with a starting frequency or starting spectral tilt corresponding to the selected stimulus of the first stage, respectively. In MD patients selecting two different stimuli in the first stage, the matching stimulus was chosen based on the patient's statement of which of the two selected stimuli was the better fit.

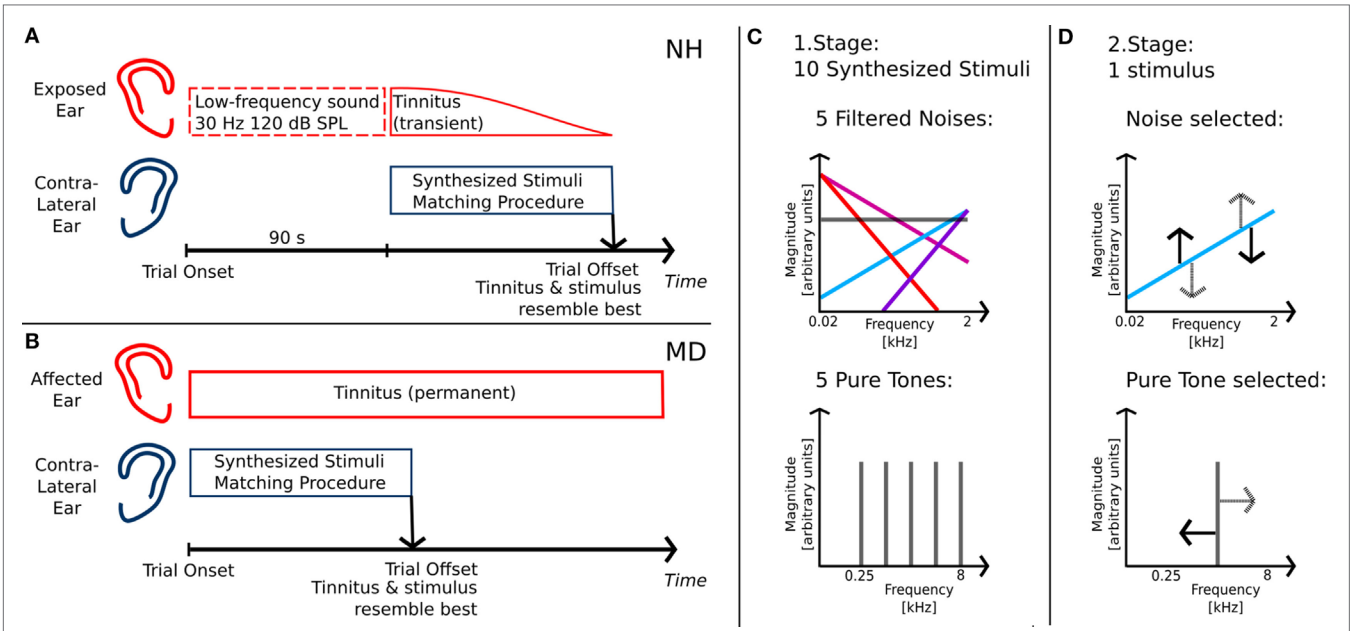

FIGURE 1 | **Tinnitus-matching procedure**. **(A)** Matching procedure for normal-hearing participants with intense low-frequency sound exposure prior to matching; **(B)** matching procedure for Ménière's disease patients with unilateral disease; **(C)** first stage of matching procedure: determining stimulus class of tinnitus percept; **(D)** second stage of matching procedure: adjusting stimulus regarding pitch/timbre and loudness of tinnitus.

The starting sound level was the mean sound level derived from the last two trials of the first stage.

During presentation of the matching stimulus, participants could continuously adjust loudness and frequency or spectral tilt (see **Figure 1D**). When participants found an adjustment for the matching stimulus that resembled their tinnitus, they stopped the adjustment procedure. Subsequently, the adjusted matching stimulus was again presented continuously and participants were required to adjust its sound level such that it was just audible. Similar to a Békésy tracking procedure, participants decreased the level of their adjusted matching stimulus until they did not perceive it anymore and then increased its intensity until they heard the stimulus again. This was done to estimate the sensation level of the matching stimulus. Adjusting the matching stimulus pitch and level and tracking the corresponding hearing threshold was considered as one trial in the second stage of the matching procedure.

In this second matching stage, NH participants always ran five trials and MD patients two to five trials depending on how many they were capable of doing due to time constraints and cognitive load.

After each trial, NH participants and MD patients were asked to describe their transient tinnitus in their own words. In NH participants, measurements were taken on two to four different days and lasted between 25 and 60 min each. In MD patients, testing was embedded in their clinical routine and carried out on 1 or 2 days. Measurements lasted 40–60 min.

All data analysis and statistics were carried out with scripts written in MATLAB 7.5 (MathWorks, Natick, MA, USA). Visualizations were done either with MATLAB 7.5 or Inkscape 0.91 (The Inkscape Team, http://www.inkscape.org).

RESULTS

Estimates of Level and Pitch of Tinnitus in NH Participants after LF Sound Exposure

All 28 NH participants exposed to the LF sound experienced a transient tinnitus percept and were able to match its pitch and level with a matching stimulus presented to the contralateral ear. Matching stimuli were adjusted after offset of the LF exposure, on average within 68.7 ± 32.5 s (mean ± SD, $N = 28$). This suggests that most NH participants concluded the matching while still hearing the transient tinnitus, which lasts about 90 s on average (30).

Conclusions drawn from questioning the NH participants after the software-based adjustment procedure are summarized as follows: 7 of the 28 NH participants described their tinnitus as tonal, 8 NH participants as a noise. The remaining 13 NH participants reported a hybrid percept consisting of noise and one or more tones. During the matching procedure, 8 out of those 13 NH participants experiencing a hybrid percept selected a matching tone and only 5 selected a matching noise. Altogether, 15 NH participants selected and adjusted a tone and 13 NH participants selected a noise (**Figure 2A**). Subjective descriptions of tinnitus perceived by NH participants after LF exposure are summarized in **Table 1**.

The loudness of the transient tinnitus percept was reported to be faint up to clearly audible with the tinnitus starting to fade out after a minute. Some participants also described qualitative changes in their tinnitus percept over time. Most of those participants reported a hybrid percept of pure tone(s) and noise. In those cases, the relative contribution of noise and tones to the tinnitus percept shifted over time. The tonal components seemed

to fade out over time, while the noise serving as a background noise at the beginning of the BP got more prominent at later time points. Participants did not complain about the percept and its loudness. At most participants stated the tinnitus to be irritating, especially in combination with a feeling of aural fullness.

Tonal Tinnitus Percepts in NH Participants after LF Exposure

Fifteen NH participants selected pure tones to characterize their tonal tinnitus. The pure tone frequency was determined by averaging all adjustments per subject. The selected matching tone frequencies (see **Figures 3A,B**) show an accumulation between 0.1 and 2 kHz, where 14 out of 15 participants selected matching tones below 2 kHz.

The mean frequency of the 15 averaged pure tones chosen was 0.96 ± 0.89 kHz (mean ± SD, $N = 15$). As the SD across adjustments in hertz is only of limited value, frequency deviations were also expressed logarithmically as fraction of an octave in cent, where 100 cents equal a semitone, so that 1,200 cents correspond to an octave.

In 13 of 15 NH participants, SDs for single participants comprised less than 1,200 cents, and for some participants, the SD was as low as 30 cents. The inter-subject mean of the sound pressure level of the adjusted matching tone was around 49.5 ± 15.9 dB SPL (mean ± SD, $N = 15$).

Sound levels for matching tones decreased with increasing frequency from 90 dB SPL at 70 Hz to 30–35 dB SPL at 2–4 kHz. Matched pure tone levels followed equal-loudness contours for human hearing within a loudness level range of 25–60 phons (see **Figure 3A**). Thus, for NH participants, matching tones at lower frequencies were not generally perceived louder than at higher frequencies, despite the difference in sound pressure levels. For an interpretation of how loud participants (NH and MD) perceived their tinnitus, sensation levels were calculated (see **Figure 4A**). The sensation level is the difference (in decibel) between the presented sound level and the absolute hearing threshold for the same sound. The mean sensation level for tonal tinnitus matches of NH participants was 15.2 ± 6.7 dB SL (mean ± SD, $N = 15$).

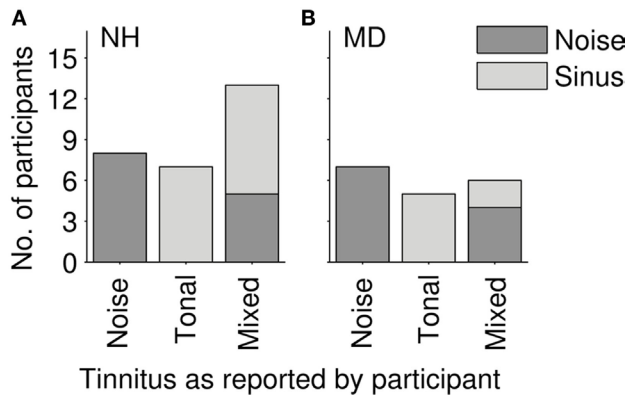

FIGURE 2 | Distribution of reported tinnitus quality. (A) Normal-hearing participants ($N = 28$). **(B)** Ménière`s disease patients ($N = 18$); gray tones code for the stimulus class that participants selected in the tinnitus-matching procedure (light gray—pure tone; dark gray—noise).

TABLE 1 | Qualitative description of transient tinnitus after intense low-frequency sound exposure (30 Hz, 120 dB SPL, 90 s) by normal-hearing participants ($N = 28$).

	N
Noise-like tinnitus	**8**
Noise—exactly like matching noise	5
Low-Pitched Noise (Roaring like a fan)	2
Noise but tonal character	1
Tonal tinnitus	**7**
Pure Tone—exactly like matching tone	2
Two Tones—alternating/simultaneously (1 tone faint + 1 tone more intense) (Ringing like church bell, Chinese meditation balls)	5
Hybrid tinnitus percept	**13**
Low-Pitched Noise (Roaring) + tone	5
High-Frequency Tone(s) + machine-like sound (Rattling, jackhammer, sewing machine)	5
Other: noise with low-frequency (LF) modulation LF tone with tinny ring Sound of pressure valve	3

Noise-Like Tinnitus Percepts in NH Participants after LF Exposure

Thirteen NH participants chose noises to match their LF-induced transient tinnitus. Adjusted spectral tilts showed a bimodal distribution slightly shifted toward negative tilts with an average adjusted spectral tilt of −1.8 ± 7.9 dB/octave (mean ± SD, $N = 13$) (see **Figures 5A,B**). Except for one participant choosing white noise (mean tilt: ~0 dB/octave) to match the tinnitus percept, all NH participants selected either noises with a clearly positive tilt (mean ± SD = 6.7 ± 3.3 dB/octave, $n = 5$) or with a clearly negative tilt (mean ± SD = −8.1 ± 3.1 dB/octave, $n = 7$).

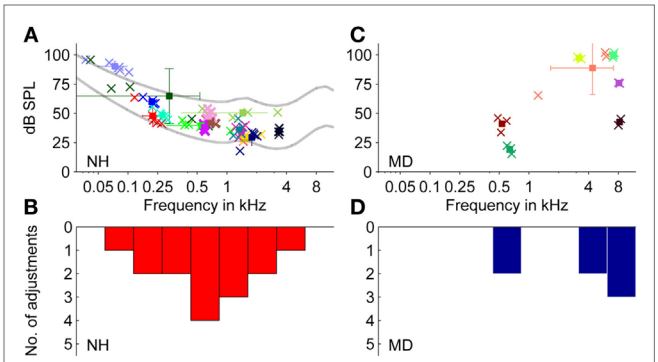

FIGURE 3 | Adjustments of level and frequency of externally presented pure tones to match a tonal tinnitus percept. (A,B) Normal-hearing participants ($N = 15$). **(C,D)** Ménière's disease patients ($N = 7$). **(A,C)** Plots show adjusted pure tones of individual participants. Data from individual participants are represented by different colors. Square symbols represent the mean of the two to five individual adjustments of one participant (indicated by cross symbols). Error bars represent the SD regarding sound pressure level (dB SPL) (vertically) and frequency (Hz) (horizontally). Lines in **(A)** represent equal-loudness contours for loudness levels at 25 and 60 phons. **(B,D)** Distribution of the selected matching tone frequencies of data shown in **(A,C)**, respectively (bin width = 1,249 cents).

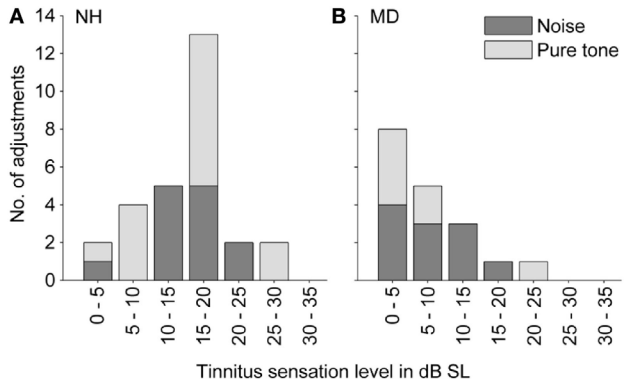

FIGURE 4 | Distribution of tinnitus loudness (expressed as sensation level). (A) Normal-hearing participants ($N = 28$). **(B)** Ménière's disease patients ($N = 18$). Colors code for the stimulus class that participants selected in the tinnitus-matching procedure (light gray – pure tone; dark gray – noise).

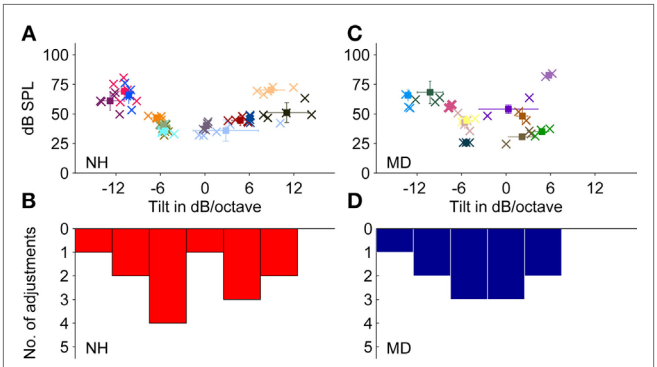

FIGURE 5 | Adjustments of level and frequency content of externally presented noises to match a noise-like tinnitus percept. (A,B) Normal-hearing participants ($N = 13$). **(C,D)** Ménière's disease patients ($N = 11$). **(A,C)** Plots show adjusted noises of individual participants. Error bars represent the SD regarding sound pressure level (dB SPL) (vertically) and frequency content (dB/octave) (horizontally). **(B,D)** Distribution of the matching noise tilts of the data shown in **(A,C)**, respectively, within the range of −17.5 to +17.5 dB/octave (bin width = 5 dB/octave). Color coding and symbols as in **Figure 3**.

Within-participant variability between adjustments was quite small (SD between 0.02 and 4.4 dB/octave).

Sound levels of the matching noises were within the range of 36–70 dB SPL with a mean of around 49.5 ± 12.9 dB SPL (mean ± SD, $N = 13$). Matching noises with highest sound pressure levels were noises with the steepest spectral tilts, either negative or positive. The mean sensation levels of matching noises were 15.3 ± 5.6 dB SL (mean ± SD, $N = 13$).

Sensation levels of both selected matching tones and matching noises in NH participants were summarized (see **Figure 4A**), and the mean was calculated as 15.2 ± 6.1 dB SL (mean ± SD, $N = 28$).

Estimates of Level and Pitch of Tinnitus in MD Patients

In all 18 MD patients, the tinnitus was a permanent sensation, sometimes varying in loudness over time. In eight patients, the loudness of the tinnitus was apparently correlated to the vertigo attacks increasing right before and/or during an attack. Three patients reported not only a loudness change correlated to the attacks but also an increase of tinnitus pitch. In this case, patients were asked to match their current tinnitus percept. Seven patients reported their tinnitus to be a noise, and five patients reported it to be a pure tone. The other six patients reported to hear a hybrid percept of pure tone(s) and noise. In the tinnitus-matching procedure, two of those patients perceiving a hybrid tinnitus matched their tinnitus with a pure tone and four of them with a noise (see **Figure 2B**). Qualitative tinnitus descriptions by the patients are summarized in **Table 2**. Descriptions included low-pitched noise, modulated noise, and high-frequency whistling.

Tonal Tinnitus in MD Patients

Seven MD patients selected pure tones in the matching procedure. Thereby, data did not show the previously suggested correlation that older patients or patients in later stages of the disease were more likely to experience tonal tinnitus of high-frequency quality instead of noise-like tinnitus (linear correlation coefficients, age: $r = -0.12$, p-value = 0.64, stage of disease $r = 0.08$, p-value = 0.76).

TABLE 2 | Qualitative description of tinnitus by Ménière's disease patients ($N = 18$) with unilateral disease and one-sided, permanent tinnitus.

	N
Noise-like tinnitus	**7**
Low-pitched noise (roaring, buzzing)	4
Noise with modulation/pulsatile (rushing blood)	2
Other: humming	1
Tonal tinnitus	**5**
Pure Tone—exactly like matching tone	2
High-frequency whistling, swishing	3
Hybrid tinnitus percept	**6**
High-frequency tone(s) + *low-pitched noise* (angle sander, whistling, and dull roaring)	4
Other: rushing blood	2
Swarm of bees	

Selected matching tone frequencies showed a bimodal distribution with peaks around 500 Hz and around 5 kHz (see **Figures 3C,D**). The mean frequency was 4.6 ± 3.3 kHz (mean ± SD, $N = 13$).

Matched sound levels increased with increasing frequency from 20 dB SPL at 0.6 kHz to 80–100 dB SPL at 7–8 kHz. Sensation levels of matching tones, however, were small (mean ± SD = 7.0 ± 7.8 dB SL, $N = 7$).

Noise-Like Tinnitus Percepts in MD Patients

Eleven of 18 MD patients selected noises to describe their tinnitus percept. The matching noise properties were averaged across the two to three trials for each patient.

Across patients, the average spectral tilt of matched noises was −2.9 ± 6.3 dB/octave (mean ± SD, $N = 11$). Matching noises showed adjusted tilts between −13 and +4 dB/octave and resulted

in an almost normal distribution with a shift toward negative tilts (see **Figures 5C,D**).

On average, the noise level adjusted by MD patients was 50.4 ± 17.3 dB SPL (mean ± SD, $N = 11$), which was comparable to noise levels of noises matched by NH participants with transient tinnitus. Due to hearing loss in the majority of MD patients, the sensation levels of the matching tones were with 8.5 ± 4.9 dB SL (mean ± SD, $N = 11$) significantly lower than in NH participants (Mann–Whitney test, p-value = 0.0002).

Sensation levels of both matched tones and matched noises in MD patients were summarized (see **Figure 4B**), and the mean was 7.9 ± 6.0 dB SL (mean ± SD, $N = 18$).

Comparison of Tinnitus Percepts in MD Patients and in NH Participants after LF-Sound Exposure

Tonal Tinnitus Percepts

Pure tones matched by MD patients and matched by NH participants differed in their distribution (see **Figure 3**) (two-sample Kolmogorov–Smirnov test, p-value = 0.019). While the majority of MD patients chose pure tones above 3 kHz, which is comparable to most tinnitus percepts of tinnitus patients (10, 16), the majority of NH participants selected pure tones below 2 kHz.

Noise-Like Tinnitus Percepts

Comparing the two subgroups of MD patients selecting noises and NH participants with BP-induced tinnitus selecting noises did not show pronounced differences. Although the distribution appeared bimodal for NH participants and rather normally distributed in MD patients, the null hypothesis stating that the two subgroups were from equal distributions could not be rejected (two-sample Kolmogorov–Smirnov test; p-value = 0.86). Both subgroups had a tendency to select noises with a negative spectral tilt, which corresponds to previous reports from the literature (16–18, 23, 30).

Test–Retest Reliability

In general, the pitch and loudness matches obtained with the current two-stage procedure showed good test–retest reliability. Intra-class correlation coefficients (ICC) were calculated for the five trials of fine matching run in one to three different sessions in NH participants (pitch matching: ICC = 0.92; loudness matching: ICC = 0.85). In MD patients, data were usually collected in one session only, but for the two to three different adjustments ICC indicated very strong test–retest reliability, too (pitch matching: ICC = 0.93; loudness matching: ICC = 0.92).

SOAE Measurements in NH Participants

In 15 of the 28 NH participants, SOAEs were measured in the same ear that was exposed to the LF sound. The first measurement was run before LF exposure to find SOAEs. The second SOAE measurement was carried out immediately after LF exposure during the BP, while NH participants perceived the corresponding transient tinnitus. During the BP, "new" SOAEs could develop and existing SOAEs increased in level. Occasionally, those SOAEs exceeded the hearing threshold determined for the same ear by audiometric testing at the beginning of the experiments. In 10 NH participants, SOAEs could be found. In all of those participants, SOAEs were slightly altered when recorded during the BP compared to baseline before LF exposure. In eight NH participants, SOAE levels exceeded the individual hearing threshold at the SOAE characteristic frequency during the BP. From these eight participants, five matched their tinnitus with a pure tone, three with a noise. **Figure 6** shows SOAEs, hearing threshold and selected tinnitus-matching tones from three participants with "audible" SOAEs. These data suggest that some NH participants might possibly have matched their tinnitus to a transiently audible SOAE.

DISCUSSION

The current study, by applying a comprehensive tinnitus-matching procedure, compared tinnitus in two groups with

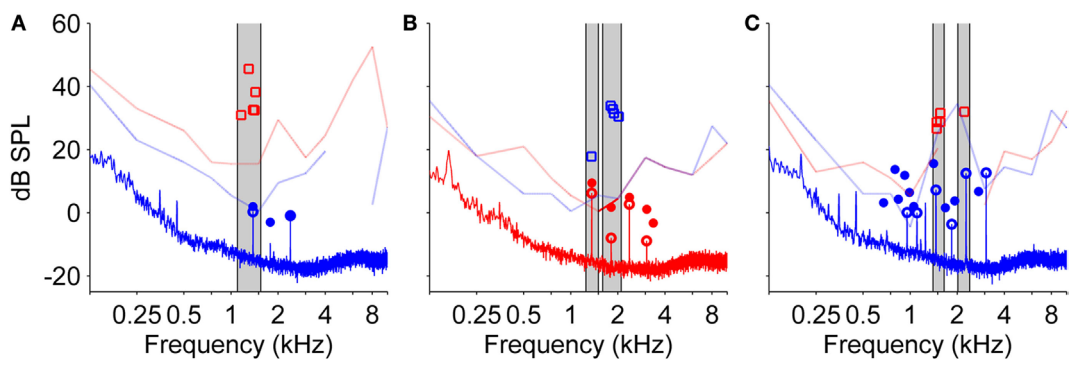

FIGURE 6 | Audiograms from both ears, spontaneous otoacoustic emissions (SOAEs), and level and frequency estimates of the tinnitus percept after low-frequency (LF) sound exposure for three different normal-hearing participants (A–C). SOAEs were recorded in ipsilateral ears before and immediately after exposure to a 30 Hz tone at 120 dB SPL for 90 s. In all three measures, red color refers to the right ear, blue color refers to the left ear. Light lines indicate audiogram thresholds (gaps within light lines are due to lack of valid data points of audiometric testing), open squares represent five adjustments of level and frequency of the matching tone presented to the contralateral ear. Solid lines represent the spectrum of the SOAE recording. Open circles represent SOAEs before LF sound exposure, filled circles represent SOAEs after LF sound exposure. Gray patches highlight SOAEs and corresponding matching tone adjustments.

chronic (MD patients) and transiently (NH participants after LF exposure) challenged cochlear homeostasis presumably resulting in hydropic conditions.

Our results show that sensation levels of tinnitus percepts were on average between 8 dB SL (MD patients) and 15 dB SL (NH participants after LF sound). Tinnitus pitch varied substantially both within and across MD patients and NH participants. While MD patients matched their tinnitus mainly with either high-pitched pure tones or noises with a tendency to low-pitched (roaring) noises, NH participants perceiving a transient tinnitus after LF-exposure matched that tinnitus with high- or low-pitched noises or low-pitched pure tones. In the few studies available in the literature, MD patients were found to perceive mainly LF tinnitus (16–21), albeit reports on MD patients with high-frequency tinnitus exist (12, 13, 36). Tinnitus percepts of MD patients in the current study were more diverse, and this is probably due to the following reasons: first, the matching procedures employed in the available literature differed from our advanced matching paradigm, in that participants could adjust matching stimuli on their own without any interference or bias caused by the examiner. Furthermore, participants could choose between two matching stimulus classes (pure tones and noises). Second, participating MD patients were heterogeneous regarding age (26–74 years). As the onset of MD symptoms generally occurs around 30–50 years (37, 38), younger MD patients without age-related hearing loss are underrepresented in our patient population. Nevertheless, contralateral, unaffected ears of all participating patients were within the normal hearing range when considering the age-corrected pure-tone average at 2, 3, and 4 kHz. This is important as the matching stimulus was delivered to the unaffected ear. All patients, despite their age, were in a stage of the disease with fluctuating symptoms.

In the following, we will dissect tinnitus percepts into tonal and noise-like tinnitus and discuss tinnitus loudness for both kinds of percepts in the end. Underlying mechanisms possibly generating the tinnitus percepts in both MD patients and NH participants after LF exposure will be proposed for tonal and noise-like tinnitus separately.

Tonal Tinnitus Generation

MD patients mainly chose high-frequency matching tones when their tinnitus was predominantly tonal. This is comparable to the tinnitus quality typically related to inner hair cell (IHC) and OHC damage (39). Here, it was suggested that the tinnitus frequency roughly corresponds to characteristic frequency of the impaired hair cells or to the frequency at the boundary between intact and impaired hair cells (40, 41). This tinnitus mechanism might generate the tonal tinnitus percepts MD patients perceive. Audiograms of MD patients often show LF hearing loss but high-frequency hearing loss also occurs, mostly in later stages of the disease (12, 13). The majority of MD patients in this study showed greatly reduced DPOAE levels as judged from measurements acquired during the clinical routine. This indicates some degree of OHC function impairment, but it is unclear if this is age-related or due to the presence of endolymphatic hydrops. OHC loss might not suffice to induce tinnitus. Therefore, damage to IHCs or the auditory nerve, which cannot be detected with DPOAE measurements, might also be required. Given the presence of both IHC and OHC dysfunction, tonal tinnitus could also be the result of endolymphatic hydrops (or its underlying pathology) combined with the pre-existing hair cell dysfunction.

Tonal tinnitus in NH participants after LF exposure showing frequencies below 2 kHz is presumably triggered by transiently challenged cochlear homeostasis. Intense, LF sound causes broad excitation patterns peaking at the apical part of the cochlea. Thus, effects on cochlear homeostasis are not restricted to the characteristic frequency region of LF sound and higher-frequency regions can be affected as well.

Temporary tinnitus in humans is generally thought to be caused by temporary decrease of OHC amplification, which results in increased firing in the central auditory system and tinnitus generation (42). Although this generation mechanism cannot be ruled out, especially for participants showing temporary worsening of hearing threshold after LF exposure, it seems to be unlikely because of concurrent, increasing OAE levels typically following LF exposure due to enhanced OHC amplification (30). Temporary enhanced amplification and increased OAE levels might contribute to a different generation mechanism.

In three participants, it could be shown that frequencies of the selected matching tones roughly corresponded to the recorded SOAE frequencies. Besides the fact that permanent tinnitus percepts originating from an SOAE are rare [between 2 and 4.5% (4, 43)], SOAEs seem to be a plausible explanation in the case of transient tonal tinnitus after LF sound exposure for the following reasons:

Tinnitus frequencies selected by NH participants in the present study correspond to typical SOAE frequencies. SOAEs in human adults are usually in the range between 0.9 and 4 kHz with distribution maxima near 1.5 and 3 kHz (44, 45).

Under normal conditions, SOAEs are relatively stable in both frequency and level (46). After intense LF exposure, however, frequency and level of SOAEs slowly cycle for a time period of about 2 min in a stereotypic manner with a level maximum typically occurring about 50 s after LF sound offset (27, 31). SOAEs presumably buried in the noise floor become detectable during that period. Typically, SOAEs are not perceived by their owner (47). It has been shown, however, that induced frequency or level changes of SOAEs can result in SOAEs becoming transiently audible (47–49), as the inhibition of SOAE perception at higher stages of auditory processing (45) might not be active anymore. Not all NH participants perceiving tonal tinnitus had SOAEs with frequencies similar to the chosen matching tone frequency. NH participants with recordable SOAEs often showed more than one SOAE, which would have resulted in a percept difficult to match with a single pure tone.

In NH participants without recordable SOAEs, SOAE levels might be significantly underestimated by external recordings after OAE back-propagation, compared to sound pressure level of OAEs within the cochlea (47). This is strongly supported by the comparison of DPOAE sound levels measured in the

gerbil meatus and the electrophysiological response to the same DPOAE in the gerbil cochlear nucleus (50). Thus, for SOAEs to be perceived by their owner, it might not be necessary that SOAE levels, as measured in the meatus, exceed the individual hearing thresholds at the SOAE characteristic frequency.

Noise-Like Tinnitus Generation

In both subject groups, a major proportion of tinnitus matches indicated a noise-like tinnitus. In experimental animals, it has been shown that the endocochlear potential increases temporarily with intense LF exposure (22, 51), resulting in a depolarization of IHCs and consequently in an increased spontaneous activity of the auditory nerve (34).

While direct recordings of the endolymphatic potential in MD patients are not feasible, a similar mechanism is nonetheless conceivable. Increased spontaneous activity of the auditory nerve could lead to the perception of a tinnitus, for which Patuzzi (34) coined the term "rate tinnitus." If a large expanse of the cochlea was affected by the above mechanism, a noise-like percept would result.

MD patients perceiving noise-like tinnitus percepts chose mainly noises with negative tilts (low pitches). This is in line with reports that MD patients typically show LF hearing loss at frequencies below 3 kHz (14), suggesting that, for hitherto unknown reasons, the apical part of the cochlea is most affected, resulting in a LF rate tinnitus. But, presumably depending on the duration of the disease, audiograms of MD patients reveal a broad range of affected frequencies (15), which is also reflected in our study population. This might explain why MD patients selected matching noises not necessarily limited to the LF range.

During intense LF stimulation, cochlear excitation is not restricted to the characteristic frequency, but the whole cochlea is excited and individual differences in cochlear excitation patterns might exist. LF stimulation affects the apical end of the cochlea strongest and LF components should be more prominent in noise-like tinnitus after intense LF exposure in NH participants (corresponding to selected noises with negative tilt). Those NH participants that chose high-pitched noises to match their LF-induced tinnitus may have done so as a compromise to represent hybrid tinnitus percepts consisting of both noise and tonal components or cochlear locations other than the apex dominate the tinnitus percept.

Tinnitus Sensation Levels

Sensation levels of tinnitus percepts were estimated with previously selected matching stimuli. In MD patients, hearing thresholds even on the contralateral, unaffected ear were slightly elevated at higher frequencies due to age-related hearing loss. Therefore, for MD patients choosing a high-pitched pure tone to match their tinnitus, loudness matches might have resulted in lower values than loudness matches using frequencies at which hearing was normal (8, 52). Loudness recruitment could also contribute: for participants suffering from cochlear hearing loss, low to moderate sensation levels may be much louder than for NH participants because the lack of cochlear compression decreases the overall dynamic range of loudness perception dramatically (52, 53). Furthermore, low sensation levels of matching tones in MD patients can be due to tinnitus loudness fluctuations. Patients were measured between vertigo attacks when tinnitus loudness was typically lower than immediately before or during attacks.

On the other hand, high loudness values in NH participants could be explained with a shift of attention toward the suddenly occurring tinnitus percept. Tinnitus sensation levels after LF exposure were on average higher than tinnitus sensation levels in MD patients and higher than tinnitus sensation levels in most patients with pathologies other than MD. Studies showed that tinnitus loudness was generally matched with stimuli below 10 dB SL, even in participants referring to their tinnitus as loud (8, 10, 54).

Procedural Limitations

Participants were presented with a limited range of sounds from which they had to select. Although this limited range of sounds was chosen to facilitate the matching procedure, participants complained about not being able to adjust amplitude modulations or about being restricted to one stimulus class when hearing hybrid percepts with both pure tones and noises. Furthermore, for both MD patients and NH participants with an LF-induced transient tinnitus, tinnitus loudness, and sometimes even tinnitus pitch were varying over time. In case of MD patients, these variations can take place over days or weeks depending on vertigo attacks or the current stage of the disease (12, 13). In NH participants, fluctuations appear within the 1–2 min of tinnitus duration. This is inherent to the transient percept after LF exposure. To guarantee consistency, NH participants were able to retrigger the tinnitus percept by turning on the LF stimulation again.

In either case, tinnitus-matching results can only represent approximations of actual tinnitus percepts, constrained by both choice of offered stimuli and experimental procedures.

CONCLUSION

Estimates of tinnitus pitch reported in the literature are heterogeneous and depend heavily on the methods used. Here, we implemented a tinnitus-matching procedure with fewer constraints than in previous, comparable studies, including noises with adjustable spectral shapes. As a consequence, our results revealed a relatively large proportion of participants with noise-like tinnitus, which might have not been detected in previous studies mostly employing sinusoidal matching tones. Contrary to reports in the literature, tinnitus pitch in NH participants after LF sound exposure and in MD patients is not exclusively LF, and hybrid percepts with noise-like and tonal components can occur.

Noise-like tinnitus cannot easily be explained with localized damage to cochlear regions. Rather, a mechanism affecting a broad frequency range is needed. An increase of spontaneous activity of the auditory nerve was suggested to cause the noise-like tinnitus observed here. However, further experiments are now required to identify unusual patterns in auditory nerve spontaneous activity as a potential tinnitus generator in chronic and induced hydropic conditions.

AUTHOR CONTRIBUTIONS

MU contributed to study design, performed data acquisition, statistical analysis and interpretation of results, drafting of the manuscript, revised the manuscript, and approved the final manuscript. LW contributed to study design and data acquisition, critically reviewed and approved the final manuscript. EK contributed to data acquisition, revised and approved the final manuscript. RG contributed to data acquisition, revised and approved the final manuscript. MD conceptualized and designed the study, contributed to data acquisition, interpretation of results, drafting of the manuscript, critically reviewed and approved the final manuscript. All authors are agreeable to be accountable for the content of the work, integrity, and accuracy of the data.

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REFERENCES

1. Meikle MB, Vernon J, Johnson RM. The perceived severity of tinnitus. Some observations concerning a large population of tinnitus clinic patients. *Otolaryngol Head Neck Surg* (1984) 92(6):689–96. doi:10.1177/019459988409200617
2. Baguley DM. Mechanisms of tinnitus. *Br Med Bull* (2002) 63:195–212. doi:10.1093/bmb/63.1.195
3. Penner MJ. An estimate of the prevalence of tinnitus caused by spontaneous otoacoustic emissions. *Arch Otolaryngol Head Neck Surg* (1990) 116(4):418–23. doi:10.1001/archotol.1990.01870040040010
4. Baskill JB, Coles RRA. Current studies of tinnitus caused by spontaneous otoacoustic emissions. In: Aran JM, Dauman R, editors. *Fourth International Tinnitus Seminar*. Amsterdam: Kugler (1992). p. 79–83.
5. Bento RF, Sanchez TG, Miniti A, Tedesco-Marchesi AJ. Continuous, high-frequency objective tinnitus caused by middle ear myoclonus. *Ear Nose Throat J* (1998) 77(10):814–8.
6. Jastreboff PJ. Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci Res* (1990) 8(4):221–54. doi:10.1016/0168-0102(90)90031-9
7. Norena A, Micheyl C, Chery-Croze S, Collet L. Psychoacoustic characterization of the tinnitus spectrum: implications for the underlying mechanisms of tinnitus. *Audiol Neurootol* (2002) 7(6):358–69.
8. Henry JA, Meikle MB. Psychoacoustic measures of tinnitus. *J Am Acad Audiol* (2000) 11(3):138–55. Available from: http://www.audiology.org/publications-resources/journal-american-academy-audiology/jaaa-archives/jaaa-archives-2000
9. Moore BC. *The Psychophysics of Tinnitus. Tinnitus*. New York: Springer (2012). p. 187–216.
10. Meikle M, Taylor-Walsh E. Characteristics of tinnitus and related observations in over 1800 tinnitus clinic patients. *J Laryngol Otol Suppl* (1984) 9:17–21. doi:10.1017/S1755146300090053
11. Ménière P. Sur une forme de surdité grave dépendant d'une lésion de l'oreille interne. *Gaz Méd de Paris* (1861) 16:29.
12. Paolino M, Ghulyan-Bedikian V. *Ménière's Disease and Tinnitus. Textbook of Tinnitus*. New York: Springer (2011). p. 477–86.
13. Ying Y-LM, Arriaga MA. *Tinnitus and Ménière's Disease. Textbook of Tinnitus*. New York: Springer (2011). p. 311–6.
14. Lopez-Escamez JA, Carey J, Chung WH, Goebel JA, Magnusson M, Mandala M, et al. Diagnostic criteria for Meniere's disease. Consensus document of the Barany Society, the Japan Society for Equilibrium Research, the European Academy of Otology and Neurotology (EAONO), the American Academy of Otolaryngology-Head and Neck Surgery (AAO-HNS) and the Korean Balance Society. *Acta Otorrinolaringol Esp* (2015) 67(1):1–7. doi:10.3233/VES-150549
15. Havia M, Kentala E, Pyykko I. Hearing loss and tinnitus in Meniere's disease. *Auris Nasus Larynx* (2002) 29(2):115–9. doi:10.1016/S0385-8146(01)00142-0
16. Reed GF. An audiometric study of two hundred cases of subjective tinnitus. *AMA Arch Otolaryngol* (1960) 71(1):84–94. doi:10.1001/archotol.1960.03770010088009
17. Graham JT, Newby HA. Acoustical characteristics of tinnitus. An analysis. *Arch Otolaryngol* (1962) 75:162–7. doi:10.1001/archotol.1962.00740040168015
18. Douek E, Reid J. The diagnostic value of tinnitus pitch. *J Laryngol Otol* (1968) 82(11):1039–42. doi:10.1017/S0022215100069838
19. Nodar RH, Graham JT. An investigation of frequency characteristics of tinnitus associated with Meniere's disease. *Arch Otolaryngol* (1965) 82(1):28–31. doi:10.1001/archotol.1965.00760010030007
20. Caparosa RJ. Medical treatment for Meniere's disease. *Laryngoscope* (1963) 73(6):666–72. doi:10.1288/00005537-196306000-00003
21. Day KM. Twenty-five years' experience with Meniere's disease. *Laryngoscope* (1963) 73(6):693–8. doi:10.1288/00005537-196306000-00006
22. Salt AN. Acute endolymphatic hydrops generated by exposure of the ear to nontraumatic low-frequency tones. *J Assoc Res Otolaryngol* (2004) 5(2):203–14. doi:10.1007/s10162-003-4032-z
23. Hirsh I, Ward W. Recovery of the auditory threshold after strong acoustic stimulation. *J Acoust Soc Am* (1952) 24:131. doi:10.1121/1.1906867
24. Hughes JR. Auditory sensitization. *J Acoust Soc Am* (1954) 26(6):1064–70. doi:10.1121/1.1907450
25. Zwicker E, Hesse A. Temporary threshold shifts after onset and offset of moderately loud low-frequency maskers. *J Acoust Soc Am* (1984) 75(2):545–9. doi:10.1121/1.390488
26. Patuzzi R, Wareing N. Generation of transient tinnitus in humans using low-frequency tones and its mechanism. In: Patuzzi R, editor. *Proceedings of the Seventh International Tinnitus Seminar*. Crawley: The University of Western Australia (2002). p. 16–24.
27. Kemp DT. Otoacoustic emissions, travelling waves and cochlear mechanisms. *Hear Res* (1986) 22:95–104. doi:10.1016/0378-5955(86)90087-0
28. Kemp DT, Brill OJ. Slow oscillatory cochlear adaptation to brief overstimulation: cochlear homeostasis dynamics. In: Cooper NP, Kemp DT, editors. *Concepts and Challenges in the Biophysics of Hearing*. Singapore: World Scientific Publ Co Pte Ltd (2009). p. 168–74.
29. Kevanishvili Z, Hofmann G, Burdzgla I, Pietsch M, Gamgebeli Z, Yarin Y, et al. Behavior of evoked otoacoustic emission under low-frequency tone exposure: objective study of the bounce phenomenon in humans. *Hear Res* (2006) 222(1–2):62–9. doi:10.1016/j.heares.2006.05.014
30. Drexl M, Uberfuhr M, Weddell TD, Lukashkin AN, Wiegrebe L, Krause E, et al. Multiple indices of the 'bounce' phenomenon obtained from the same human ears. *J Assoc Res Otolaryngol* (2014) 15(1):57–72. doi:10.1007/s10162-013-0424-x
31. Kugler K, Wiegrebe L, Grothe B, Kössl M, Gürkov R, Krause E, et al. Low-frequency sound affects active micromechanics in the human inner ear. *R Soc Open Sci* (2014) 1(2). doi:10.1098/rsos.140166
32. Kemp DT. Stimulated acoustic emissions from within the human auditory system. *J Acoust Soc Am* (1978) 64(5):1386–91. doi:10.1121/1.382104
33. Kirk DL, Patuzzi RB. Transient changes in cochlear potentials and DPOAEs after low-frequency tones: the 'two-minute bounce' revisited. *Hear Res* (1997) 112(1–2):49–68. doi:10.1016/S0378-5955(97)00105-6

34. Patuzzi R, editor. Outer hair cells, EP regulation and tinnitus. *Proceedings of the Seventh International Tinnitus Seminar*. Crawley: The University of Western Australia (2002).
35. Nakashima T, Naganawa S, Sugiura M, Teranishi M, Sone M, Hayashi H, et al. Visualization of endolymphatic hydrops in patients with Meniere's disease. *Laryngoscope* (2007) 117(3):415–20. doi:10.1097/MLG.0b013e31802c300c
36. Herraiz C, Tapia MC, Plaza G. Tinnitus and Ménière's disease: characteristics and prognosis in a tinnitus clinic sample. *Eur Arch Otorhinolaryngol* (2006) 263(6):504–9. doi:10.1007/s00405-006-0019-9
37. Watanabe Y, Mizukoshi K, Shojaku H, Watanabe I, Hinoki M, Kitahara M. Epidemiological and clinical characteristics of Meniere's disease in Japan. *Acta Otolaryngol Suppl* (1995) 519:206–10. doi:10.3109/00016489509121906
38. Gates GA. Meniere's disease review 2005. *J Am Acad Audiol* (2006) 17(1):16–26. doi:10.3766/jaaa.17.1.3
39. Jastreboff PJ, Hazell JW. A neurophysiological approach to tinnitus: clinical implications. *Br J Audiol* (1993) 27(1):7–17. doi:10.3109/03005369309077884
40. Robertson D, Irvine DR. Plasticity of frequency organization in auditory cortex of guinea pigs with partial unilateral deafness. *J Comp Neurol* (1989) 282(3):456–71. doi:10.1002/cne.902820311
41. Eggermont J. Cortical tonotopic map reorganization and its implications for treatment of tinnitus. *Acta Otolaryngol* (2006) 126(sup 556):9–12. doi:10.1080/03655230600895259
42. Nuttall AL, Meikle MB, Trune DR. Peripheral processes involved in tinnitus. In: Snow JB, editor. *Tinnitus: Theory and Management*. Shelton: PMPH-USA (2004). p. 52–68.
43. Penner M. Spontaneous otoacoustic emissions and tinnitus. In: Tyler RS, editor. *Tinnitus Handbook*. San Diego: Singular (2000). p. 203–20.
44. Yongbing S, Martin W. Spontaneous otoacoustic emissions in tinnitus patients. *J Otol* (2006) 1(1):35–9. doi:10.1016/S1672-2930(06)50006-6
45. Braun M. A retrospective study of the spectral probability of spontaneous otoacoustic emissions: rise of octave shifted second mode after infancy. *Hear Res* (2006) 215(1–2):39–46. doi:10.1016/j.heares.2006.03.008
46. Burns EM. Long-term stability of spontaneous otoacoustic emissions. *J Acoust Soc Am* (2009) 125(5):3166–76. doi:10.1121/1.3097768
47. Long G. Perceptual consequences of the interactions between spontaneous otoacoustic emissions and external tones. I. Monaural diplacusis and after-tones. *Hear Res* (1998) 119(1–2):49–60. doi:10.1016/S0378-5955(98)00032-X
48. Long GR, Tubis A. Modification of spontaneous and evoked otoacoustic emissions and associated psychoacoustic microstructure by aspirin consumption. *J Acoust Soc Am* (1988) 84(4):1343–53. doi:10.1121/1.396633
49. Long GR, Tubis A. Investigations into the nature of the association between threshold microstructure and otoacoustic emissions. *Hear Res* (1988) 36(2–3):125–38. doi:10.1016/0378-5955(88)90055-X
50. Faulstich M, Kossl M. Neuronal response to cochlear distortion products in the anteroventral cochlear nucleus of the gerbil. *J Acoust Soc Am* (1999) 105(1):491–502. doi:10.1121/1.424586
51. Salt AN, Lichtenhan JT, Gill RM, Hartsock JJ. Large endolymphatic potentials from low-frequency and infrasonic tones in the guinea pig. *J Acoust Soc Am* (2013) 133(3):1561–71. doi:10.1121/1.4789005
52. Goodwin PE, Johnson RM. The loudness of tinnitus. *Acta Otolaryngol* (1980) 90(5–6):353–9. doi:10.3109/00016488009131736
53. Buus S, Florentine M. Growth of loudness in listeners with cochlear hearing losses: recruitment reconsidered. *J Assoc Res Otolaryngol* (2002) 3(2):120–39. doi:10.1007/s101620010084
54. Fowler EP. The "illusion of loudness" of tinnitus – its etiology and treatment. *Laryngoscope* (1942) 52(4):275–85.

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Analysis of Audiometric Differences of Patients with and without Tinnitus in a Large Clinical Database

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Human hearing loss (HL) and comorbidities like tinnitus pose serious problems for people's daily life, which in most severe cases may lead to social isolation, depression, and suicide. Here, we investigate the relationship between hearing deficits and tinnitus. To this end, we conducted a retrospective study on anonymized pure tone and speech audiometric data from patients of the ENT hospital Erlangen in which we compare audiometric data between patients with and without tinnitus. Overall data from 37,661 patients with sensorineural (SHL) or conductive HL (CHL) with (T, 9.5%) or without (NT, 90.5%) a tinnitus percept in different age groups and with different tinnitus pitches were included in this study. The results of the pure tone audiometry comparisons showed significant differences in T patients compared to NT patients. In young patients, we generally found lower hearing thresholds in T compared to NT patients. In adult patients, differences were more heterogeneous: hearing thresholds in T patients were lower in low frequency ranges, while they were higher at high frequencies. Furthermore, lower thresholds were more often found in CHL patients and could rarely be detected in SHL patients. In speech audiometry, only CHL patients with high-pitched tinnitus showed lower thresholds compared to NT patients' thresholds. The results of this study may point to a biologically plausible functional benefit on hearing thresholds in HL tinnitus patients. We hypothesize that the physiological mechanism of stochastic resonance counteracts HL by adding neuronal noise to the system. This neuronal noise may induce changes in the auditory pathway and finally—as a side effect of threshold improvement—lead to the development of a tinnitus percept. We propose a general model of changed hearing thresholds in T patients, being either decreased or increased compared to NT patients.

Keywords: human, pure tone audiometry, speech audiometry, sensorineural hearing loss, conductive hearing loss, tinnitus model

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INTRODUCTION

Hearing is crucial for audio-verbal communication in humans and by that essential for social interactions. Consequently, hearing impairment pose serious problems for the people's daily life, which in most severe cases may lead to social isolation, depression, and suicide (1, 2). The fact that our

western societies face increasing noise exposure in daily routine, work, and spare time adds to the problem and results in continuously increasing numbers of people suffering from hearing impairments. Studies in the United States revealed about 9% of the general population being affected by hearing deficits in 2000 (3)—with an overrepresentation of 15% in children (4)—and an increase of cases over 10 years when assessed with more advanced testing procedures (5).

In general, factors leading to this high prevalence of hearing impairments are diverse. Consequently, the cause for hearing impairment may be located within different anatomical structures. Hearing disorders may have their origin in outer or middle ear, causing conductive hearing loss (CHL), or sensorineural structures (starting in the cochlea and including all structures along the auditory pathway), causing sensorineural hearing impairments. Of course patients can also suffer from a combination of conductive and sensorineural hearing loss (HL), either with unilateral or bilateral impairments. Probably, the most common cause for sensorineural hearing impairments is noise-induced HL (NIHL), which can lead to a number of secondary symptoms like tinnitus [for review, see Ref. (6)], depression (7), or hyperacusis (8). With increasing HL over time, the capability of speech discrimination is affected, generating difficulties in everyday life communication (9). As Cruickshanks and colleagues (10) demonstrated that the risk of HL is increased by almost 90% every 5 years, the demographic changes in most industrialized western countries will make age-dependent HL even more relevant in the future.

Tinnitus is a widespread, but poorly understood symptom often seen in HL patients. The prevalence in the general population is assumed to be at around 10–15% (11). Men seem to be more often affected then women up to the age of 75, when prevalence is about equal for both genders (12). About 1–2% of the tinnitus patients state their quality of life being significantly decreased by their phantom percept (13). Although HL and the prevalence of tinnitus are increasing with age (14), it is still under debate if age-related HL or age-related changes in physiological processes in the central and peripheral auditory system are the source of the increasing prevalence of tinnitus (15).

Population studies revealed that individuals with tinnitus on average suffer from stronger HL in high frequencies; patients with low-pitched tinnitus (below 1,500 Hz) show stronger low frequency HL than patients with middle- or high-pitched tinnitus [e.g., Ref. (16–19)]. In any case, the question remains why in some patients with HL subjective tinnitus is developing at all. In a recent study (20), we put forward a model for the physiological improvement of hearing thresholds, which as side effect also explains the development of tinnitus. The model is based on the idea that the auditory system tries to compensate for a HL by means of stochastic resonance (SR) at the receptor level. SR refers to the phenomenon that weak signals that are subthreshold for a given sensor still can be detected and transmitted by that sensor if noise (internal or external) is added to the sensor input, both in technical and physiological systems (21–24). We further assume that HL leads to an unequal distribution of spectral input into the auditory system, with a reduced input from the affected spectral ranges. Our model proposes that the impaired hearing thresholds within those frequency channels may be improved again (at least to a certain degree) by means of SR. Obviously, for SR to work, internal noise has to be generated within the auditory system and fed back to the receptor level (20). We propose that this internal noise is reflected in neuronal hyperactivity. If the HL is permanent, the neuronal hyperactivity that enables SR to compensate for increased thresholds may subsequently cause neuronal plasticity along the auditory pathway and finally may lead to the development of a phantom percept, i.e., subjective tinnitus. In that sense, the model views tinnitus as a side effect of a mechanism within the auditory system that seeks to optimize signal transmission at the receptor level. If this model would be true, we would expect that, in tinnitus patients, initial HL should be compensated to a certain degree, resulting in overall better hearing thresholds in tinnitus patients compared to non-tinnitus patients with comparable damage in the auditory system.

In this retrospective study, we search for data in support of this hypothesis by performing a fine-grained analysis of the audiometric data of over 37,000 patients with different forms of HL, and with or without a tinnitus percept.

MATERIALS AND METHODS

We performed a retrospective study on anonymized audiometric data from patients with HL who came to the ENT hospital in Erlangen for medical examination. Therefore, no declaration of consent was required by German law. All data were collected between the years 2000 and 2015, HL patients who complained about experiencing pure-tone tinnitus percepts were classified as tinnitus patients (group T) and patients without complains about any form of tinnitus were classified as non-tinnitus patients (group NT). Patients with other forms of tinnitus (e.g., noise-like tinnitus) were not included in this study. Applied audiometric methods were pure-tone and speech audiometry for multisyllabic numbers. Data from 37,661 patients (74,976 ears) including all groups of age [median (25, 75% quantil): 42 (21, 58)] were investigated. Patients were not characterized by their gender or by former or current pathologies not affecting hearing.

Testing Procedures

Standardized audiometric testing instruments of an audiological clinic were used for this study. All devices fulfilled the necessary requirements according to ISO 8253-1 and 8253-3. The following audiometric methods were performed: pure-tone audiometry: air conduction hearing level thresholds were measured for both ears separately for every patient. Analyzed frequencies were 250; 500; 750; 1,000; 1,500; 2,000; 3,000; 4,000; 6,000; and 8,000 Hz and HL was calculated (range: −10–130 dB). Speech Audiometry: using the Freiburger test, multisyllabic numbers were presented to each ear separately. For these data, acoustic levels at 50% understanding were calculated and used for further analysis (range: 0–120 dB). Audibility was directly linked to the acoustic level (range: 0–100%). The Freiburg multisyllabic numbers were used since it belongs to our standard procedure to determine the level of 50% understanding. This value usually correlates with the

hearing threshold in the low frequency region and is less affected by high-frequency HL. We did not use the monosyllabic words since monosyllabic perception is usually measured at higher levels.

Tinnitus characteristics were determined in terms of signal type and signal level measured in decibel HL by comparison of the internal tinnitus with external sound from the audiometer. Three types of signals were possible: broadband noise, narrow band noise (1/3 octave bandwidth), and pure tones between 0.25 and 8 kHz. Tinnitus loudness was determined by increasing the signal level above hearing threshold slowly in steps of 1 dB and asked the subjects for a comparison with their tinnitus percept.

Data Preprocessing and Statistical Analysis

Data preprocessing was performed with a custom-made Matlab 2008 program (MathWorks, MA, USA). For statistical analysis Statistica 2007 (StatSoft. Inc., OK, USA) was used. Patients were classified within the preprocessing into groups based on their audiometric data: mild to medium symmetric sensorineural HL in adults [SHL, mean air-bone–gap ≤5 dB across all frequencies, HL difference between both ears <20 dB, maximal HL ≤40 dB; number of ears: n(T) = 4,390, n(NT) = 26,142], symmetric CHL in adults [CHL, mean air-bone gap >5 dB across all frequencies, HL difference between both ears <20 dB, maximal HL ≤40 dB; n(T) = 2,538, n(NT) = 24,726], and a group of children and adolescents under 18 years with symmetric HL [HL difference between both ears <20 dB, maximal HL ≤40 dB; n(T) = 234, n(NT) = 16,946] without further subdivision in SHL or CHL patients.

Furthermore, adult subjects were grouped by age: young adults 18–39 years [SHL: n(T) = 1,418, n(NT) = 8,540; CHL: n(T) = 630, n(NT) = 7,642], elder adults 40–60 years [SHL: n(T) = 2,164; n(NT) = 11,236; CHL: n(T) = 1,182; n(NT) = 9,254], and seniors >60 years [SHL: n(T) = 808; n(NT) = 6,366; CHL: n(T) = 726; n(NT) = 7,830]. The subjective pure-tone tinnitus frequencies were grouped by pitch: low-pitched [<1,000 Hz; n(SHL) = 507; n(CHL) = 387], medium-pitched [1,000–4,000 Hz; n(SHL) = 875; n(CHL) = 662], and high-pitched [>4,000 Hz; n(SHL) = 3,008; n(CHL) = 1,489].

In pure tone audiometry, we aimed to quantify the potential threshold decrease or increase tinnitus may have on hearing thresholds in each single tested hearing frequency. To this end, the difference of mean HL in each frequency was calculated for each age and tinnitus pitch group and its age matched NT patients (mean HL difference, where positive values indicate a threshold decrease, negative values indicate an increase of hearing thresholds by tinnitus in decibel) and compared by multifactorial ANOVAs. Generally, for statistical population analysis, parametrical tests like Students t-test and multifactorial ANOVAs were used. Tukey *post hoc* tests enabled detailed analysis within the ANOVAs. Additionally, we compared the paired pure tone and speech audiometry data of 2,548 patients in which both audiometric methods were allied by multiple linear regressions and 2-factorial ANOVA (Figure S1 in Supplementary Material).

RESULTS

Pure-Tone Audiometry in Patients with and without Tinnitus Percepts

Hearing Thresholds of Children and Adolescents

The HL of young patients without (NT) and with reports of tinnitus (T) was compared. To rule out any age bias due to audiometric limitations in infants, we tested first if any difference in hearing thresholds of very young children (aged 1–9 years) and adolescents (aged 10–17 years) could be found. Neither in NT (t-test, mean ± SD: children 20.5 ± 17.3 dB, adolescents 19.2 ± 21.6 dB, $p = 0.15$) nor in T patients group (t-test: children 16.0 ± 12.6 dB, adolescents 14.3 ± 19.8 dB, $p = 0.17$) any significant differences were found. Therefore, all subjects were pooled and HL was analyzed by a 2-factorial ANOVA with the factors frequency and group.

Figure 1A depicts this variance analysis. The upper panel shows an increase of mean HL toward higher frequencies in all patients (**Figure 1A**, upper panel). Interestingly, young people with tinnitus generally suffered less from HL than non-tinnitus patients (**Figure 1A**, inset), overall showing about 5 dB lower mean hearing thresholds. They were especially less affected at frequencies below 4,000 Hz and above 6,000 Hz (**Figure 1A**, lower panel, Tukey *post hoc* tests, always $p < 0.05$). In **Figure 1B**, the mean HL difference of NT and T patients is given as a function of frequency. Each green bar indicates the significant difference (tested by single sample t-tests) of the HL at a given stimulation frequency. Again, all frequencies except 4 and 6 kHz show significantly positive values indicating lower hearing thresholds in T compared to NT patients.

Note that this young patients group is not representative for patients affected by tinnitus in the "general population" where usually only adults are considered (1, 25). For that reason, further analyses were focused on adult groups with SHL and CHL. In these patients, we were able to analyze a significantly larger number of patients' ears.

Hearing Thresholds in Adults with and without Tinnitus

In a first overview, NT and T patients' audiograms separated for their cause of HL (sensorineural or conductive) were analyzed by 2-factorial ANOVAs with the factors frequency and group as depicted in **Figures 2A,C** and further illustrated by the HL difference in **Figures 2B,D**, respectively.

In adult patients, we generally found that T patients' thresholds in lower frequency ranges were lower compared to NT patients, while in other frequency ranges there was no difference or, especially above 2 kHz, those patients showed higher thresholds compared to NT patients: a detailed analysis revealed that for both groups, SHL (**Figure 2A**, upper panel) and CHL (**Figure 2C**, upper panel), HL significantly increased from low to high frequencies. HL in general was higher for CHL patients (30.9 ± 20.0 dB) than for subjects suffering from SHL (18.3 ± 16.7 dB; t-test, $p < 0.001$).

In patients with SHL, the analysis revealed the opposite result seen in young patients, namely mean thresholds across all

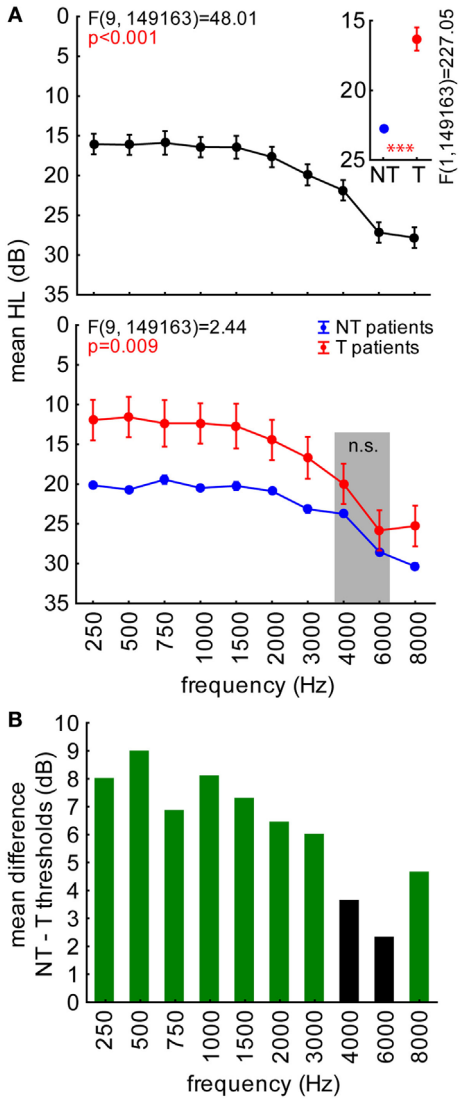

FIGURE 1 | Hearing loss (HL in decibel) in young patients (aged 1–17 years) with and without tinnitus. (A) Results of a 2-factorial ANOVA on HL with factors frequency (upper panel) and patient group (inset), interaction plot at the bottom. Symbols depict mean values; whiskers give 95% confidence intervals. Asterisks depict significance value of the 1-factorial ANOVA part: ***$p < 0.001$. Gray-shaded area indicates two frequencies not significantly different between groups (Tukey *post hoc* tests). **(B)** Mean differences between the HL of NT [blue in **(A)**] and T patients [red in **(A)**] quantifying the threshold difference referred to as mean HL difference, where positive values indicate lower, negative values higher hearing thresholds in decibel. Values significantly different from 0 are colored green (single sided *t*-tests).

frequencies being higher in patients with tinnitus compared to patients without such a mispercept by roughly 1 dB (**Figure 2A**, inset). In the interaction of both factors, it became clear (Tukey *post hoc* tests, $p < 0.001$) that T patients only showed significantly higher thresholds compared to NT patients at frequencies above 2 kHz (**Figure 2A**, lower panel). This result is further illustrated in **Figure 2B** showing the mean HL difference, which also revealed a significant threshold increase at frequencies above 2 kHz for SHL patients with tinnitus.

In patients with CHL, the analysis of the factor group showed no significant difference between T and NT patients when thresholds were averaged across all frequencies (**Figure 2C**, inset). Nevertheless, the interaction analysis (**Figure 2C**, lower panel) revealed significantly lower thresholds in T patients for frequencies below 750 Hz but higher thresholds compared to NT patients in the range of 3–4 kHz (Tukey *post hoc* tests, $p < 0.05$), which was further supported by the HL difference analysis (**Figure 2D**).

Next, we analyzed different aspects of our patient cohort to outline some additional characteristics of the threshold differences between T and NT patients. First, we focused on the age dependency of thresholds as a function of the existence of a tinnitus percept: SHL patients of all ages showed higher hearing thresholds when affected by tinnitus, while CHL patients with tinnitus only showed such higher thresholds in the age group of 40–60 years. This was assessed by 3-factorial ANOVAs with the factors frequency, age and group as depicted in **Figures 3A,C**. Lower hearing thresholds in T patients only reached significance when analyzed across frequency ranges (low = < 1 kHz, mid = < 4 kHz, high = ≥ 4 kHz; green shaded areas in **Figures 3A,C**) but could be further identified when analyzed by HL differences for specific age groups (**Figures 3B,D**).

Patients with SHL (**Figure 3A**) replicated the results shown in **Figure 2** averaged over all three age groups when factors frequency, group, or the corresponding interaction were analyzed. For the factor age [$(F2, 291,140) = 11,515.0$, $p < 0.001$], we found lower HL in 18- to 39-year-old adults (12.0 ± 0.2 dB) compared to 40- to 60-year-old adults (19.6 ± 0.15 dB) and adults above 60 years (30.4 ± 0.2 dB). The interaction of age and frequency is given in the upper left panel of **Figure 3A** and the interaction of age and group in the right upper panel. There, T patients showed generally higher thresholds than NT patients (Tukey *post hoc* tests), which is carved out in the three-way interaction in the lower part of **Figure 3A**: in SHL, Tinnitus patients' higher frequencies showed significantly larger thresholds than those of non-tinnitus patients (cf. red shaded areas; statistical tests were performed separately for every stimulation frequency). When 2-factorial ANOVAs [factors frequency range (low, mid, high) and group] were performed, significantly lower hearing thresholds in T patients could be detected for low frequencies in the above 60 years group only (green shaded area in **Figure 3A**). The HL difference (**Figure 3B**) for the three different age groups showed corresponding frequency ranges affected, with higher values at high frequency ranges of the tinnitus patients and lower values at 750 Hz in the above 60 years tinnitus patients.

In CHL patients (**Figure 3C**), we found a similar age-dependent HL as above [$F(2, 262,589) = 5,794.5$, $p < 0.001$; 18- to 39-year-old adults: 18.7 ± 0.15 dB, 40- to 60-year-old adults: 31.7 ± 0.3 dB, adults above 60 years: 46.5 ± 0.4 dB]. Again, a significant interaction of age and frequency as well as age and group was found (**Figure 3C**, upper panels). Across all frequencies, CHL T patients' hearing thresholds differed by age, as we found higher threshold in 40- to 60-year-old adults but lower threshold in 18- to 39-year-old adults. No overall significant differences were seen in adults above 60 years (Tukey *post hoc*

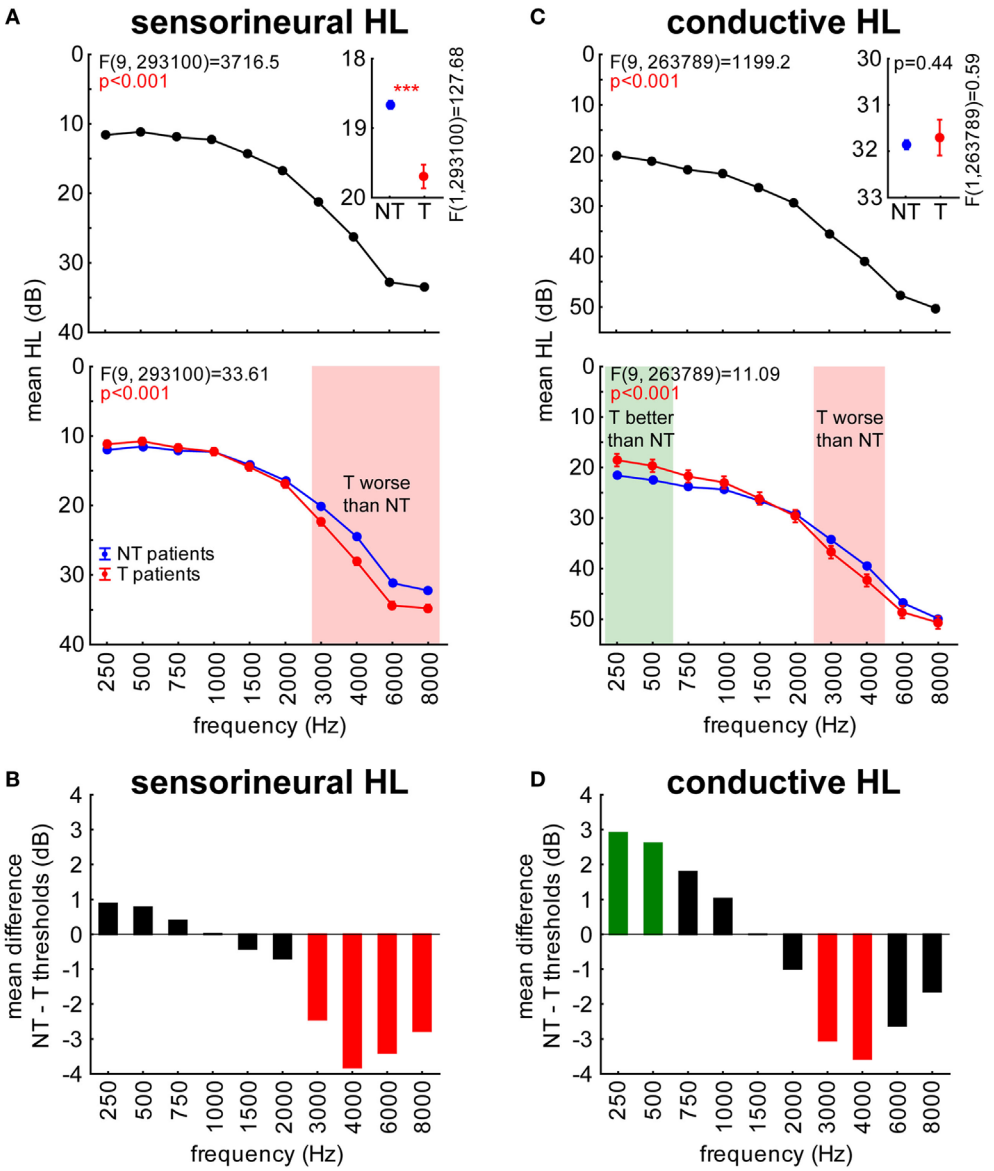

FIGURE 2 | Hearing loss (HL in decibel) in sensorineural (A,B) and conductive HL (C,D) patients. Symbols as in **Figure 1**. **(A,C)** Red areas indicate frequencies where T patients exhibit higher hearing thresholds compared to NT patients, the green area indicates frequencies where T patients show lower thresholds than NT patients (Tukey *post hoc* tests). **(B,D)** Red bars indicate significantly higher thresholds, green bars significantly lower thresholds.

tests; **Figure 3C**, upper right panel). In the three-way interaction (**Figure 3C**, lower panel) higher thresholds in T patients were found at frequencies above 1 kHz in 40- to 60-year-old adults (cf. red shaded area, statistical tests were performed separately for every stimulation frequency), while significantly lower hearing thresholds in T patients could be detected at low frequencies in 18- to 39-year-old adults and the adults above 60 years of age only [2-factorial ANOVAs for frequency ranges (low, mid, and high) and group, green shaded area in **Figure 3C**]. Correspondingly, in the analyses of HL differences (**Figure 3D**) of CHL patients, we found significantly lower values for low frequency thresholds in 18- to 39-year-old adults and adults above 60 years of age with tinnitus while 40- to 60-year-old adults with tinnitus showed significantly higher values in the mid- to high-frequency range.

From **Figures 2** and **3**, it became clear that potential effects of the perception of tinnitus on hearing thresholds follow a frequency-specific pattern: in T compared to NT patients, lower thresholds were only observed in the low-frequency range while higher thresholds were exclusively seen in the high-frequency range. As pitches of tonal tinnitus percepts most frequently are located in the high-frequency range, we hypothesized that the higher hearing thresholds in T patients may be due to masking of the perception of tones in that frequency range. To test this hypothesis, we analyzed if there is a relation between the perceived

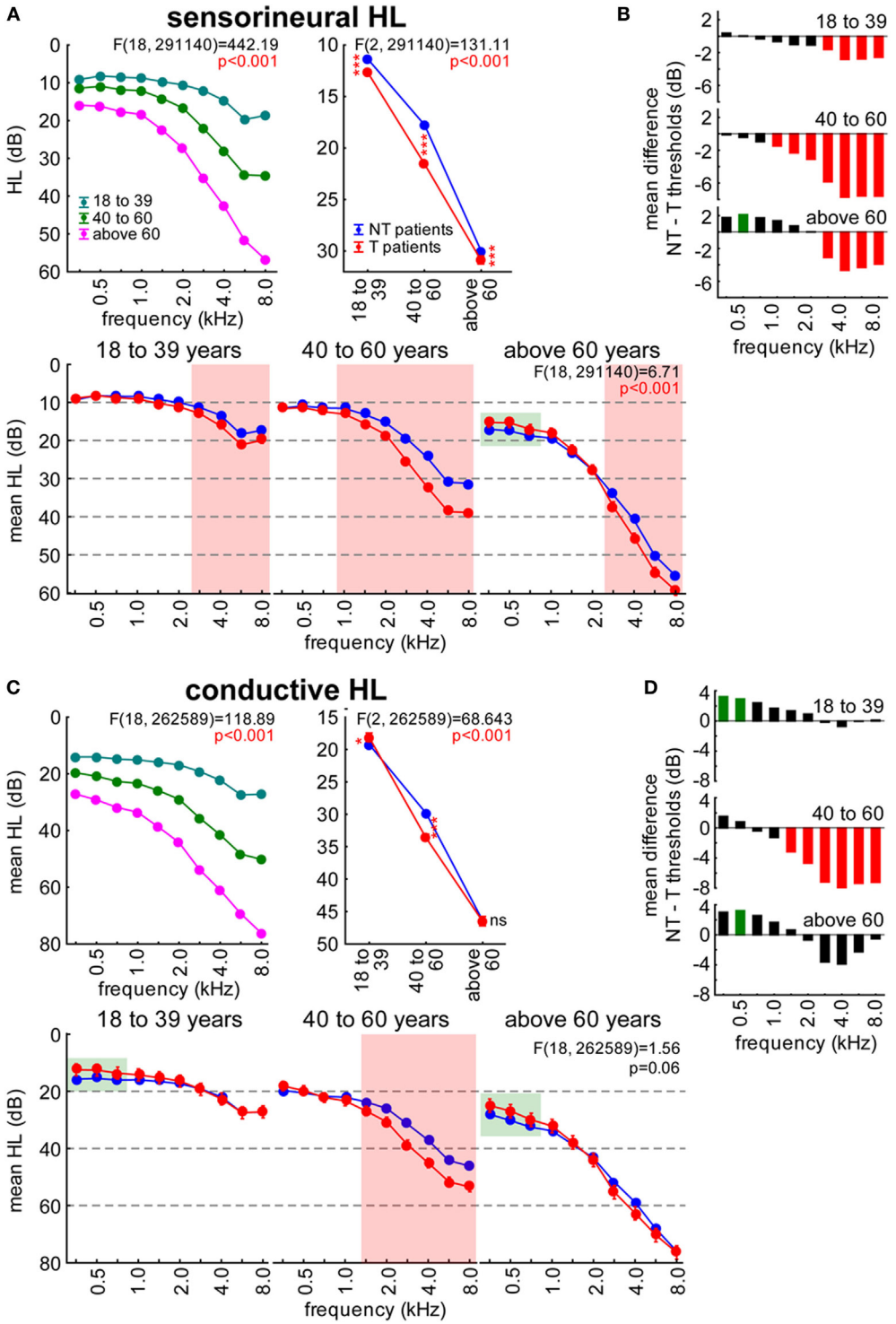

FIGURE 3 | Age dependency of hearing loss (HL in decibel) in sensorineural (A,B) and conductive HL (C,D) patients. **(A,C)** show the interaction plots of the 3-factorial ANOVAs of HL with factors age, patient group, and frequency. Asterisks depict significance levels of the Tukey *post hoc* tests in the interaction plot of age X patient group: ns, not significant, $^{*}p < 0.05$, $^{***}p < 0.001$. Red shaded areas indicate frequencies with T patients exhibiting higher hearing thresholds compared to NT patients (Tukey *post hoc* tests separately for each frequency). Green shaded areas indicate frequency ranges (low: <1 kHz, mid: <4 kHz, high: ≥4 kHz) with T patients exhibiting lower hearing thresholds compared to NT patients (2-factorial ANOVAs for frequency ranges and patient groups). **(B,D)** give the mean HL difference of NT and T patients with red bars indicating significantly higher thresholds, green bars significantly lower thresholds in T patients compared to NT patients.

tinnitus pitch and the hearing thresholds of patients by 2-factorial ANOVAs with the factors frequency and perceived tinnitus pitch (NT, low, medium, and high tinnitus pitch). The results of these analyses are depicted in **Figure 4**. We found higher thresholds in SHL T patients rather being independent of tinnitus pitch. Lower threshold in CHL T patients on the other hand were most prominent in patients with high-pitched tinnitus percepts.

In detail, in SHL patients' data (**Figure 4A**, center panel), we found higher thresholds in T compared to the NT patients being most prominent but frequency unspecific in low-pitch tinnitus patients (Tukey *post hoc* tests), but also significant in the medium and high-pitch tinnitus patients, especially for frequencies above 2 kHz (**Figure 4B**). In CHL patients, a somewhat different picture emerged (**Figure 4C**). While low- and medium-pitch tinnitus patients showed generally higher thresholds than NT patients when averaged across all stimulation frequencies, the high-pitch tinnitus patients did show lower thresholds compared to the NT patients' thresholds (**Figure 4C**, center panel). This difference (**Figure 4D**) was most prominent for frequencies below 1,500 Hz in high-pitched T groups, while the higher values in tinnitus patients with medium-pitched tinnitus frequencies started at 1,500 Hz and reached up to the highest tested frequency of 8 kHz (Tukey *post hoc* tests). For low-pitched T patients, thresholds were again generally higher compared to NT patients.

In summary, the results of the pure tone audiometry comparisons showed significant differences in hearing thresholds of T compared to NT patients in both directions. In young patients with tinnitus, we generally found significantly lower thresholds almost independent of stimulation frequency (**Figure 1**) or perceived tinnitus pitch (not shown). In adult patients (**Figures 2–4**), data were more heterogeneous: in T compared to NT patients, lower hearing thresholds were found at low frequencies only, while higher thresholds were generally found at high frequencies. Furthermore, lower thresholds in T patients were more often found in CHL patients and could rarely be detected in SHL patients.

Differences in Speech Audiometry between Patients with and without Tinnitus

In order to examine the potential differences in speech comprehension between both patient groups, we analyzed the results of the Freiburger test, which examines the intelligibility of multisyllabic numbers. **Figure 5** shows the 2-factorial ANOVA of the speech reception thresholds with factors age group and tinnitus pitch group, tested for the two major patients groups separately.

Variance analysis of speech audiometry in SHL patients (**Figure 5A**) showed the expected dependency of speech reception thresholds on age (left panel), but in addition, a stronger impairment of speech intelligibility in patients with low tinnitus pitch in comparison to all other groups (Tukey *post hoc* tests), the medium and high tinnitus frequency perceivers were not significantly different in their understanding of numbers compared to each other and to the NT patients. We did find a significant interaction of both factors (age group and tinnitus pitch), with Tukey *post hoc* tests revealing stronger impairments in adults above 39 years of age and with tinnitus pitches below 1 kHz (**Figure 6**, right panel).

In CHL patients (**Figure 5B**), we found a comparable age dependency of the speech reception thresholds (**Figure 5B**, left panel) as in SHL patients as well as a decrease of speech intelligibility in low-pitch tinnitus patients. In contrast to SHL patients, CHL high-pitched tinnitus patients showed an increase of speech intelligibility thresholds of 7 dB compared to NT patients' thresholds (**Figure 5B**, middle panel). No interaction of both factors was found, indicating a threshold decrease in high-pitched tinnitus patients at all ages (**Figure 5B**, right panel).

To control for any direct effect of the individual hearing threshold on speech reception threshold, we analyzed the relation of both thresholds by multiple linear regressions, center of gravity comparisons, and a 2-factorial ANOVA of the individual threshold differences of the two, pooled across all patients. The results of these analyses are depicted in Figure S1 in Supplementary Material and show no qualitative differences between the age or tinnitus groups in the first two analyses. Nevertheless, the differences between low and medium-pitched tinnitus patients and high-pitched tinnitus and NT patients emerge in the 2-factorial ANOVA and further support the finding, that low/medium pitch tinnitus patients show a different pattern of threshold loss compared to high-pitched tinnitus and NT patients, namely a lower distance between speech reception and pure tone thresholds.

Analysis of Tinnitus Loudness

The subjective tinnitus loudness perceived by the patients of the two groups relative to their hearing thresholds turned out to be not a good predictor of speech intelligibility. An analysis of this relative tinnitus loudness by a 3-factorial ANOVA with the factors age group, HL group, and tinnitus pitch is given in **Figure 6**. The tinnitus perception was loudest in 40- to 60-year-old adults (**Figure 6**, upper left panel, Tukey *post hoc* tests, always $p < 0.001$) and in the SHL patients (**Figure 6**, upper center panel), particularly in the high-frequency pitched pure tone tinnitus percepts (**Figure 6**, upper right panel, Tukey *post hoc* tests, always $p < 0.001$). No interaction of age and HL group indicated a parallel shift of tinnitus loudness (**Figure 6**, center left panel). Furthermore, we found significant interactions of age and pitch (**Figure 6**, center panel) with a decrease of tinnitus loudness with age for low frequencies and increase for high frequencies. We also found a significant interaction of pitch and HL group (**Figure 6**, center right panel) with louder low-pitched tinnitus percepts in SHL patients (tukey *post hoc* test, $p < 0.001$) and louder high-pitched tinnitus in CHL patients (tukey *post hoc* test, $p < 0.01$). Most interestingly, the lack of a significant three-way interaction (**Figure 6**, bottom panel) indicated that the identified differences of tinnitus loudness dependency on age and pitch seemed to be similar in both HL groups.

DISCUSSION

Hearing Thresholds in Patients with and without Tinnitus

In this retrospective study, we compared audiometric data of a total of more than 37,000 patients (74,976 ears) with sensorineural

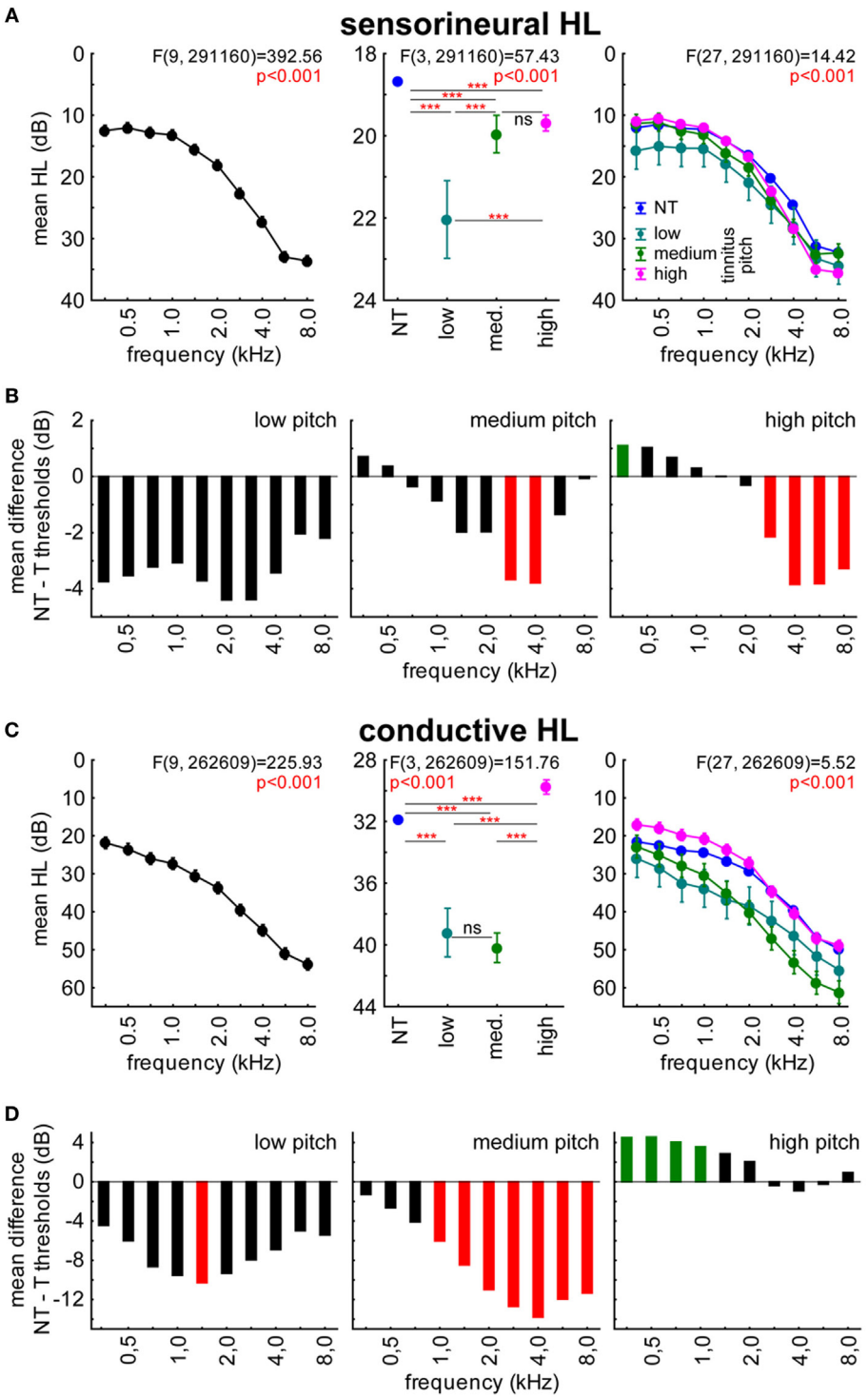

FIGURE 4 | **Relation between hearing loss in (HL in decibel) and tinnitus pitch in sensorineural (A,B) and conductive HL (C,D) patients**. **(A,C)** show the results of the 2-factorial ANOVAs of HL with factors frequency and perceived tinnitus pitch (including NT patients) in the two patient groups. Asterisks depict significance levels of the Tukey *post hoc* tests: ns, not significant; ***$p < 0.001$. **(B,D)** The threshold difference between T and NT patients is given, with red bars indicating significantly higher thresholds, green bars significantly lower thresholds in T patients compared to NT patients.

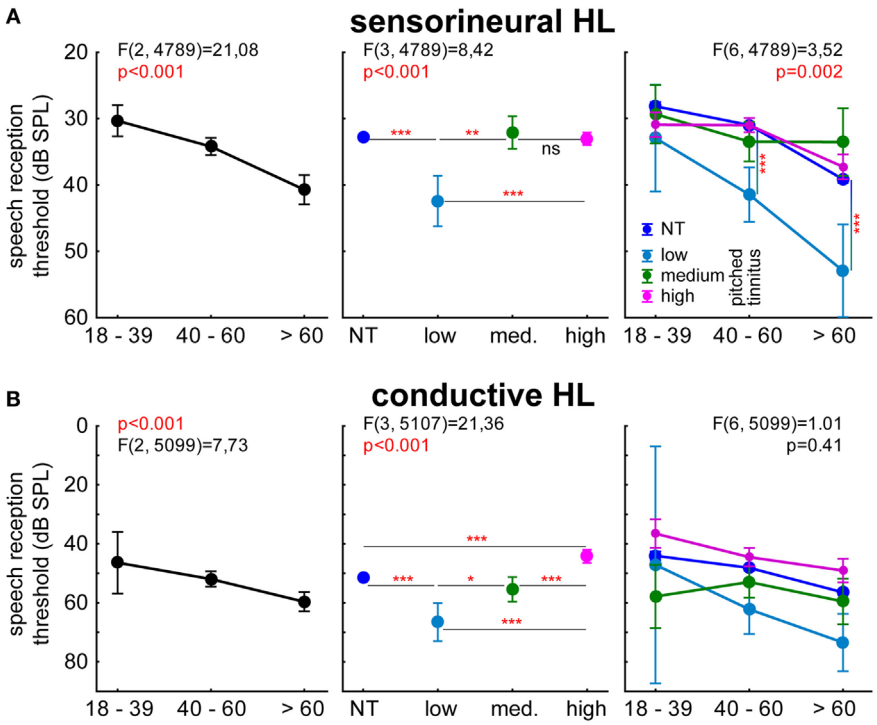

FIGURE 5 | Speech reception thresholds (decibel SPL) of sensorineural (A) and conductive HL (B) patients in the Freiburger speech intelligibility test (multisyllable numbers). 2-factorial ANOVAs of the 50% threshold with the factors age and perceived tinnitus pitch (including NT patients). Asterisks depict significance levels of the Tukey *post hoc* tests: $*p < 0.05$, $*p < 0.01$, $***p < 0.001$.

or CHL with or without tinnitus. We hypothesize that tinnitus is an epiphenomenon of a neuronal process normalizing impaired hearing thresholds. The audiometric results and tinnitus frequency distributions of tinnitus patients of different age groups are in line with the literature [e.g., Ref. (17, 26, 27)] even though the sample groups investigated there were much smaller than in our patient cohort. Also, the hearing thresholds of non-tinnitus patients are comparable to other cohorts (28, 29). Only few studies compared both patient groups directly (30, 31) and found either no change or a worsening of speech perception in tinnitus patients. To our knowledge, no peer reviewed study compared the pure tone hearing thresholds of both patient groups the way we did.

Here, we report specific differences in hearing thresholds between T and NT patients that may be interpreted as gains or losses in patients with tinnitus compared to non-tinnitus patients. These differences emerged on the population level of over 37,000 patients and in the paired analysis of a smaller subpopulation of roughly 2,500 patients in both pure tone and speech audiometry that seemed to follow a certain systematic (**Figure 7**, center panel): in T compared to NT adults, lower thresholds were seen in the low frequency range only, while higher thresholds were observed the high frequency range. In addition, lower thresholds were more often seen in CHL patients, while in SHL patients, higher thresholds predominated. Finally, the individually perceived tinnitus pitch seemed to be correlated with systematic alterations to the differences in hearing thresholds (**Figure 4**), which obviously are more complex than the simple masking we had first hypothesized to explain threshold increasing effects (see Hearing Thresholds in Adults with and without Tinnitus). In the case of paired data (Figure S1 in Supplementary Material), it becomes clear additionally that the most frequent (high) tinnitus pitch leads to the strongest improvement in speech intelligibility. Children and adolescents did not seem to follow this systematic, as only lower thresholds were seen there (**Figure 1**).

Based on our observations, we here put forward a model of tinnitus impact on hearing thresholds as shown in **Figure 7**: the basic feature of the model (**Figure 7**, center panel) is that thresholds in the low frequency range are lowered in tinnitus patients, i.e., in the range where HL is typically mild (**Figure 7**, center panel, green shaded area). Here, high frequency thresholds are higher in tinnitus patients, i.e., in the range where HL is typically more severe (**Figure 7**, center panel, red shaded area). This general scheme of hearing threshold change in tinnitus patients may be shifted by two main factors: sensorineural HL can shift the threshold function down (**Figure 7**, upper row)—CHL (**Figure 7**, lower row) has no effect on the function, while tinnitus pitch may shift the function along the abscissa (**Figure 7**, columns), resulting in a larger frequency range of lower thresholds (green shaded areas) for high-pitched tinnitus and a larger frequency range of higher thresholds (red shaded areas) for low-pitched tinnitus.

This model qualitatively reproduces the results of our retrospective data analysis (see **Figures 2B,D** and **4B,D**). Interestingly,

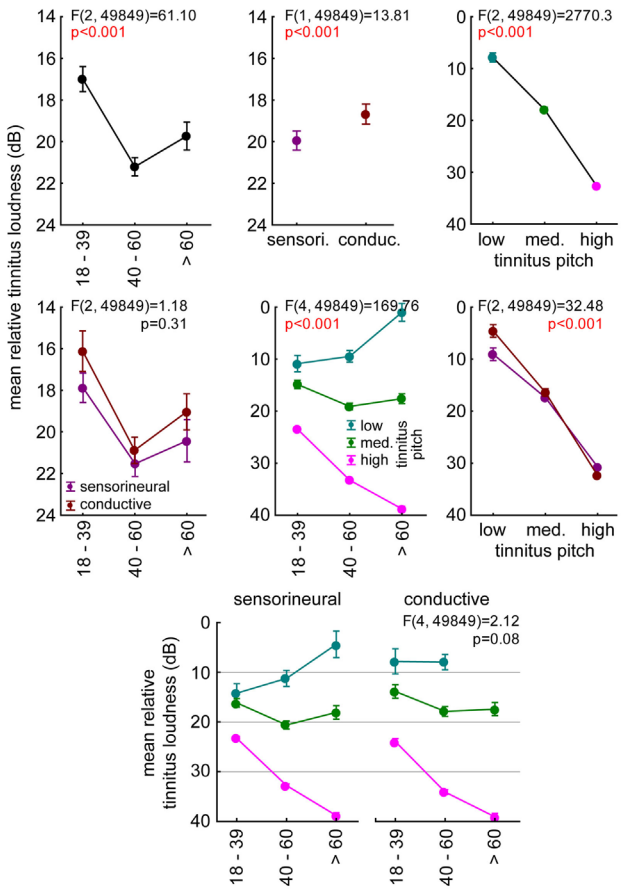

FIGURE 6 | Relation between mean relative tinnitus loudness (dB) and age, tinnitus pitch, and hearing loss (HL). The individual tinnitus loudness was calculated relative to the threshold at that frequency. Results of the 3-factorial ANOVA of tinnitus loudness relative to hearing threshold with the three factors described above. The upper three panels represent the 1-factorial part of the ANOVA results (age, HL, tinnitus pitch), the center line panels show the 2-factorial ANOVA interaction plots (age vs. HL, age vs. tinnitus pitch, tinnitus pitch vs. age), and the bottom line panel shows the interaction plot of all three factors.

when analyzing the relative frequency of the different tinnitus–threshold-interaction types postulated here (percent values in panels of **Figure 7**), it became obvious that interaction types with predominantly higher hearing thresholds are less frequent (adding up to 25.5%) than those types with lower thresholds (adding up to 74.4%). As these lower thresholds were exclusively observed for pure tone measurements in the low frequency range, we here put forward the hypothesis that *the biological function of the neuronal mechanism that finally also leads to tinnitus is to improve speech perception in the case of hearing loss*. This seems to work particularly well for high-pitched tinnitus which was encountered in about 2/3 of the patients (64.9%). In other words, patients suffering from tinnitus might be affected by this phantom percept as a side effect of their auditory system compensating for HL (cf. below).

Models of Tinnitus Development

Recent tinnitus models (32–37) all postulate damage to the peripheral receptor epithelium to be etiologic for the development of tinnitus. In response to the decreased input into the auditory system caused by such damage, one class of models further suggest an increased neuronal gain to provide homeostatic plasticity, while other suggest misbalanced lateral inhibition or a failure of a compensatory mechanism as the source of the phantom percept. Both types of models lack explanatory power since the potential biological function of homeostatic plasticity (in terms of information processing) or misbalanced lateral inhibition remains unclear.

To overcome these conceptual problems, we have recently put forward a model for tinnitus-related development of neuronal hyperactivity that is based on SR to restore hearing thresholds after HL (20). SR refers to the phenomenon that weak signals that are sub-threshold for a given sensor still can be detected and transmitted by that sensor if noise is added to the sensor input (21–24). In that way, SR serves to lift signals above a given hearing threshold and most probably is a mechanism that already works in the healthy system (38–40). One could interpret earlier results in a way that SR (while not mentioned explicitly) may also counteract increased hearing thresholds (17, 41). In this interpretation, tinnitus would be a (condoned) side effect of threshold restoration (42). In this context, recent studies have shown that patients with tinnitus show poorer listening performance in noise than patients without tinnitus (30). This is in line with our results, as the researchers focused mainly on sensorineural HL patients with and without tinnitus where we find comparable effects in our large patient cohort.

The analysis of audiometric patient data presented here are perfectly in line with the view of our model: first, the decreasing effects of SR on hearing threshold predicted by our model were observed in all adolescents and child HL patients with tinnitus as well as 65% of the adult HL patients with tinnitus. Second, threshold decreasing effects in tinnitus patients were observed in the low frequency range where HL is mild, that is, in a range where SR should be more effective as the amount of internal noise to be added to the signal to lift it above threshold must not be too high. Third, the threshold decreasing effects were most frequently observed in CHL tinnitus patients and were rather uncommon in SHL patients, which is in line with the idea that an intact neuronal system (as in CHL) more likely is able to compensate for HL by means of a neuronal SR mechanism than a damaged system (as in SHL). Note that still not all CHL patients may show lower thresholds, as patients suffering from CHL and SHL would be classified as CHL based on the diagnostic criteria (mean air-bone gap >5 dB; see Materials and Methods) and, therefore, may reduce the effect in the group. Finally, our model seems biologically plausible in that tinnitus development is condoned as a side effect of threshold restoration, as it particularly improves the speech intelligibility (improved thresholds in the speech relevant frequency range, most frequent (high) tinnitus pitch leads to strongest improvement of speech intelligibility), and communication by speech is crucial for social interaction and communication between

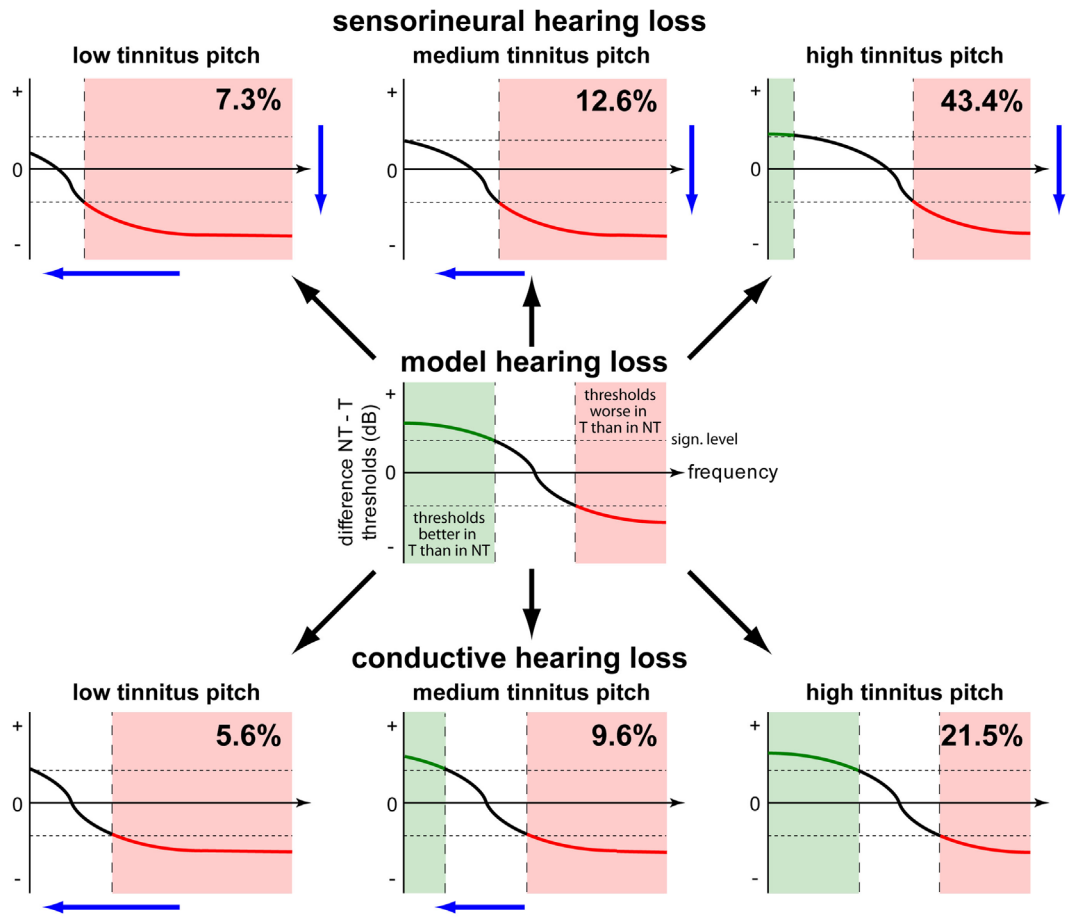

FIGURE 7 | **Model of decreasing (green) or increasing (red) effects of tinnitus on hearing thresholds**. In the center panel, the general effect of tinnitus on hearing threshold is given. Tinnitus is beneficial for thresholds in the low frequency range and detrimental in the high frequency range. The horizontal broken lines symbolize the significance levels. Two main factors alter this relationship by shifting the function either down (SHL) or left (tinnitus pitch). For more explanation, refer to the text.

humans (2, 25, 43). This view is supported by the fact that high pitch tinnitus patients show the largest distance of speech and corresponding pure tone thresholds, which are even higher than those of NT patients. An alternative interpretation of the data presented here is that steeper audiogram slopes due to better hearing thresholds at low frequencies and worse hearing thresholds at high frequencies are more likely to lead to the development of tinnitus (17).

One drawback of this retrospective study is the lack of information on etiology, comorbidities or medication of the 37,000 patients pooled together here. As tinnitus is only a symptom resulting from different diseases, its specific characteristic may differ from case to case. The fact that our analysis still yielded highly systematic results further strengthens our interpretation of the functional benefit of tinnitus on hearing thresholds.

In conclusion, we think that within an evolutionary frame of reference it makes perfectly sense to optimize hearing and information extraction ability on the cost of generating a phantom percept off the frequencies of interest, i.e., high-pitched tinnitus. Why some patients suffer from low or medium-pitched tinnitus remains unclear and may have different reasons than the threshold decreasing high pitch tinnitus. Another open question why only a minority of CHL patients is developing a tinnitus percept has to be addressed in a more detailed, possibly prospective, follow-up study.

AUTHOR CONTRIBUTIONS

DG analyzed the data and wrote a part of the manuscript. KT wrote a part of the manuscript, interpreted the data, and performed the statistical analysis. PK and AS provided assistance in the statistical analysis and interpretation of the data. UH provided the data. HS wrote a part of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fneur.2017.00031/full#supplementary-material.

REFERENCES

1. Coles R. Epidemiology of tinnitus: (2) demographic and clinical features. *J Laryngol Otol* (1984) 98:195–202. doi:10.1017/S1755146300090466
2. Lewis JE, Stephens SD, Mckenna L. Tinnitus and suicide. *Clin Otolaryngol Allied Sci* (1994) 19:50–4. doi:10.1111/j.1365-2273.1994.tb01147.x
3. Ruben RJ. Redefining the survival of the fittest: communication disorders in the 21st century. *Laryngoscope* (2000) 110:241–5. doi:10.1097/00005537-200002010-00010
4. Niskar AS, Kieszak SM, Holmes AE, Esteban E, Rubin C, Brody DJ. Estimated prevalence of noise-induced hearing threshold shifts among children 6 to 19 years of age: the Third National Health and Nutrition Examination Survey, 1988-1994, United States. *Pediatrics* (2001) 108:40–3. doi:10.1542/peds.108.1.40
5. Holube I, Fredelake S, Vlaming M, Kollmeier B. Development and analysis of an International Speech Test Signal (ISTS). *Int J Audiol* (2010) 49:891–903. doi:10.3109/14992027.2010.506889
6. Daniel E. Noise and hearing loss: a review. *J Sch Health* (2007) 77:225–31. doi:10.1111/j.1746-1561.2007.00197.x
7. Li CM, Zhang XZ, Hoffman HJ, Cotch MF, Themann CL, Wilson MR. Hearing impairment associated with depression in US Adults, National Health and Nutrition Examination Survey 2005-2010. *JAMA Otolaryngol Head Neck Surg* (2014) 140:293–302. doi:10.1001/jamaoto.2014.42
8. Katzenell U, Segal S. Hyperacusis: review and clinical guidelines. *Otol Neurotol* (2001) 22:321–6;discussion326–327.doi:10.1097/00129492-200105000-00009
9. Gates GA, Mills JH. Presbycusis. *Lancet* (2005) 366:1111–20. doi:10.1016/S0140-6736(05)67423-5
10. Cruickshanks KJ, Wiley TL, Tweed TS, Klein BE, Klein R, Mares-Perlman JA, et al. Prevalence of hearing loss in older adults in Beaver Dam, Wisconsin. The Epidemiology of Hearing Loss Study. *Am J Epidemiol* (1998) 148:879–86. doi:10.1093/oxfordjournals.aje.a009713
11. Baguley D, Mcferran D, Hall D. Tinnitus. *Lancet* (2013) 382:1600–7. doi:10.1016/S0140-6736(13)60142-7
12. Hoffman HJ, Reed GW. Epidemiology of tinnitus. In: Snow JB, editor. *Theory and Management*. Hamilton: BC Decker (2004). p. 24–7.
13. Hebert S, Canlon B, Hasson D, Magnusson Hanson LL, Westerlund H, Theorell T. Tinnitus severity is reduced with reduction of depressive mood – a prospective population study in Sweden. *PLoS One* (2012) 7:e37733. doi:10.1371/journal.pone.0037733
14. Lockwood AH, Salvi RJ, Burkard RF. Tinnitus. *N Engl J Med* (2002) 347:904–10. doi:10.1056/NEJMra013395
15. Møller AR. Epidemiology of tinnitus in adults. 1st ed. In: Møller AR, Langguth B, De Ridder D, Kleinjung T, editors. *Textbook of Tinnitus*. New York: Springer (2011). p. 29–37.
16. Henry JA, Meikle M, Gilbert A. Audiometric correlates of tinnitus pitch: insights from the Tinnitus Data Registry. In: Hazell J, editor. *Proceedings of the Sixth International Tinnitus Seminar*. London: The Tinnitus and Hyperacusis Centre (1999). p. 51–7.
17. König O, Schaette R, Kempter R, Gross M. Course of hearing loss and occurrence of tinnitus. *Hear Res* (2006) 221:59–64. doi:10.1016/j.heares.2006.07.007
18. Moore BC, Vinay, Sandhya. The relationship between tinnitus pitch and the edge frequency of the audiogram in individuals with hearing impairment and tonal tinnitus. *Hear Res* (2010) 261:51–6. doi:10.1016/j.heares.2010.01.003
19. Sereda M, Hall DA, Bosnyak DJ, Edmondson-Jones M, Roberts LE, Adjamian P, et al. Re-examining the relationship between audiometric profile and tinnitus pitch. *Int J Audiol* (2011) 50:303–12. doi:10.3109/14992027.2010.551221
20. Krauss P, Tziridis K, Metzner C, Schilling A, Hoppe U, Schulze H. Stochastic resonance controlled upregulation of internal noise after hearing loss as a putative cause of tinnitus-related neuronal hyperactivity. *Front Neurosci* (2016) 10:597. doi:10.3389/fnins.2016.00597
21. Benzi R, Sutera A, Vulpiani A. The mechanism of stochastic resonance. *J Phys A Math Gen* (1981) 14:L453. doi:10.1088/0305-4470/14/11/006
22. Collins JJ, Imhoff TT, Grigg P. Noise-enhanced information transmission in rat SA1 cutaneous mechanoreceptors via aperiodic stochastic resonance. *J Neurophysiol* (1996) 76:642–5.
23. Levin JE, Miller JP. Broadband neural encoding in the cricket cercal sensory system enhanced by stochastic resonance. *Nature* (1996) 380:165–8. doi:10.1038/380165a0
24. Gammaitoni L, Hänggi P, Jung P, Marchesoni F. Stochastic resonance. *Rev Mod Phys* (1998) 70:223. doi:10.1103/RevModPhys.70.223
25. Coles RR. Epidemiology of tinnitus: (1) prevalence. *J Laryngol Otol Suppl* (1984) 9:7–15. doi:10.1017/S1755146300090041
26. Axelsson A, Sandh A. Tinnitus in noise-induced hearing loss. *Br J Audiol* (1985) 19:271–6. doi:10.3109/03005368509078983
27. Savastano M. Tinnitus with or without hearing loss: are its characteristics different? *Eur Arch Otorhinolaryngol* (2008) 265:1295–300. doi:10.1007/s00405-008-0630-z
28. Nadol JB Jr. Hearing loss. *N Engl J Med* (1993) 329:1092–102. doi:10.1056/NEJM199310073291507
29. Schreiber BE, Agrup C, Haskard DO, Luxon LM. Sudden sensorineural hearing loss. *Lancet* (2010) 375:1203–11. doi:10.1016/S0140-6736(09)62071-7
30. Moon I, Won J, Kang H, Kim D, An Y, Shim H. Influence of tinnitus on auditory spectral and temporal resolution and speech perception in tinnitus patients. *J Neurosci* (2015) 35:14260–9. doi:10.1523/JNEUROSCI.5091-14.2015
31. Gilles A, Schlee W, Rabau S, Wouters K, Fransen E, Van De Heyning P. Decreased speech-in-noise understanding in young adults with tinnitus. *Front Neurosci* (2016) 10:288. doi:10.3389/fnins.2016.00288
32. Gerken GM. Central tinnitus and lateral inhibition: an auditory brainstem model. *Hear Res* (1996) 97:75–83. doi:10.1016/S0378-5955(96)80009-8
33. Eggermont JJ. Central tinnitus. *Auris Nasus Larynx* (2003) 30(Suppl):S7–12. doi:10.1016/S0385-8146(02)00122-0
34. Eggermont JJ, Roberts LE. The neuroscience of tinnitus. *Trends Neurosci* (2004) 27:676–82. doi:10.1016/j.tins.2004.08.010
35. Schaette R, McAlpine D. Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J Neurosci* (2011) 31:13452–7. doi:10.1523/JNEUROSCI.2156-11.2011
36. Knipper M, Van Dijk P, Nunes I, Ruttiger L, Zimmermann U. Advances in the neurobiology of hearing disorders: recent developments regarding the basis of tinnitus and hyperacusis. *Prog Neurobiol* (2013) 111:17–33. doi:10.1016/j.pneurobio.2013.08.002
37. Ruttiger L, Singer W, Panford-Walsh R, Matsumoto M, Lee SC, Zuccotti A, et al. The reduced cochlear output and the failure to adapt the central auditory response causes tinnitus in noise exposed rats. *PLoS One* (2013) 8:e57247. doi:10.1371/journal.pone.0057247
38. Zeng F-G, Fu Q-J, Morse R. Human hearing enhanced by noise. *Brain Res* (2000) 869:251–5. doi:10.1016/S0006-8993(00)02475-6
39. Long Z-C, Shao F, Zhang Y-P, Qin Y-G. Noise-enhanced hearing sensitivity. *Phys Lett A* (2004) 323:434–8. doi:10.1016/j.physleta.2004.02.019
40. Ries DT. The influence of noise type and level upon stochastic resonance in human audition. *Hear Res* (2007) 228:136–43. doi:10.1016/j.heares.2007.01.027
41. Hebert S, Fournier P, Norena A. The auditory sensitivity is increased in tinnitus ears. *J Neurosci* (2013) 33:2356–64. doi:10.1523/JNEUROSCI.3461-12.2013
42. Parra LC, Pearlmutter BA. Illusory percepts from auditory adaptation. *J Acoust Soc Am* (2007) 121:1632–41. doi:10.1121/1.2431346

43. Langguth B, Landgrebe M, Kleinjung T, Sand GP, Hajak G. Tinnitus and depression. *World J Biol Psychiatry* (2011) 12:489–500. doi:10.3109/15622975.2011.575178

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Different Patterns of Hearing Loss among Tinnitus Patients: A Latent Class Analysis of a Large Sample

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Background: The heterogeneity of tinnitus is a major challenge for tinnitus research. Even if a complex interaction of many factors is involved in the etiology of tinnitus, hearing loss (HL) has been identified as the most relevant etiologic factor. Here, we used a data-driven approach to identify patterns of hearing function in a large sample of tinnitus patients presenting in a tinnitus clinic.

Methods: Data from 2,838 patients presenting at the Tinnitus Center of the University Regensburg between 2007 and 2014 have been analyzed. Standard audiometric data were frequency-wise categorized in four categories [a: normal hearing (0–20 dB HL); b: moderate HL (25–50 dB HL; representing outer hair cell loss); c: severe HL (>50 dB HL; representing outer and inner hair cell loss); d: no data available] and entered in a latent class analysis, a statistical method to find subtypes of cases in multivariate categorical data. To validate the clinical relevance of the identified latent classes, they were compared with respect to clinical and demographic characteristics of their members.

Results: The classification algorithm identified eight distinct latent classes with an excellent separation. Patient classes differed with respect to demographic (e.g., age, gender) and clinical characteristics (e.g., tinnitus location, tinnitus severity, gradual, or abrupt onset, etc.).

Discussion: Our results demonstrate that data-driven categorization of hearing function seems to be a promising approach for profiling tinnitus patients, as it revealed distinct subtypes that reflect prototypic forms of HL and that differ in several relevant clinical characteristics.

Keywords: chronic tinnitus, hearing loss, cluster analysis, latent classes, audiometry

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INTRODUCTION

Tinnitus, the perception of sound in the absence of a corresponding auditory stimulus, is a frequent disorder (1). Clinically, tinnitus can be very heterogeneous with respect to the perceived sound characteristics (e.g., tonal vs. broadband noise), its localization (in one or both ears, in the head, etc.), its time course (continuous, intermittent, fluctuating), its modifying factors (e.g., reduction by

masking), and its comorbidities (hyperacusis, depression, insomnia). This clinical heterogeneity is paralleled by heterogeneity in tinnitus pathophysiology. Recent pathophysiological models assume that tinnitus emerges as a clinical symptom as result of abnormal activation of different overlapping and interacting brain networks (2). Abnormally activated networks in tinnitus patients include the auditory, attention, salience, and distress networks. The activation pattern varies from patient to patient and reflects the individuals' symptoms (3, 4). As an example, distressed and not distressed tinnitus patients differ in their activation of the cortical stress-related network (5).

Among other factors, the large heterogeneity of the tinnitus patient population represents a major barrier for the development of effective tinnitus treatments [see, e.g., Ref. (4) for a review]. The heterogeneity of tinnitus can be described on various dimensions such as its etiology, perceptual characteristics of the sound (i.e., pitch and loudness), time since onset, continuous or intermittent, levels of conscious awareness and perceived distress, and comorbidities (6). One approach to address this challenge is the establishment of large databases for enabling data-driven identification of subtypes (7–9).

Even if current etiologic models assume a complex interplay of various factors, several lines of evidence indicate that hearing loss (HL) is the most relevant etiologic factor for tinnitus development (10, 11). First, epidemiological studies have identified HL as a major risk factor for tinnitus (12). Second, induction of HL in animals induces reliably increased neuronal activity and synchronicity (11, 13) as well as behavioral evidence of tinnitus (14). Third, the tinnitus spectrum of most tinnitus patients is clearly related to their pattern of HL (15, 16). If, for example, somebody experiences tinnitus at 4 kHz at the left ear, typically a HL at 4 kHz on the left ear can be detected in the audiogram.

Because of the high etiological relevance of HL for tinnitus, hearing function is presumably one of the relevant criteria for classifying tinnitus patients. Currently, this factor is not receiving major attention in the classification of tinnitus patients. In the description of study samples in tinnitus research rarely details are given about the hearing function of participants. If anything, the mean audiogram (averaged over all participants) is displayed. Whenever statistical analyses are performed relating hearing function to other aspects of tinnitus, typically either HL averaged over both ears and all measured frequencies or the maximum HL is used to characterize patients' incapacities.

Both indicators are of only limited value, as there exist different patterns of the quantity of HL, e.g., related to outer or inner hair cell damage, which might be highly relevant for a comprehensive characterization of a tinnitus patient. However, the information about specific patterns of HL quantity is getting lost, if the audiograms of participants are averaged.

Here, we used a data-driven approach to identify patterns of hearing function in a large sample of tinnitus patients presenting in a specialized Tinnitus Center. The goal of the study was to clarify, whether specific patterns of HL quantity can be established from patients' audiograms and how stable different tinnitus patients can be classified using these patterns of HL. In case, this data-driven approach for pattern identification reveals a statistically sound and medically well interpretable solution, one could assume that this new categorization of hearing function would be a reasonable alternative to current approaches to deal with audiogram data in the characterization of tinnitus patients.

MATERIALS AND METHODS

Patient Assessment

The analysis has been based on data from all patients who presented between 2007 and 2014 at the Interdisciplinary Tinnitus Center at the University of Regensburg, Regensburg, Germany and who gave informed consent for data collection in the Tinnitus Research Initiative database (TRI Database). The TRI Database is an international patient database that has been established for facilitating research efforts toward the identification of tinnitus subtypes and outcome predictors (8). For these purposes, patients presenting in tinnitus clinics and undergoing specific, well-defined treatment interventions, both in clinical trials or in clinical routine, are systematically assessed and their data are preprocessed for plausibility in standardized protocols (8). In addition to audiometric data, the database includes demographic and clinical data, data about tinnitus handicap, tinnitus severity, and quality of life [for a detailed description of the datasets, see Ref. (8)]. Collection of data for the TRI Database has been approved by the local ethics committee of the University of Regensburg, Germany. All patients completed various tinnitus questionnaires, underwent a microscopy of the ear and received an audiological examination including pure-tone audiometry (125–8,000 Hz). Sample size for this classificatory part of our analysis thus reached $n = 2{,}838$.

Due to item-wise missing values of the 17 variables used to describe demographic or clinical characteristics of patients beyond the results of the audiometry, effective sample sizes in comparisons of emerging latent classes (see below) vary. Most variables reach effective sample sizes clearly above 2,000 patients. Only Beck Depression Inventory (BDI) ($n = 1{,}544$) and TBF-total score ($n = 1{,}464$), which both were added later to the assessment program, and patients' CGI-ratings ($n = 807$), which were assessed only in patients entering a treatment program at their first day of treatment, reached smaller sample sizes.

Classification of Hearing Function

As HL presenting at different threshold levels can be linked to different possible pathological mechanisms (17), patients' audiogram data were not treated as a "naive" continuous metric of intensity of HL, but classified into four different states. At each of seven pre-specified frequencies (125 Hz, 250 Hz, 500 Hz, 1 kHz, 2 kHz, 4 kHz, and 8 kHz) the measured grade of HL was classified into one of the following four categories: (1) normal hearing (0–20 dB HL); (2) mild/moderate HL (25–50 dB HL), representing mostly outer hair cell loss; (3) severe/profound HL (>50 dB HL), representing outer and inner hair cell damage; and (4) no data available (either due to technical restrictions or because of physician based abbreviations of the audiogram assessment). This categorization tries to better reflect the physiological condition of patients' hearing and to avoid a potentially misleading interpretation of HL as homogenously quantifiable risk factor (18). Additionally, by introducing a fourth category of "not measured" into the analysis,

a potential selection bias due to technical equipment or due to physicians' practices in assessing HL can be avoided.

Statistical Methods

Latent class analysis (LCA) is a statistical approach for identifying groups of cases in multivariate categorical data. These groups are called latent classes. Our assumption is that HL of the left ear does not predetermine HL for the right side, and *vice versa*. Therefore, a vector of 14 variables (each frequency with 4 HL categories) per patient defines the starting point of statistical analysis. Theoretically, over 250 million combinations (exactly 4**14 = 268,435,456) are possible. Observed were 590 answering vectors in 2,838 patients. LCA tries to reproduce the empirical frequencies of these answering vectors by estimating two different kinds of model parameters: π_g, i.e., the relative sizes of G latent classes (G has to be determined *a priori*), and the probability π_{isg} for an answer s ($s = 1, \ldots, 4$) on each item i ($i = 1, \ldots, 14$), given the membership in a certain latent class g. The class-specific answering probabilities $\pi_{ix|g}$ thus indicate the nearness between this specific answer and membership in the respective latent class g. LCA therefore results in G membership probabilities per person to each of the latent classes (see Supplementary Material for further details). Strong solutions with little overlap between different latent profiles provide for each person one unequivocal high membership probability and $m - 1$ very low membership probabilities. Classification then is based on the modal value of these probabilities.

Visualization of membership probabilities is an intuitively appealing method of model evaluation. Alternatively, so-called fit indices can be calculated for each number of latent classes chosen. Clearly, a perfect model fit must be reached, if (in our case) 590 classes are introduced to the model. By introducing a penalty term for adding new latent classes, a decision for the optimal number of classes can be drawn choosing the model with the best fit. We used the BIC index as criteria to decide on the number of latent classes. Calculations were performed using WinMIRA by von Davier (19).

Differences between latent classes on continuous variables (like age) were assessed using SAS PROC GLM to perform analysis of variance for unequal cell sizes. Differences on qualitative variables (like sex) were assessed using chi-square test (SAS PROC FREQ). Due to the exploratory character of this study, no adjustment for type-I error inflation was performed.

RESULTS

The sample comprised 2,838 patients (mean age 51.7 ± 12.9 years, 67.6% male). In 1,925 of them, audiometric data were available.

In order to avoid local maxima of the estimation function, 50 starting values for parameter estimation were randomly chosen for each model covering 2 up to 12 latent classes. According to the BIC fit index, eight latent classes represent an optimal solution for the given data set. Posterior probabilities of class membership display excellent separation of groups of HL as indicated by a mean membership probability above 0.9 for all latent classes (**Table 1**) (see Supplementary Material for details about the calculation of latent classes). Detailed clinical and demographic data of the sample are given in **Table 2**.

The largest class (LC1; **Figure 1** upper left chart) comprises nearly one-third (32.2%) of the sample and represents patients with lacking audiometry. By holding these untested patients in a separate group it is possible to scrutinize potential selection biases between clinical characteristics and audiometry. Therefore, it is meaningful to analyze these patients as a specific "pattern of hearing loss."

The 21.6% of the sample suffers from mild to moderate HL probably due to primarily outer hair cell damage especially for frequencies above 4 kHz (LC2; **Figure 1**, upper right chart). This group was entitled "bilateral high frequency (HF) hearing loss." Tinnitus patients with nearly normal audiogram (LC3; **Figure 1**, lower left chart) comprise about 20.6% of the total sample. Here, in rare cases (about 10% of this group), only frequencies above 4 kHz are involved with mild/moderate HL for both ears. A large proportion of patients with at least moderate HL in higher frequencies (2 kHz and above) for both ears can be observed in LC4. Twenty to thirty percent of this latent class were measured with thresholds over 50 dB above 4 kHz. Lower frequencies (below 500 Hz) are mostly not affected by HL. The proportion of this group is 13% of the total sample. The group was entitled "bilateral medium-high frequency HL."

Figure 2 displays patterns of HL with much smaller proportion among tinnitus patients (all <5%). LC5 (upper left chart in **Figure 2**) was called "severe pantonal HL" and is characterized by high proportions of at least moderate HL at all measured frequencies. Almost half of the patients of this group have thresholds over 50 dB above 4 kHz. Both ears are concerned quite similarly.

By contrast, latent classes 6, 7, and 8 all display asymmetric patterns of HL. In LC6 (3.8% of total sample; upper right chart in **Figure 2**), most patients have normal hearing at the right ear below 4 kHz but severely impaired hearing at their left ear at all frequencies. Damage on the left ear is mostly a HL between 25 and 50 dB ("mild to moderate"). We therefore named this group "left-sided pantonal medium HL."

It is noteworthy, that LC7 (2.9%, lower left chart of **Figure 2**) is not a symmetrical counterpart to LC6, though in this group mostly the right ear is affected by HL. But whereas LC6 members displayed mild to moderate HL at their affected left ear, members of LC7 have a broadband severe HL at their right ears. Already at 125 Hz, more than 20% of this group suffer from threshold elevations above 50 dB. This proportion is continuously increasing with increasing frequencies up to nearly 60% at 8 kHz. We therefore called this group of patients "right-sided pantonal severe HL."

The smallest group of patients isolated by LCA can be depicted from the lower right chart of **Figure 2** (LC8). In 1.2% of all patients, a pattern was observed with considerable proportion of normal hearing at the right ear in lower frequencies (e.g., 66% at 125 kHz), continuously decreasing to 20% at 8 kHz. But at patients' left ears, nearly nobody was measured with normal hearing at any frequency: mostly, a severe HL (thresholds >50 dB) could be observed across all frequencies, or the left ear was not assessed (especially at higher frequencies). As practically, all patients had a full examination of the right ear, it can be assumed that missing values for left ears in this group do not mean "unknown" degree of HL, but represent a physician's or audiologist's decision to stop the assessment, because complete deafness of the left ear impede

TABLE 1 | Mean membership probabilities for latent classes.

Patient is classified into		Mean membership probabilities for latent classes							
Class no.	Relative class size	LC1	LC2	LC3	LC4	LC5	LC6	LC7	LC8
LC1	0.322	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
LC2	0.216	0.000	0.910	0.058	0.030	0.000	0.001	0.000	0.000
LC3	0.206	0.000	0.054	0.943	0.003	0.000	0.001	0.000	0.000
LC4	0.133	0.000	0.057	0.004	0.914	0.009	0.012	0.004	0.000
LC5	0.048	0.000	0.000	0.000	0.018	0.967	0.009	0.003	0.004
LC6	0.037	0.000	0.002	0.000	0.019	0.012	0.963	0.004	0.000
LC7	0.029	0.000	0.006	0.000	0.009	0.013	0.004	0.968	0.000
LC8	0.011	0.000	0.000	0.000	0.000	0.002	0.000	0.000	0.998

a full measurement of all frequencies at the left side. This group therefore was named "left-sided pantonal severe HL."

Latent class 1 (lacking audiometry) during all statistical comparisons of clinical and demographic parameters yielded results very similar to the total average or proportions of the total sample (see **Table 2**). Therefore, no hints are present that the lack of an audiometric test was related to a certain subgroup of tinnitus patients. The relative size of the remaining seven HL-patterns thus could be adjusted to the proportions given in **Table 2**. These reflect the expected latent class sizes in our patient sample, if all patients had received threshold measurements.

Mean age of patients (see **Table 2**) was 51.7 years (SD 12.9) with the youngest patients presenting in LC3 (normal hearing) and the oldest patients in LC5 (severe pantonal HL). Female patients (total: 32.4%, see **Table 2**) were under-represented in LC2 (bilateral HF HL) and LC4 (bilateral medium-HF HL), and at largest over-represented in LC5 (severe pantonal HL) and LC6 (left-sided pantonal medium HL).

Age at onset of tinnitus was on average 43.2 years (SD 13.9; **Table 2**). Thus, there was a mean delay of nearly 9 years until presenting at the Regensburg Tinnitus Center. Patients with more or less normal hearing (LC3) had virtually no delay in help seeking. The small difference between age at onset as compared to biographical mean age (41.7 vs. 41.9 years) is probably confounded by missing values about the onset. Patients with bilateral medium-to-severe HF HL (LC4) and with severe pantonal HL (LC5) reported the latest onset of tinnitus.

Symptom load as measured by the Tinnitus Questionnaire (TQ, 20) was highest in LC5 (severe pantonal HL), and lowest in LC3 (normal hearing). Signs of depression as measured by BDI were also most prominent in LC5, and quite lenient in all other patient groups. For the TBF Questionnaire, a short form of the Tinnitus Handicap Inventory, mean scores 2 points above the total average were found for LC5 and LC8 (left-sided pantonal severe HL). Whereas patients' self-ratings of tinnitus severity on the clinical global impression scale of tinnitus (CGI self-rating) revealed no significant differences between the different latent classes, the answers to the question "How much of a problem is your tinnitus?" differed significantly. But this effect was very small with membership in latent classes of HL explaining only less than 3% of the variance of patients' ratings.

Characteristics of tinnitus manifestation over groups of HL are also given in **Table 2**. The different latent classes differed in most but not all characteristics (see **Table 2**). Whether or not tinnitus started abruptly, differed most between LC4 (43% abrupt start) and LC5 (40%) on the one side, and over 60% for latent classes 6 (left-sided pantonal medium HL) and 7 (right-sided pantonal severe HL). A non-pulsatile tinnitus was in total reported by 79.6% of all patients. Only in LC7 (right-sided pantonal severe HL) this proportion was considerably smaller (66%).

Handedness of patients could not be shown to covary with patterns of HL (and therefore was also not connected to side of HL). On which side patients experienced their tinnitus (exclusively on one side or with dominance of one side vs. bilateral symptoms) was clearly connected to the asymetrical patterns of HL. Members of LC6 (left-sided pantonal HL) as well as members of LC8 (left-sided pantonal severe HL) reported tinnitus more often for their left side. Accordingly, patients in LC7 (right-sided pantonal severe HL) clearly reported more often to experience their tinnitus on the right side (48% as compared to 12.3% in the total sample).

For LC8 (left-sided pantonal severe HL), their tinnitus was more frequently experienced as constant over time. If patients suffered from HF HL (LC2) or had quite normal audiometric results (LC3), they tended more often to describe their tinnitus as a tonal event. Patients with severe pantonal HL (LC5) by contrast had much higher proportions of tinnitus experienced as a kind of noise. The tinnitus pitch did not differ too much across the LCs except a smaller proportion of very high pitch (19% as compared to 28% for the whole sample) in LC7 (right-sided pantonal severe HL), and more prominence of low pitch tinnitus in LC3 (normal hearing) and LC6 (left-sided pantonal medium HL) with 4.4 and 6.3% (total sample: 2.5%).

DISCUSSION

In the present study, we performed a data-driven analysis of a large sample to identify clinically meaningful subtypes of HL patterns among tinnitus patients. Information about hearing function was derived from standard pure-tone audiograms, which were all performed in the context of clinical routine assessment according to standard procedures in one clinical center.

A potential limitation of our study is the limited sensitivity of the standard audiogram. The database only contained audiogram data from air conduction measurements, which made it impossible to differentiate cochlear from conductive HL. Moreover measurement of otoacoustic emissions might provide a more exact measurement of outer hair cell function than audiometric

TABLE 2 | Patterns of HL and related demographic and clinical data.

Description of patterns of HL											
Variable	**Pattern of hearing loss (HL)**	**LC2: bilateral high frequency (HF) HL**	**LC3: normal hearing**	**LC4: bilateral medium-HF HL**	**LC5: severe pantonal HL**	**LC6: left-sided pantonal medium HL**	**LC7: right-sided pantonal severe HL**	**LC8: left-sided pantonal severe HL**	**LC1: lacking audiometry**	**Total**	**Prob. *F*-test**
	N[a]	614	581	378	135	104	81	32	913	2.838	
Prevalence (adjusted)	(without LC1)	0.319	0.302	0.196	0.070	0.054	0.042	0.017	n.a.	1.00	n.a.
Age	Mean	53.099	41.878	58.706	61.186	53.799	57.026	57.372	51.763	51.694	$p < 0.0001$
	SD	9.76	11.32	10.94	12.78	10.92	12.51	12.69	13.00	12.94	
Age at onset	Mean	45.201	41.726	47.869	47.944	45.765	46.908	44.630	43.053	43.213	$p < 0.0001$
	SD	11.92	11.98	13.51	16.61	10.92	14.66	16.91	14.08	13.85	
Tinnitus Questionnaire total score (at screening)	Mean	40.178	36.813	41.894	50.944	44.768	42.519	46.500	41.639	40.910	$p < 0.0001$
	SD	17.35	17.19	16.83	17.09	17.35	17.95	17.88	18.31	17.76	
Beck Depression Inventory total score (at screening)	Mean	10.117	10.835	10.086	14.356	10.970	11.188	11.409	11.532	10.905	$p < 0.01$
	SD	8.04	8.62	7.70	9.84	10.11	7.95	8.74	9.05	8.60	
TBF-12 total score (at screening)	Mean	12.772	11.970	13.158	14.971	13.563	13.083	14.733	12.706	12.801	$p < 0.01$
	SD	5.10	5.58	5.28	4.90	0.96	5.76	5.20	5.68	5.42	
Severity rating (patient, at screening)	Mean	3.407	3.219	3.548	3.813	3.437	3.472	3.567	3.486	3.431	$p < 0.0001$
	SD	0.84	0.90	0.81	0.83	0.84	0.87	0.82	0.90	0.88	
CGI rating (patient, at first visit)	Mean	3.880	3.978	3.769	3.800	4.094	3.920	4.133	3.909	3.901	n.s.
	SD	0.79	0.78	0.80	0.94	0.96	0.91	0.92	0.80	0.82	
Categorical variables											**Prob. chi-square test**
Sex	% Female	24.9	36.7	27.0	50.4	50.0	46.9	46.9	30.6	32.4	$p < 0.001$
Handedness	% Right hand	83.3	84.1	83.1	87.9	80.6	73.8	78.1	83.8	83.3	n.s.
Abrupt beginning of tinnitus	% Abrupt	48.8	56.8	43.1	40.0	60.4	60.3	57.1	52.3	51.1	$p < 0.001$
Tinnitus pulsation	% No	82.2	80.0	82.3	72.7	81.5	66.2	75.0	77.9	79.6	$p < 0.01$
Location of tinnitus[b]	% Right	8.6	14.3	10.0	9.9	4.0	48.2	6.3	12.9	12.3	$p < 0.001$
	% Left	18.2	13.1	15.2	15.2	37.6	11.1	37.5	17.7	17.1	
	% Both sides (worse left)	18.2	22.6	26.3	21.2	21.8	6.2	34.4	18.7	20.6	
	% Both sides (worse right)	16.7	16.6	17.6	21.2	12.9	23.5	6.3	17.2	17.1	
	% Both sides equally	27.8	24.5	20.6	24.2	13.9	8.6	6.3	23.8	23.4	
Tinnitus manifestation over time	% Constant	87.9	82.8	88.8	92.0	88.5	80.8	93.3	84.4	86.1	$p < 0.05$

(*Continued*)

TABLE 2 | Continued

Description of patterns of HL											
Variable	**Pattern of hearing loss (HL)**	**LC2: bilateral high frequency (HF) HL**	**LC3: normal hearing**	**LC4: bilateral medium-HF HL**	**LC5: severe pantonal HL**	**LC6: left-sided pantonal medium HL**	**LC7: right-sided pantonal severe HL**	**LC8: left-sided pantonal severe HL**	**LC1: lacking audiometry**	**Total**	**Prob. *F*-test**
Loudness variation	% Yes	60.4	61.1	55.5	64.0	63.8	59.2	63.3	61.5	60.4	n.s.
Tonal characteristic of tinnitus[c]	% Tone	64.8	65.6	58.3	45.2	50.5	53.3	33.3	63.7	61.3	$p < 0.001$
	% Noise	5.7	10.6	11.0	32.5	19.0	23.4	33.3	11.3	11.9	
	% Crickets	21.5	15.6	20.7	12.7	15.8	13.0	16.7	16.1	17.7	
Tinnitus pitch	% Very high	30.8	27.8	23.6	27.4	28.1	18.7	30.0	29.1	27.9	$p < 0.001$
	% High	56.1	51.8	55.8	53.0	42.2	41.3	46.7	52.0	52.8	
	% Medium	11.9	16.0	19.4	16.2	22.9	40.0	20.0	16.6	16.8	
	% Low	1.3	4.4	1.2	3.4	6,3	0.0	3.3	2.3	2.5	

[a]*Due to missing values, sample sizes might vary.*
[b]*Lacking categories to 100% are "inside head," "elsewhere."*
[c]*Lacking category is "other."*

thresholds and it also remains to be determined whether the cut-offs for categorization of HL used in this study (<20 dB HL, 25–50 dB HL, >50 dB HL) are most appropriate. Finally, cochlear damage in the HF range above 8 kHz (21), dead regions between tested frequencies (22) and synaptopathy of high-threshold fibers (23) can all be relevant for tinnitus development but are not detected by the standard audiogram.

Presumably the different forms of "hidden hearing loss" are particularly relevant in LC3, the group with normal audiogram. However, in spite of the fact that patient categorization was only based on the audiogram which represents a rather rough information of cochlear function, our analysis revealed several relevant findings.

First, the classification algorithm identified eight distinct latent classes with an excellent separation. This means that all patients could be almost unambiguously allocated to a given class.

Second, the HL patterns of the different classes reflected typical clinical patterns of HL: bilateral normal hearing (LC3), bilateral HF HL (LC2), bilateral medium to HF HL (LC4), bilateral pantonal HL (LC5), medium (LC6), severe (LC8) pantonal HL left, and medium-to-severe pantonal HL right (LC7).

Third, patients of the various latent classes differed in most demographic and clinical tinnitus characteristics indicating the clinical relevance of our categorization and confirming HL as a relevant criterion for profiling of tinnitus patients.

As expected from the fact that bilateral HL frequently develops over the life span, patients with normal audiogram (LC3) were younger, whereas patients with pantonal pronounced HL (LC5) were older than average. This fits with the pattern of HL in LC5 which is typical for age-related HL. Another expected finding was that tinnitus laterality was related to the side of unilateral HL (LC6, LC7, LC8). Patients with unilateral HL (LC6, LC7) reported also more frequently abrupt tinnitus onset, which fits with sudden HL as a frequent cause of unilateral hearing impairment. By contrast, bilateral hearing impairment (LC4, LC5), which is typically developing slowly over time, was more often related to gradual onset.

Patients with HL exclusively in the HF range (LC2) and patients with normal audiogram (LC3), who frequently have hearing impairment in the extended HF range (21) had more often tonal tinnitus, which fits with their circumscribed hearing impairment. Accordingly, noise-like tinnitus was more frequently observed in patients with pantonal hearing impairment (LC5). Patients with pantonal impairment (LC5) were also characterized by higher tinnitus severity and more depressive symptoms, as reflected by increased scores in TQ, TBF-12, and BDI. This finding is in line with earlier investigations demonstrating that the degree of HL is associated with tinnitus severity (24).

In summary, our findings revealed distinct subtypes that reflect prototypic forms of HL and that differ in several relevant clinical characteristics. Further research should aim at further refinement by taking into account more detailed audiological informations. Finally, other possible approaches for cluster analyses should be tested and compared. One possible approach would be to weight differences between adjacent frequencies, as they might be particularly relevant for tinnitus generation (25). Such an approach may help to better identify relevant characteristics of hearing function (for example, the 4 kHz dip as a typical pattern of HL

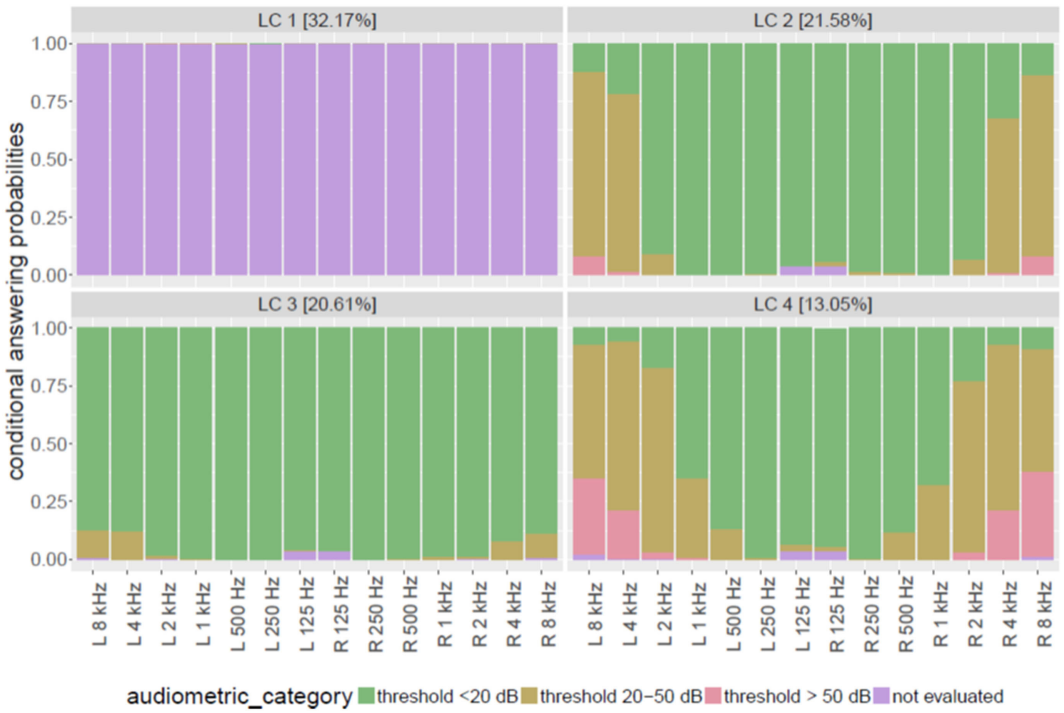

FIGURE 1 | **Patterns of hearing loss with high prevalence in tinnitus patients**.

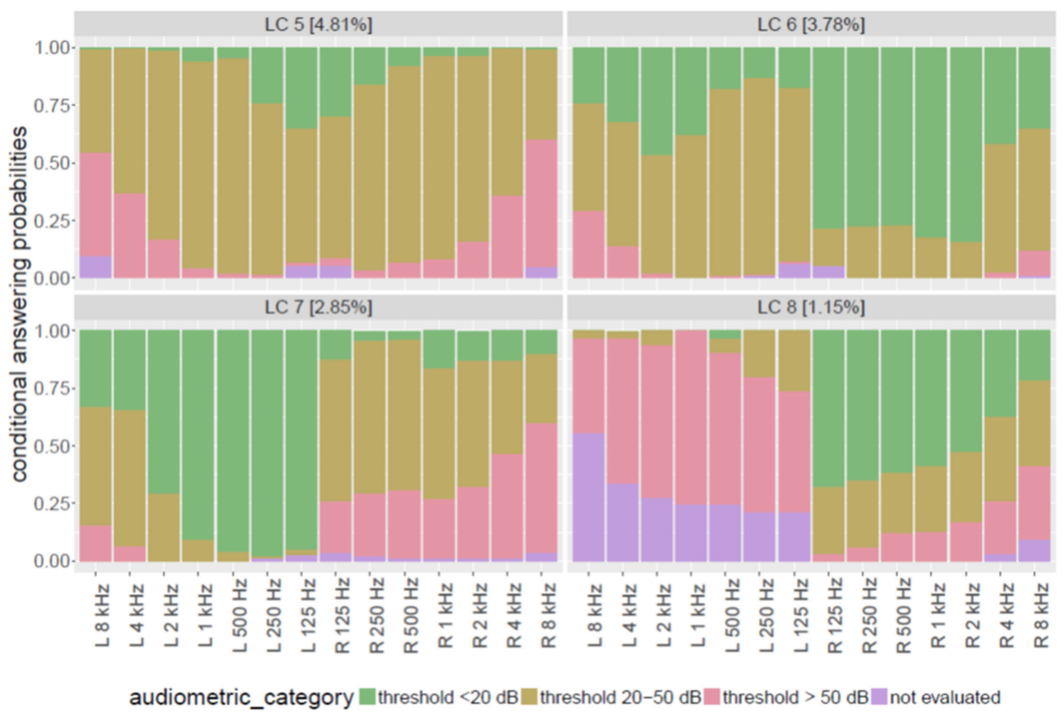

FIGURE 2 | **Patterns of hearing loss with low prevalence in tinnitus patients**.

among tinnitus patients), which may remain insufficiently represented by a classification algorithm in which all tested frequencies are weighted equally.

AUTHOR CONTRIBUTIONS

BL, ML, HF, and UF designed the study; ML, MS, VV, TS, and SS were responsible for data management; HF and UF performed the statistical analysis; BL, ML, WS, and ML interpreted the results; BL and UF drafted the manuscript; all the authors contributed to the manuscript and approved first final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fneur.2017.00046/full#supplementary-material.

REFERENCES

1. Gallus S, Lugo A, Garavello W, Bosetti C, Santoro E, Colombo P, et al. Prevalence and determinants of tinnitus in the Italian adult population. *Neuroepidemiology* (2015) 45:12–9. doi:10.1159/000431376
2. De Ridder D, Elgoyhen AB, Romo R, Langguth B. Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc Natl Acad Sci U S A* (2011) 108:8075–80. doi:10.1073/pnas.1018466108
3. De Ridder D, Vanneste S, Weisz N, Londero A, Schlee W, Elgoyhen AB, et al. An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci Biobehav Rev* (2014) 44C:16–32. doi:10.1016/j.neubiorev.2013.03.021
4. Elgoyhen AB, Langguth B, De Ridder D, Vanneste S. Tinnitus: perspectives from human neuroimaging. *Nat Rev Neurosci* (2015) 16(10):632–42. doi:10.1038/nrn4003
5. Vanneste S, Plazier M, Van der Loo E, Van de Heyning P, Congedo M, De Ridder D. The neural correlates of tinnitus-related distress. *Neuroimage* (2010) 52:470–80. doi:10.1016/j.neuroimage.2010.04.029
6. Baguley D, McFerran D, Hall D. Tinnitus. *Lancet* (2013) 382:1600–7. doi:10.1016/S0140-6736(13)60142-7
7. Tyler R, Coelho C, Tao P, Ji H, Noble W, Gehringer A, et al. Identifying tinnitus subgroups with cluster analysis. *Am J Audiol* (2008) 17:S176–84. doi:10.1044/1059-0889(2008/07-0044)
8. Landgrebe M, Zeman F, Koller M, Eberl Y, Mohr M, Reiter J, et al. The Tinnitus Research Initiative (TRI) database: a new approach for delineation of tinnitus subtypes and generation of predictors for treatment outcome. *BMC Med Inform Decis Mak* (2010) 10:42. doi:10.1186/1472-6947-10-42
9. Witsell DL, Schulz KA, Moore K, Tucci DL. Implementation and testing of research infrastructure for practice-based research in hearing and communication disorders. *Otolaryngol Head Neck Surg* (2011) 145:565–71. doi:10.1177/0194599811406369
10. Langguth B, Kreuzer PM, Kleinjung T, De Ridder D. Tinnitus: causes and clinical management. *Lancet Neurol* (2013) 12:920–30. doi:10.1016/S1474-4422(13)70160-1
11. Shore SE, Roberts LE, Langguth B. Maladaptive plasticity in tinnitus – triggers, mechanisms and treatment. *Nat Rev Neurol* (2016) 12:150–60. doi:10.1038/nrneurol.2016.12
12. Hoffman HJ, Reed GW. Epidemiology of tinnitus. In: Snow JB, editor. *Tinnitus: Theory and Management*. London: BC Decker (2004). p. 16–41.
13. Eggermont JJ, Roberts LE. The neuroscience of tinnitus. *Trends Neurosci* (2004) 27:676–82. doi:10.1016/j.tins.2004.08.010
14. Turner JG. Behavioral measures of tinnitus in laboratory animals. *Prog Brain Res* (2007) 166:147–56. doi:10.1016/S0079-6123(07)66013-0
15. Norena A, Micheyl C, Chery-Croze S, Collet L. Psychoacoustic characterization of the tinnitus spectrum: implications for the underlying mechanisms of tinnitus. *Audiol Neurootol* (2002) 7:358–69. doi:10.1159/000066156
16. Schecklmann M, Vielsmeier V, Steffens T, Landgrebe M, Langguth B, Kleinjung T. Relationship between audiometric slope and tinnitus pitch in tinnitus patients: insights into the mechanisms of tinnitus generation. *PLoS One* (2012) 7:e34878. doi:10.1371/journal.pone.0034878
17. Shekhawat GS, Searchfield GD, Stinear CM. The relationship between tinnitus pitch and hearing sensitivity. *Eur Arch Otorhinolaryngol* (2014) 271:41–8. doi:10.1007/s00405-013-2375-6
18. Stebbins WC, Hawkins JE Jr, Johnson LG, Moody DB. Hearing thresholds with outer and inner hair cell loss. *Am J Otolaryngol* (1979) 1:15–27. doi:10.1016/S0196-0709(79)80004-6
19. von Davier M. *WINMIRA—A Program System for Analyses with the Rasch-Model, with the Latent Class Analysis and with the Mixed-Rasch Model*. Kiel: IPN (1999).
20. Goebel G, Hiller W. [The tinnitus questionnaire. A standard instrument for grading the degree of tinnitus. Results of a multicenter study with the tinnitus questionnaire]. *HNO* (1994) 42(3):166–72. German.
21. Vielsmeier V, Lehner A, Strutz J, Steffens T, Kreuzer PM, Schecklmann M, et al. The relevance of the high frequency audiometry in tinnitus patients with normal hearing in conventional pure-tone audiometry. *Biomed Res Int* (2015) 2015:302515. doi:10.1155/2015/302515
22. Weisz N, Hartmann T, Dohrmann K, Schlee W, Norena A. High-frequency tinnitus without hearing loss does not mean absence of deafferentation. *Hear Res* (2006) 222:108–14. doi:10.1016/j.heares.2006.09.003
23. Kujawa SG, Liberman MC. Synaptopathy in the noise-exposed and aging cochlea: primary neural degeneration in acquired sensorineural hearing loss. *Hear Res* (2015) 330:191–9. doi:10.1016/j.heares.2015.02.009
24. Mazurek B, Olze H, Haupt H, Szczepek AJ. The more the worse: the grade of noise-induced hearing loss associates with the severity of tinnitus. *Int J Environ Res Public Health* (2010) 7:3071–9. doi:10.3390/ijerph7083071
25. Konig O, Schaette R, Kempter R, Gross M. Course of hearing loss and occurrence of tinnitus. *Hear Res* (2006) 221:59–64. doi:10.1016/j.heares.2006.07.007

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Decreased Speech-In-Noise Understanding in Young Adults with Tinnitus

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Objectives: Young people are often exposed to high music levels which make them more at risk to develop noise-induced symptoms such as hearing loss, hyperacusis, and tinnitus of which the latter is the symptom perceived the most by young adults. Although, subclinical neural damage was demonstrated in animal experiments, the human correlate remains under debate. Controversy exists on the underlying condition of young adults with normal hearing thresholds and noise-induced tinnitus (NIT) due to leisure noise. The present study aimed to assess differences in audiological characteristics between noise-exposed adolescents with and without NIT.

Methods: A group of 87 young adults with a history of recreational noise exposure was investigated by use of the following tests: otoscopy, impedance measurements, pure-tone audiometry including high-frequencies, transient and distortion product otoacoustic emissions, speech-in-noise testing with continuous and modulated noise (amplitude-modulated by 15 Hz), auditory brainstem responses (ABR) and questionnaires.Nineteen students reported NIT due to recreational noise exposure, and their measures were compared to the non-tinnitus subjects.

Results: No significant differences between tinnitus and non-tinnitus subjects could be found for hearing thresholds, otoacoustic emissions, and ABR results.Tinnitus subjects had significantly worse speech reception in noise compared to non-tinnitus subjects for sentences embedded in steady-state noise (mean speech reception threshold (SRT) scores, respectively −5.77 and −6.90 dB SNR; $p = 0.025$) as well as for sentences embedded in 15 Hz AM-noise (mean SRT scores, respectively −13.04 and −15.17 dB SNR; $p = 0.013$). In both groups speech reception was significantly improved during AM-15 Hz noise compared to the steady-state noise condition ($p < 0.001$). However, the modulation masking release was not affected by the presence of NIT.

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Conclusions: Young adults with and without NIT did not differ regarding audiometry, OAE, and ABR.However, tinnitus patients showed decreased speech-in-noise reception. The results are discussed in the light of previous findings suggestion NIT may occur in the absence of measurable peripheral damage as reflected in speech-in-noise deficits in tinnitus subjects.

Keywords: noise-induced tinnitus, homeostatic plasticity, young adults, recreational noise exposure, ABR, speech-in-noise testing, otoacoustic emissions, speech-in-noise understanding

INTRODUCTION

Due to the large amount of social activities in which adolescents are exposed to high music levels such as concerts, night clubs, sports events, pubs, bars, etc., the younger population is at risk to develop noise-induced symptoms such as hearing loss, hyperacusis, and tinnitus (Smith et al., 2000; Serra et al., 2005; Beach et al., 2013) of which noise-induced tinnitus (NIT) is the symptom most frequently reported by adolescents (Widen and Erlandsson, 2004; Gilles A. et al., 2013). Tinnitus is often perceived temporarily after noise exposure, usually disappearing within a few hours. Prevalence numbers of temporary tinnitus vary from 45 to 85% depending on the definition of temporary tinnitus used by the authors (Mercier and Hohmann, 2002; Chung et al., 2005; Quintanilla-Dieck et al., 2009; Gilles A. et al., 2013). However, a significant amount of young people perceive permanent NIT for which prevalence numbers range from 3 to 15% (Widen and Erlandsson, 2004; Gilles A. et al., 2013). Several studies suggest that the amount of young people suffering from noise-induced symptoms has increased over the years (Niskar et al., 1998, 2001; Henderson et al., 2011).

Often, tinnitus is perceived in the absence of measurable hearing loss (Schaette and McAlpine, 2011). Spiral ganglion neurons are the first neural structures of the auditory system. The ganglion is almost entirely (95%) composed of type I neurons which receive synaptic input from a single inner hair cell. On the other hand, each inner hair cell forms synapses with 10–30 type I neurons (Davis and Liu, 2011). Previous research has shown that the characteristics of the sensory receptors largely determine the fundamental parameters but that the intrinsic properties of primary afferent neurons also contribute (Scroggs and Fox, 1992). Spiral ganglion neurons possess tonotopic specializations due to the tonotopically varying soma and axon diameter of putative type I neurons with the largest neurons situated toward the basal regions (Liberman and Oliver, 1984). As such, frequency coding and intensity coding is modulated by the spiral ganglion neurons by respectively gradation of the spiral ganglion neuron soma along the cochlear axis and spiral ganglion axon diameter variations around the inner hair cell circumference (Davis and Liu, 2011). Kujawa and Liberman presented an animal model showing that noise exposure caused suprathreshold response decrements (measured by auditory brainstem responses) while auditory threshold sensitivity recovered. In addition, degeneration of both the pre- and post-synaptic elements of the inner hair cell occurred throughout the basal part of the cochlea despite normal hair cell populations. It was shown that the loss of peripheral terminals of the cochlear neurons occurs almost instantly after noise exposure but that cell death and disappearance of the somata were extremely slow with a decrease of spiral ganglion cells of around 50% over a time period of 2 years (Kujawa and Liberman, 2009).

Pure-tone audiometry and otoacoustic emissions (OAEs) are well-known audiological measurements used in the daily clinical practice investigating the presence of hearing damage. Sometimes, auditory brainstem responses (ABR) are also measured in order to provide more information. However, the sensitivity of these tests in an early stage of noise damage is still under debate as it is suggested that OAEs and ABR thresholds are sensitive metrics of hair cell damage, they are insensitive to neuronal degeneration in cases loss of cochlear neurons occur in the absence of hair cell loss (Kujawa et al., 1995). Lately the focus has been on the use of speech tests for this purpose. The sensitivity of speech tests in quiet and in noise might have a higher sensitivity for subtle changes in hearing function (Jansen et al., 2013). Some studies reported that subjects with sensorineural hearing loss have worse speech reception compared to normal-hearing people during speech-in-noise testing using a steady state noise (Bacon et al., 1998; Lorenzi et al., 2006a). The use of amplitude-modulated noise during speech-in-noise testing might add important information concerning the underlying pathology in subclinical noise-induced damage. Many mechanisms are involved in the phenomenon of the so-called "masking release effect" but it is well known that "dip listening" plays an important role (Fullgrabe et al., 2006). Dip listening or "valley listening" comprises the ability to take advantage of relatively short temporal minima in the fluctuating background noise to detect important speech cues and normal hearing subjects benefit from it to a greater extent than hearing impaired persons (Festen and Plomp, 1990; Gustafsson and Arlinger, 1994; Dubno et al., 2002; Fullgrabe et al., 2006). It was suggested that this loss of ability may be attributed to an impaired temporal resolution in the hearing function of the patient group. However, studies have shown that the reduction of masking release is rather caused by impaired suprathreshold processing of the temporal and spectral domain such as abnormal coding of temporal fine-structure information and degraded frequency selectivity, rather than less-than-normal audibility (Gustafsson and Arlinger, 1994; Fullgrabe et al., 2006; Lorenzi et al., 2006a,b). Furthermore, some studies focused on the effects of tinnitus on speech reception in noise. Newman et al. showed that a group of hearing impaired tinnitus patients had significantly worse speech

reception abilities compared to a control group with similar hearing impairment (Newman et al., 1994). In addition, the presence of tinnitus in the deaf ear of patients with single-sided deafness and tinnitus in the deaf ear, also affects speech reception in the non-tinnitus ear (Mertens et al., 2013). It is however still unclear whether subjects with NIT (even with normal hearing thresholds) also show decreased masking release during speech-in-noise testing. To our knowledge, amplitude-modulated noise has not yet been used in the assessment of early noise-induced hearing damage.

To date, little is known about the early signs of noise damage and it is unclear which audiological tests can detect and localize early noise-induced damage in adolescents caused by recreational noise exposure. Therefore, an extensive test protocol on a group of students was performed comprising: otoscopy, reflex measurements, tympanometry, pure-tone audiometry including high-frequency audiometry, otoacoustic emissions, speech-in-noise testing (with two types of noise masker: continuous and amplitude modulated), auditory brainstem responses, and tinnitus analysis and questionnaires in cases tinnitus was present (see **Table 1**). The present study aimed to reveal early signs of recreational noise damage in noise-exposed young adults by use of audiological tests available in clinical settings. It is hypothesized that young adults with tinnitus show more peripheral deficit of the auditory system compared to non-tinnitus subjects which might be expressed by poorer auditory thresholds, decreased or absent otoacoustic emissions and/or deviating ABR results. Furthermore, it might be the case that tinnitus subjects perform worse compared to the control group at a suprathreshold level reflected in poorer masking release during speech-in-modulated-noise testing.

METHODS

A test protocol was developed in order to detect early-stage noise-induced damage in a young population in an early stage. The test protocol comprised the following audiological measurements: otoscopy, impedance and reflex measurements, pure-tone audiometry (including high-frequency audiometry), tinnitus analysis (in cases where tinnitus was present), otoacoustic emissions, speech-in-noise testing, auditory brainstem responses and questionnaires concerning hyperacusis and tinnitus (when present). This study was approved by the ethics committee of the University Hospital Antwerp (identification: 11/12/108). Written informed consent was obtained of all subjects. The original informed consents were archived and added to the personal medical document of the patients.

TABLE 1 | Demographic information concerning the study subjects.

	Mean age (years)	Total of subjects (N)	Tinnitus (N)	No tinnitus (N)
Male	23.1 ± 3.9	23	11	12
Female	23.5 ± 1.9	64	8	56

Subjects

Subjects were recruited by sending an email for participation to all Medicine students of the University Antwerp ($N = 650$) of which 91 students replied for participation. Exclusion criteria were: the presence of pulsatile tinnitus, middle ear pathology, known neurologic diseases, history of depression, and asymmetric sensorineural hearing loss. Students had to be below the age of 30 years old and should attend parties, concerts or festivals on a weekly basis. It was however stated that students could not attend such events 2 days prior to the testing date in order to control for temporary symptoms at the time of testing. A brief and limited questionnaire concerning tinnitus presence, tinnitus etiology, and noise exposure was answered by all subjects. Nineteen students perceived permanent tinnitus defined as tinnitus present for more than 3 months at the time of testing. All tinnitus subjects indicated recreational noise exposure as the most likely causal factor of their tinnitus (next to other possibilities: occupational noise exposure, head trauma, recurrent middle ear infections, other). Students were only included when going to a party/concert for at least once a week and/or when they used personal listening devices (PLDs) several times a week at a volume level of 70% or more of the maximum capacity of the device.

In total, four students were excluded from the study due to middle ear pathology. **Table 1** shows the demographic data on the test population. The following paragraphs elucidate on the methodology of the various audiological tests performed in the current protocol. ABR testing and speech-in-amplitude-modulated-noise (AM noise) was added to the test protocol in a later phase. As such, after initial testing, all participants were invited a second time for additional testing. Fifty two percent of the participants returned for the second testing moment. **Table 2** provides information on the amount of subjects who underwent the various audiological measurements. In all cases the ear of each participant with the worst PTA was included for statistical analysis. In the control group (non-tinnitus group) 43 subjects showed the worst PTA score on the right side and 25 on the left side. In the tinnitus group 8 subjects showed the worst PTA score on the right side and 11 on the left side. In the latter group, the included ears also corresponded with the ear in which the tinnitus was (most loudly) perceived.

Pure Tone Audiometry

Pure tone audiometry was measured in all participating subjects according to the clinical standards (ISO 8253-1, 1989) using a two-channel Interacoustics AC-40 audiometer in a soundproof booth. Air conduction thresholds were measured at 125 Hz, 250 Hz, 500 Hz, 1 kHz, 2 kHz, 3 kHz, 4 kHz, 6 kHz, and 8 kHz. In addition, extended HFA was performed including 9, 10, 11.2, 12.5, 14, and 16 kHz. Bone conduction thresholds were measured between 250 Hz and 4 kHz.

The auditory thresholds of tinnitus and non-tinnitus subjects were compared by use of Mann–Whitney U-tests. A $p \leq 0.05$ was considered as significant. Bonferroni–Holm was applied in order to correct for multiple testing. In addition, to test whether the tinnitus group contained a significantly larger number of clinically relevant outliers, the phenotype was recoded into

TABLE 2 | Overview of the audiological test protocol performed in subjects with and without NIT.

Audiological measurement		Number of subjects included	
		Tinnitus	No Tinnitus
Otoscopy		19	68
Impedance and reflex measurement		19	68
Tinnitus analysis		19	n.a.
Pure tone audiometry	Classical	19	68
	High-frequency	19	68
Otoacoustic emissions	TEOAEs	19	68
	DPOAEs	19	68
Speech-in-noise testing	Steady-state noise	19	68
	AM-noise	19	23
Auditory brainstem responses		19	23
Questionnaires	Hyperacusis questionnaire	18	50
	Tinnitus questionnaire	19	n.a.
	Visual analogue scale for tinnitus loudness	19	n.a.

HQ, hyperacusis questionnaire; TQ, Tinnitus Questionnaire and VAS-L; n.a., not applicable.

two groups: normal (<25 dB HL) and hearing loss (≥25 dB HL), and this recoded variable was tested for association with the presence/absence of tinnitus using a Chi Square test or a Fisher's exact test (in cases where the conditions did not fit the requirements for the Chi Square test).

Tinnitus Analysis and Questionnaires

To rate the personal tinnitus disturbance two instruments were used: the Tinnitus Questionnaire (TQ) for annoyance grading and a Visual Analogue Scale (VAS) for loudness grading. The TQ is an instrument which differentiates between emotional and cognitive distress, auditory perceptual difficulties, and self-experienced intrusiveness caused by the tinnitus (Goebel and Hiller, 1994). Looking at the total score going from 0 to 84, subjects are assigned to a distress category: slight (score = 0–30, grade 1), moderate (score = 31–46, grade 2), severe (score = 47–59, grade 3), and very severe (score = 60–84, grade 4). In the present study, a Dutch validated version of the TQ was used (41). In addition, tinnitus loudness was also assessed by a VAS (VAS-L) going from 0 to 10 (0 = tinnitus is not heard at all, 10 = an extremely loud tinnitus).

The type of tinnitus was evaluated by asking whether one perceived a pulsatile or non-pulsatile tinnitus, whether the tinnitus was perceived constantly or not, unilaterally or bilaterally, and whether the tinnitus sound was a pure-tone (ringing), a noise (hissing) or a mixture of different sounds (polyphonic). Tinnitus duration was questioned during the tinnitus analysis by asking the participants for how long they have had experienced constant tinnitus.

The presence of hyperacusis was evaluated by a Flemish validated version of Khalfa's Hyperacusis Questionnaire (HQ; Khalfa et al., 2002). According to Khalfa's HQ one is diagnosed with hyperacusis when the score on the HQ is 28 or more. While validating the Dutch version of the HQ, Meeus also compared the HQ scores with other hyperacusis measurements and found one can already speak of clinically relevant hyperacusis with a score of 22 on the Flemish HQ (Meeus et al., 2010). Therefore, the score of 22 was applied as a cut-off score for the presence of hyperacusis in the present study.

Otoacoustic Emissions

Transient evoked otoacoustic emissions (TEOAEs) as well as distortion product otoacoustic emissions (DPOAEs) were measured in all subjects. TEOAEs were elicited using biphasic click sounds of 80 μs presented at an intensity level of 80 dB SPL and recorded over a frequency range of 500–4000 Hz.

DPOAEs were elicited by use of a pair of two pure tone frequencies (f1 and f2) closely spaced and presented simultaneously at a level of 55 dB SPL for f1 and 65 dB SPL for f2 (frequency ratio f2/f1 = 1.22). The largest and most robust distortion product is 2f1–f2 and can be detected in almost all normal ears.

Non-parametric tests (Mann–Whitney U) were applied to assess possible differences between tinnitus subjects and non-tinnitus subjects for TEOAEs as well as DPOAEs. A $p \leq 0.05$ was considered as significant and the Bonferroni–Holm method was used to correct for multiple testing. In addition, to test whether the tinnitus group contained a significantly larger number of clinically relevant TEOAE and DPOAE outliers (SNR <3 dB and SNR <6 dB, respectively), the phenotype was recoded into two groups. For TEOAE analysis OAEs were considered as present when the SNR exceeded 3 dB and were considered as absent when the SNR was below 2.99 dB. For DPOAE analysis OAE were considered present when the SNR ≥6 dB. The presence/absence of TEOAEs/DPOAEs were tested for association with the presence/absence of tinnitus using a Pearson Chi Square test or a Fisher's exact test (in cases where the conditions did not fit the requirements for the Chi Square test).

Speech-in-Noise Testing

The Leuven Intelligibility Sentence Test (LIST; Van Wieringen and Wouters, 2008), a Dutch sentence test, was applied. The LIST consists of 35 lists of 10 sentences that are a reflection of daily communication and are of equivalent difficulty. An adaptive procedure is used with the noise at a fixed level of 65 dB SPL. The procedure starts at a signal-to-noise ratio (SNR) of 0 dB meaning that speech and noise are presented equally loud (65 dB SPL). Subsequently, the intensity level within a list of sentences is varied in steps of 2 dB adaptively in a 1-down (when the keywords in the sentence are correctly repeated), 1-up (when the keywords in the sentence are incorrectly repeated) procedure to determine the 50% correct identification point which is called the speech reception threshold (SRT), expressed in dB SNR. Before starting the actual procedure, one list was performed as a training list. Speech reception was calculated as the mean SNR obtained by the subject. For example, a score of −5 dB SNR means that the

speech could be 5 dB quieter than the noise which is fixed at 65 dB SPL.

During the presentation of the sentences two kinds of noise masker were applied. A first noise masker was a steady-state noise spectrally matched with the long-term average speech spectrum so that the SNR would be, on average, approximately equal at all frequencies (Van Wieringen and Wouters, 2008). For the other masker used during the second testing moment, the stationary noise was amplitude-modulated by 15 Hz with a modulation depth of 100%, from now on referred to as AM noise.

Subjects were seated in a quiet room and the sentences were presented monaurally through headphones. The sentences were played directly from a computer using software interface TigerSpeech Technology (2012) and passed through an audiometer. Sentence levels were adjusted by the software during adaptive testing depending whether the keywords in the sentences were repeated correctly or incorrectly. Also the noise was played from the same software passing through the audiometer and was presented to the ipsilateral ear at a fixed level of 65 dB SPL. The levels of speech and noise were calibrated by a licensed company prior to the commencement of the experiment.

In order to test for normal distributions of the components, Shapiro–Wilk test of normality was applied and Q-Q plots were visually inspected. All variables were normally distributed ($p > 0.05$ for Shapiro–Wilk) in both groups. Consequently, parametric tests (student-t) were performed in order to find differences between speech reception in tinnitus and non-tinnitus subjects for steady-state noise as well as AM noise. In addition, parametric tests were performed to reveal possible differences in performance between speech reception during stationary noise and during AM noise for tinnitus as well as non-tinnitus subjects separately. Bonferroni–Holm correction was applied to correct for multiple testing.

In addition, a repeated measures ANOVA analysis was performed in order to see whether there was a different masking release effect of AM-noise for tinnitus subjects and non-tinnitus subjects when compared to the steady-state noise condition.

Auditory Brainstem Responses

Auditory brainstem responses (ABR) were recorded in a subset of tinnitus and non-tinnitus patients with the Bio-Logic Auditory Evoked Potentials system (version 6.2.1.1) and a Bio-Logic Navigator Pro® interface. The skin was prepared by use of a Nuprep gel in order to lower the skin impedance which had to be below 5 kOhm and inter-electrode impedance had to be below 2 kOhm. Electrodes were placed on both mastoids and on the high forehead with the common electrode on the lower forehead. Subjects laid down on a comfortable bed and the light was subdued. Subjects were also instructed to keep the eyes closed during the measurements and to minimize all muscle activity as much as possible.

100 μs-duration clicks were presented with alternating polarity through ER-3A earphones at a rate of 31.0 stimuli/s and a level of 80 dBnHL (= dB normalized hearing level). Contralateral white noise masking was applied with an intensity of 55 dBnHL. The signal was high pass filtered from 100 Hz and low pass filtered from 3000 Hz. Artifact rejection was set at 23.8 μV and a maximum of 2000 averages was recorded. In order to obtain the best possible outcome of the ABR testing, all recordings were repeated 3 times to ensure reproducibility.

ABR component amplitudes (baseline-to-peak) and latencies were determined by visual inspection of the waveforms I–V. Wave V is the most robust waveform in an adult population. Other waveforms may not always occur or be accurately identified by the clinician. In addition, latency ratios I–III, I–V, and III–V were calculated as well as the interpeak ratios I–III, I–V, and III–V.

Previous research has shown that the exact matching for pure-tone audiometry thresholds (with maximum differences of 5 dB HL) of groups is a prerequisite in order to be able to correctly analyze ABR data (Gu et al., 2012). As such, two separate analyses were performed. The first analysis was based on the data matched at a group level. Subsequently, a more precise matching was performed where every tinnitus subject was age- and gender-matched as well as matched until the level of 5 dB HL for pure-tone thresholds from 1 to 4 kHz (as this is the maximum frequency spectrum tested by the ABR). Therefore, in the second analysis, less participants were included to ensure the exact matching and only 10 pairs of perfectly matched subjects were obtained. The latter analysis was performed in addition to the group analysis in order to obtain reliable results. If Shapiro–Wilk test for normality showed normal data distributions, parametric testing was used.

RESULTS

Nineteen subjects had permanent tinnitus, corresponding with 22% of the sample. Noise exposure was briefly evaluated by a short questionnaire. All students with tinnitus attributed their tinnitus to recreational noise exposure. Party/concert attendance of students was approximately once a week. Sixty four percent of the control group and 52% of the tinnitus group attended a musical venue more than once a week.

Details on the tinnitus type, side and severity are shown in **Tables 3, 4**. Most young adults perceived a bilateral, pure-tone tinnitus with only limited tinnitus distress (on average a grade 1 on the TQ). The mean tinnitus duration was 2 years ($SD = 1.2$ years) and none of the tinnitus subjects reported tinnitus from childhood. The HQ was filled out by most subjects showing a clear difference between non-tinnitus and tinnitus subjects. Tinnitus subjects scored significantly higher on the HQ (mean = 15.39; $SD = 6.65$) compared to non-tinnitus subjects (mean = 7.71; $SD = 7.96$; $p = 0.001$). Only few subjects were diagnosed with hyperacusis though this symptom was relatively more prevalent in the tinnitus group. In the non-tinnitus group ($N = 68$) three subjects had a score >22 on the HQ while already four subjects of the tinnitus group ($N = 19$) had scores above 22. The sample of students with hyperacusis was too small to perform further statistical analysis.

Figures 1, **2** illustrate the median and the variability for the conventional audiometry and the HFA respectively as well as the differences between the tinnitus and non-tinnitus subjects.

TABLE 3 | Distribution of tinnitus type and tinnitus side characteristics assessed in the tinnitus group.

Tinnitus characteristic		*N* subjects
Type	Pure-tone	13
	Noise	6
	Polyphonic	0
Side	Unilateral	2
	Bilateral	16
	Central	1

TABLE 4 | Mean scores for the TQ and VAS-L, including standard deviations for the tinnitus subjects.

Tinnitus questionnaires	Mean	*SD*
TQ score	27.72	15.23
VAS-L score	5.44	2.46

No significant differences in hearing thresholds between tinnitus subjects and non-tinnitus subjects were apparent (see **Table 5**). Some outliers could be noted where the hearing thresholds exceeded 25 dB HL which no longer could be considered as normal hearing. It was investigated whether such outliers were more prevalent in the tinnitus group compared to the controls by use of a Fisher's exact test. Within the power of the current study, there is no indication that the tinnitus group contained a significantly larger fraction of outliers compared to the control group. More insight into the audiometric data is provided in the Supplementary Materials section.

TEOAE and DPOAE were compared between tinnitus and non-tinnitus subjects for each frequency band. **Tables 6, 7** summarize the otoacoustic emissions data which is also plotted in **Figures 3**, **4**. No significant differences in OAE strength were found between groups for the measured TEOAE as well as DPOAE frequency bands. By the use Mann–Whitney *U*-tests to compare OAEs between the tinnitus and the control group it is likely to miss crucial differences between the two groups. In particular, clinically relevant outliers in the case group may be missed, since nonparametric tests transform the observed values into ranks. Therefore, additional dichotomizing was performed where TEOAEs were considered as present when the SNR was equal to or exceeded 3 dB SNR and DPOAEs were considered as present when $\geq$6 dB SNR. Chi square tests and Fisher's exact tests did not show—within the power of the current study—significant differences in the prevalence of present/absent OAEs in both groups. More insight into the latter analyses is provided in the Supplementary Material section.

Concerning the speech-in-noise testing, tinnitus subjects had significantly worse SRT scores compared to non-tinnitus subjects for sentences embedded in steady-state noise (mean SRT scores, respectively −5.77 and −6.90 dB SNR; $p = 0.025$) as well as for sentences embedded in 15 Hz AM-noise (mean SRT scores, respectively −13.04 and −15.17 dB SNR; $p = 0.013$) as illustrated in **Figure 5**. In the repeated measures ANOVA the between subject effect was "group" and the within subject effect was "noise type." In addition, the interaction between noise type and group was investigated. Significant effects were shown for group and speech-in-noise ($p < 0.001$) but no interaction effect was apparent ($p = 0.162$) meaning that the increase of masking release when going from steady-state noise to AM noise were quite similar for both groups with the difference that tinnitus subjects had a worse starting point. Also, a logistic regression was performed in order to explain the variance provided by speech-in-noise testing in NIT subjects. By use of this analysis it was confirmed that speech-in-noise testing by use of steady-state noise was worse in NIT subjects ($p = 0.018$) as well as in AM-15 Hz noise ($p = 0.011$). In addition, it was found that speech-in-noise testing explains 40% of the variance (Nagelkerke $R^2 = 0.403$).

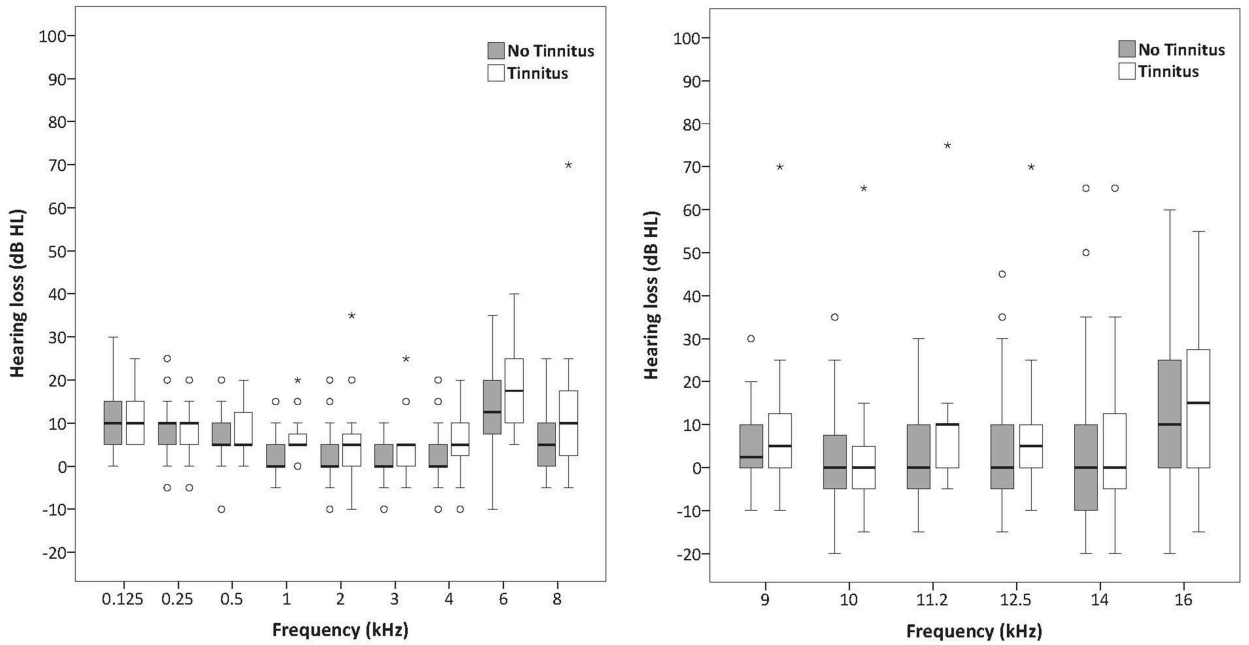
Visual inspection of the ABR waveforms were performed. Wave V is the most robust wave in an adult population. Other waves may not always occur or be accurately identified by the clinician by use of visual inspection. Independent student's *t*-tests were performed in order to reveal differences between the tinnitus and control group for the latency and/or amplitude of the different ABR waveforms. **Table 8** provides an overview of the mean latencies, amplitudes, interpeak latencies/amplitudes, standard deviations, and the outcome of the independent student's *t*-test between tinnitus subjects and controls. After correction for multiple testing by Bonferroni–Holm, no statistical differences could be shown between tinnitus subjects and controls within the power of the current study.

For the second analysis, for which a very thorough matching was applied (see ABR description in the Methods Section), paired student's *t*-tests were performed but again did not show any statistical different ABR results between tinnitus and non-tinnitus subjects concluding the ABR data did not differ between groups in the present dataset.

DISCUSSION AND CONCLUSIONS

Audiological Tests Assessing the Peripheral Pathway

The current study examined a group of 87 recreationally noise-exposed university students. In total 19 students, corresponding to 22% of the study population, experienced NIT which was present for more than 3 months at the time of testing. Earlier epidemiological studies on noise-induced symptoms in Belgian adolescents has shown a high prevalence of NIT in this population in line with the current findings (Gilles et al., 2012; Gilles A. et al., 2013; Degeest et al., 2014). The present study compared test results of various audiological tests between the students with and without tinnitus. The testing consisted of pure-tone audiometry (including high frequencies), otoacoustic emissions, ABR, and speech-in-noise testing (with steady-state noise and amplitude-modulated noise). No significant differences in audiometric thresholds between tinnitus and control subjects could be observed. Most had normal hearing thresholds and the prevalence of outliers (thresholds >25 dB HL) was equal in both groups.



FIGURES 1, 2 | Boxplots representing pure tone audiometry for conventional frequencies (125–8 kHz) and high frequencies (9–16 kHz) for tinnitus subjects and non-tinnitus subjects. The box length is the interquartile range (IQR). A circle represents outliers with values between 1.5 and 3 box lengths from the upper or lower edge of the box. Asterisks represent extreme outliers (more than 3 times the IQR).

Furthermore, TEOAEs, DPOAEs, and ABRs did not show significant differences between the groups. However, speech-in-noise reception was significantly decreased in tinnitus subjects. The following paragraphs discuss these findings in the light of previous findings suggesting the presence of (noise-induced) tinnitus may occur in the absence of measurable peripheral damage and might cause more central plasticity than expected.

It has been suggested that extended high frequency audiometry (HFA) testing might reveal cochlear damage at higher frequencies than investigated by a conventional audiogram (Yildirim et al., 2010; Mehrparvar et al., 2011). Concerning the application of HFA in young people exposed to recreational noise, only limited research has been performed so far. However, it has been shown that, when no signs of noise-induced hearing damage can be detected on the conventional audiometry (125 Hz to 8 kHz), hearing thresholds at the higher frequencies (9–16 kHz) can be significantly increased (Sulaiman et al., 2013). However, the current study shows that adolescents with NIT do not necessarily have decreased hearing thresholds on conventional nor high-frequency audiometry. Also, audiometric outliers (>25 dB HL) were not more prevalent in the tinnitus group compared to controls indicating that there were no significant differences in hearing thresholds between the groups. This fact raises questions concerning the applicability of pure-tone audiometry as an assessment tool for the evaluation of early noise-induced damage. Besides HFA, evidence for the clinical use of OAEs in the early detection of noise-induced damage is growing (Sliwinska-Kowalska and Kotylo, 1997; Prasher and Sulkowski, 1999). It has been suggested that OAEs might reveal outer hair cell damage before it is reflected in the audiogram (Sliwinska-Kowalska and Kotylo, 1997, 2001). Sulaiman et al. showed increased high-frequency thresholds and decreased DPOAE amplitudes in a group of PLD users in the absence of measurable hearing loss between 125 Hz and 8 kHz (Sulaiman et al., 2013). Also McKee and Stephens showed decreased OAEs in tinnitus subjects with normal hearing (McKee and Stephens, 1992). In the current study, TEOAEs as well as DPOAEs were performed in all students but no significant differences between tinnitus and non-tinnitus subjects could be observed. Similar results were obtained in a younger population in a recent study by Sanchez et al. assessing 168 adolescents by use of pure-tone audiometry (250 Hz to 16 kHz), TEOAEs and DPOAEs. 28.6% of the sample experienced permanent tinnitus, 28% sporadic tinnitus and the remaining 43.4% did not have tinnitus. No significant differences were observed between the groups regarding audiometric thresholds and TEOAEs/DPOAEs (Sanchez et al., 2015). Considering the present results as well as findings from previous studies, the use of OAEs in noise-exposed subjects is still under debate. Possibly OAEs might render more information in cases of acute acoustic trauma with temporary threshold shift in order to more precisely investigate specific frequency regions such as the 3–6 kHz region (Buchler et al., 2012). However, as a tool for early noise-induced hearing damage screening, the overall results of studies are rather inconclusive at this point (Shupak et al., 2007), suggesting that the addition of OAE measurements to the golden standard of audiometry, is not

TABLE 5 | Audiometric differences between the tinnitus and the non-tinnitus group for all measured frequencies.

Audiometric frequency	*p*-value (uncorrected)	*p*-value (corrected)
125 Hz	0.52	1.00
250 Hz	0.75	1.00
500 Hz	0.52	1.00
1 kHz	<0.01	0.06
2 kHz	0.24	1.00
3 kHz	0.34	1.00
4 kHz	0.02	0.25
6 kHz	0.07	0.86
8 kHz	0.19	1.00
9 kHz	0.76	1.00
10 kHz	0.95	0.95
11.2 kHz	0.16	1.00
12.5 kHz	0.33	1.00
14 kHz	0.26	1.00
16 kHz	0.64	1.00

Bonferroni-Holm correction was applied for multiple testing correction but uncorrected values are provided as well.

TABLE 6 | Differences in TEOAE band-frequency strength [signal-to-noise ratio (SNR)] between tinnitus and non-tinnitus subjects.

TEOAE band-frequency (kHz)	Median TEOAE strength (dB SNR)		*p*-value (uncorrected)	*p*-value (corrected)
	Tinnitus	No tinnitus		
1	3.90	1.95	0.83	1.0
1.4	6.90	7.50	0.98	0.98
2	7.90	5.20	0.86	1.0
2.8	5.30	5.90	0.63	1.0
4	2.60	3.85	0.25	1.0

Uncorrected p-values are given and were corrected for multiple testing by use of Bonferroni–Holm.

TABLE 7 | Differences in DPOAE band-frequency strength between tinnitus and control subjects.

DPOAE band-frequency (kHz)	Median DPOAE strength (dB SNR)		*p*-value (uncorrected)	*p*-value
	Tinnitus	No tinnitus		
1	6.10	9.10	0.80	0.80
1.4	15.00	12.40	0.15	0.45
2	13.00	10.60	0.28	0.57
2.8	10.40	7.40	0.01	0.07
4	14.20	10.45	0.10	0.41

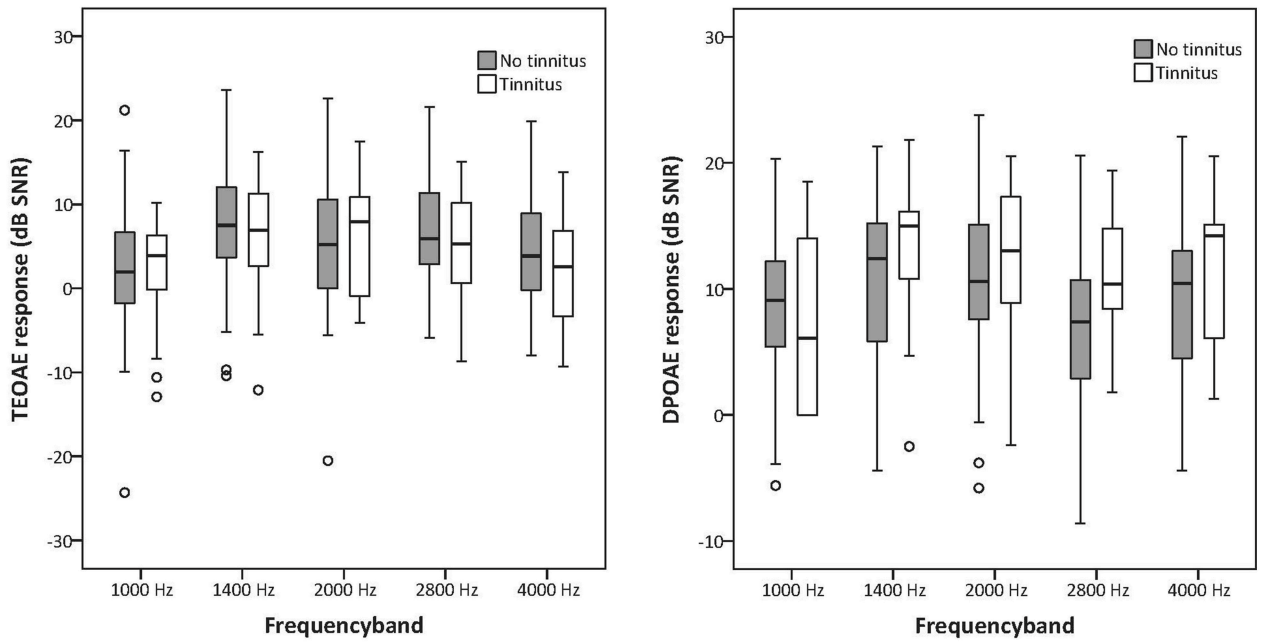
Uncorrected p-values are given and were corrected for multiple testing by use of Bonferroni–Holm.

sufficient in detecting early-staged noise-induced hearing damage.

The current study did not find any differences in ABR results between tinnitus subjects and controls. This is in line with the study by Barnea et al. who performed HFA and ABR testing on a tinnitus group with normal hearing sensitivity in the range of 125 Hz to 8 kHz compared to an age- and gender-matched control group. Similar to the present study, high-frequency and ABR audiometric data did not differ between the considered groups (Barnea et al., 1990). Although not found in the present study, an I–V amplitude ratio alteration was previously reported. Schaette and McAlpine found reduced wave I potentials in normal-hearing female tinnitus subjects but normal amplitude of the more centrally generated wave V. The authors concluded that the deviation of wave I, which is generated by the primary auditory nerve fibers, provides direct evidence for "hidden hearing loss" that manifests as reduced neural output coming from the cochlea followed by renormalization of neural response magnitude within the brainstem reflected by normal wave V amplitudes (Schaette and McAlpine, 2011). Similar results were obtained for male subjects in another study where tinnitus subjects also showed reduced wave I amplitudes but, in addition, enhanced wave V reflecting elevated input to the inferior colliculi. Also elevated I–III and I/V amplitude ratios were apparent implicating disproportionally high activity in spherical bush cells in the ventral cochlear nucleus (Gu et al., 2012). Intergender differences exist in auditory brainstem response amplitudes and latencies (Durrant et al., 1990). Therefore, the current study also investigated possible differences in ABR results within male and female subjects for tinnitus subjects vs. controls (see also Supplementary Material). No gender differences were apparent in the current data set. However, it has to be pointed out that the control group contained more female subjects than males and therefore these results should be interpreted with caution.

Peripheral vs. Central Deficits

The deteriorating effects of hearing loss on speech reception in noise have been thoroughly investigated in previous research (Festen and Plomp, 1990; Bacon et al., 1998; Peters et al., 1998; Bernstein and Grant, 2009; Rhebergen et al., 2010). The decreased release of masking that occurs in hearing impaired subjects could only be partly explained by reduced audibility (Bacon et al., 1998). The ability to correctly detect the temporal fine structure of speech (Fullgrabe et al., 2006; Lorenzi et al., 2006a) and to process spectral details (Nelson et al., 2003; Nelson and Jin, 2004), seems critical for dip-listening. As loss of ability to use temporal and spectral cues in speech is highly associated with sensorineural hearing loss (Bacon et al., 1998) the question arises why speech reception in noise is also sometimes decreased in normal hearing subjects in the absence of hearing loss (Middelweerd et al., 1990). In the present study, tinnitus subjects had significantly worse speech reception in steady-state noise as well as in AM noise. Ryu et al. also found decreased speech perception ability (in quiet as well as in noise) in normal-hearing tinnitus subjects compared to controls (Ryu et al., 2012) in line with earlier findings by Huang et al. (2007). A recent study including tinnitus subjects with and without hearing loss and a control group, measured spectral and temporal



FIGURES 3, 4 | Boxplots representing TEOAE and DPOAE data for tinnitus subjects and controls. The box length is the interquartile range (IQR). A circle represents outliers with values between 1.5 and 3 box lengths from the upper or lower edge of the box.

resolution as well as speech-in-noise reception. No significant differences between tinnitus subjects and controls were found concerning spectral and temporal abilities. However, SRT scores were significantly worse in tinnitus subjects (Moon et al., 2015). It is discussed that normal temporal and spectral resolution in tinnitus subjects reflect the undisturbed functionality of OHCs in the cochlea. In case of cochlear damage, the basilar membrane response would be more linear and broadly tuned resulting in reduced compression and broadening of the auditory filters which would negatively affect both frequency selectivity and temporal resolution (Moore and Glasberg, 1986; Oxenham and Bacon, 2003). The findings of decreased SRT scores in the absence of temporal/spectral resolution deficits imply that tinnitus can occur without OHC damage and might depend more on plastic changes in the central auditory system (Moon et al., 2015). The present results confirm the latter findings as no differences in peripheral functioning, tested by pure-tone audiometry, and OAEs, could be observed. In this study however, spectral and temporal resolution was not specifically tested but speech-in-amplitude-modulated noise testing gives a robust idea of temporal encoding when listening in the gaps. Speech reception in AM noise was significantly worse in tinnitus patients compared to controls but to the same extent as speech reception in steady-state noise. Hence, no additional temporal deficits are apparent in the tinnitus group. It can be suggested that speech reception testing in AM noise does not add useful information to the classical speech reception test in steady-state noise. However, in line with previous findings by Moon et al. the suggestion can be made that the decreased speech reception in subjects with NIT, in the absence of measurable cochlear lesions, might be due to a more central deficit.

The presence of tinnitus in the absence of measurable cochlear hearing loss, as is the case in the present study, forms a serious challenge to the model of cortical hyperactivity. However, Kujawa and Liberman earlier reported that in an animal experiment 50–60% of the auditory nerve fibers in the high-frequency region of the cochlea were deafferented after mild acoustic trauma without any permanent auditory threshold elevation suggesting that normal hearing thresholds do not necessarily exclude the possibility of cochlear damage (Kujawa and Liberman, 2009). It is posited that acoustic overexposure can produce a rapid and irreversible loss of cochlear nerve peripheral terminals on IHCs and a slow degeneration of spiral ganglion cells, despite full recovery of cochlear thresholds and no loss of IHCs or OHCs (Kujawa and Liberman, 2009; Lin et al., 2011). The phenomenon of noise-induced cochlear neuronal degeneration in mice independent of auditory threshold changes, described by Kujawa and Liberman is recently described as "cochlear synaptopathy" (Liberman et al., 2015). Recently, it has been shown that a deficiency in pejvakin protein can cause exceptional vulnerability to sound as pejvakin deficient cochleae exhibit features of marked oxidative stress and impaired antioxidant defenses (Delmaghani et al., 2015). Although aforementioned studies are animal studies, a study by Weisz et al. supported the presence of deafferentiation in the absence of audiometrically detectable hearing loss in humans with tinnitus by use of the Threshold Equalizing Noise test (TEN test; Weisz et al., 2006). However, these results were not confirmed in one of our

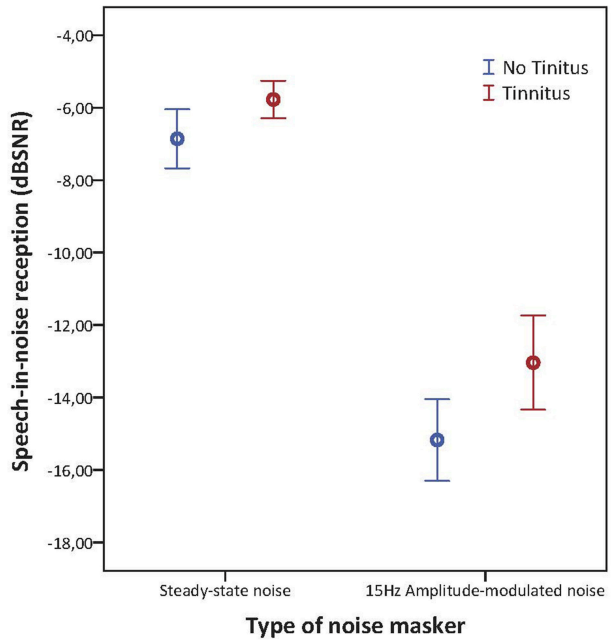


FIGURE 5 | Error barchart illustrating speech-in-noise reception in steady state noise and AM-15 Hz noise of tinnitus and non-tinnitus subjects. The bars represents the 95% confidence interval and the standard error is depicted by the whiskers.

previous studies (Gilles A. D. et al., 2013). The authors believe that in case of presence of cochlear synaptopathy or pejvakin deficiency in the tinnitus subjects, ABR data of the tinnitus subjects would have shown amplitude and latency decrements which were not apparent in the present study. Not-withstanding, the results of the current study does not exclude the possible interference of (currently unmeasurable) peripheral damage in NIT.

However, it can also be argued that when all audiometric findings show normal results in NIT subjects, retrocochlear deficits are present which result into the tinnitus percept on one hand but may also influence performance on a broader scale. The neural correlates of tinnitus have been described as auditory as well as non-auditory (Langers and Melcher, 2011; Langguth et al., 2012; Vanneste and De Ridder, 2012) suggesting that speech performance may also be altered as a consequence of cortical reorganization. It was shown by a previous study that brainstem responses evoked by speech in subjects with speech message decoding difficulties, may reveal miscoding in subcortical structures as an origin (Kraus and Nicol, 2005). In addition, deficiencies in higher-order cortical networks have been found in tinnitus subjects with normal hearing thresholds (Melcher et al., 2009).

CONCLUSIONS AND FUTURE RESEARCH

The current study examined a group of 87 recreationally noise-exposed university students. The argument can be made that, ideally, also a group of non-exposed students should be included in order to investigate their audiological characteristics and compare them to the subject groups with occasional recreational noise exposure. However, as recreational noise exposure is an undeniable part of the current society, it is rather impossible to find young subjects who did not have any kind of noise exposure during their lifespan. Therefore, the decision was made to only include young adults with a certain amount of noise exposure. Noise exposure was evaluated by use of a very limited questionnaire broadly evaluating the current frequency of social events with high music levels as well as noise exposure caused by PLDs. However, noise exposure during the lifespan was not assessed meaning that, inevitably, there were possibly (small to large) differences in the total amount of noise exposure. As such, it cannot be ruled out by the current analysis that tinnitus subjects did not have more noise exposure. Nevertheless, the scope of the present study was not to investigate nor calculate noise exposure in adolescents but the focus was on the effects of recreational noise on the audiological characteristics. As some students experienced NIT, which can be considered as a symptom of noise damage, this symptom was used to make a comparison in audiological characteristics between tinnitus and non-tinnitus subjects.

No peripheral lesions could be observed in the current study evaluated by pure-tone audiometry, OAEs, and ABR. Speech-in-noise testing however was significantly decreased in tinnitus subjects possibly suggesting more centrally located deficits in tinnitus subjects. In addition, it can be said that cortical reorganization may occur due to frequent exposure to recreational noise exposure in the absence of any measurable peripheral hearing loss. The present article underlines the need for further testing besides the conventional audiometry. The sensitivity of pure-tone audiometry as well as OAE measurements might be insufficient to detect peripheral noise-induced damage at an early stage and one must interpret normal outcome results with this technique with caution. The authors like to mention the theory of "homeostatic plasticity" described by Gourévitch et al. (2014). These authors showed that reversible noise-induced threshold shifts may mask progressive underlying neuropathology that likely has profound long-term consequences on auditory processing. A normal audiogram is considered as the "golden standard." However, although the peripheral parts of the auditory system seem to be functioning normal (expressed by normal audiogram and OAE measurements in the present study), substantial changes may occur in the auditory brain post noise exposure. The mechanism of "central gain" at the level of the auditory brainstem, or more cortically located, causes initially a decrease in synaptic efficacy in central parts of the auditory system in the noise-exposed frequency region. Long-lasting changes in synaptic efficacy after prolonged noise exposure could affect the expression of inhibition (Gourévitch et al., 2014). Gourevitch et al. provided evidence that the peripheral auditory system can be harmed without decreased auditory thresholds and that long-lasting disturbance of the excitation-inhibition balance in the central auditory system may eventually lead to cortical reorganization as a result of homeostatic plasticity (Gourévitch et al., 2014). Homeostatic plasticity mechanisms may regulate

TABLE 8 | overview of the detectability of each wave (= N), mean values for wave latency (in ms) and amplitude (in μV) and standard deviations.

Variable	Group	N	Mean	*SD*	*p*-value for independent *t*-test (uncorrected)	*p*-value for independent *t*-test (corrected)
LATENCY						
Wave I	Controls	23	1.57	0.11	0.63	1.00
	Tinnitus	19	1.60	0.17		
Wave II	Controls	17	2.74	0.17	0.57	1.00
	Tinnitus	7	2.78	0.11		
Wave III	Controls	23	3.75	0.24	0.17	1.00
	Tinnitus	19	3.67	0.18		
Wave IV	Controls	9	5.01	0.16	0.07	0.98
	Tinnitus	5	4.81	0.19		
Wave V	Controls	23	5.51	0.23	0.88	1.00
	Tinnitus	19	5.53	0.33		
INTERPEAK LATENCY						
Wave I–III	Controls	23	2.18	0.17	0.16	1.00
	Tinnitus	19	2.06	0.28		
Wave III–V	Controls	23	1.78	0.12	0.05	0.75
	Tinnitus	19	1.91	0.31		
Wave I–V	Controls	23	3.94	0.18	0.54	1.00
	Tinnitus	19	3.98	0.22		
AMPLITUDE						
Wave I	Controls	23	0.10	0.08	0.12	1.00
	Tinnitus	19	0.14	0.08		
Wave II	Controls	17	0.06	0.06	0.09	1.00
	Tinnitus	7	0.11	0.07		
Wave III	Controls	23	0.20	0.10	0.18	1.00
	Tinnitus	19	0.26	0.15		
Wave IV	Controls	10	0.10	0.06	0.38	1.00
	Tinnitus	5	0.07	0.05		
Wave V	Controls	23	0.23	0.13	0.14	1.00
	Tinnitus	19	0.18	0.07		
INTERPEAK AMPLITUDE						
Wave I–III	Controls	23	0.10	0.15	0.77	1.00
	Tinnitus	19	0.12	0.15		
Wave III–V	Controls	23	0.02	0.14	0.04	0.64
	Tinnitus	19	0.08	0.17		
Wave I–V	Controls	23	0.13	0.19	0.08	1.00
	Tinnitus	19	0.04	0.12		

Corrected and uncorrected p-values are shown for the independent samples t-test.

a central gain mechanism, and tinnitus might be a side-effect of these changes (Norena and Farley, 2013; Brotherton et al., 2015). In addition, it has to be noted that hyperacusis was more prevalent in the tinnitus sample than in the controls. Psychoacoustic measurements in the tinnitus subjects might have rendered additional, important information and it can be considered as a limitation of the study that these measurements were not performed. Further research is required in order to investigate the role of homeostatic plasticity in recreational noise exposure.

In conclusion, speech-in-noise testing forms a reliable and clinically feasible technique to assess noise-induced damage in patients with normal peripheral function but with complaints of NIT. The present study shows promising results concerning the use of speech-in-noise testing in detection of noise-induced damage in adolescents. The use of amplitude-modulated noise however, requires more research in order to further investigate the mechanism of homeostatic plasticity in recreational noise exposure as well as the peripheral involvement. Furthermore, late-auditory evoked potentials might provide additional information on higher-order cortical processing of (speech) sounds (Joos et al., 2014) and might therefore also render useful information in normal-hearing tinnitus subjects.

AUTHOR CONTRIBUTIONS

AG: This author has substantially contributed to the design of the study, the acquisition, analysis, and interpretation of the data, is the main author for drafting the manuscript. WS: This author has substantially contributed to the interpretation of the study and the writing of the manuscript. He agrees to be accountable for all aspects of the work. SR: This author has substantially contributed to the collection of the data and the final version of the manuscript. KW: This author has substantially contributed to the analysis of the data and reviewed the final draft very carefully. EF: This author has substantially contributed to the analysis of the data and reviewed the final draft very carefully. PV: This author was involved in the design, analysis and interpretation of the study and has contributed to the writing of the manuscript. All authors gave final approval of this version to be published as such and agrees to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Bacon, S. P., Opie, J. M., and Montoya, D. Y. (1998). The effects of hearing loss and noise masking on the masking release for speech in temporally complex backgrounds. *J. Speech Lang. Hear. Res.* 41, 549–563. doi: 10.1044/jslhr.4103.549

Barnea, G., Attias, J., Gold, S., and Shahar, A. (1990). Tinnitus with normal hearing sensitivity: extended high-frequency audiometry and auditory-nerve brain-stem-evoked responses. *Audiology* 29, 36–45. doi: 10.3109/00206099009081644

Beach, E., Williams, W., and Gilliver, M. (2013). Estimating young Australian adults' risk of hearing damage from selected leisure activities. *Ear Hear.* 34, 75–82. doi: 10.1097/AUD.0b013e318262ac6c

Bernstein, J. G., and Grant, K. W. (2009). Auditory and auditory-visual intelligibility of speech in fluctuating maskers for normal-hearing and hearing-impaired listeners. *J. Acoust. Soc. Am.* 125, 3358–3372. doi: 10.1121/1.3110132

Brotherton, H., Plack, C. J., Maslin, M., Schaette, R., and Munro, K. J. (2015). Pump up the volume: could excessive neural gain explain tinnitus and hyperacusis? *Audiol. Neurootol.* 20, 273–282. doi: 10.1159/000430459

Büchler, M., Kompis, M., and Hotz, M. A. (2012). Extended frequency range hearing thresholds and otoacoustic emissions in acute acoustic trauma. *Otol. Neurotol.* 33, 1322. doi: 10.1097/MAO.0b013e318263d598

Chung, J. H., Des Roches, C. M., Meunier, J., and Eavey, R. D. (2005). Evaluation of noise-induced hearing loss in young people using a web-based survey technique. *Pediatrics* 115, 861–867. doi: 10.1542/peds.2004-0173

Davis, R. L., and Liu, Q. (2011). Complex primary afferents: what the distribution of electrophysiologically-relevant phenotypes within the spiral ganglion tells us about peripheral neural coding. *Hear. Res.* 276, 34–43. doi: 10.1016/j.heares.2011.01.014

Degeest, S., Corthals, P., Vinck, B., and Keppler, H. (2014). Prevalence and characteristics of tinnitus after leisure noise exposure in young adults. *Noise Health* 16, 26–33. doi: 10.4103/1463-1741.127850

Delmaghani, S., Defourny, J., Aghaie, A., Beurg, M., Dulon, D., Thelen, N., et al. (2015). Hypervulnerability to sound exposure through impaired adaptive proliferation of peroxisomes. *Cell* 163, 894–906. doi: 10.1016/j.cell.2015.10.023

Dubno, J. R., Horwitz, A. R., and Ahlstrom, J. B. (2002). Benefit of modulated maskers for speech recognition by younger and older adults with normal hearing. *J. Acoust. Soc. Am.* 111, 2897–2907. doi: 10.1121/1.1480421

Durrant, J. D., Sabo, D. L., and Hyre, R. J. (1990). Gender, head size, and ABRs examined in large clinical sample. *Ear Hear.* 11, 210–214. doi: 10.1097/00003446-199006000-00008

Festen, J. M., and Plomp, R. (1990). Effects of fluctuating noise and interfering speech on the speech-reception threshold for impaired and normal hearing. *J. Acoust. Soc. Am.* 88, 1725–1736. doi: 10.1121/1.400247

Füllgrabe, C., Berthommier, F., and Lorenzi, C. (2006). Masking release for consonant features in temporally fluctuating background noise. *Hear. Res.* 211, 74–84. doi: 10.1016/j.heares.2005.09.001

Gilles, A., De Ridder, D., Van Hal, G., Wouters, K., Kleine Punte, A., and Van de Heyning, P. (2012). Prevalence of leisure noise-induced tinnitus and the attitude toward noise in university students. *Otol. Neurotol.* 33, 899–906. doi: 10.1097/mao.0b013e31825d640a

Gilles, A., Van Hal, G., De Ridder, D., Wouters, K., and Van de Heyning, P. (2013). Epidemiology of noise-induced tinnitus and the attitudes and beliefs towards noise and hearing protection in adolescents. *PLoS ONE.* 8:e70297. doi: 10.1371/journal.pone.0070297

Gilles, A. D., Ridder, D., and Van de Heyning, P. (2013). No cochlear dead regions detected in non-pulsatile tinnitus patients: an assessment with the threshold equalizing noise (sound pressure level) test. *Noise Health 15*, 129–133. doi: 10.4103/1463-1741.110297

Goebel, G., and Hiller, W. (1994). The tinnitus questionnaire. A standard instrument for grading the degree of tinnitus. Results of a multicenter study with the tinnitus questionnaire. *HNO* 42, 166–172.

Gourevitch, B., Edeline, J. M., Occelli, F., and Eggermont, J. J. (2014). Is the din really harmless? *Long-term effects of non-traumatic noise on the adult auditory system. Nat. Rev. Neurosci.* 15, 483–491. doi: 10.1038/nrn3744

Gu, J. W., Herrmann, B. S., Levine, R. A., and Melcher, J. R. (2012). Brainstem auditory evoked potentials suggest a role for the ventral cochlear nucleus in tinnitus. *J. Assoc. Res. Otolaryngol.* 13, 819–833. doi: 10.1007/s10162-012-0344-1

Gustafsson, H. A., and Arlinger, S. D. (1994). Masking of speech by amplitude-modulated noise. *J. Acoust. Soc. Am.* 95, 518–529. doi: 10.1121/1.408346

Henderson, E., Testa, M. A., and Hartnick, C. (2011). Prevalence of noise-induced hearing-threshold shifts and hearing loss among US youths. *Pediatrics* 127, e39–e46. doi: 10.1542/peds.2010-0926

Huang, C. Y., Lee, H. H., Chung, K. C., Chen, H. C., Shen, Y. J., and Wu, J. L. (2007). Relationships among speech perception, self-rated tinnitus loudness and disability in tinnitus patients with normal pure-tone thresholds of hearing. *ORL J. Otorhinolaryngol. Relat. Spec.* 69, 25–29. doi: 10.1159/000096713

Jansen, S., Luts, H., Dejonckere, P., van Wieringen, A., and Wouters, J. (2013). Efficient hearing screening in noise-exposed listeners using the digit triplet test. *Ear Hear. 34*, 773–778. doi: 10.1097/AUD.0b013e318297920b

Joos, K., Gilles, A., Van de Heyning, P., De, R. D., and Vanneste, S. (2014). From sensation to percept: the neural signature of auditory event-related potentials. *Neurosci. Biobehav. Rev.* 42C, 148–156. doi: 10.1016/j.neubiorev.2014.02.009

Khalfa, S., Dubal, S., Veuillet, E., Perez-Diaz, F., Jouvent, R., and Collet, L. (2002). Psychometric normalization of a hyperacusis questionnaire. *ORL J. Otorhinolaryngol. Relat. Spec.* 64, 436–442. doi: 10.1159/000067570

Kraus, N., and Nicol, T. (2005). Brainstem origins for cortical 'what' and 'where' pathways in the auditory system. *Trends Neurosci.* 28, 176–181. doi: 10.1016/j.tins.2005.02.003

Kujawa, S. G., Fallon, M., and Bobbin, R. P. (1995). Time-varying alterations in the f2-f1 DPOAE response to continuous primary stimulation. *I: response characterization and contribution of the olivocochlear efferents. Hear. Res.* 85, 142–154. doi: 10.1016/0378-5955(95)00041-2

Kujawa, S. G., and Liberman, M. C. (2009). Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. *J. Neurosci.* 29, 14077–14085. doi: 10.1523/JNEUROSCI.2845-09.2009

Langers, D. R., and Melcher, J. R. (2011). Hearing without listening: functional connectivity reveals the engagement of multiple nonauditory networks during basic sound processing. *Brain Connect.* 1, 233–244. doi: 10.1089/brain.2011.0023

Langguth, B., Schecklmann, M., Lehner, A., Landgrebe, M., Poeppl, T. B., Kreuzer, P. M., et al. (2012). Neuroimaging and neuromodulation: complementary approaches for identifying the neuronal correlates of tinnitus. *Front. Syst. Neurosci.* 6:15. doi: 10.3389/fnsys.2012.00015

Liberman, L. D., Suzuki, J., and Liberman, M. C. (2015). Dynamics of cochlear synaptopathy after acoustic overexposure. *J. Assoc. Res. Otolaryngol.* 16, 205–219. doi: 10.1007/s10162-015-0510-3

Liberman, M. C., and Oliver, M. E. (1984). Morphometry of intracellularly labeled neurons of the auditory nerve: correlations with functional properties. *J. Comp. Neurol.* 223, 163–176. doi: 10.1002/cne.902230203

Lin, H. W., Furman, A. C., Kujawa, S. G., and Liberman, M. C. (2011). Primary neural degeneration in the Guinea pig cochlea after reversible noise-induced threshold shift. *J. Assoc. Res. Otolaryngol.* 12, 605–616. doi: 10.1007/s10162-011-0277-0

Lorenzi, C., Gilbert, G., Carn, H., Garnier, S., and Moore, B. C. (2006a). Speech perception problems of the hearing impaired reflect inability to use temporal fine structure. *Proc. Natl. Acad. Sci. U.S.A.* 103, 18866–18869. doi: 10.1073/pnas.0607364103

Lorenzi, C., Husson, M., Ardoint, M., and Debruille, X. (2006b). Speech masking release in listeners with flat hearing loss: effects of masker fluctuation rate on identification scores and phonetic feature reception. *Int. J. Audiol.* 45, 487–495. doi: 10.1080/14992020600753213

McKee, G. J., and Stephens, S. D. (1992). An investigation of normally hearing subjects with tinnitus. *Audiology* 31, 313–317. doi: 10.3109/00206099209072919

Meeus, O. M., Spaepen, M., Ridder, D. D., and Heyning, P. H. (2010). Correlation between hyperacusis measurements in daily ENT practice. *Int. J. Audiol.* 49, 7–13. doi: 10.3109/14992020903160868

Mehrparvar, A. H., Mirmohammadi, S. J., Ghoreyshi, A., Mollasadeghi, A., and Loukzadeh, Z. (2011). High-frequency audiometry: a means for early diagnosis of noise-induced hearing loss. *Noise Health* 13, 402–406. doi: 10.4103/1463-1741.90295

Melcher, J. R., Levine, R. A., Bergevin, C., and Norris, B. (2009). The auditory midbrain of people with tinnitus: abnormal sound-evoked activity revisited. *Hear. Res.* 257, 63–74. doi: 10.1016/j.heares.2009.08.005

Mercier, V., and Hohmann, B. W. (2002). Is electronically amplified music too loud? what do young people think? *Noise Health* 4, 47–55.

Mertens, G., Punte, A. K., De Ridder, D., and Van de Heyning, P. (2013). Tinnitus in a single-sided deaf ear reduces speech reception in the nontinnitus ear. *Otol. Neurotol.* 34, 662–666. doi: 10.1097/mao.0b013e31828779f0

Middelweerd, M. J., Festen, J. M., and Plomp, R. (1990). Difficulties with speech intelligibility in noise in spite of a normal pure-tone audiogram. *Audiology* 29, 1–7. doi: 10.3109/00206099009081640

Moon, I. J., Won, J. H., Kang, H. W., Kim, D. H., An, Y. H., and Shim, H. J. (2015). Influence of tinnitus on auditory spectral and temporal resolution and speech perception in tinnitus patients. *J. Neurosci.* 35, 14260–14269. doi: 10.1523/JNEUROSCI.5091-14.2015

Moore, B. C., and Glasberg, B. R. (1986). Comparisons of frequency selectivity in simultaneous and forward masking for subjects with unilateral cochlear impairments. *J. Acoust. Soc. Am.* 80, 93–107. doi: 10.1121/1.394087

Nelson, P. B., and Jin, S. H. (2004). Factors affecting speech understanding in gated interference: cochlear implant users and normal-hearing listeners. *J. Acoust. Soc. Am.* 115, 2286–2294. doi: 10.1121/1.1703538

Nelson, P. B., Jin, S. H., Carney, A. E., and Nelson, D. A. (2003). Understanding speech in modulated interference: cochlear implant users and normal-hearing listeners. *J. Acoust. Soc. Am.* 113, 961–968. doi: 10.1121/1.1531983

Newman, C. W., Wharton, J. A., Shivapuja, B. G., and Jacobson, G. P. (1994). Relationships among psychoacoustic judgments, speech understanding ability and self-perceived handicap in tinnitus subjects. *Audiology* 33, 47–60. doi: 10.3109/00206099409072954

Niskar, A. S., Kieszak, S. M., Holmes, A., Esteban, E., Rubin, C., and Brody, D. J. (1998). Prevalence of hearing loss among children 6 to 19 years of age: the Third National Health and Nutrition Examination Survey. *JAMA* 279, 1071–1075. doi: 10.1001/jama.279.14.1071

Niskar, A. S., Kieszak, S. M., Holmes, A. E., Esteban, E., Rubin, C., and Brody, D. J. (2001). Estimated prevalence of noise-induced hearing threshold shifts among children 6 to 19 years of age: the Third National Health and Nutrition Examination Survey, 1988-1994, United States. *Pediatrics* 108, 40–43. doi: 10.1542/peds.108.1.40

Noreña, A. J., and Farley, B. J. (2013). Tinnitus-related neural activity: theories of generation, propagation, and centralization. *Hear. Res.* 295, 161–171. doi: 10.1016/j.heares.2012.09.010

Oxenham, A. J., and Bacon, S. P. (2003). Cochlear compression: perceptual measures and implications for normal and impaired hearing. *Ear Hear.* 24, 352–366. doi: 10.1097/01.AUD.0000090470.73934.78

Peters, R. W., Moore, B. C., and Baer, T. (1998). Speech reception thresholds in noise with and without spectral and temporal dips for hearing-impaired and normally hearing people. *J. Acoust. Soc. Am.* 103, 577–587. doi: 10.1121/1.421128

Prasher, D., and Sulkowski, W. (1999). The role of otoacoustic emissions in screening and evaluation of noise damage. *Int. J. Occup. Med. Environ. Health* 12, 183–192.

Quintanilla-Dieck, M. L., Artunduaga, M. A., and Eavey, R. D. (2009). Intentional exposure to loud music: the second MTV.com survey reveals an opportunity to educate. *J. Pediatr.* 155, 550–555. doi: 10.1016/j.jpeds.2009.04.053

Rhebergen, K. S., Versfeld, N. J., de Laat, J. A., and Dreschler, W. A. (2010). Modelling the speech reception threshold in non-stationary noise in hearing-impaired listeners as a function of level. *Int. J. Audiol.* 49, 856–865. doi: 10.3109/14992027.2010.498446

Ryu, I. S., Ahn, J. H., Lim, H. W., Joo, K. Y., and Chung, J. W. (2012). Evaluation of masking effects on speech perception in patients with unilateral chronic tinnitus using the hearing in noise test. *Otol. Neurotol.* 33, 1472–1476. doi: 10.1097/mao.0b013e31826dbcc4

Sanchez, T. G., Oliveira, J. C., Kii, M. A., Freire, K., Cota, J., and Moraes, F. V. (2015). Tinnitus in adolescents: the start of the vulnerability of the auditory pathways. *Codas* 27, 5–12. doi: 10.1590/2317-1782/20152013045

Schaette, R., and McAlpine, D. (2011). Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J. Neurosci.* 31, 13452–13457. doi: 10.1523/JNEUROSCI.2156-11.2011

Scroggs, R. S., and Fox, A. P. (1992). Multiple Ca2+ currents elicited by action potential waveforms in acutely isolated adult rat dorsal root ganglion neurons. *J. Neurosci.* 12, 1789–1801.

Serra, M. R., Biassoni, E. C., Richter, U., Minoldo, G., Franco, G., Abraham, S., et al. (2005). Recreational noise exposure and its effects on the hearing of adolescents. *Part I: an interdisciplinary long-term study. Int. J. Audiol.* 44, 65–73. doi: 10.1080/14992020400030010

Shupak, A., Tal, D., Sharoni, Z., Oren, M., Ravid, A., and Pratt, H. (2007). Otoacoustic emissions in early noise-induced hearing loss. *Otol. Neurotol.* 28, 745–752. doi: 10.1097/MAO.0b013e3180a726c9

Sliwinska-Kowalska, M., and Kotylo, P. (1997). [Is otoacoustic emission useful in the differential diagnosis of occupational noise-induced hearing loss?]. *Med. Pr.* 48, 613–620.

Sliwinska-Kowalska, M., and Kotylo, P. (2001). Otoacoustic emissions in industrial hearing loss assessment. *Noise Health* 3, 75–84.

Smith, P. A., Davis, A., Ferguson, M., and Lutman, M. E. (2000). The prevalence and type of social noise exposure in young adults in England. *Noise Health* 2, 41–56.

Sulaiman, A. H., Husain, R., and Seluakumaran, K. (2013). Evaluation of early hearing damage in personal listening device users using extended high-frequency audiometry and otoacoustic emissions. *Eur. Arch. Otorhinolaryngol.* 8, 710–715.

TigerSpeech Technology (2012). TigerSpeech Technology. Los Angeles: Innovative Speech Software.

Vanneste, S., and De Ridder, D. (2012). The auditory and non-auditory brain areas involved in tinnitus. *An emergent property of multiple parallel overlapping subnetworks. Front. Syst. Neurosci.* 6:31. doi: 10.3389/fnsys.2012.00031

Van Wieringen, A., and Wouters, J. (2008). LIST and LINT: sentences and numbers for quantifying speech understanding in severely impaired listeners for Flanders and the Netherlands. *Int. J. Audiol.* 47, 348–355. doi: 10.1080/14992020801895144

Weisz, N., Hartmann, T., Dohrmann, K., Schlee, W., and Norena, A. (2006). High-frequency tinnitus without hearing loss does not mean absence of deafferentation. *Hear. Res.* 222, 108–114. doi: 10.1016/j.heares.2006.09.003

Widen, S. E., and Erlandsson, S. I. (2004). Self-reported tinnitus and noise sensitivity among adolescents in Sweden. *Noise Health* 7, 29–40.

Yildirim, G., Berkiten, G., Kuzdere, M., and Ugras, H. (2010). High frequency audiometry in patients presenting with tinnitus. *J. Int. Adv. Otol.* 6, 401–407.

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Impairments of Speech Comprehension in Patients with Tinnitus—A Review

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Tinnitus describes the subjective perception of a sound despite the absence of external stimulation. Being a sensory symptom the majority of studies focusses on the auditory pathway. In the recent years, a series of studies suggested a crucial involvement of the limbic system in the manifestation and development of chronic tinnitus. Regarding cognitive symptoms, several reviews addressed the presence of cognitive impairments in tinnitus as well and concluded that attention and memory processes are affected. Despite the importance for social communication and the reliance on a highly functional auditory system, speech comprehension remains a largely neglected field in tinnitus research. This is why we review here the existing literature on speech and language functions in tinnitus patients. Reviewed studies suggest that speech comprehension is impaired in patients with tinnitus, especially in the presence of competing noise. This is even the case in tinnitus patients with normal hearing thresholds. Additionally, speech comprehension measures seem independent of other measures such as tinnitus severity and perceived tinnitus loudness. According to the majority of authors, the speech comprehension difficulties arise as a result of central processes or dysfunctional neuroplasticity.

Keywords: tinnitus, speech comprehension, speech processing, cognition, review, aging

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INTRODUCTION

Impairment of General Cognitive Functions in Tinnitus

Tinnitus describes the perception of a sound in the absence of physical stimulation and is also called a phantom percept. As tinnitus is characterized as a sensory and auditory phenomenon, most studies addressed dysfunctions of the auditory pathways. Several studies suggested that not only peripheral, but also central auditory systems and dysfunctional neuroplastic processes as a consequence of deafferentation contribute to the development of the impairment (Møller, 2007). Consequently, therapeutic approaches focused on the auditory system (e.g., Jastreboff et al., 1996; Eysel-Gosepath et al., 2004; Stein et al., 2016). However, research provided evidence that not only auditory pathways are affected, but also the limbic system which is involved in affective processing (see e.g., Husain, 2016). The support for this was not only provided by imaging studies, but was also gained from clinical reports stressing the high comorbidity of tinnitus with psychiatric disorders (Andersson, 2002).

Reviews on Cognitive Functions

Due to neuroplasticity and the strong linkage of auditory pathways, the limbic system and central processes, it is not surprising that a range of cognitive processes are impaired in tinnitus patients. To date, there are three reviews with each having a slightly different focus (Andersson and McKenna, 2006; Mohamad et al., 2016; Tegg-Quinn et al., 2016).

In their review, Andersson and McKenna (2006) grouped studies in three areas: (1) neuropsychological measures evidenced that control of attention is impaired under conditions when attentional demands are neither high nor low, but average; (2) regarding cognitive biases, selective attention bias was described as an emphasis on disorder-related information (e.g., found in patients suffering from anxiety disorders), while memory bias has been found among depressive patients, who tend to remember negative information better than positive. Such biases were present in tinnitus patients as well, but there may be two subgroups of tinnitus patients with one exhibiting a focus on tinnitus related information (similar to anxiety disorders) and the other displaying memory distortions as seen in depression; and (3) finally, Andersson and McKenna (2006) report the literature on the conscious appraisal of tinnitus (i.e., in terms of remembering and reporting the intrusiveness of tinnitus) and they stress the prominent role of complaints, worries and anxious thoughts.

In a systematic literature search on tinnitus and cognition with a focus on attention and memory, Mohamad et al. (2016) reviewed nine studies. While there is mixed evidence for the notion that working memory and selective attention are affected in tinnitus patients, a clearer picture evolved for executive attention. This function encompasses the engagement and disengagement to a specific stimulus, as well as the switch to a different stimulus to reach a specific goal. These processes enhance processing of relevant stimuli and in turn achieve a better representation of information in working memory. For instance, tinnitus patients were generally slower than controls in Stroop tasks (Andersson et al., 2000; Jackson et al., 2014) and also exhibited a deficit in an "attention network test" which is used as an index measure for executive control (Heeren et al., 2014). Additionally, self-reported tinnitus symptoms correlated with behavioral measures for executive control (Heeren et al., 2014; Jackson et al., 2014). Reviewing this evidence, Mohamad et al. (2016) concluded that there is at least preliminary evidence that tinnitus interferes with executive attention.

Recently Tegg-Quinn et al. (2016) performed a similar systematic review, however, with a broader search criterion and a focus on clinical management of invasive tinnitus. They report 18 studies employing a variety of measures on cognitive functions in tinnitus. Based on nine studies reporting attentional impairments, the authors state that executive control of attention is impaired and impacts on cognitive function in tinnitus patients.

Apart from these reviews, Roberts et al. (2015) also stress the crucial involvement of attentional processes in the generation of chronic tinnitus and provide a model for these dysfunctional processes. In normal hearing persons, neural patterns from actual inputs are compared to predicted representations generated from long-term memory. If the factual and the predicted inputs match, the comparison leads to a cancellation and no mismatch signal is generated. In tinnitus patients, cochlear damage and/or subsequent neuroplastic processes lead to a mismatch between the two inputs and the comparison process draws attention towards the auditory input to generate a better representation of the acoustic environment.

Taken together, several reviews agree that tinnitus is accompanied by cognitive impairments particularly affecting attentional executive functions. Given the reliance of speech comprehension on detailed and stable representations of the acoustic environment, it seems surprising that speech is not a more dominant topic regarding cognitive impairments. This is even more so, when considering the tight connections between attention and language. We will briefly describe these processes before we review the studies on speech, language and tinnitus.

Language Comprehension, Production, Working Memory and Attention

There is a strong link between language and attention at several levels and a comprehensive overview would go far beyond the scope of the current review. The best example for this link is the role of joint attention during language acquisition when infants want that their communication partners devote their attention to the same object in space as they do (Moore and Dunham, 1995). Similarly, speakers generally look at objects before they name them and so the order of naming becomes evident from the order of looking (e.g., Meyer and Dobel, 2004; Dobel et al., 2011) and listeners look at objects that are mentioned by a speaker (e.g., Henderson et al., 2003). Besides this overt behavior, there are earlier states of processing when attention plays an important role. The connection between language comprehension, working memory capacity (WMC) and attention is possibly best captured by the *Ease of Language Understanding* (ELU) model (for a recent overview of the model see Rönnberg et al., 2013). With regard to early encoding of auditory stimuli, the interplay of attention and WMC ensures a fine-tuning of processing even under adverse conditions. Several neurophysiological studies suggest that attentional processes interact with WMC already at subcortical levels (Zouridakis et al., 1998; Sörqvist et al., 2012; Tsuchida et al., 2012). Next to these early effects, the interplay of attention with WMC and their influence on short-term retention was evidenced by studies on persons wearing hearing aids, where short-term memory performance correlated with the degree of hearing impairment under conditions of divided attention (Tun et al., 2009; Rönnberg et al., 2011). Thus, on one hand, hearing aids reduce attentional costs when persons listen to speech in noise and can improve speech comprehension (Rönnberg et al., 2013). On the other hand, tinnitus is likely to put strong demands on speech processing in everyday listening situations, or more explicitly: "signal distortion will tax WMC during speech understanding" (Rönnberg et al., 2013; p. 10).

On this background, i.e., the presence of cognitive and particularly attentional deficits in tinnitus patients and the strong link between attention and speech, we predict that speech comprehension impairments might be a prominent symptom in patients with chronic tinnitus, especially under difficult

listening conditions. Here, we want to substantiate this claim by a systematic review. Second, we want to find out if this impairment is caused by peripheral cochlear damage and is as such a rather trivial phenomenon. Finally, we would like to give some recommendations for future research based on our findings.

METHODS

Search Strategy

A literature search with no date restriction using the search terms (((tinnitus) AND ("speech perception" OR "speech recognition")) NOT (implant* OR transplant*) NOT review NOT Schwannoma NOT cancer NOT sudden NOT Menière) was undertaken in the PubMed and PsycINFO databases as well as the Cochrane Library in September 2016. Additional articles were obtained through the references of studies identified during the initial search (see **Figure 1**). The search resulted in $N = 13$ publications reported below and summarized in **Table 1**.

RESULTS

With regard to our hypothesis, we will briefly outline the overall results (for an overview see **Table 1**). From the 13 studies reported here, 12 evidenced impairments of speech perception in patients suffering from chronic tinnitus. Across studies a variety of tests was used with and without competing noise. Patients were native speakers of rather different languages. As the only exception, Tugumia et al. (2016) did not report impaired speech perception in tinnitus patients. Six studies contrasted speech comprehension under easy (e.g., no competing noise) and difficult conditions. Five of those reported stronger impairments under difficult conditions. In 8 of the 13 studies, the authors interpreted their results in terms of central contributions to the generation of speech comprehension impairments. In the remaining studies, the authors did not comment on this. In the following we will present the reviewed studies in more detail and focus on the hypotheses outlined in the "Introduction" Section.

Newman et al. (1994) investigated the relationship between psychoacoustic measures and speech perception abilities in patients with hearing loss and tinnitus (THL group) compared to patients with hearing loss only (HL group; $N = 23$ in each group). Even though peripheral hearing loss was similar in both groups, the THL group performed worse than the HL group in speech-in-noise tasks, particularly for difficult items. There was no correlation between speech perception abilities and perceived handicap. The authors suggested that tinnitus should be considered as a symptom which may also have central causes rather than only peripheral damage. Hearing loss does not even have to be present for the occurrence of impairment in speech perception as reported by Soalheiro et al. (2012). This study evidenced impaired speech perception measured without competing noise in 81% of almost 300 workers exposed to occupational stress.

Goldstein and Shulman (1999) investigated 25 individuals with severely disturbing tinnitus. About half of these participants (52%) displayed low scores in at least one of the central auditory speech tests. More specifically, in the low-pass filtered speech test and the competing sentence test, patients complaining about interference of tinnitus with daily communication displayed low performance. Similar to Newman et al. (1994), Goldstein and Shulman stressed the contribution of central processes to the generation of chronic tinnitus. Huang et al. (2007) investigated speech perception in 20 chronic tinnitus patients speaking Mandarin. Compared to controls, tinnitus patients displayed generally lower scores in perceiving sentences in noise and, as it appears (though it was not analyzed), this was more so when sentences were hard to predict, i.e., with low contextual cues. As above, these measures did not correlate with tinnitus loudness or disability, again arguing for a contribution of central processes to the symptomatology. A corroborating result for the influence of hearing condition was obtained with 19 Portuguese patients suffering from tinnitus and hyperacusis (Hennig et al., 2011). Compared to normal hearing controls they demonstrated lower abilities to comprehend sentences in noise, but performed normally without noise. Using the Korean version of the hearing in noise test, Ryu et al. (2012) showed that tinnitus patients with normal pure tone audiometry ($N = 20$), thus arguing for no peripheral impairment, performed worse than normal hearing controls, both when sentences were displayed in silence and with competing noise, i.e., there was no influence of hearing condition. Similar findings were reported on 15 Dutch speakers wearing cochlear implants suffering from one-sided tinnitus (Mertens et al., 2013). Importantly, this study evidenced impaired speech-in-noise comprehension in the unaffected ear which argues again for impairment with strong contributions from central processes (note that publications studying patients with hearing aids were excluded from this review; this study was included, however, because the unaffected ear was tested). Similarly arguing for an involvement of central processes, Cuny et al. (2004) reported left hemispheric dominance (i.e., a right ear advantage) in controls employing a word dichotic listening and lateralized decision task. In contrast, tinnitus patients did not show such a hemispheric asymmetry for language processing. Importantly, tinnitus was simulated in normal hearing participants by a tinnitus-like noise and thus the difference between groups could not be attributed to the tinnitus noise *per se* (note that the inclusion of white band noise worsens speech intelligibility even in normal hearing participants, Paglialonga et al., 2011). Consequently, the authors argued for functional reorganization as a consequence of the peripheral damage. The same conclusion was reached by Moon et al. (2015) who measured the speech recognition threshold in noise in 21 patients suffering from tinnitus. Patients performed worse than a control group in this test, but not in a variety on tests on auditory spectral and temporal resolution. This dissociation argues again against the hypothesis that tinnitus depends on damage to outer hair cells, but rather on plastic changes in the central auditory pathways after damage to the cochlea.

To further investigate the dissociation between inconspicuous pure tone audiograms and speech perception, Jain and Sahoo (2014) tested 20 patients suffering from mild to moderate tinnitus in comparison to a control group. Tests

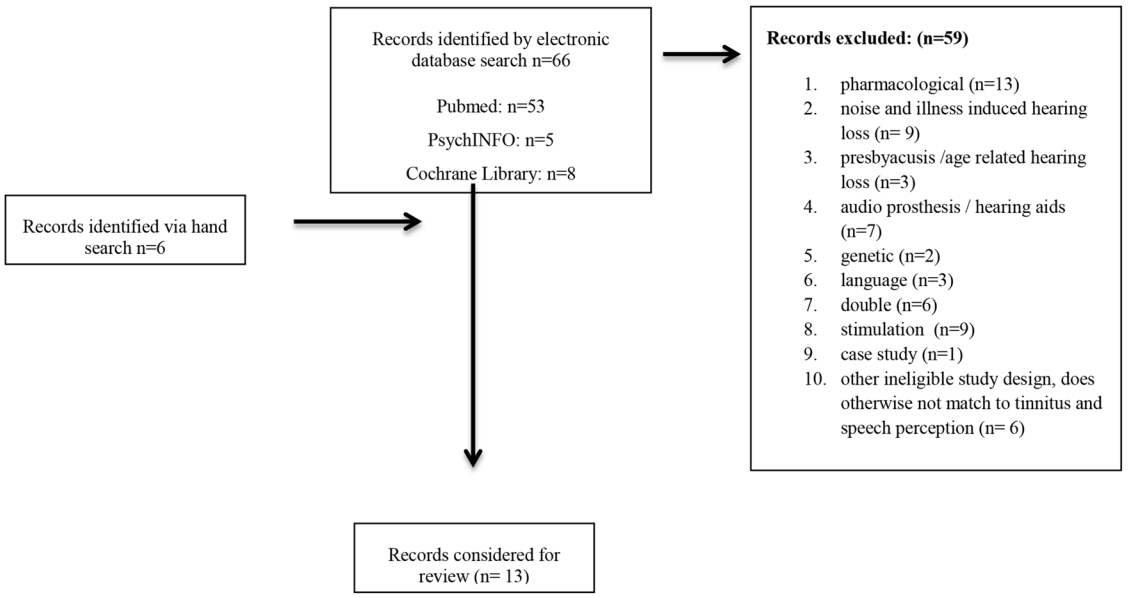

FIGURE 1 | Flow chart for the identification of studies included in the review.

included speech perception in noise, frequency discrimination, differential limen of intensity and frequency, gap detection and modulation detection thresholds. The results demonstrated that tinnitus patients were impaired in various aspects of auditory perception such as frequency discrimination, temporal resolution and speech recognition in noise, i.e., aspects that are not reflected in a pure tone audiogram. Corroborating results were presented from 19 young adults exposed to leisure noise who developed noise-induced tinnitus (Gilles et al., 2016). While these participants did not show differences to a control group with regard to hearing thresholds, otoacoustic emissions and ABR, participants with noise induced tinnitus displayed worse abilities for sentence comprehension in noise. Thus, even if measures indicating peripheral damage are inconspicuous, speech perception tests argue for impairment.

DISCUSSION AND CONCLUSION

Given the small but recently increasing literature on problems with speech perception in tinnitus, it appears that these functions are impaired in tinnitus patients across a wide variety of languages. Despite the small number of studies, the reported evidence for tinnitus-related impairments of speech perception goes far beyond just a few cases. From an experimental viewpoint, in most studies the investigated group sizes allowed to make robust conclusions (all $N > 14$). The only study that did not find evidence for speech comprehension impairments is by Tugumia et al. (2016). They used a Portuguese version of a speech-in-Noise test (Pereira and Schochat, 2011) as a validation of an auditory training procedure. Speech comprehension did not improve due to training and was already in the normal range before training. Reasons for this divergence to other studies might be the rather mild condition of the studied patients as well as their young age.

As a particularly important finding in several studies, speech measures were impaired even if audiological measures were inconspicuous (e.g., Ryu et al., 2012; Mertens et al., 2013; Gilles et al., 2016). Thus, a speech impairment was even demonstrated when no peripheral damage was evidenced. We take this as an argument that reduced speech comprehension is a phenomenon *per se* and not only attributable to impaired hearing. Consequently, it is not a trivial problem as one might have quite understandably suggested.

Nevertheless, the underlying mechanism is far from understood. Despite the strong link between attention and speech comprehension as well as language in general, it is not yet clear what the nature of their relation is. Are the impairments in speech processing a direct consequence of attentional impairments or are they both the consequence of underlying common functions? The majority of authors agree that for the development of chronic tinnitus peripheral damage is accompanied by neuroplastic processes of central functions (e.g., Newman et al., 1994; Cuny et al., 2004; Paglialonga et al., 2011; Mertens et al., 2013). If attentional functions and/or working memory and/or language processing are *per se* affected, then this should become obvious independent from the domain of input. This assumption is to some degree supported by the very high percentage of tinnitus patients reporting difficulties with concentration (Watts et al., 2016) and by evidence for poorer reading performance in these patients (Sanchez and Stephens, 1997). As a working hypothesis, we propose that difficulties in speech comprehension arise via a central mechanism. The phantom noise attracts attention and as a consequence these resources are not available for

TABLE 1 | Overview of reviewed studies: basic participant characteristics, employed speech comprehension tests, main results and conclusions regarding our hypothesis language.

	Methods			Results		Conclusion
Study	**Participants**	**Speech comprehension (SC) test(s)**	**Language**	**Impairment in SC for T**	**Impact of hearing condition**	**Involvement of central processes**
Newman et al. (1994)	*N* = 23: UT + BHL *N* = 23: BHL	Harvard Psychoacoustic Laboratory word test, synthetic sentence identification test, speech perception in noise test, dichotic sentence identification test	English	yes	yes	yes
Goldstein and Shulman (1999)	*N* = 25: severe T + NH	Monaural low-pass filtered speech, binaural fusion test, rapid alternating speech test, competing sentence test, staggered spondaic word test	English	yes	yes	yes
Cuny et al. (2004)	*N* = 20: UT *N* = 10: BT *N* = 30: healthy controls	Dichotic listening, lateralized lexical decision	French	yes	n.a.	yes
Huang et al. (2007)	*N* = 20: T + NH	Mandarin speech perception in noise test	Mandarin	yes	yes	yes
Hennig et al. (2011)	*N* = 19: T + NH + hyperacusis *N* = 23: controls	Sentences recognition threshold in silence and in noise	Portuguese	yes	yes	n.s.
Paglialonga et al. (2011)	*N* = 10: NH	Speech in noise test with three-alternative, forced-choice paradigm	Italian	yes	yes	n.s.
Ryu et al. (2012)	*N* = 20: T + NH *N* = 20: healthy controls	Korean version of hearing in noise test	Korean	yes	no	n.s.
Soalheiro et al. (2012)	*N* = 495: T	Logoaudiometric thresholds without competing noise	Portuguese	yes	n.a.	n.s.
Mertens et al. (2013)	*N* = 15: UHL and ipsilateral T	Speech reception threshold using the Leuven intelligibility sentences test	Dutch	yes	n.a.	yes
Jain and Sahoo (2014)	*N* = 10: mild T *N* = 10: moderate T *N* = 20: controls	Speech perception in noise based on sentences from the Kannada quick speech in noise test	Canarese	yes	n.a.	yes
Moon et al. (2015)	*N* = 9: UT + NH *N* = 12: UT + HL *N* = 9: BT + HL *N* = 15: healthy controls	Speech recognition in noise	Korean	yes	n.a.	yes
Gilles et al. (2016)	*N* = 19: T *N* = 68: controls	Speech-in-noise testing with continuous and modulated noise based on the Leuven intelligibility sentence test	Dutch	yes	n.a.	yes
Tugumia et al. (2016)	*N* = 12: UT + BT	Speech-in-noise test (not specified)	Portuguese	no	n.a.	n.s.

U, unilateral; B, bilateral; T, Tinnitus; HL, hearing loss; NH, normal hearing; SC, speech comprehension; n.a., not applicable; n.s, not stated.

other processes. This becomes expressed as deficits of divided or selective attention. As described in the introduction and emphasized by the ELU model, language processing depends on all levels on attentional capacities and is consequently impaired. We outline below some possibilities to investigate this on a detailed functional level. As an alternative hypothesis, this mechanism could be mediated via stress responses to the tinnitus. Such a possibility seems plausible regarding the recent evidence for the involvement of the limbic system (e.g., Husain, 2016).

Outlook

We propose that future research should aim at disentangling the various factors that contribute to impaired speech perception in tinnitus as well as the causal relationships between attention and speech comprehension. By comparing complex sentences (with e.g., relative clauses) to simpler sentences, the interaction with working memory can be tested. The use of text passages requiring frequent or rare shifts of attention could evidence the interplay of speech processing with attentional requirements. Eye-tracking proved as a useful technique to measure shifts and the locus of attention and could therefore be applied in tinnitus-research to investigate the interplay of speech processing and attentional focus (e.g., Andersson et al., 2011). Moreover, keeping in mind that different phonemes are plotted on different frequency areas in an audiogram, it would be interesting to investigate which phonemes are harder to understand than others. As an example on the single word level, it is highly plausible that words with fricatives are particularly hard to process, especially with competing white noise. While these are only a few suggestions, they necessitate the development of systematic, standardized test batteries employing speech stimuli involving not only tinnitus-researchers but linguists, phoneticians and neuroscientists alike. The multitude and variability of symptoms demand that tinnitus research becomes even more interdisciplinary than it already is.

AUTHOR CONTRIBUTIONS

DI, BM and CD: literature search, conception of review; wrote the manuscript. OG-L, GFV and GS: conception of review; wrote the manuscript.

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REFERENCES

Andersson, G. (2002). Psychological aspects of tinnitus and the application of cognitive-behavioral therapy. *Clin. Psychol. Rev.* 22, 977–990. doi: 10.1016/s0272-7358(01)00124-6

Andersson, G., Eriksson, J., Lundh, L. G., and Lyttkens, L. (2000). Tinnitus and cognitive interference: a stroop paradigm study. *J. Speech Lang. Hear. Res.* 43, 1168–1173. doi: 10.1044/jslhr.4305.1168

Andersson, G., and McKenna, L. (2006). The role of cognition in tinnitus. *Acta Otolaryngol. Suppl.* 126, 39–43. doi: 10.1080/03655230600895226

Andersson, R., Ferreira, F., and Henderson, J. M. (2011). I see what you're saying: the integration of complex speech and scenes during language comprehension. *Acta Psychol.* 137, 208–216, doi: 10.1016/j.actpsy.2011.01.007

Cuny, C., Chéry-Croze, S., Bougeant, J. C., and Koenig, O. (2004). Investigation of functional hemispheric asymmetry of language in tinnitus sufferers. *Neuropsychology* 18, 384–392. doi: 10.1037/0894-4105.18.2.384

Dobel, C., Glanemann, R., Kreysa, H., Zwitserlood, P., and Eisenbeiss, S. (2011). "Visual encoding of meaningful and meaningless scenes," in *Event Representation in Language and Cognition*, eds E. Pedersen, and J. Bohnemeyer (Cambridge: Cambridge University Press), 189–215.

Eysel-Gosepath, K., Gerhards, F., Schicketanz, K.-H., Teichmann, K., and Benthien, M. (2004). Aufmerksamkeitslenkung in der Tinnitustherapie. Vergleich von Effekten unterschiedlicher Behandlungsmethoden. *HNO* 52, 431–439. doi: 10.1007/s00106-003-0929-4

Gilles, A., Schlee, W., Rabau, S., Wouters, K., Fransen, E., and Van de Heyning, P. (2016). Decreased speech-in-noise understanding in young adults with tinnitus. *Front. Neurosci.* 10:288. doi: 10.3389/fnins.2016.00288

Goldstein, B., and Shulman, A. (1999). Central auditory speech test findings in individuals with subjective idiopathic tinnitus. *Int. Tinnitus J.* 5, 16–19.

Heeren, A., Maurage, P., Perrot, H., De Volder, A., Renier, L., Araneda, R., et al. (2014). Tinnitus specifically alters the top-down executive control sub-component of attention: evidence from the attention network task. *Behav. Brain Res.* 269, 147–154. doi: 10.1016/j.bbr.2014.04.043

Henderson, J. M., Williams, C. C., Castelhano, M. S., and Falk, R. J. (2003). Eye movements and picture processing during recognition. *Percept. Psychophys.* 65, 725–734. doi: 10.3758/bf03194809

Hennig, T. R., Costa, M. J., Urnau, D., Becker, K. T., and Schuster, L. C. (2011). Recognition of speech of normal-hearing individuals with tinnitus and hyperacusis. *Int. Arch. Otorhinolaryngol.* 15, 21–28. doi: 10.1590/S1809-48722011000100003

Huang, C. Y., Lee, H. H., Chung, K. C., Chen, H. C., Shen, Y. J., and Wu, J. L. (2007). Relationships among speech perception, self-rated tinnitus loudness and disability in tinnitus patients with normal pure-tone threshold s of hearing. *ORL* 69, 25–29. doi: 10.1159/000096713

Husain, F. T. (2016). Neural networks of tinnitus in humans: elucidating severity and habituation. *Hear Res.* 334, 37–48. doi: 10.1016/j.heares.2015.09.010

Jackson, J. G., Coyne, I. J., and Clough, P. J. (2014). A preliminary investigation of potential cognitive performance decrements in non-help-seeking tinnitus sufferers. *Int. J. Audiol.* 53, 88–93. doi: 10.3109/14992027.2013.846481

Jain, C., and Sahoo, J. P. (2014). The effect of tinnitus on some psychoacoustical abilities in individuals with normal hearing sensitivity. *Int. Tinnitus J.* 19, 28–35. doi: 10.5935/0946-5448.20140004

Jastreboff, P. J., Gray, W. C., and Gold, S. L. (1996). Neurophysiological approach to tinnitus patients. *Am. J. Otol.* 17, 236–240.

Mertens, G., Kleine Punte, A., De Ridder, D., and Van de Heyning, P. (2013). Tinnitus in a single-sided deaf ear reduces speech reception in the nontinnitus ear. *Otol. Neurotol.* 34, 662–666. doi: 10.1097/MAO.0b013e31828779f0

Meyer, A. S., and Dobel, C. (2004). "Application of eye tracking in speech production research," in *The Mind's Eye: Cognitive and Applied Aspects of Eye Movement Research*, eds J. Hyöna, J. R. Radach, and H. Deubel (Oxford: Elsevier Science), 253–272.

Mohamad, N., Hoare, D. J., and Hall, D. A. (2016). The consequences of tinnitus and tinnitus severity on cognition: A review of the behavioural evidence. *Hear. Res.* 332, 199–209. doi: 10.1016/j.heares.2015.10.001

Møller, A. R. (2007). The role of neural plasticity in tinnitus. *Prog. Brain Res.* 166, 37–45. doi: 10.1016/S0079-6123(07)66003-8

Moon, I. J., Won, J. H., Kang, H. W., Kim, D. H., An, Y. H., and Shim, H. J. (2015). Influence of tinnitus on auditory spectral and temporal resolution and speech perception in tinnitus patients. *J. Neurosci.* 35, 14260–14269. doi: 10.1523/JNEUROSCI.5091-14.2015

Moore, C., and Dunham, P. J. (1995). *Joint Attention: Its Origins and Role in Development*. Hillsdale, NJ: Lawrence Erlbaum Associates.

Newman, C. W., Wharton, J. A., Shivapuja, B. G., and Jacobson, G. P. (1994). Relationships among psychoacoustic judgments, speech understanding ability and self-perceived handicap in tinnitus subjects. *Audiology* 33, 47–60. doi: 10.3109/00206099409072954

Paglialonga, A., Fiocchi, S., Del Bo, L., Ravazzani, P., and Tognola, G. (2011). Quantitative analysis of cochlear active mechanisms in tinnitus subjects with normal hearing sensitivity: time-frequency analysis of transient evoked otoacoustic emissions and contralateral suppression. *Auris Nasus Larynx* 38, 33–40. doi: 10.1016/j.anl.2010.04.006

Pereira, L. D., and Schochat, E. (2011). *Testes Auditivos e Comportamentais para Avaliação do Processamento Auditivo Central*. São Paulo: Pró Fono.

Roberts, L. E., Bosnyak, D. J., Bruce, I. C., Gander, P. E., and Paul, B. T. (2015). Evidence for differential modulation of primary and nonprimary auditory cortex by forward masking in tinnitus. *Hear. Res.* 327, 9–27. doi: 10.1016/j.heares.2015.04.011

Rönnberg, J., Danielsson, H., Rudner, M., Arlinger, S., Sternäng, O., Wahlin, A., et al. (2011). Hearing loss is negatively related to episodic and semantic long-term memory but not to short-term memory. *J. Speech Lang. Hear. Res.* 54, 705–726. doi: 10.1044/1092-4388(2010/09-0088)

Rönnberg, J., Lunner, T., Zekveld, A., Sörqvist, P., Danielsson, H., Lyxell, B., et al. (2013). The ease of language understanding (ELU) model: theoretical, empirical and clinical advances. *Front. Syst. Neurosci.* 7:31. doi: 10.3389/fnsys.2013.00031

Ryu, I. S., Ahn, J. H., Lim, H. W., Joo, K. Y., and Chung, J. W. (2012). Evaluation of masking effects on speech perception in patients with unilateral chronic tinnitus using the hearing in noise test. *Otol. Neurotol.* 33, 1472–1476. doi: 10.1097/MAO.0b013e31826dbcc4

Sanchez, L., and Stephens, D. (1997). A tinnitus problem questionnaire in a clinic population. *Ear Hear.* 18, 210–217.doi: 10.1097/00003446-199706000-00004

Soalheiro, M., Rocha, L., do Vale, D. F., Fontes, V., Valente, D., and Teixeira, L. R. (2012). Speech recognition index of workers with tinnitus exposed to environmental or occupational noise: a comparative study. *J. Occup. Med. Toxicol.* 22:6. doi: 10.1186/1745-6673-7-26

Sörqvist, P., Stenfelt, S., and Rönnberg, J. (2012). Working memory capacity and visual-verbal cognitive load modulate auditory-sensory gating in the brainstem: toward a unified view of attention. *J. Cogn. Neurosci.* 24, 2147–2154. doi: 10.1162/jocn_a_00275

Stein, A., Wunderlich, R., Lau, P., Engell, A., Wollbrink, A., Shaykevich, A., et al. (2016). Clinical trial on tonal tinnitus with tailor-made notched music training. *BMC Neurol.* 16:38. doi: 10.1186/s12883-016-0558-7

Tegg-Quinn, S., Bennett, R. J., Eikelboom, R. H., and Baguley, D. M. (2016). The impact of tinnitus upon cognition in adults: A systematic review. *Int. J. Audiol.* 55, 533–540. doi: 10.1080/14992027.2016.1185168

Tsuchida, Y., Katayama, J., and Murohashi, H. (2012). Working memory capacity affects the interference control of distractors at auditory gating. *Neurosci. Lett.* 516, 62–66. doi: 10.1016/j.neulet.2012.03.057

Tugumia, D., Samelli, A. G., Matas, C. G., Magliaro, F. C., and Rabelo, C. M. (2016). Auditory training program in subjects with tinnitus. *Codas* 28, 27–33. doi: 10.1590/2317-1782/20162015113

Tun, P. A., McCoy, S., and Wingfield, A. (2009). Aging, hearing acuity and the attentional costs of effortful listening. *Psychol. Aging* 24, 761–766. doi: 10.1037/a0014802

Watts, E., Fackrell, K., Smith, S., Sheldrake, J., and Hoare, D. (2016). "Why is tinnitus a problem? A qualitative analysis of problems reported by tinnitus patients," in *Poster presented at 10th International Tinnitus Research Initiative Conference and 1st EU Cost Action (TINNET) Conference, 16th–18th March 2016* (Nottingham).

Zouridakis, G., Simos, P. G., and Papanicolaou, A. C. (1998). Multiple bilaterally asymmetric cortical sources account for the auditory N1m component. *Brain Topogr.* 10, 183–189.

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Speech Comprehension Difficulties in Chronic Tinnitus and Its Relation to Hyperacusis

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Objective: Many tinnitus patients complain about difficulties regarding speech comprehension. In spite of the high clinical relevance little is known about underlying mechanisms and predisposing factors. Here, we performed an exploratory investigation in a large sample of tinnitus patients to (1) estimate the prevalence of speech comprehension difficulties among tinnitus patients, to (2) compare subjective reports of speech comprehension difficulties with behavioral measurements in a standardized speech comprehension test and to (3) explore underlying mechanisms by analyzing the relationship between speech comprehension difficulties and peripheral hearing function (pure tone audiogram), as well as with co-morbid hyperacusis as a central auditory processing disorder.

Subjects and Methods: Speech comprehension was assessed in 361 tinnitus patients presenting between 07/2012 and 08/2014 at the Interdisciplinary Tinnitus Clinic at the University of Regensburg. The assessment included standard audiological assessments (pure tone audiometry, tinnitus pitch, and loudness matching), the Goettingen sentence test (in quiet) for speech audiometric evaluation, two questions about hyperacusis, and two questions about speech comprehension in quiet and noisy environments ("How would you rate your ability to understand speech?"; "How would you rate your ability to follow a conversation when multiple people are speaking simultaneously?").

Results: Subjectively-reported speech comprehension deficits are frequent among tinnitus patients, especially in noisy environments (cocktail party situation). 74.2% of all investigated patients showed disturbed speech comprehension (indicated by values above 21.5 dB SPL in the Goettingen sentence test). Subjective speech comprehension complaints (both for general and in noisy environment) were correlated with hearing level and with audiologically-assessed speech comprehension ability. In contrast, co-morbid hyperacusis was only correlated with speech comprehension difficulties in noisy environments, but not with speech comprehension difficulties in general.

Conclusion: Speech comprehension deficits are frequent among tinnitus patients. Whereas speech comprehension deficits in quiet environments are primarily due to

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peripheral hearing loss, speech comprehension deficits in noisy environments are related to both peripheral hearing loss and dysfunctional central auditory processing. Disturbed speech comprehension in noisy environments might be modulated by a central inhibitory deficit. In addition, attentional and cognitive aspects may play a role.

Keywords: chronic tinnitus, speech perception problems, Goettingen sentence test, hearing loss, hyperacusis

INTRODUCTION

Subjective tinnitus is the perception of sound in the absence of a corresponding acoustic signal (Baguley et al., 2013). Hearing loss is a recognized risk factor for tinnitus (Hoffmann and Reed, 2004). It is assumed that tinnitus is generated by plastic changes in the central nervous system as a reaction to reduced auditory input similar to mechanisms associated with phantom perceptions after limb amputation (De Ridder et al., 2011). Accordingly, the majority of tinnitus patients show elevated hearing thresholds in the standard pure tone audiogram (Norena et al., 2002). And many tinnitus patients with normal or nearly normal hearing thresholds in the pure tone audiogram exhibit cochlear damage (Shore et al., 2016). Hair cell loss at frequencies between the tested frequencies ("dead regions") (Weisz et al., 2006) or at high frequencies (Vielsmeier et al., 2015) have been observed in tinnitus patients with normal standard audiograms. There is also evidence for damage of high-threshold auditory nerve fibers in tinnitus patients with normal standard audiograms (Schaette and McAlpine, 2011). Speech comprehension difficulties are frequently reported by tinnitus patients (Tyler and Baker, 1983; Newman et al., 1994), even by those with normal pure tone audiograms. Particular difficulties in following a conversation are reported in situations when multiple people are speaking simultaneously, e.g., in a classical "cocktail party situation" (Jones and Litovsky, 2008).

Speech comprehension difficulties are for many tinnitus patients among the leading causes for their tinnitus-related handicap and therefore clinically, highly relevant. In contrast, knowledge about prevalence, assessment, pathogenetic mechanisms, and management of speech comprehension impairment in tinnitus patients is still very limited. Only few studies have addressed specifically impaired speech comprehension abilities in tinnitus patients. An early study demonstrated that 13 out of 25 normal hearing patients with tinnitus have deficits in at least one out of several conducted speech comprehension tests (Goldstein and Shulman, 1999). The investigation of a large sample of 495 workers who suffered from tinnitus showed speech intelligibility deficits as compared to workers without tinnitus (Soalheiro et al., 2012). A small study investigating 20 Chinese speaking patients with tinnitus revealed that speech comprehension was impaired as compared to a control group, particularly in noisy environments (Huang et al., 2007). Another study revealed that masking of tinnitus by sound improved speech comprehension (Ryu et al., 2012). An improvement of speech comprehension in noise was also observed in patients with unilateral cochlear implants when tinnitus loudness was reduced by activation of cochlear implants (Mertens et al., 2013a) or in patients whose tinnitus was successfully suppressed by rTMS treatment (Barwood et al., 2013). Wearing hearing aids over a time of 3 months showed amelioration of the performance in two speech comprehension tests for elderly patients (Araujo and Iório, 2016). Speech comprehension was worse in patients with tinnitus at the beginning of the trial, improved in patients with and without tinnitus during the trial, but speech comprehension abilities remained reduced in all evaluated parameters at the end of the treatment.

These findings suggest that tinnitus and impaired speech comprehension in noise share a common pathophysiological substrate in the central auditory pathways, a notion supported by a recent study demonstrating that ears with and without tinnitus did not differ in auditory spectral and temporal resolution abilities, but in speech comprehension in noise (Moon et al., 2015). It seems also likely that speech comprehension deficits among tinnitus patients are not solely explainable by cochlear damage, as speech comprehension deficits are also reported by patients with normal audiograms.

One of the most common comorbidities with a prevalence of 40–55% in chronic tinnitus is hyperacusis (Schecklmann et al., 2014). Hyperacusis is in general defined as abnormal intolerance to sounds. For this work we define hyperacusis as stated in the Tinnitus Sample Case History Questionnaire (TSCHQ) from the TRI (Tinnitus Research Initiative) database (refer to the methods; Schecklmann et al., 2014, 2015). Two studies investigated the relationship between hyperacusis and speech comprehension: Ten patients with auditory processing disorder, but normal audiogram, showed elevated hyperacusis scores and worse transient evoked otoacoustic emission suppression test results in contrast to 12 age-matched controls (Spyridakou et al., 2012). Strong correlations were found for a speech in babble test targeting the right ear and self-described speech in noise understanding, and also for a transient evoked otoacoustic emission suppression test targeting the right ear and hyperacusis. Nineteen normal-hearing patients with tinnitus and hyperacusis showed similar performance in speech comprehension in silence but lower performance in a communication scenario in contrast to 23 normal hearing subjects without hearing complaints (Hennig et al., 2012). The authors speculated that the common pathophysiologic substrate of tinnitus, hyperacusis, and speech comprehension (in noise) may be a dysfunction in the medial olivary cochlear system. This is an efferent inhibitory system from the primary auditory cortex along the auditory pathway to the outer and inner hair cells of the cochlea with the function to filter out irrelevant noise (Harkrider and Bowers, 2009).

In summary, the available data suggest that both peripheral and central auditory dysfunction is involved in speech comprehension difficulties in tinnitus (and hyperacusis)

patients. However, most available data come from small and heterogeneous samples and there is only very limited information about how many tinnitus patients suffer from speech comprehension difficulties in quiet and in noisy environments, and how these communication difficulties relate to audiometric findings and to tinnitus characteristics. Moreover no established standard exists for the clinical assessment of speech comprehension difficulties. The mechanisms of this phenomenon are still incompletely understood and no specific evidence-based treatments exist.

The primary aim of the present work was to investigate how many patients with tinnitus suffer from subjective speech comprehension in quiet and in noisy environments. The second aim was to investigate how subjective impairments in speech comprehension relate to audiological findings in the standard audiogram and in a validated sentence comprehension test. The third aim was to explore the relationship between speech comprehension difficulties and co-morbid hyperacusis.

MATERIALS AND METHODS

Subjects/Sample

All patients presenting with subjective chronic tinnitus at the Interdisciplinary Tinnitus Center at the University of Regensburg (Regensburg, Germany, a tertiary referral center) between July, 1st 2012 and August, 31th 2014 were invited to participate in this exploratory prospective study.

Assessment

All patients completed various tinnitus questionnaires including the Tinnitus Sample Case History Questionnaire (TSCHQ; Langguth et al., 2007) and the German version of the Tinnitus Questionnaire (TQ; Goebel and Hiller, 1994; range: 0–84 with higher scores presenting higher distress), underwent microscopy of the ear and received an audiological examination including pure tone audiometry (125–8000 Hz), stapedius reflex testing, and tympanometry.

In addition to this standard assessment, speech comprehension was prospectively assessed by a specific German sentence test ["Goettingen Satztest" (Kollmeier and Wesselkamp, 1997)] and by asking two subjective questions about speech comprehension impairment.

The Goettingen sentence test was chosen as it reflects a realistic comprehension situation and has a high ecological validity (Kollmeier and Wesselkamp, 1997; Arweiler-Harbeck et al., 2011; May-Mederake and Shehata-Dieler, 2013). This test was performed in silence via air conduction by using headphones. A total of 20 sentences were presented starting at 35 dB SPL. Based on the results given by the patient the loudness was elevated or reduced accordingly in the upcoming sentences. The result is expressed as the 50% perception-threshold in dB and can range from 0 to 100 dB SPL.

Subjective speech comprehension impairment was assessed by the following two questions: "How would you rate your ability to understand speech? (German: Wieviele Probleme haben Sie, Sprache zu verstehen?)" and "How would you rate your ability to follow a conversation when multiple people are speaking simultaneously (e.g., in restaurant situation)? [German: Wieviele Probleme haben Sie, Gesprächen zu folgen, wenn mehrere Personen gleichzeitig sprechen (z.B. in einer Gaststätte)?]" on a scale ranging between 0 and 5 points (0, no problems; 1, minimal problems; 2, minor problems; 3, moderate problems; 4, significant problems; 5, massive problems; German: 0, keine Probleme; 1, sehr wenig Probleme; 2, wenig Probleme; 3, mäßig; 4, starke Probleme; 5, sehr starke Probleme). Patients gave written informed consent that data were gathered and analyzed for the Tinnitus Research Initiative Database which was approved by the Ethics Committee of the University Hospital of Regensburg (Germany; reference number 08/046).

For descriptive analyses, "normal" was defined by the cut-off criterion that the hearing thresholds at all frequencies measured in the standard pure tone audiogram (125, 250, 500 Hz, 1, 2, 4, 6, 8 kHz) were equal or below 20 dB hearing loss. The mean value over all frequencies (125, 250, 500 Hz, 1, 2, 4, 6, 8 kHz) and both ears was calculated for each patient. Patients with normal or disturbed speech comprehension in accordance to the Goettingen sentence test were determined by the cut-off criterion $\leq$ 21.5 dB SPL.

Moreover we explored whether tolerability of loud sounds (hyperacusis) was related to subjectively perceived speech comprehension deficits. For this purpose, we used two questions ["Do you have a problem tolerating sounds because they often seem much too loud? That is, do you often find sounds too loud or hurtful, which other people around you find quite comfortable?" (answers: never, rarely, sometimes, usually, always; rated with 1, 2, 3, 4, 5, respectively); "Do sounds cause you pain or physical discomfort?" (answers: no, yes, I don't know; rated with 0, 1, 2, respectively)], that have recently been validated as useful screening questions for hyperacusis (Schecklmann et al., 2015). Notably, these questions focus particularly on symptoms of fear/pain-related hyperacusis and do not diagnosis hyperacusis comprehensively.

Data were collected within the framework of the Tinnitus Research Initiative Database (Landgrebe et al., 2010).

Statistical Analysis

For statistical analyses, we initially present descriptive data and the number of patients with speech comprehension problems. Speech comprehension ratings are also shown compared to normal values for the audiogram and Goettingen test results. To evaluate possible factors contributing to subjective speech comprehension deficits (dependent variables) we analyzed possible associations by using Pearson correlation coefficients for metric variables (hearing level, Goettingen Satztest, and hyperacusis question 1 with speech comprehension questions 1 and 2) and Student *t*-tests for independent samples for dichotomous variables (patients with yes vs. no answers in hyperacusis question 2 with respect to speech comprehension questions 1 and 2). For these analyses, we firstly used the average hearing level and the Goettingen test score. We assumed that these audiologic variables were related to the subjectively perceived deficit in speech comprehension. To assess the influence of sound tolerability on speech comprehension

we correlated the answers for the question "Do you have a problem tolerating sounds because they often seem much too loud? That is, do you often find sounds too loud or hurtful, which other people around you find quite comfortable?" and "Do sounds cause you pain or physical discomfort?" with the speech comprehension scores and compared the speech comprehension scores between patients who experienced physical discomfort or pain to sounds and those who did not (**Table 1**). All association analyses were repeated with the Tinnitus severity (Tinnitus Questionnaire Score) as co-variate by using partial correlations for the Pearson correlations and analyses of covariance for the Student *t*-test. Spearmen correlations revealed the same results. As partial correlations are only possible for parametric tests we provide results of the Pearson correlations. The significance threshold was set to 5%. No corrections for multiple comparisons were performed because of the explorative character of the study. Strength of correlations was indicated as small ($r = 0.1$), medium ($r = 0.3$), and high ($r = 0.5$). Effect sizes for Student *t*-tests were indicated by Cohen's d with 0.2 as small, 0.5 as medium and 0.8 as large effect size. Statistical analyses were performed with SPSS (SPSS Inc., USA, version 22).

RESULTS

Complete data sets (audiogram, Goettingen sentence test, speech comprehension questions) were available from 361 patients. One hundred and thirty-one (36.3%) were female, 51 (14.1%) reported right-sided tinnitus, 92 (25.5%) left-sided tinnitus, and 216 (59.8%) tinnitus in both ears or within the head. Age was 52.4 ± 12.5 (mean ± SD) years, tinnitus distress was 39.8 ± 17.3 as indicated by the tinnitus questionnaire (Hiller and Goebel, 1992; Goebel and Hiller, 1994; Hiller et al., 1994), and tinnitus duration was 93.8 ± 97.0 months. 296 (82%) patients had hearing loss (>20 dB HL in one or more frequencies of the standard audiogram) and 268 (74.2%) showed disturbed speech comprehension as indicated by values above 21.5 dB SPL in the Goettingen sentence test. One hundred and fifty-two (42.1%) patients reported problems with speech comprehension in general (score of at least one in question 1) and 288 (79.8%) problems with speech comprehension when multiple people were speaking simultaneously (speech comprehension in group conversation or noisy environment; score of at least one in question 2). For detailed distribution of answers to the two questions see **Figure 1**. As can be seen in **Figure 2** there is a quite

TABLE 1 | (A) Association of variables of audiometry, Goettingen sentence test, and hyperacusis with subjective speech comprehension.

	General speech comprehension problems (0–5)	Speech comprehension problems in noisy environment (0–5)
Average hearing loss (dB HL) ($n = 361$)	$r = 0.474$; $p < 0.001$	$r = 0.517$; $p < 0.001$
Goettingen sentence test (dB SPL) ($n = 361$)	$r = 0.351$; $p < 0.001$	$r = 0.387$; $p < 0.001$
Do you have a problem tolerating sounds because they often seem much too loud? That is, do you often find too loud or hurtful sounds which other people around you find quite comfortable? ($n = 349$)	$r = 0.071$; $p = 0.188$	$r = 0.268$; $p < 0.001$
Do sounds cause you pain or physical discomfort? (yes: $n = 188$; no = 126)	yes: 0.96 ± 1.25; no: 0.83 ± 1.17; $t = 0.982$; $df = 312$; $p = 0.327$; $d = 0.107$	yes: 2.66 ± 1.54; no: 2.10 ± 1.62; $t = 3.140$; $df = 312$; $p = 0.002$; $d = 0.354$

r, Pearson correlations coefficient; t, Student t-test; xx ± xx, mean ± sd.

TABLE 1 | (B) Association of variables of audiometry, Goettingen sentence test, and hyperacusis with subjective speech comprehension with the tinnitus questionnaire as covariate.

	General speech comprehension problems (0–5)	Speech comprehension problems in noisy environment (0–5)
Average hearing loss (dB HL) ($n = 344$)	$r = 0.443$; $p < 0.001$	$r = 0.467$; $p < 0.001$
Goettingen sentence test (dB SPL) ($n = 344$)	$r = 0.310$; $p < 0.001$	$r = 0.330$; $p < 0.001$
Do you have a problem tolerating sounds because they often seem much too loud? That is, do you often find too loud or hurtful sounds which other people around you find quite comfortable? ($n = 344$)	$r = -0.025$; $p = 0.644$	$r = 0.167$; $p = 0.002$
Do sounds cause you pain or physical discomfort? (yes: $n = 186$; no = 125)	yes: 0.97 ± 1.25; no: 0.82 ± 1.17; $F = 0.296$; $df = 1308$; $p = 0.645$	yes: 2.66 ± 1.55; no: 2.07 ± 1.61; $F = 1.685$; $df = 1308$; $p = 0.195$

r, Pearson correlations coefficient; F, analysis of covariance; xx ± xx, mean ± sd.

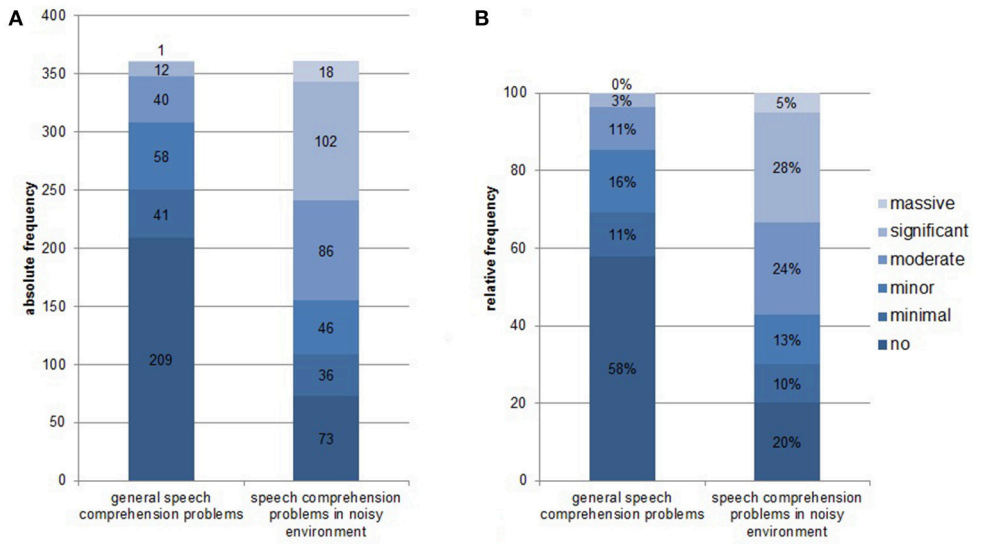

FIGURE 1 | Distribution of answers from the speech comprehension questions ["How would you rate your ability to understand speech?" and "How would you rate your ability to follow a conversation when multiple people are speaking simultaneously (e.g., in restaurant situation)? on a scale ranging between 0, no problems; 1, minimal problems; 2, minor problems; 3, moderate problems; 4, significant problems; to 5, massive problems]. (A) Absolute frequency of distribution; **(B)** Relative frequency of distribution.

good overlap of audiometry results or results from the Goettingen sentence test with subjective ratings of speech comprehension. This is also evident by correlating these variables showing significant positive correlations of the mean audiogram and the comprehension test with subjective speech comprehension in general and in noisy environment with medium to high correlations coefficients (**Table 1A**).

Moreover we assessed the influence of co-morbid hyperacusis (low tolerability of loud sounds) on speech comprehension (**Table 1A**). Patients with hyperacusis (Schecklmann et al., 2015) showed an association with speech comprehension problems in noisy environments (negligible effects sizes), but not in quiet environments (small effect sizes correlation coefficient). For the first hyperacusis question, patients reporting greater problems with sound tolerance showed greater problems with speech comprehension in noisy environments and vice versa. For hyperacusis question two, patients with discomfort to sounds report significant higher speech comprehension deficits in noisy environments in contrast to patients without discomfort to sounds.

All correlations in **Table 1A** were also repeated with the Tinnitus severity (Tinnitus Questionnaire Score) as co-variate. Apart from the correlation between the categorical hyperacousis question "Do sounds cause you pain or physical discomfort?" and the speech comprehension in noise score, which lost significance after inclusion of the TQ score as co-variate, results remained unchanged (**Table 1B**), indicating that the main findings are not driven by global tinnitus severity.

DISCUSSION

The study of speech comprehension difficulties in a large sample of tinnitus patients revealed several main findings. First, with a prevalence of about 40%, subjectively-reported speech comprehension deficits are frequent among tinnitus patients. This number substantially increased when patients were asked about speech comprehension in noisy environments (cocktail party situation) where almost 80% of all investigated tinnitus patients reported difficulties. We are aware that there may be a selection bias as the sample comes from a tertiary referral clinic. However, our sample is much larger than previously investigated samples (Huang et al., 2007) and consisted of unselected patients who consecutively presented because of tinnitus in our clinic. Moreover, there are similar reports of high prevalence of speech intelligibility deficits in workers with exposure to noise and tinnitus (Soalheiro et al., 2012). A further aspect that was not considered in this study is the potential influence of tinnitus-related cognitive or attentional impairment on speech comprehension difficulties. Therefore population-based studies and studies involving cognitive testing will be needed for a valid estimation of the prevalence of speech comprehension difficulties among tinnitus patients. Future studies should also consider the use of more specific diagnostic tests for the presence of hyperacusis beyond the screening questions used in this study. In order to identify the relevance of tinnitus in the interplay between hearing loss and speech comprehension, future research should also include people without tinnitus.

Subjectively-reported speech comprehension difficulties were correlated with the Goettingen sentence test scores. The highly-significant correlation between the subjective perception reports and the behavioral audiological measurements with the Goettingen sentence test provides some validation for the speech comprehension-related questions used for this study. On the other hand, the score in the Goettingen sentence test only explained about 12% of the variance (r^2) of the subjective

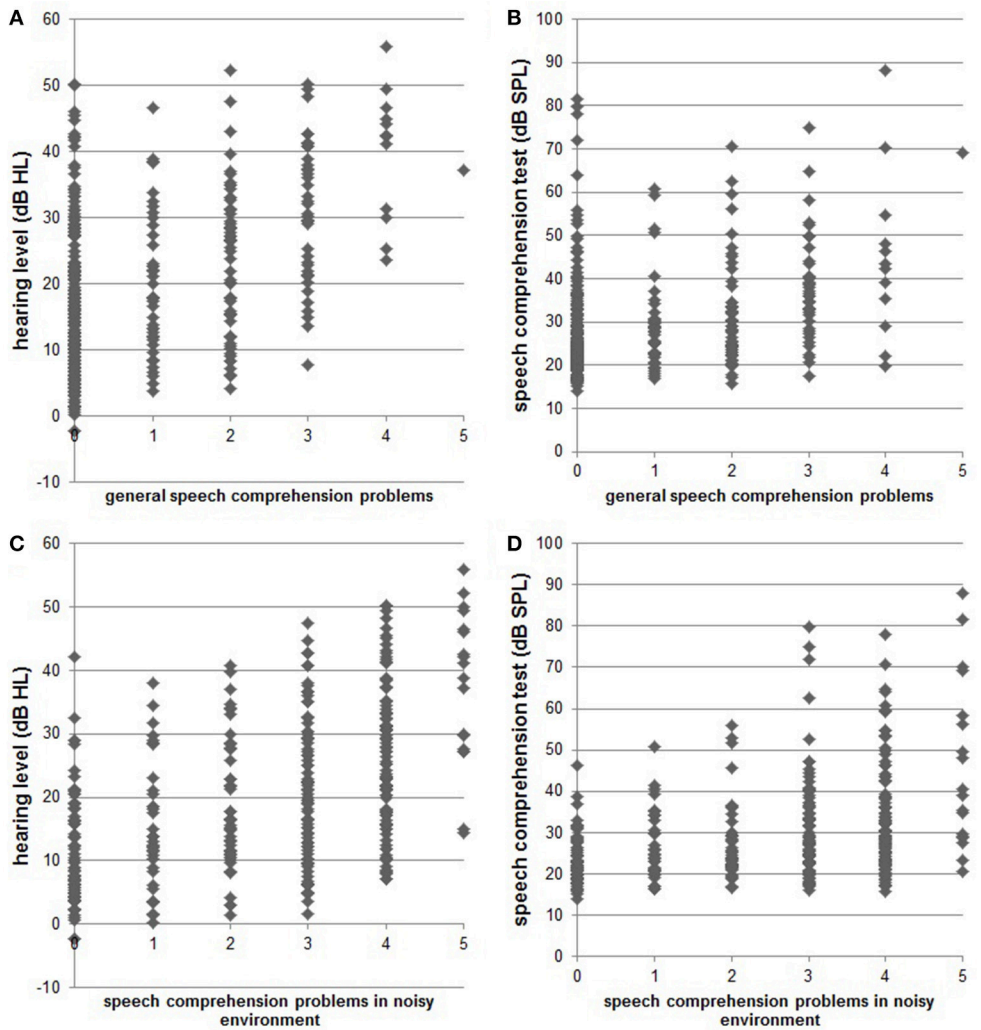

FIGURE 2 | Scatter plots indicate single subject data for hearing status [pure tone audiogram; (A): $r = 0.474$; **(C)**: $r = 0.517$] and speech comprehension status [results from Goettingen sentence test; **(B)**: $r = 0.351$, **(D)**: $r = 0.387$] compared to the subjective speech comprehension report in quiet and in noisy environments, respectively.

speech comprehension impairment. This suggests that the two measurements reflect different aspects and arguing for the usefulness of both subjective and behavioral assessment of speech comprehension.

As most people with tinnitus have some form of cochlear damage, both hearing impairment and tinnitus can contribute to impaired speech comprehension in tinnitus patients (Tyler and Baker, 1983). Here, we found a high correlation between audiometrically-determined hearing threshold shifts and the subjectively-perceived speech comprehension difficulties. This clearly suggests that the degree of cochlear damage has a significant impact of speech comprehension difficulties—both in general (per question 1) and in "cocktail party" situations (per question 2). On the other hand, both our data and previous studies show that there are also tinnitus patients with normal hearing who complain about speech comprehension deficits (Goldstein and Shulman, 1999; Soalheiro et al., 2012) highlighting the role of non-peripheral modulating factors of speech comprehension deficits.

We also investigated the relationship between co-morbid hyperacusis and speech comprehension difficulties. Answers to the two screening questions for hyperacusis (Schecklmann et al., 2015) were significantly related to speech comprehension in the cocktail party situation, but not to speech comprehension in general. As hyperacusis is presumably due to increased central gain as a consequence of deficient central inhibitory mechanisms (Brotherton et al., 2015), our findings suggest that this inhibitory dysfunction of the auditory pathway has a specific impact on speech comprehension difficulties in the cocktail party situation.

According to current knowledge, a central inhibitory deficit is occurring both in tinnitus and hyperacusis, but is especially pronounced in hyperacusis (Noreña, 2011; Hébert et al., 2013). This central inhibitory deficit seems to be particularly relevant for speech comprehension deficits in noisy environments. One

could speculate that speech comprehension in cocktail party situations requires functional inhibitory systems in the neural auditory pathways to actively filter out irrelevant sounds and this function is impaired in tinnitus patients and particularly in tinnitus patients with hyperacusis. This "Central Inhibitory Deficit"—hypothesis would fit with the reported improvement of speech-in-noise perception after successful tinnitus reduction (Barwood et al., 2013; Mertens et al., 2013b).

The medial olivary cochlear system is involved in the auditory efferent system originating in the auditory cortex with connections to the inner and outer hair cells (Hennig et al., 2012). This filter system for peripheral input (Harkrider and Bowers, 2009) takes part in the processing of interaural time differences for sound localization (Myoga et al., 2014), which is necessary for conversation in noisy environment. Moreover, the medial olivary cochlear system has anti-masking functions to adjust cochlear amplification in situations of listening to speech-in-noise (Bidelman and Bhagat, 2015). This might be involved in hyperacusis as shown by decreased distortion-product otoacoustic emissions, as elicited by noise presented to the contralateral ear in patients with tinnitus and low sound level tolerance (Knudson et al., 2014).

Previously, an association between otoacoustic emissions and hyperacusis (but not with hearing in noise) was found (Spyridakou et al., 2012). This study showed elevated hyperacusis scores and reduced otoacoustic emissions of 10 patients with auditory processing disorder but normal hearing in contrast to 12 age-matched controls. Nineteen normal-hearing patients with tinnitus and hyperacusis showed similar performance in speech comprehension in silence, but lower performance in a communication scenario in contrast to 23 normal hearing subjects without hearing complaints (Hennig et al., 2012). In conclusion, a central inhibitory deficit (e.g., an impairment of the medial olivary cochlear system) might represent the common pathophysiologic substrate of tinnitus, hyperacusis, and speech comprehension (in noise) (Hennig et al., 2012).

Electroencephalographic studies have demonstrated that auditory alpha oscillations, which are reduced in tinnitus patients, are critically involved in speech comprehension(Weisz et al., 2011), suggesting that reduced alpha oscillations in the temporal cortex could represent a neuronal correlate of deficient central inhibitory activity in the auditory system, which can manifest as tinnitus and as deficient speech-in-noise comprehension.

In summary, our data suggest that speech comprehension difficulties occur frequently among tinnitus patients and are caused by both peripheral hearing loss and deficient central inhibitory mechanisms. The latter are probably important for speech comprehension difficulties in noisy environments.

The presented work has several implications for clinical management and research. First, our data indicate that speech comprehension difficulties occur frequently among tinnitus patients and should be regularly explored in clinical routine. Second, the proposed screening questions can be used for a simple and easily feasible standardized assessment of speech comprehension difficulties in tinnitus patients. Third, our data also suggest that it would be worthwhile to investigate whether improvement of tinnitus severity and hyperacousis, e.g., by cognitive behavioral therapy, might also improve speech comprehension in noise. Fourth, the effects of hearing aids and specific forms of hearing training should be evaluated in clinical trials.

AUTHOR CONTRIBUTIONS

VV and BL: conception, acquisition, interpretation, manuscript. PK and FH: acquisition, manuscript. TS and WS: interpretation, analysis, manuscript. PS: acquisition, interpretation, manuscript. TK: conception, interpretation, manuscript. MS: acquisition, interpretation, analysis, manuscript.

REFERENCES

Araujo, T. M., and Iório, M. C. M. (2016). Effects of sound amplification in self-perception of tinnitus and hearing loss in the elderly. *Braz. J. Otorhinolaryngol.* 82, 289–296. doi: 10.1016/j.bjorl.2015.05.010

Arweiler-Harbeck, D., Janeschik, S., Lang, S., and Bagus, H. (2011). Suitability of auditory speech sound evaluation (A§E®) in German cochlear implant patients. *Eur. Arch. Otorhinolaryngol.* 268, 1259–1266. doi: 10.1007/s00405-011-1505-2

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Barwood, C. H., Wilson, W. J., Malicka, A. N., McPherson, B., Lloyd, D., Munt, K., et al. (2013). The effect of rTMS on auditory processing in adults with chronic, bilateral tinnitus: a placebo-controlled pilot study. *Brain Stimul.* 6, 752–759. doi: 10.1016/j.brs.2013.01.015

Bidelman, G. M., and Bhagat, S. P. (2015). Right-ear advantage drives the link between olivocochlear efferent 'antimasking' and speech-in-noise listening benefits. *Neuroreport* 26, 483–487. doi: 10.1097/WNR.0000000000000376

Brotherton, H., Plack, C. J., Maslin, M., Schaette, R., and Munro, K. J. (2015). Pump up the volume: could excessive neural gain explain tinnitus and hyperacusis? *Audiol. Neurootol.* 20, 273–282. doi: 10.1159/000430459

De Ridder, D., Elgoyhen, A. B., Romo, R., and Langguth, B. (2011). Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8075–8080. doi: 10.1073/pnas.1018466108

Goebel, G., and Hiller, W. (1994). [The tinnitus questionnaire. A standard instrument for grading the degree of tinnitus. Results of a multicenter study with the tinnitus questionnaire]. *HNO* 42, 166–172. German.

Goldstein, B., and Shulman, A. (1999). Central auditory speech test findings in individuals with subjective idiopathic tinnitus. *Int. Tinnitus J.* 5, 16–19.

Harkrider, A. W., and Bowers, C. D. (2009). Evidence for a cortically mediated release from inhibition in the human cochlea. *J. Am. Acad. Audiol.* 20, 208–215. doi: 10.3766/jaaa.20.3.7

Hébert, S., Fournier, P., and Noreña, A. (2013). The auditory sensitivity is increased in tinnitus ears. *J. Neurosci.* 33, 2356–2364. doi: 10.1523/JNEUROSCI.3461-12.2013

Hennig, T. R., Costa, M. J., Rossi, A. G., and Moraes, A. B. (2012). Auditory rehabilitation effects on the temporal ordering ability in elderly hearing aids users. *J. Soc. Bras. Fonoaudiol.* 24, 26–33. doi: 10.1590/S2179-64912012000100006

Hiller, W., and Goebel, G. (1992). A psychometric study of complaints in chronic tinnitus. *J. Psychosom. Res.* 36, 337–348.

Hiller, W., Goebel, G., and Rief, W. (1994). Reliability of self-rated tinnitus distress and association with psychological symptom patterns. *Br. J. Clin. Psychol.* 33 (Pt 2), 231–239.

Hoffmann, H. J., and Reed, G. W. (2004). "Epidemiology of tinnitus," in *Tinnitus: Theory and Management*, ed J. Snow (London: BC Decker), 16–41.

Huang, C. Y., Lee, H. H., Chung, K. C., Chen, H. C., Shen, Y. J., and Wu, J. L. (2007). Relationships among speech perception, self-rated tinnitus loudness and disability in tinnitus patients with normal pure-tone thresholds of hearing. *ORL J. Otorhinolaryngol. Relat. Spec.* 69, 25–29. doi: 10.1159/000096713

Jones, G. L., and Litovsky, R. Y. (2008). Role of masker predictability in the cocktail party problem. *J. Acoust. Soc. Am.* 124, 3818–3830. doi: 10.1121/1.2996336

Knudson, I. M., Shera, C. A., and Melcher, J. R. (2014). Increased contralateral suppression of otoacoustic emissions indicates a hyperresponsive medial olivocochlear system in humans with tinnitus and hyperacusis. *J. Neurophysiol.* 112, 3197–3208. doi: 10.1152/jn.00576.2014

Kollmeier, B., and Wesselkamp, M. (1997). Development and evaluation of a German sentence test for objective and subjective speech intelligibility assessment. *J. Acoust. Soc. Am.* 102, 2412–2421.

Landgrebe, M., Zeman, F., Koller, M., Eberl, Y., Mohr, M., Reiter, J., et al. (2010). The Tinnitus Research Initiative (TRI) database: a new approach for delineation of tinnitus subtypes and generation of predictors for treatment outcome. *BMC Med. Inform. Decis. Mak.* 10:42. doi: 10.1186/1472-6947-10-42

Langguth, B., Goodey, R., Azevedo, A., Bjorne, A., Cacace, A., Crocetti, A., et al. (2007). Consensus for tinnitus patient assessment and treatment outcome measurement: Tinnitus Research Initiative meeting, Regensburg, July 2006. *Prog. Brain Res.* 166, 525–536. doi: 10.1016/S0079-6123(07)66050-6

May-Mederake, B., and Shehata-Dieler, W. (2013). A case study assessing the auditory and speech development of four children implanted with cochlear implants by the chronological age of 12 months. *Case Rep. Otolaryngol.* 2013:359218. doi: 10.1155/2013/359218

Mertens, G., Kleine Punte, A., De Ridder, D., and Van de Heyning, P. (2013b). Tinnitus in a single-sided deaf ear reduces speech reception in the nontinnitus ear. *Otol. Neurotol.* 34, 662–666. doi: 10.1097/MAO.0b013e31828779f0

Mertens, G., Punte, A. K., and Van de Heyning, P. (2013a). Self-assessment of hearing disabilities in cochlear implant users using the SSQ and the reduced SSQ5 version. *Otol. Neurotol.* 34, 1622–1629. doi: 10.1097/MAO.0b013e31829ce980

Moon, I. J., Won, J. H., Kang, H. W., Kim, D. H., An, Y. H., and Shim, H. J. (2015). Influence of tinnitus on auditory spectral and temporal resolution and speech perception in tinnitus patients. *J. Neurosci.* 35, 14260–14269. doi: 10.1523/JNEUROSCI.5091-14.2015

Myoga, M. H., Lehnert, S., Leibold, C., Felmy, F., and Grothe, B. (2014). Glycinergic inhibition tunes coincidence detection in the auditory brainstem. *Nat. Commun.* 5, 3790. doi: 10.1038/ncomms4790

Newman, C. W., Wharton, J. A., Shivapuja, B. G., and Jacobson, G. P. (1994). Relationships among psychoacoustic judgments, speech understanding ability and self-perceived handicap in tinnitus subjects. *Audiology* 33, 47–60.

Noreña, A. J. (2011). An integrative model of tinnitus based on a central gain controlling neural sensitivity. *Neurosci. Biobehav. Rev.* 35, 1089–1109. doi: 10.1016/j.neubiorev.2010.11.003

Norena, A., Micheyl, C., Chéry-Croze, S., and Collet, L. (2002). Psychoacoustic characterization of the tinnitus spectrum: implications for the underlying mechanisms of tinnitus. *Audiol. Neurootol.* 7, 358–369. doi: 10.1159/000066156

Ryu, I. S., Ahn, J. H., Lim, H. W., Joo, K. Y., and Chung, J. W. (2012). Evaluation of masking effects on speech perception in patients with unilateral chronic tinnitus using the hearing in noise test. *Otol. Neurotol.* 33, 1472–1476. doi: 10.1097/MAO.0b013e31826dbcc4

Schaette, R., and McAlpine, D. (2011). Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J. Neurosci.* 31, 13452–13457. doi: 10.1523/JNEUROSCI.2156-11.2011

Schecklmann, M., Landgrebe, M., Langguth, B., and TRI Database Study Group. (2014). Phenotypic characteristics of hyperacusis in tinnitus. *PLoS ONE* 9:e86944. doi: 10.1371/journal.pone.0086944

Schecklmann, M., Lehner, A., Schlee, W., Vielsmeier, V., Landgrebe, M., and Langguth, B. (2015). Validation of screening questions for hyperacusis in chronic tinnitus. *Biomed. Res. Int.* 2015:191479. doi: 10.1155/2015/191479

Shore, S. E., Roberts, L. E., and Langguth, B. (2016). Maladaptive plasticity in tinnitus–triggers, mechanisms and treatment. *Nat. Rev. Neurol.* 12, 150–160. doi: 10.1038/nrneurol.2016.12

Soalheiro, M., Rocha, L., do Vale, D. F., Fontes, V., Valente, D., and Teixeira, L. R. (2012). Speech recognition index of workers with tinnitus exposed to environmental or occupational noise: a comparative study. *J. Occup. Med. Toxicol.* 7:26. doi: 10.1186/1745-6673-7-26

Spyridakou, C., Luxon, L. M., and Bamiou, D. E. (2012). Patient-reported speech in noise difficulties and hyperacusis symptoms and correlation with test results. *Laryngoscope* 122, 1609–1614. doi: 10.1002/lary.23337

Tyler, R. S., and Baker, L. J. (1983). Difficulties experienced by tinnitus sufferers. *J. Speech Hear. Disord.* 48, 150–154.

Vielsmeier, V., Lehner, A., Strutz, J., Steffens, T., Kreuzer, P. M., Schecklmann, M., et al. (2015). The relevance of the high frequency audiometry in tinnitus patients with normal hearing in conventional pure-tone-audiometry. *Biomed. Res. Int.* 2015:302515. doi: 10.1155/2015/302515

Weisz, N., Hartmann, T., Dohrmann, K., Schlee, W., and Norena, A. (2006). High-frequency tinnitus without hearing loss does not mean absence of deafferentation. *Hear. Res.* 222, 108–114. doi: 10.1016/j.heares.2006.09.003

Weisz, N., Hartmann, T., Müller, N., Lorenz, I., and Obleser, J. (2011). Alpha rhythms in audition: cognitive and clinical perspectives. *Front. Psychol.* 2:73. doi: 10.3389/fpsyg.2011.00073

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REVIEW
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Misophonia and Potential Underlying Mechanisms: A Perspective

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There is a growing research interest in the diagnosis rate of misophonia, a condition characterized by a negative emotional/autonomic reaction to specific everyday sounds. Diagnosis of misophonia requires a thorough case history and audiological test procedures. Associative and non-associative learning models for understanding the underlying mechanisms of misophonia have been presented. Currently, there is no cure or pharmaceutical agent for misophonia; however, therapy programs addressing misophonia and its characteristics do exist. Investigation of comorbid conditions and other psychological therapy strategies might help to reveal more about the underlying mechanisms and potentially lead to a successful treatment method.

Keywords: misophonia, auditory system, limbic system, associative learning, classical conditioning, sensitization

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INTRODUCTION

Misophonia is a condition where patients experience a negative emotional reaction and dislike (e.g., anxiety, agitation, and annoyance) to specific sounds (e.g., ballpoint pen clicking (repeatedly), tapping, typing, chewing, breathing, swallowing, tapping foot, etc.) (Jastreboff and Jastreboff, 2002). Misophonia is a derivate from the Greek words misos (hate) and phónè (voice), and means hate of sound. Each patient's reaction is unique as it depends on the specific conditions under which the sound was experienced and any previous evaluations of that sound. Prior to Jastreboff introducing the term misophonia, there have been different terms to describe the condition, such as soft sound sensitivity symptom, select sound sensitivity syndrome, decreased sound tolerance, and sound-rage (Schwartz et al., 2011; Neal and Cavanna, 2013). A patient's negative reaction and dislike may occur in response to sound at any level. Although hyperacusis and misophonia can coexist, hyperacusis refers specifically to an increased sensitivity to certain frequencies and volume ranges of sound (Song et al., 2014). Misophonia can be distinguished from hyperacusis by its sensitivity to the subjective response provoked (Pienkowski et al., 2014). A subtype of misophonia is phonophobia, when fear to a specific sound is the dominant factor (Jastreboff and Hazell, 1999; Henry et al., 2002; Jastreboff and Jastreboff, 2015). It is important to recognize that "subtype" implies that the class of sounds that elicit phonophobia are drawn from misophonic sounds or that they share a similar mechanism, neither of which is necessarily true. From a phenomenological viewpoint, while fear is the dominant emotion in phonophobia, anger is the dominant emotion in misophonia. However, more recent research suggest that other than anger there is at least four other dominant emotions present in misophonia (i.e., irritation, stress and anxiety, aggravation, feeling trapped, and impatience) (Rouw and Erfanian, 2018).

INCIDENCE

In a recent study, Wu et al. (2014) investigated the incidence, correlates, and impairments associated with misophonia in a student population. Out of 483 undergraduate students (mean age = 21.4 years), 22.8% were often or always sensitive to or annoyed by specific sounds (e.g., eating, repetitive tapping, or nasal noises). Dislike of throat sounds, rustling papers, and environmental sounds were reported by 19.5, 16.1, and 14% of respondents, respectively. Literature suggests that 60% of patients with tinnitus also have misophonia (Jastreboff and Jastreboff, 2002; Jastreboff and Hazell, 2004) and 86% of tinnitus patients have hyperacusis, 25–30% of which requiring treatment (Anari et al., 1999; Jastreboff and Jastreboff, 2006). Jastreboff deduced that 1.75% of the general population has hyperacusis without tinnitus, but it is still difficult to differentiate those who have hyperacusis alone, misophonia alone, and those who have both (Jastreboff, 2015).

CHARACTERISTICS

Misophonia usually begins during childhood or adolescence, sometimes affecting academic performance (Edelstein et al., 2013; Schroder et al., 2013). An intense negative emotional reaction is usually triggered by bodily sounds (e.g., chewing, breathing, swallowing, and foot tapping, etc.) and may be connected to a particular person creating that sound (Edelstein et al., 2013; Schroder et al., 2013). In addition to the emotional aversion, patients sometimes report physical pressure building in the chest, the desire to stop the person from making the sound, and other autonomic reactions (Moller, 2011). Sometimes patients will mimic the sound to cancel it out. Rarely do physical reactions, such as assaulting the person making the sound, occur. However, because the patient is never sure when the trigger sound might be heard, the patient often lives in a perpetual state of anxiety. Patients are hyper-focused on listening for that trigger; they will avoid certain situations, people, and foods that they think will cause the sound (Edelstein et al., 2013). Overall, patients may suffer physical and emotional discomfort, contributing to a reduced quality of life (Edelstein et al., 2013).

According to Jastreboff and Jastreboff (2015), only 7 cases (2.2%) out of 318 misophonic patients exhibited a psychiatric disorder. Some researchers argue that misophonia and psychiatric disorders are unrelated. However, others tend to believe that psychiatric disorders and misophonia might coexist. Schroder et al. (2013) conducted a study to classify misophonia as its own form of psychiatric disorder. Their results showed a pattern of intense reactions to specific stimuli, avoidance, and worry that matched with traits of other psychiatric disorders, i.e., social phobia, post-traumatic stress disorder, personality disorders with impulsive aggression, intermittent explosive disorder, autism spectrum disorder, sensory processing disorders, antisocial personality disorder, and phonophobia (Schroder et al., 2013). Although the nosological nature of misophonia is still a topic of debate, Schroder's findings seem to call for misophonia to be classified as a subtype of a discrete psychiatric disorder.

DIAGNOSIS

Clinically, diagnosing misophonia requires a detailed case history to determine onset, triggers, reactions, and co-morbid conditions. Questionnaires may also be useful when determining the severity and uniqueness of each patient's case. Although certain questionnaires have been proposed to evaluate the severity of misophonia (Khalfa et al., 2002; Dauman and Bouscau-Faure, 2005), their validity needs to be confirmed. On the other hand, no one questionnaire has been consistently used across studies for the evaluation of misophonia. Examples of some of the questionnaires currently being used to evaluate misophonia are: (1) the Misophonia Questionnaire (MQ), which is a three-part self-report questionnaire developed by Wu et al. (2014) to assess the presence of misophonia symptoms as well as related emotions and behaviors; and (2) the Amsterdam Misophonia Scale (A-MISO-S), a concept scale based on the already validated Yale-Brown Obsessive-Compulsive Scale (Y-BOCS) (Schroder et al., 2013). The A-MISO-S is a six-item scale that evaluates different areas affected by misophonia such as: the time spent focusing on misophonia; interference with social functions; level of anger; impulse control; control over thoughts and anger; and time spent avoiding situations contributing to misophonia.

The audiological assessment of misophonia is complex. To date there is no agreement on a specified protocol to assess misophonia. However, audiological assessment includes pure tone thresholds and loudness discomfort levels (LDL). Patients with misophonia may have hearing loss or normal hearing. LDLs have been reported at normal and reduced levels (Jastreboff and Jastreboff, 2013). There is no precise description of how to test LDLs in patients with misophonia. It is therefore possible that variations can occur in the results obtained due to the specific method administrated and differences in the way patients are instructed (Hawkins et al., 1987; Sherlock and Formby, 2005; Jastreboff and Jastreboff, 2015). Nevertheless, Jastreboff and Jastreboff (2015) indicated that when misophonia is present with hyperacusis, LDL values can range from 30 to 120 dB HL. This further emphasizes that LDLs alone are insufficient to accurately diagnose hyperacusis and/or misophonia (Jastreboff and Jastreboff, 2015). Differences in auditory late potentials may be present when patients are tested using an oddball paradigm. Schroder et al. (2014) concluded that the deviant tone evoked a smaller N100 in misophonia patients than in healthy controls. Such responses might have been because of deficits in processing auditory information at low intensities or because of coexisting mood and psychiatric conditions. This study supports the recommendation of a thorough case history and use of questionnaires to understand all aspects of the patient's life that may contribute to misophonia (Ferreira et al., 2013; Schroder et al., 2013).

Anecdotal Cases in the Literature

A 36-year-old woman, Ms. A, raised in foster homes, had a mother suffering from depression and a father who did not provide support and affection (Veale, 2006). At the time that she visited the clinic, she was married and had one son.

Ms. A was a solitary person who desired to be deaf. Her desire was so intense that she would block her ears with cotton balls soaked in oil. Veale (2006) had the patient complete a series of questionnaires and found that she was extremely averse to sounds of any kind and fulfilled criteria for multiple personality disorders. In this case, repeated avoidance of sound might have led to increased auditory gain and worsening of symptoms.

Neal and Cavanna (2013) offered a case study of a 52-year-old man suffering from Tourette syndrome and misophonia. Neuropsychiatric examination revealed multiple motor ticks (e.g., facial grimacing and shoulder shrugging) and phonic ticks (e.g., yelping and barking) since age 11. The man also had mild obsessive-compulsive behaviors, depression, and sleep problems. Interestingly, this man noticed his aversion to sounds (e.g., father chewing food, sounds when riding a bus) developed about 1 year before his tics started. The authors thus speculated that there may be a pathophysiological association between the two conditions.

Webber et al. (2014) reported a pediatric case of misophonia and Tourette syndrome. The young female patient was also diagnosed with comorbid obsessive-compulsive spectrum disorder (OCD) and attention deficit hyperactivity disorder (ADHD). At 6 years of age, she developed frequent motor and vocal tics. During an interview, the young girl reacted strongly to certain auditory and visual stimuli and demanded that the sound stop. These findings are like those reported by Neal and Cavanna (2013), i.e., obsessive compulsive tendencies, an early age of onset, and the presence of both motor and vocal tics. The neural circuitry involved in OCD and Tourette syndrome may be similar to that of misophonia (Husted et al., 2006; Neal and Cavanna, 2013). A methodical screening for misophonia in disorders such as Tourette syndrome and OCD might uncover a pathophysiological connection between sounds and anomalies within the limbic system and its connections with the auditory cortex and the autonomic nervous system.

Kluckow et al. (2014) interviewed 15 patients being treated for eating disorders about possible misophonia symptoms. Three of the 15 patients met the criteria for misophonia. The first patient recalled her misophonia trigger to high-pitched voices starting around age six. Hearing the sound would cause her to binge eat. Coping mechanisms included ear plugs, music for distraction, and digging her fingernail into her hand to cause pain but not draw blood. The second patient had misophonia triggered by the eating habits of family friends. Following the development of her aversion, the patient started to increase exercise and decrease eating. The third patient presented with misophonia caused by the sound of family members eating cereal out of a bowl; she found the clinking spoon in the bowl and the sound of cereal being chewed repulsive. Aversion to these stimuli was so strong that she was unable to eat. In all three cases, severe aversion to eating sounds preceded the development of an eating disorder. Kluckow et al. (2014) suggested that the co-presentation of misophonia and eating disorders should be investigated during a thorough case history.

TREATMENT

Currently, there are no research studies that have investigated pharmaceutical options to treat misophonia. Anecdotal information suggests the prescription of antidepressants and anxiolytics to address the reactions and co-morbid conditions associated with misophonia. Despite the lack of pharmaceutical remedies, a variety of therapies have been considered, with some showing signs of potential success.

Tinnitus retraining therapy (TRT) is a therapy program designed by Jastreboff to manage tinnitus and, as a secondary effect, hyperacusis and misophonia (Jastreboff and Jastreboff, 2006). TRT assumes that altering conditioned reflexes at the subconscious level will reduce or eliminate the connection between the auditory system and the limbic and autonomic nervous systems (Kiessling, 1980). Relating these auditory conditions to conditioned reflexes also helps to understand how loudness correlates with severity (Tyler et al., 2007). The connection is reinforced by the two stimuli and the auditory characteristics are of less importance. The therapy protocol consists of directive counseling and sound therapy, tailored to each patient's specific situation. This model emphasizes the need for the patient to understand the underlying mechanisms of the condition. The patient will adhere to the treatment assigned and make conscious efforts to alter the underlying mechanisms once s/he understands how they operate. A clear review of TRT and individual goals between patient and clinical provider will ensure the patient has realistic expectations. For sound therapy, TRT works to reinforce positive sounds and reduce exposure to sounds causing a negative reaction. Exposure to background noise and avoidance of silence work to desensitize certain sounds. If the patient also has hearing loss, s/he may benefit from ear-level combination devices, which provide amplification and tranquil background sounds. Overall, TRT should take about 9–18 months to complete (Jastreboff and Jastreboff, 2002). If the patient is suffering from hyperacusis and misophonia, Jastreboff and Jastreboff (2015) recommend first treating hyperacusis. Once misophonia is isolated, the goal is to change the relationship between the auditory, limbic, and autonomic nervous systems and to eliminate the conditioned reflex. Although the conditioned-reflex model of TRT has been challenged in theory (Tyler et al., 2006), TRT should work better with misophonia patients than with tinnitus patients because misophonia involves an external trigger, which can be manipulated to potentially eliminate the conditioned response (Jastreboff and Jastreboff, 2002). Misophonia patients undergoing TRT are encouraged to avoid silence and ear overprotection. Decreasing patients' reactions to their trigger sounds by introducing pleasant sounds and constant low intensity sounds is thought to improve these patients' conditions. Reclassifying the sounds plus heavy counseling are recommended to help these patients. TRT outcomes were reported by Jastreboff et al., as 152 out of 184 patients with misophonia with hyperacusis, and patients with misophonia without hyperacusis (139 patients out of 167) showed improvements after TRT (Jastreboff and Jastreboff, 2015).

Although the development of a therapeutic approach such as TRT often applied it has not proven to be clinically effective. This has led to some controversy regarding the application of this theory as a therapeutic approach, partially because the underlying mechanism of misophonia is not well understood. Besides TRT, other neuropsychiatric therapies might be effective for misophonia patients. Schneider and Arch (Schneider and Arch, 2015) reviewed potential treatments for misophonia based on specific characteristics of the condition. Since one of the common responses to a misophonia trigger is anger, they suggested focusing on therapies that work to decrease anger, such as cognitive restructuring and stress inoculation training (Blake and Hamrin, 2007). A literature review of therapy and training programs for anger in youth populations indicated that Cognitive-Relaxation Coping Skills Training and Multicomponent Cognitive-Behavioral Therapy (CBT) were able to reduce anger-related behaviors and improve control over the expression of anger (Blake and Hamrin, 2007). Similarly, a stress inoculation training program for high school students significantly decreased negative stress events and reduced overall anger (Hains, 1992). Since misophonic symptoms often arise in adolescence, these anger therapies may also apply to the misophonia population.

Other ways to address misophonia could be through compassion training, distress-tolerance, and acceptance-based treatments (Schneider and Arch, 2015). Mindfulness (moment-to-moment awareness) may enhance traditional CBT programs in psychologically distraught patients (Hofmann et al., 2011). In a group of moderately to severely distressed tinnitus patients, internet-based CBT and acceptance and commitment therapy (ACT) were both shown to reduce the negative impact of tinnitus on quality of life, as measured by tinnitus severity and tinnitus-related distress (Hesser et al., 2014). Techniques used in these programs included applied relaxation, positive imagery, attention training, cognitive restructuring, exposure, and background sounds to cope with tinnitus. There are only two case studies reported in the literature that show the effects of CBT for youths with misophonia (McGuire et al., 2015). After completing psychoeducation, reviewing how to appropriately respond when a trigger is present, and gradually increasing exposure to the trigger sounds, the two female patients had reduced symptoms and expressed that they now had the tools to cope. Although two case studies alone do not constitute concrete evidence that CBT will work with misophonia patients, it does invite further research to determine the efficacy of such a therapy program.

The potential therapies listed above look to address emotional reactions caused by misophonia triggers. In order to have a truly effective treatment, more research is needed to better define a diagnostic protocol, rule out comorbid conditions, and to provide evidence for the underlying mechanisms of misophonia (Webber and Storch, 2015). Since misophonia triggers can be from many sources, people, and situations, it is likely that one therapy program will not address every manifestation of misophonia, indicating a need for individualized therapy.

UNDERLYING MECHANISMS

Auditory information ascends through the brainstem to the cerebral cortices in two parallel pathways, mainly known as the classical and non-classical auditory pathways (Moller and Rollins, 2002). The anatomy of the non-classical pathway differs from that of the classical pathway mainly in the thalamic relay nuclei (Moller and Rollins, 2002). The classical pathway is intermittent in the ventral portion of the medial geniculate body, while the non-classical pathway is interrupted in nuclei located in the medial and dorsomedial geniculate body (Moller and Rollins, 2002; Moller et al., 2005). Recall that misophonia is described as a negative reaction to sound results from enhanced limbic and autonomic responses without abnormal enhancement of the auditory system (Jastreboff, 1999; Jastreboff and Hazell, 1999). Since it is known that classical and non-classical auditory pathways interact with the limbic system, a breakdown in such processes may contribute to an increased association between auditory stimuli and emotional and autonomic reactions (Jastreboff and Hazell, 2004; Langguth and Landgrebe, 2011).

The literature suggests that the majority of patients with misophonia have normal hearing sensitivity (Schroder et al., 2014), while the limbic and autonomic nervous systems are in a heightened state of excitation and thus react abnormally to normal auditory input (Moller, 2011). A recent functional and structural MRI study has revealed that trigger sounds elicited increased responses in the anterior insular cortex (AIC) and abnormal functional connectivity between the AIC and medial frontal, medial parietal, and medial temporal regions (Kumar et al., 2017). The findings of Kumar et al. (2017) implied that there was abnormal myelination in the medial frontal cortex that shows abnormal functional connectivity, and that the aberrant neural response mediates the emotional coloring and physiological arousal that accompany misophonic experiences.

A focal point of rebuttal by the Kumar et al. (2017), study lies in their experimental designs where general annoyance elicited by one stimulus condition (i.e., baby crying, a person screaming) was disassociate from a specifically misophonic reaction elicited by another stimulus condition (i.e., eating and breathing sounds). In a commentary (Schroder et al., 2017), Schroder et al. argued that it was unclear whether the subjects in the Kumar et al. (2017) study actually suffered from misophonia, as Schroder et al. promote the idea that misophonia is a distinct form of a psychiatric disorder with specific and well-defined diagnostic criteria (Schroder et al., 2013). Secondly, the validity of the questionnaire used to select subjects with misophonia were put into question. In addition, anger, which is an essential component in the diagnosis of misophonia, was overlooked and instead the focus was on annoyance. As such, part of Schroder et al.'s argument was that the observed brain differences in the Kumar et al. (2017) study might be correlated to general annoyance rather than anger specifically. A final comment was about the design of the study and how it might have put subjects at risk of sensitization to sound by repeated exposure (Kumar and Griffiths, 2017; Schroder et al., 2017).

In response, Kumar et al. (2017) stated that, to date, there are no diagnostic criteria for misophonia in ICD 10 or DSM-5, as

subjects were selected based on having stable typical response to trigger sounds over years which are usually anger but can also come in the form of anxiety. This argument was supported by findings of a large scale study involving more than 300 subjects with misophonia, who primary reported emotional responses in the form of irritation/annoyance and not anger (Kumar and Griffiths, 2017). Finally, regarding Schroder et al.'s last comment, Kumar argued that it was not clear how re-exposure to sounds that have been producing a typical misophonic reaction for years might have any bearing on the reaction produced (Kumar and Griffiths, 2017). Despite the controversy surrounding these findings, the AIC is one of the core components of limbic and autonomic nervous system activity control.

Learning involves associating events happening at different times, a process that is of fundamental importance for a number of perceptual and cognitive processes (Wallenstein et al., 1998; Fuster et al., 2000). There are two forms of learning, associative and non-associative, which we will briefly describe and then use to elucidate misophonia. Associative learning involves one stimulus presented simultaneously with another stimulus, creating a specific reaction. Conditioning to stimuli can be either through classical or operant conditioning (Vlaeyen, 2015). Non-associative learning is a change in behavior after repeated presentations of a stimulus, but there is no reinforcement via a second stimulus like there is in associative learning. In response to a single stimulus, an individual can either experience habituation or sensitization. Habituation is a decrease in response to a stimulus following multiple identical presentations (Ursin, 2014). In healthy systems, habituation and sensitization counteract one another and allow the individual to stay in a neutral state (Ursin, 2014). Associative learning, particularly classical conditioning, and non-associative learning, particularly sensitization, may help to explain the underlying mechanisms of misophonia.

Associative and Non-associative Learning

Jastreboff and Jastreboff (2002) developed a model to explain the neural mechanisms governing tinnitus, hyperacusis, and misophonia (Jastreboff and Jastreboff, 2002). Associative learning via classical conditioning supports their theory. Since classical conditioning works in anticipation of a change to the environment (Vlaeyen, 2015), they hypothesized that patients suffering from one or all three of these problems have enhanced connections between their auditory system and their limbic and autonomic nervous systems (Jastreboff and Hazell, 2004). Misophonia is a form of conditioned behavior that develops as a physical reflex through classical conditioning with a misophonia trigger (e.g., eating noises, lip-smacking, pen clicking, tapping and typing . . .) as the conditioned stimulus, and anger, irritation or stress the unconditioned stimulus. The involvement of the limbic system helps to explain the emotional component to this condition and suggests that the connection is controlled by a conditioned reflex. Three components of the limbic system (the amygdala, parahippocampus, and insula) have been shown to activate more strongly in other conditions such as tinnitus (Song et al., 2013, 2015; Carpenter-Thompson et al., 2014). Moller (2013) also suggested that the amygdala's control of fear, depression, and anxiety may explain a connection to the auditory system in patients with phonophobia.

Activating the limbic and autonomic nervous systems triggers irrational reactions to stimuli (Molini et al., 2014). Hyperacusis patients have increased auditory sensitivity, which is passed on to the autonomic nervous system and results in increased activation (Jastreboff and Jastreboff, 2015). Similarly, misophonia patients have an increase in activation between the auditory pathways and autonomic nervous system, resulting in negative emotional reaction to sound. Similarly, pain processing involves an association with intrusion, long-term exposure, depression, anxiety, defensive responses, and prolonged avoidance (Vlaeyen, 2015). Misophonia patients have triggers that cause annoyance, anxiety, and depression. They respond by trying to ignore or escape the stimulus. Prolonged avoidance can exacerbate the condition. Misophonia patients may plug their ears with cotton balls or live a life of silence (Veale, 2006; Edelstein et al., 2013). As in pain, the trigger is not life-threatening, and yet it leads to worry, fear, and anxiety. Although these points should not be treated as specific to misophonia, further investigation into tinnitus, hyperacusis, and pain mechanisms may also help to understand misophonia.

This begs the question of why some people learn this type of association (classical conditioning) and not others. Classical conditioning or associative learning elicits reflexive, automatic, and involuntary behavior (Jarius and Wildemann, 2015; Kotchoubey and Pavlov, 2017). The only responses that can be elicited out of classical conditioning are those that rely on responses that are naturally made by the individual with misophonia or, for that matter, any other condition. These responses are often involuntary and occurring below the level of conscious awareness (Jarius and Wildemann, 2015). The hallmark of misophonia is an extreme emotional response to the trigger stimulus. As a result, for misophonic patients, these emotional responses might create a classical conditioning paradigm that maintains or strengthens the misophonic physical reflex (Schroder et al., 2013). With that said, individual differences in associative learning do exist, in part due to psychological and individual personality variables (Murphy and Msetfi, 2014).

On the other hand, non-associative learning can result in habituation or sensitization. The symptoms of misophonia arise from enhanced sensitized functional connections or shortcuts between the limbic, auditory, and autonomic nervous system (Schwartz et al., 2011). Sensitization is defined as increased neuronal activity in response to a stimulus (Jimenez et al., 2017). Before stimuli or neural activity reach the brain, they are classified and evaluated by complex neuronal pathways. If the signal becomes actively connected to previous emotions or memories, there will be an overlap in the auditory signal and emotions, creating a complex neural signal. That complex neural signal is what finally reaches the level of conscious awareness; hence, the anxiety and stress related to the auditory signal are incorporated subconsciously and revealed at the conscious level. After repeated cycles of this process, the neuronal reaction

threshold decreases and allows the complex hyper-responses to reach the brain more easily (Zenner et al., 2006). The underling mechanism for sensitization in misophonia is unknown, but has typical been associated to strengthening of synaptic signals, a process known as long-term potentiation, or "kindling," repeated stimulation of specific neurons in the limbic system. In a recent publication showed that misophonia patients can have enhanced autonomic reactivity to a sound, but not to other sensory stimuli (Edelstein et al., 2013). The subjective experiences described by these misophonia patients to trigger sounds share qualitative features with the sensory symptoms reported by patients with tic disorders such as Tourette syndrome. Recent reports have also suggested that misophonic symptoms can be found in the context of two of the most common psychiatric comorbidities of Tourette syndrome, in addition to obsessive-compulsive disorder, generalized anxiety disorder, and schizotypal personality disorder (Ferreira et al., 2013; Neal and Cavanna, 2013; Cavanna and Martino, 2014). Overall, although there is preliminary evidence supporting the suggestion that the underlying mechanism can occur in misophonia due to sensitization more research is needed.

Association Between Misophonia and Synesthesia

The brain constantly integrates signals across different modalities. To that extent, a defining aspect of misophonia occurs when the disturbing sound produced by others provoke an emotional response. This emotional response exhibits a marked connection between the auditory system and other limbic and autonomic systems (Bruxner, 2016). This process resembles another phenomenon: synesthesia which is the occurrence of a particular sensory stimulus can evoke additional sensations and associations (Galton, 1883; Harrison and Baron-Cohen, 1995; Baron-Cohen et al., 1996; Barnett et al., 2008; Edelstein et al., 2013). It is proposed that synesthesia results from an increase of neural connections and interactions between different sensory modalities (Brang and Ramachandran, 2011; Mylopoulos and Ro, 2013).

There may be a connection between misophonia and synesthesia. In synesthesia, as in misophonia, a pathological distortion of connections between the auditory cortex and limbic structures can cause a form of sound-emotion synesthesia (Edelstein et al., 2013). Furthermore, in both phenomena, an external sound can produce internal perceptual and sensational experiences (Barratt and Davis, 2015). There are also reports suggesting that the two phenomena are linked by their affective components, in addition to their perceptual similarities (Edelstein et al., 2013). For example, negative autonomic reactions are associated with the experience of misophonia. Specifically, sufferers report that noises of any volume made by others such as breathing, swallowing, or foot tapping can elicit feelings of disgust, anger, or hatred (Schroder et al., 2013). Misophonic experiences are also similar to synesthetic associations in that they are both automatic and cross-modal. Further exploration of the similarities between these two conditions is needed to discover whether and how these two phenomena are related.

CONCLUSION

Certain characteristics of misophonia that follow rules from both associative and non-associative learning principles could possibly be used to better understand the underlying mechanisms. If non-associative learning does help to explain the underlying mechanisms of misophonia, then there needs to be research investigating this connection. To date, research has made only weak speculation, with little evidence to support the theory. TRT seems to be an effective treatment option for patients with sound sensitivity disorders such as misophonia. Although the majority of patients do find relief through TRT, there are still cases that receive no relief. Finally, Vlaeyen (2015) suggested a connection between non-associative and associative learning. The anxiety evoked by a stimulus may induce negative effects, causing sensitization. Perhaps this is the key to a successful misophonia treatment. By combining TRT and sensitization strategies, those few patients who do not receive relief via either method alone might benefit from a combined method. Future studies should focus on further examining the relationship between associative and non-associative learning and misophonia.

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REFERENCES

Anari, M., Axelsson, A., Eliasson, A., and Magnusson, L. (1999). Hypersensitivity to sound–questionnaire data, audiometry and classification. *Scand. Audiol.* 28, 219–230. doi: 10.1080/010503999424653

Barnett, K. J., Finucane, C., Asher, J. E., Bargary, G., Corvin, A. P., Newell, F. N., et al. (2008). Familial patterns and the origins of individual differences in synaesthesia. *Cognition* 106, 871–893. doi: 10.1016/j.cognition.2007.05.003

Baron-Cohen, S., Burt, L., Smith-Laittan, F., Harrison, J., and Bolton, P. (1996). Synaesthesia: prevalence and familiality. *Perception* 25, 1073–1079. doi: 10.1068/p251073

Barratt, E. L., and Davis, N. J. (2015). Autonomous Sensory Meridian Response (ASMR): a flow-like mental state. *PeerJ* 3:e851. doi: 10.7717/peerj.851

Blake, C. S., and Hamrin, V. (2007). Current approaches to the assessment and management of anger and aggression in youth: a review. *J. Child Adolesc. Psychiatr. Nurs.* 20, 209–221. doi: 10.1111/j.1744-6171.2007.00102.x

Brang, D., and Ramachandran, V. S. (2011). Survival of the synesthesia gene: why do people hear colors and taste words? *PLoS Biol.* 9:e1001205. doi: 10.1371/journal.pbio.1001205

Bruxner, G. (2016). 'Mastication rage': a review of misophonia - an under-recognised symptom of psychiatric relevance? *Australas. Psychiatry*24, 195–197. doi: 10.1177/1039856215613010

Carpenter-Thompson, J. R., Akrofi, K., Schmidt, S. A., Dolcos, F., and Husain, F. T. (2014). Alterations of the emotional processing system may underlie preserved rapid reaction time in tinnitus. *Brain Res.* 1567, 28–41. doi: 10.1016/j.brainres.2014.04.024

Cavanna, A. E., and Martino, D. (2014). How many Gilles de la Tourette syndromes? *Eur. J. Neurol.* 21, 685–686. doi: 10.1111/ene.12282

Dauman, R., and Bouscau-Faure, F. (2005). Assessment and amelioration of hyperacusis in tinnitus patients. *Acta Otolaryngol.* 125, 503–509. doi: 10.1080/00016480510027565

Edelstein, M., Brang, D., Rouw, R., and Ramachandran, V. S. (2013). Misophonia: physiological investigations and case descriptions. *Front. Hum. Neurosci.* 7:296. doi: 10.3389/fnhum.2013.00296

Ferreira, G. M., Harrison, B. J., and Fontenelle, L. F. (2013). Hatred of sounds: misophonic disorder or just an underreported psychiatric symptom? *Ann. Clin. Psychiatry* 25, 271–274.

Fuster, J. M., Bodner, M., and Kroger, J. K. (2000). Cross-modal and cross-temporal association in neurons of frontal cortex. *Nature* 405, 347–351. doi: 10.1038/35012613

Galton, F. (1883). *Inquiries into Human Faculty and its Development*. London: Macmillan. doi: 10.1037/14178-000

Hains, A. A. (1992). A stress inoculation training program for adolescents in a high school setting: a multiple baseline approach. *J. Adolesc.* 15, 163–175. doi: 10.1016/0140-1971(92)90045-7

Harrison, J., and Baron-Cohen, S. (1995). Synaesthesia: reconciling the subjective with the objective. *Endeavour* 19, 157–160. doi: 10.1016/0160-9327(96)82878-X

Hawkins, D. B., Walden, B. E., Montgomery, A., and Prosek, R. A. (1987). Description and validation of an LDL procedure designed to select SSPL90. *Ear Hear.* 8, 162–169. doi: 10.1097/00003446-198706000-00006

Henry, J. A., Jastreboff, M. M., Jastreboff, P. J., Schechter, M. A., and Fausti, S. A. (2002). Assessment of patients for treatment with tinnitus retraining therapy. *J. Am. Acad. Audiol.* 13, 523–544.

Hesser, H., Westin, V. Z., and Andersson, G. (2014). Acceptance as a mediator in internet-delivered acceptance and commitment therapy and cognitive behavior therapy for tinnitus. *J. Behav. Med.* 37, 756–767. doi: 10.1007/s10865-013-9525-6

Hofmann, S. G., Grossman, P., and Hinton, D. E. (2011). Loving-kindness and compassion meditation: potential for psychological interventions. *Clin. Psychol. Rev.* 31, 1126–1132. doi: 10.1016/j.cpr.2011.07.003

Husted, D. S., Shapira, N. A., and Goodman, W. K. (2006). The neurocircuitry of obsessive-compulsive disorder and disgust. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 30, 389–399.

Jarius, S., and Wildemann, B. (2015). And Pavlov still rings a bell: summarising the evidence for the use of a bell in Pavlov's iconic experiments on classical conditioning. *J. Neurol.* 262, 2177–2178. doi: 10.1007/s00415-015-7858-5

Jastreboff, M., and Jastreboff, P. (2002). Decreased sound tolerance and tinnitus retraining therapy (TRT). *Aust. N. Z. J. Audiol.* 24, 74–84. doi: 10.1375/audi.24.2.74.31105

Jastreboff, P., and Hazell, J. (2004). *Tinnitus Retraining Therapy: Implementing the Neurophysiological Model*. Cambridge: Cambridge University Press. doi: 10.1017/CBO9780511544989

Jastreboff, P., and Jastreboff, M. (2013). Using TRT to treat hyperacusis, misophonia and phonophobia. *ENT Audiol. News* 21, 88–90.

Jastreboff, P. J. (1999). Tinnitus retraining therapy. A clinical implementation of the neurophysiological model of tinnitus. *Verhaltenstherapie* 9:33. doi: 10.1007/s00106-014-2979-1

Jastreboff, P. J. (2015). 25 years of tinnitus retraining therapy. *HNO* 63, 307–311. doi: 10.1007/s00106-014-2979-1

Jastreboff, P. J., and Hazell, W. P. J. (1999). Tinnitus retraining therapy. *Br. J. Audiol.* 33, 68–69.

Jastreboff, P. J., and Jastreboff, M. M. (2006). Tinnitus retraining therapy: a different view on tinnitus. *ORL J. Otorhinolaryngol. Relat. Spec.* 68, 23–29; discussion 29–30. doi: 10.1159/000090487

Jastreboff, P. J., and Jastreboff, M. M. (2015). Decreased sound tolerance: hyperacusis, misophonia, diplacousis, and polyacousis. *Handb. Clin. Neurol.* 129, 375–387. doi: 10.1016/B978-0-444-62630-1.00021-4

Jimenez, X. F., Abousssouan, A., Mandell, D., and Huffman, K. L. (2017). Additional evidence supporting the central sensitization inventory (CSI) as an outcome measure among chronic pain patients in functional restoration program care. *Spine J.* 17:1765. doi: 10.1016/j.spinee.2017.08.225

Khalfa, S., Dubal, S., Veuillet, E., Perez-Diaz, F., Jouvent, R., and Collet, L. (2002). Psychometric normalization of a hyperacusis questionnaire. *ORL J. Otorhinolaryngol. Relat. Spec.* 64, 436–442. doi: 10.1159/000067570

Kiessling, J. (1980). [Masking of tinnitus aurium by maskers and hearing aids (author's transl)]. *HNO* 28, 383–388.

Kluckow, H., Telfer, J., and Abraham, S. (2014). Should we screen for misophonia in patients with eating disorders? A report of three cases. *Int. J. Eat. Disord.* 47, 558–561. doi: 10.1002/eat.22245

Kotchoubey, B., and Pavlov, Y. G. (2017). Name conditioning in event-related brain potentials. *Neurobiol. Learn. Mem.* 145, 129–134. doi: 10.1016/j.nlm.2017.09.009

Kumar, S., and Griffiths, T. D. (2017). Response: commentary: the brain basis for misophonia. *Front. Behav. Neurosci.* 11:127. doi: doi: 10.3389/fnbeh.2017.00127 doi: 10.3389/fnbeh.2017.00127

Kumar, S., Tansley-Hancock, O., Sedley, W., Winston, J. S., Callaghan, M. F., Allen, M., et al. (2017). The brain basis for misophonia. *Curr. Biol.* 27, 527–533. doi: 10.1016/j.cub.2016.12.048

Langguth, B., and Landgrebe, M. (2011). "Tinnitus and depression," in *Textbook of Tinnitus*, eds A. R. Moller, B. Langguth, D. De Ridder, and T. Kleinjung (New York, NY: Springer), 493–498. doi: 10.1007/978-1-60761-145-5_63

McGuire, J. F., Wu, M. S., and Storch, E. A. (2015). Cognitive-behavioral therapy for 2 youths with misophonia. *J. Clin. Psychiatry* 76, 573–574. doi: 10.4088/JCP.14cr09343

Molini, E., Faralli, M., Calzolaro, L., and Ricci, G. (2014). Impact of identifying factors which trigger bothersome tinnitus on the treatment outcome in tinnitus retraining therapy. *ORL J. Otorhinolaryngol. Relat. Spec.* 76, 81–88. doi: 10.1159/000360994

Moller, A. R. (2011). "Misophonia, phonophobia, and "exploding head" syndrome," in *Textbook of Tinnitus*, eds A. R. Moller, B. Langguth, D. De Ridder, and T. Kleinjung (New York, NY: Springer), 25–26. doi: 10.1007/978-1-60761-145-5_4

Moller, A. R. (2013). *Hearing: Anatomy, Physiology, and Disorders of the Auditory System*. San Diego, CA: Plural Publishing, Inc.

Moller, A. R., Kern, J. K., and Grannemann, B. (2005). Are the non-classical auditory pathways involved in autism and PDD? *Neurol. Res.* 27, 625–629.

Moller, A. R., and Rollins, P. R. (2002). The non-classical auditory pathways are involved in hearing in children but not in adults. *Neurosci. Lett.* 319, 41–44. doi: 10.1016/S0304-3940(01)02516-2

Murphy, R. A., and Msetfi, R. M. (2014). Individual differences in associative learning. *Front. Psychol.* 5:466. doi: doi: 10.3389/fpsyg.2014.00466 doi: 10.3389/fpsyg.2014.00466

Mylopoulos, M. I., and Ro, T. (2013). Synesthesia: a colorful word with a touching sound? *Front. Psychol.* 4:763. doi: 10.3389/fpsyg.2013.00763

Neal, M., and Cavanna, A. E. (2013). Selective sound sensitivity syndrome (misophonia) in a patient with Tourette syndrome. *J. Neuropsychiatry Clin. Neurosci.* 25:E01. doi: 10.1176/appi.neuropsych.11100235

Pienkowski, M., Tyler, R. S., Roncancio, E. R., Jun, H. J., Brozoski, T., Dauman, N., et al. (2014). A review of hyperacusis and future directions: part I Measurement, I., mechanisms, and treatment. *Am. J. Audiol.* 23, 420–436. doi: 10.1044/2014_AJA-13-0037

Rouw, R., and Erfanian, M. (2018). A large-scale study of misophonia. *J. Clin. Psychol.* 74, 453–479. doi: 10.1002/jclp.22500

Schneider, R. L., and Arch, J. J. (2015). Letter to the editor: potential treatment targets for misophonia. *Gen. Hosp. Psychiatry* 37, 370–371. doi: 10.1016/j.genhosppsych.2015.03.020

Schroder, A., van Diepen, R., Mazaheri, A., Petropoulos-Petalas, D., Soto de Amesti, V., Vulink, N., et al. (2014). Diminished n1 auditory evoked potentials to oddball stimuli in misophonia patients. *Front. Behav. Neurosci.* 8:123. doi: 10.3389/fnbeh.2014.00123

Schroder, A., van Wingen, G., Vulink, N. C., and Denys, D. (2017). Commentary: the brain basis for misophonia. *Front. Behav. Neurosci.* 11:111. doi: 10.3389/fnbeh.2017.00111

Schroder, A., Vulink, N., and Denys, D. (2013). Misophonia: diagnostic criteria for a new psychiatric disorder. *PLoS One* 8:e54706. doi: 10.1371/journal.pone.0054706

Schwartz, P., Leyendecker, J., and Conlon, M. (2011). Hyperacusis and misophonia: the lesser-known siblings of tinnitus. *Minn. Med.* 94, 42–43.

Sherlock, L. P., and Formby, C. (2005). Estimates of loudness, loudness discomfort, and the auditory dynamic range: normative estimates, comparison of procedures, and test-retest reliability. *J. Am. Acad. Audiol.* 16, 85–100. doi: 10.3766/jaaa.16.2.4

Song, J. J., De Ridder, D., Schlee, W., Van de Heyning, P., and Vanneste, S. (2013). "Distressed aging": the differences in brain activity between early- and late-onset tinnitus. *Neurobiol. Aging* 34, 1853–1863. doi: 10.1016/j.neurobiolaging.2013.01.014

Song, J. J., De Ridder, D., Weisz, N., Schlee, W., Van de Heyning, P., and Vanneste, S. (2014). Hyperacusis-associated pathological resting-state brain oscillations in the tinnitus brain: a hyperresponsiveness network with paradoxically inactive auditory cortex. *Brain Struct. Funct.* 219, 1113–1128. doi: 10.1007/s00429-013-0555-1

Song, J. J., Vanneste, S., Schlee, W., Van de Heyning, P., and De Ridder, D. (2015). Onset-related differences in neural substrates of tinnitus-related distress: the anterior cingulate cortex in late-onset tinnitus, and the frontal cortex in early-onset tinnitus. *Brain Struct. Funct.* 220, 571–584. doi: 10.1007/s00429-013-0648-x

Tyler, R. S., Coelho, C., and Noble, W. (2006). Tinnitus: standard of care, personality differences, genetic factors. *ORL J. Otorhinolaryngol. Relat. Spec.* 68, 14–19; discussion 20–22. doi: 10.1159/000090486

Tyler, R. S., Oleson, J., Noble, W., Coelho, C., and Ji, H. (2007). Clinical trials for tinnitus: study populations, designs, measurement variables, and data analysis. *Prog. Brain Res.* 166, 499–509. doi: 10.1016/S0079-6123(07)66048-8

Ursin, H. (2014). Brain sensitization to external and internal stimuli. *Psychoneuroendocrinology* 42, 134–145. doi: 10.1016/j.psyneuen.2014.01.008

Veale, D. (2006). A compelling desire for deafness. *J. Deaf Stud. Deaf Educ.* 11, 369–372. doi: 10.1093/deafed/enj043

Vlaeyen, J. W. (2015). Learning to predict and control harmful events: chronic pain and conditioning. *Pain* 156(Suppl. 1), S86–S93. doi: 10.1097/j.pain.0000000000000107

Wallenstein, G. V., Eichenbaum, H., and Hasselmo, M. E. (1998). The hippocampus as an associator of discontiguous events. *Trends Neurosci.* 21, 317–323. doi: 10.1016/S0166-2236(97)01220-4

Webber, T. A., Johnson, P. L., and Storch, E. A. (2014). Pediatric misophonia with comorbid obsessive-compulsive spectrum disorders. *Gen. Hosp. Psychiatry* 36.e1–2. doi: 10.1016/j.genhosppsych.2013.10.018

Webber, T. A., and Storch, E. A. (2015). Toward a theoretical model of misophonia. *Gen. Hosp. Psychiatry* 37, 369–370. doi: 10.1016/j.genhosppsych.2015.03.019

Wu, M. S., Lewin, A. B., Murphy, T. K., and Storch, E. A. (2014). Misophonia: incidence, phenomenology, and clinical correlates in an undergraduate student sample. *J. Clin. Psychol.* 70, 994–1007. doi: 10.1002/jclp.22098

Zenner, H. P., Pfister, M., and Birbaumer, N. (2006). Tinnitus sensitization: sensory and psychophysiological aspects of a new pathway of acquired centralization of chronic tinnitus. *Otol. Neurotol.* 27, 1054–1063. doi: 10.1097/01.mao.0000231604.64079.77

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Salivary Stress-Related Responses in Tinnitus: A Preliminary Study in Young Male Subjects with Tinnitus

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Objective: This preliminary study examined if baseline measures of stress-related biomarkers as measured by salivary secretions of specific autonomic [measured by salivary α-amylase (sAA)], endocrine (measured by salivary cortisol), and immune (measured by salivary neopterin) responses are greater in male subjects with tinnitus in response to an induced-stress task.

Method: Twenty male subjects with no significant hearing loss, 10 with tinnitus, and 10 without tinnitus were enrolled in this study.Salivary secretions were collected before and after the induced stress task at four different time intervals.

Results: sAA levels were lower in the tinnitus group in comparison to subjects without tinnitus, suggesting impaired sympathetic activity in the subjects with tinnitus although these levels remained stable throughout the stress experiment.While no significant effects could be obtained for salivary cortisol or neopterin, salivary neopterin levels were trending toward significance over all measurements. Behavioral measures of stress were found to correlate negatively with measures of sAA and salivary neopterin.

Conclusion: The results of this study suggest impaired stress-related sAA mechanisms in male subjects with tinnitus, as evidenced by the different stress reactions induced in the endocrine system (as measured by salivary cortisol) and the immune system (as measured by salivary neopterin).

Keywords: tinnitus, stress-related responses, salivary alpha amylase, salivary cortisol, salivary neopterin

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INTRODUCTION

Tinnitus is a sound that is perceived in the absence of an external acoustic stimulus (Jastreboff and Hazell, 1993). The complexity in identifying the underlying mechanism of tinnitus generation is in part related to the subjective nature of this condition, along with the cascade of associated emotional and psychological reactions accompanying its occurrence. Patients with chronic tinnitus can experience a disabling sense of depression, anxiety, stress, and sleep difficulties (Halford and Anderson, 1991; Folmer et al., 1999; Folmer and Griest, 2000; Shargorodsky et al., 2010).

Stress is widely acknowledged as a predisposing and precipitating factor in different neurological and psychiatric illnesses. While acute stress promotes adaptation, prolonged stress over time leads to wear-and-tear on the body—so called the allostatic load, which comprises the negative physiological and psychological effects of stress. Stress induces secretion of corticotropin-releasing hormone from the hypothalamus, which stimulates the secretion of adrenocorticotropin from the pituitary gland. That release of adrenocorticotropin to blood

causes the adrenal gland to secrete stress hormones, such as cortisol (Hebert and Lupien, 2007). In addition, several studies have indicated that the sympathetic nervous system (SNS) plays an important role in stress (Cohen and Khalaila, 2014; Lupis et al., 2014; McKune et al., 2014; Carnuta et al., 2015; Het et al., 2015). Given that secretions from salivary glands are modulated by direct sympathetic innervations, salivary α-amylase (sAA) is proposed to be a surrogate marker of SNS activity, with changes in sAA reflecting sympathetic influences on salivary glands.

Reaction to acute stress is not only limited to activity that involves the activation of sympathetic and endocrine systems, but can also be observed in the immune system (Fuchs et al., 1993a; Moynihan, 2003). In the course of an immune response, a whole spectrum of active substances is generated, one of them being neopterin. Neopterin is released into the body by the kidneys and produced by monocytes/macrophages after stimulation by the cytokine interferon-γ (Widner et al., 2002). Both animal and human research has demonstrated that an acute stressor does change neopterin levels (Breinekova et al., 2007; Lindsay et al., 2015). Particularly, stress is found to impact hormonal regulatory function, causing immune cells to become insensitive to hormones and consequently resulting in suppression of immune system responses, triggering increased susceptibility to diseases (Cohen et al., 1991; van West and Maes, 2001).

Taken as a whole, alteration of individual physiological status as a result of a stressor is generally accepted as an underlying mechanism for stress generation (Chrousos and Gold, 1992; Chrousos, 2000; Tsigos and Chrousos, 2002; Kudielka and Wust, 2010). This mechanism involves activation of both the hypothalamic-pituitary-axis (HPA) and SNS. Given that there is a clear association between stress and tinnitus (Hinton et al., 2006; Shargorodsky et al., 2010; Nondahl et al., 2011; Kim et al., 2015), similar HPA and SNS salivary reactions can be expected in subjects with tinnitus.

With this in mind, the aim of this preliminary study is to examine autonomic, immune, and endocrine stress-related markers in male subjects with tinnitus in comparison to healthy controls (i.e., subjects without tinnitus). We wanted to examine if the concentrations of circulating sAA and salivary cortisol in male subjects with tinnitus differ from healthy controls. In addition, since increase in immune system function can lead to deregulation of the endocrine and immune systems, we also looked at salivary neopterin as an immune system marker in relation to tinnitus. As such, we hypothesized that salivary stress related markers could be used to distinguish stress influences and variations between the two groups.

MATERIALS AND METHODS

Participants

Male subjects were invited to participate in a research study by posting flyers at the University of North Carolina at Greensboro (UNCG) campus and surrounding areas. Subjects were excluded if they were smokers, had any dental work within the 48 h prior to sample collection, were prescribed or currently taking any medications for depression, anxiety, stress, bipolar disorder, epilepsy, thyroid dysfunction, schizophrenia, or insomnia, or any other neurological diseases. Because of monthly hormonal fluctuation (specifically cortisol) due to the menstrual cycle, only males were included in this study. In addition, due to the hormonal changes, which accompany increased age, the age limits were set from 18 to 35 years.

In total, 16 male subjects with tinnitus and 20 male subjects without tinnitus applied to participate in the study. However, 6 male subjects with tinnitus and 10 male subjects without tinnitus were excluded because they did not fit the inclusion criteria. Twenty male subjects with no significant hearing loss, 10 with tinnitus (mean age: 23.9 years; $Sd = 3.78$) and 10 without tinnitus (mean age: 23.6 years; $Sd = 4.47$), were enrolled in this study. All research involved in this study was approved by the authors' Institutional Review Board (The university of North Carolina at Greensboro IRB #12-0223), and all subjects gave signed informed consent prior to enrollment. All clinical investigation was conducted according to the principles expressed in the Declaration of Helsinki. Informed consent, written or oral, was obtained from the participants.

Questionnaires and Audiometric Testing

Male subjects with tinnitus completed all questionnaires, while those without tinnitus only filled in four out of the six questionnaires (tinnitus medical history intake questionnaire and tinnitus severity index excluded). All questionnaires were mailed out to subjects prior to their scheduled lab visit. In addition, audiometric testing was administrated to determine subjects hearing level. Subjects with tinnitus had their tinnitus pitch and loudness match measured.

Stress Test

To induce stress, we adapted the mental arithmetic task, part of the the Trier Social Stress Test (TSST), which has proven to be effective in inducing physiological changes in response to stress (Kirschbaum et al., 1993). After collecting the baseline measure, subjects were asked to perfrom a mental arthmetic task, by counting backwards, starting at 5000, in intervals of seven, for up to 5 min.

Clinical Assessment

The neuromeric rating scale (NRS) and saliva collections were assessed before beginning the stress task as well as 5, 30, and 60 min after inducing stress.

Numeric Rating Scale (NRS) of Acute Stress

Numeric Rating Scale (NRS) of acute stress was assessed subjectively by asking subjects to rate how stressed they were before collecting each of the four saliva samples on a scale from 0 (=no stress) to 10 (=extremely stressed).

Saliva Collection

Commercial collection aids were used from the Salimetrics laboratory (Salimetrics, LLC, USA). All samples were measured using a kinetic kit. The collection aid was used to collect unstimulated saliva samples via a passive drool method. All saliva samples obtained were stored in a 2 ml cryovials, and immediately stored in an −80°C laboratory freezer. Collected saliva sample were de-identified, and subjects were assigned a

number that was used in the saliva testing and in subsequent psychometric scores analysis. Prior to saliva collections, all subjects were instructed to avoid food, dental surgery, sugary drinks, alcohol, caffeine, nicotine, acidic drinks, and excessive napping or exercising on scheduled lab visits, for at least an hour before collecting the saliva samples. In addition, subjects' were instructed not to brush their teeth within 45 min prior to sample collection in order to avoid any risk of lowering pH levels and influencing bacterial growth. Due to the sensitive nature of some of the targeted biomarkers and to minimize possible influence of circadian rhythm, all collection procedures took place at the same time of day: 6:00 p.m.

Flow Rate Correction

If correction for saliva flow rate is not made, there will be variation in the concentration per volume unit from subject-to-subject. This variation could cause problems in statistical analysis that might make it difficult to reveal a biomarker-behavior-relationship.

The correction method explained below enabled us to correct for sAA concentration per minute for each trial result:

$$\textit{Flow rate (ml/min)} = \textit{((saliva (ml)/(time (min)))} \quad (1)$$

There was no need to correct sAA activity for flow rate since the alpha amylase levels were not correlated with flow rate at each measurement.

Saliva Processing

Upon completion of the collection procedures, a total of 80 saliva samples were packed in dry ice and sent to the Salimetrics laboratory (Salimetrics, LLC, USA) for analysis. Salivary cortisol and neopterin were measured in duplicates, while sAA was measured in singulate.

Statistics

All parameters, along with each subject's demographic and psychometric data, were analyzed using descriptive statistics. A Mann–Whitney test was conducted to determine whether there was a difference in the Pittsburgh sleep quality index (PSQI) and the perceived stress scale (PSS) between subjects, with and without tinnitus. In addition, Spearman's Rho correlation was computed to determine associations between baseline measures of PSS and NRS.

To test if there is a significant difference between sAA, salivary cortisol and salivary neopterin between subjects, with and without tinnitus at baseline, a Mann–Whitney test was conducted. In addition Spearman's Rho correlation was computed to determine associations between PSS and baseline measures of sAA, salivary cortisol, and salivary neopterin, respectively.

A Kruskal–Wallis test was applied using the values of the different time intervals (baseline and post-test) as the test variables, and the groups, control vs. tinnitus, as the grouping variables, for each of the three stress-related markers (sAA, salivary cortisol, and salivary neopterin) to examine any significant effect. When significance value was obtained a follow-up with Mann–Whitney test was conducted. To avoid type I error a Bonferroni correction was made adjusting the p-value by dividing 0.05/(# comparison). Effect size was computed manually using the following formula:

$$r = Z/\sqrt{(N)} \quad (2)$$

Similarly, a Kruskal–Wallis test was conducted to examine any evidence of difference on each of the three biomarkers for the follow-up measures at 30 and 60 min. When significance value was obtained a follow-up with Mann–Whitney test was conducted.

We applied a Mann–Whitney test to determine whether there was a difference in flow rate measures for sAA at baseline, 5, 30, and 60 min post-test. In addition, Spearman's Rho was conducted between flow rate and sAA at baseline, and 5, 30, and 60 min.

To confirm the experimental validation of the stress test we applied a Kruskal–Wallis test using values of NRS of the different time intervals as the test variables, and the groups, control vs. tinnitus, as the grouping variables. A similar analysis was applied for post-measurements.

To look for the association or dissociation between sAA and salivary cortisol, we calculated area under the curve (AUC) (Pruessner et al., 2003; Ali and Pruessner, 2012). AUC, with respect to ground, was first calculated for sAA, cortisol, and neopterin using the trapezoid formula described previously (Pruessner et al., 2003):

$$AUC = \sum_{i=1}^{n-1} \frac{\left(m_{(i+1)} + m_i\right) \cdot t_i}{2}$$

m = measurements

t = denoting the distance (time) between the measurements

This score incorporates information regarding both baseline and reactivity individually for sAA and cortisol within one score. We then divided the AUC of sAA by the AUC of salivary cortisol to derive an overall ratio (Ali and Pruessner, 2012), representing the variable of amylase over cortisol based on the individual AUC variables, which we named AOC. This variable can be thought of as the variation in sAA levels after correcting for variations in salivary cortisol. Complimentarily, the ratio variable cortisol over amylase (COA) was calculated by dividing the AUC of salivary cortisol by the AUC of sAA. This variable can be thought of as the variation of cortisol corrected for the variation in amylase. We also divided the AUC of sAA and the AUC of salivary neopterin to derive an overall ratio variable of amylase over neopterin (AON) as well as the AUC of salivary neopterin by the AUC of sAA (NOA). In addition, we also divided the AUC of salivary cortisol and the AUC of salivary neopterin (CON) as well as the AUC of salivary neopterin by the AUC of cortisol (NOC). These various ratio computations are based on Ali and Pruessner's work on assessing sAA over cortisol ratio as a marker to assess deregulation of the stress system (Ali and Pruessner, 2012).

AUC of sAA, AUC of salivary cortisol, alpha amylase over cortisol (AOC), cortisol over alpha amylase (COA), alpha amylase over neopterin (AON), neopterin over alpha amylase (NOA), cortisol over neopterin (CON), and neopterin

over cortisol (NOC) were then z-transformed to standardized measurements.

A Mann–Whitney test was applied to evaluate differences of AUC of sAA, AUC of salivary cortisol, and AUC of salivary neopterin between subjects with and without tinnitus. Spearman's Rho were computed between AUC of salivary cortisol and AUC of salivary neopterin and AUC of sAA and AUC of salivary cortisol.

A logistic regression including AUC of sAA, AUC of salivary cortisol and AUC of salivary neopterin as independent variables and group (subjects with tinnitus vs. subjects without tinnitus) as dependent variables was also conducted.

Spearman's correlations were conducted to examine the associations between the standardized AUC of sAA, AUC of salivary cortisol, α-amylase over cortisol, cortisol over α-amylase, α-amylase over neopterin, neopterin over α-amylase, cortisol over neopterin, and neopterin over cortisol with subjective indexes of stress. A 5% level of significance and a Spearman's Rho two-tailed analyses were adopted for all analyses. All statistical analyses were completed using the SPSS software package 22.

RESULTS

Descriptive Characteristics

Overall Subjects

Both subjects with tinnitus and subjects without tinnitus reported to have no serious health conditions, which required immediate or continuous medical attention. When given the choice to rate their overall health, 60% of subjects without tinnitus described their health as "excellent." The majority of subjects with tinnitus (80%) indicated their overall health as "good" or "excellent." Four subjects with tinnitus experienced one to three headaches per week in the month prior to experimentation; however, 80% reported these headaches not to be a significant problem. Exposure to noise was reported as one out of the two main causes of tinnitus. The remaining subjects reported exposure to stressful events as the primary cause of their tinnitus.

A Mann–Whitney test indicated that the PSQI scores were significantly greater for subjects with tinnitus ($M = 4.9$, $Sd = 2.18$, $Md = 5.50$) than subjects without tinnitus ($M = 2.70$, $Sd = 1.05$, $Md = 2$), $U = 20.50$, $p = 0.02$, $r = 0.51$. Similarly, the PSS score for subjects with tinnitus ($M = 20.30$, $Sd = 6.86$, $Md = 20$) were greater than subjects without tinnitus ($M = 10.10$, $Sd = 2.80$, $Md = 10$), $U = 6.50$, $p = 0.001$, $r = 0.73$. Pure tone audiometry results indicated that all subjects had normal hearing thresholds of 20 dB HL or better (with tinnitus, right ear $M = 16$, $Sd = 10.72$; without tinnitus, right ear $M = 19.66$, $Sd = 12.24$) and (with tinnitus, left ear $M = 22$, $Sd = 9.30$; without tinnitus, left ear $M = 14.50$, $Sd = 10.20$). A comparison between the pure tone averages revealed no significant effect between groups. No significant difference between groups was revealed for the pure tone averages.

Tinnitus Characteristics

Five tinnitus subjects reported to have a gradual onset of tinnitus, while five subjects reported to have a sudden onset. The average duration of tinnitus was 3.30 years ($Sd = 1.63$). Six subjects perceived their tinnitus bilaterally, while four perceived it unilaterally. The average pitch match for both the left and right ears were approximately 3 kHz (right ear $M = 3525$, $Sd = 2180$; left ear $M = 3050$, $Sd = 1978$), while average loudness match ranged from 13 to 58 dB HL. The range of loudness match varied from 13 to 44 dB HL in the right ear and 13 to 58 dB HL in the left ear (right ear $M = 30.50$, $Sd = 11.24$; left ear $M = 31.20$, $Sd = 13.33$).

According to the TSI scores ($M = 19.20$, $Sd = 6.90$), tinnitus was not considered to be a bothersome or debilitating problem for subjects with tinnitus. Pure tone ringing was the most common tinnitus sound described by the tinnitus subjects (70%), while hissing and music were the least described tinnitus sounds (20%); 10% reported their tinnitus to be a combination of different tones.

Baseline Measures

A Mann–Whitney test revealed significant difference for baseline sAA (μ/ml) which demonstrated lower score for subjects with tinnitus ($M = 42.84$, $Sd = 24.38$, $Md = 42.65$) than subjects without tinnitus ($M = 98.60$, $Sd = 61.51$, $Md = 65.45$), $U = 20$, $p = 0.023$, $r = -0.50$. Baseline concentrations of salivary cortisol (mg/dl) (subjects with tinnitus $M = 0.10$, $Sd = 0.09$, $Md = 0.07$; subjects without tinnitus $M = 0.09$, $Sd = 0.06$, $Md = 0.09$), $U = 49$, $p = 0.94$, $r = 0.01$, yielded no statistical significant difference. Similarly, salivary neopterin (ng/ml) yielded no significant difference (subjects with tinnitus $M = 2.35$, $Sd = 0.75$, $Md = 2.54$; subjects without tinnitus $M = 1.71$, $Sd = 0.99$, $Md = 1.79$), $U = 28$, $p = 0.09$, $r = 0.37$. See **Figures 1A–C**.

There was a significant correlation between scores the PSS and baseline measures of sAA (μ/ml) ($r_s = -0.49$ $p = 0.02$), indicating that higher stress rates corresponded to lower concentrations of sAA (see **Figure 2A**). The correlation between the related-stress psychometric scores of the PSS and baseline measures of salivary cortisol (mg/dl) ($r_s = -0.05$, $p = 0.81$) and salivary neopterin (ng/ml) ($r_s = 0.34$, $p = 0.14$) yielded no statistical significance. See **Figures 2B,C**.

Experiment

Stress Test

A Kruskal–Wallis test was conducted to determine whether baseline salivary stress-related measures of subjects with tinnitus and subjects without tinnitus varied from post-test measure. The test, which was corrected for tied ranks, showed that in regard to sAA (μ/ml) ($x^2 = 5.14$, $p = 0.02$) results yielded a significant effect from post-test measure. Specifically, baseline measures of sAA for subjects with tinnitus were significantly lower ($M = 42.84$, $Sd = 24.38$, $Md = 42.65$) than subjects without tinnitus ($M = 98.60$, $Sd = 61.51$, $Md = 65.45$), $U = 20$, $p = 0.02$, $r = -0.50$.

No statistical significant difference was found for salivary cortisol (mg/dl) at baseline ($x^2 = 0.006$, $p = 0.09$), and post-test measure ($x^2 = 0.07$, $p = 0.79$). Similarly, there was no statistical significance difference demonstrated for salivary

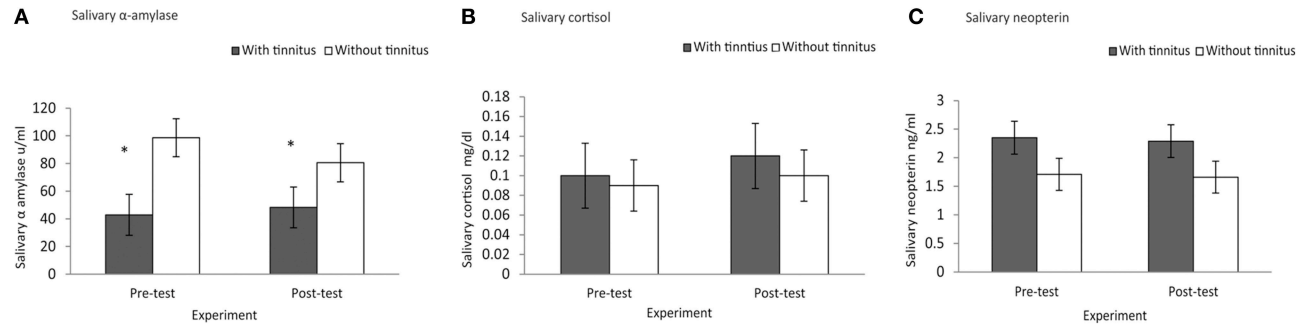

FIGURE 1 | Experimental effect of group differences at baseline vs.post-test for (A) salivary α amylase, (B) salivary cortisol, and (C) salivary neopterin. **(A)** Subjects with tinnitus ($M = 42.84$, $Sd = 24.38$) had lessened salivary α amylase scores than those without tinnitus ($M = 98.60$, $Sd = 61.51$). **(A)** *Indicates significant differences. **(B)** Salivary cortisol, and **(C)** salivary neopterin yielded no statistical significant differences between baseline and post-test measure.

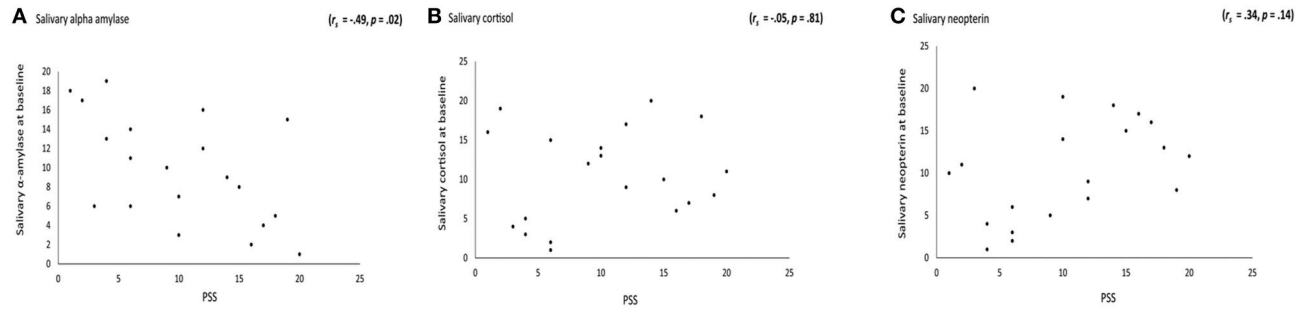

FIGURE 2 | Correlation between the perceived stress scale (PSS) and stress-related markers. (A) A significant negative correlation ($r_S = -0.49$ $p = 0.02$) between PSS and baseline measures of salivary α amylase, indicating the higher the stress the lower salivary α amylase concentrations. **(B)** No correlation between PSS scores and baseline measures of salivary cortisol was obtained ($r_S = -0.05$ $p = 0.81$). Similarly, **(C)** PSS with baseline measures of salivary neopterin ($r_S = 0.34$ $p = 0.14$), yielded no statistical significance.

neopterin (ng/ml) at baseline ($x^2 = 2.76$, $p = 0.09$), and post-test measure ($x^2 = 1.75$, $p = 0.18$).

Follow-Up

With regard to sAA (μ/ml) there was no statistical significant difference between post-test measure and 30 min fellow-up ($x^2 = 4.80$, $p = 0.02$). However, a significant difference was obtained at 60 min follow-up ($x^2 = 7$, $p = 0.008$). Specifically, subjects with tinnitus ($M = 40.21$, $Sd = 30.06$, $Md = 22.95$) had lower measures of sAA than subjects without tinnitus ($M = 87.81$, $Sd = 48.04$, $Md = 80.20$), $U = 15$, $p = 0.008$, $r = -0.59$. See **Figure 3A**.

No statistical significant difference was demonstrated for salivary cortisol (mg/dl) at 30 min ($x^2 = 0.20$, $p = 0.65$), and 60 min ($x^2 = 14$, $p = 0.70$). Similarly, there was no significant difference demonstrated for salivary neopterin (ng/ml) at 30 min ($x^2 = 1.12$, $p = 0.29$), and 60 min follow up ($x^2 = 1.28$, $p = 0.25$). See **Figures 3B,C**.

Control of Flow Rate of Salivary α-Amylase

A Mann–Whitney test of sAA flow rate reveled no statistical significant difference of flow rate measures in (ml/min) at baseline (subjects with tinnitus $M = 1.85$, $Sd = 0.85$, $Md = 1.50$; subjects without tinnitus $M = 2.02$, $Sd = 1.20$, $Md = 1.75$), $U = 47$, $p = 0.81$, $r = -0.05$, at 5 min post-test measure (subjects with tinnitus $M = 2.21$, $Sd = 0.78$, $Md = 2$; subjects without tinnitus $M = 1.79$, $Sd = 0.88$, $Md = 1.62$), $U = 30.50$, $p = 0.13$, $r = -0.30$, at 30 min follow-up measure (subjects with tinnitus $M = 2.02$, $Sd = 0.45$, $Md = 2$; subjects without tinnitus $M = 1.79$, $Sd = 0.84$, $Md = 1.50$), $U = 31$, $p = 0.13$, $r = -0.30$, as well as at 60 min follow-up measure (subjects with tinnitus $M = 1.77$, $Sd = 0.40$, $Md = 2$; subjects without tinnitus $M = 1.59$, $Sd = 0.80$, $Md = 1.20$), $U = 34$, $p = 0.21$, $r = -0.27$. In addition, Spearman's Rho revealed no significant correlation between flow rate and sAA activity at baseline ($r_s = -0.21$, $p = 0.35$), 5 min ($r_s = 0.08$, $p = 0.73$), 30 min ($r_s = 0.22$, $p = 0.34$), and 60 min ($r_s = -0.07$, $p = 0.75$) post-test.

Experimental Validation (Baseline vs. 5 min Post-test)

A Kruskal–Wallis test was conducted for NRS at baseline and 5 min post-test, and revealed statistical difference at the edge of significance ($x^2 = 3.69$, $p = 0.05$), A Mann–Whitney test showed that subjects with tinnitus had a greater increase in NRS scores post-test ($M = 4.40$, $Sd = 2.50$, $Md = 4.50$) than subjects without tinnitus ($M = 2.80$, $Sd = 2.39$, $Md = 2.50$), $U = 25$, $p = 0.05$, $r = -0.42$. See **Figure 4**.

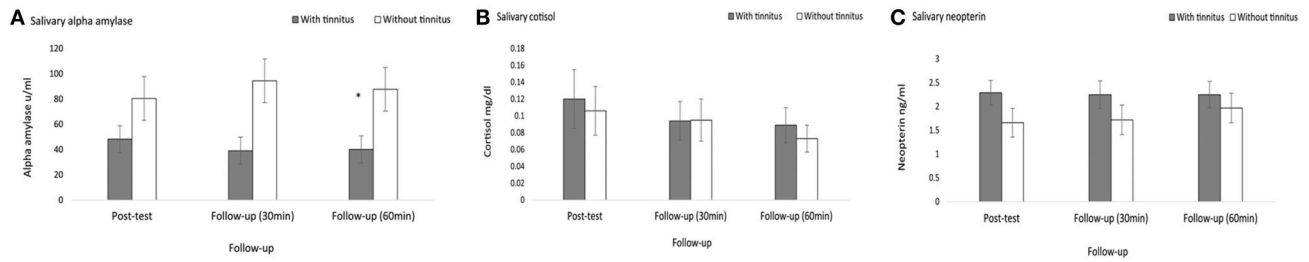

FIGURE 3 | Follow-up effect of group differences at 5, 30, and 60 min post-test measures. (A) Salivary α amylase yielded no statistical significant difference for 30 min post-test, however a significant difference was obtained for 60 min post-test Specifically, subjects with tinnitus ($M = 40.21$, $Sd = 30.06$) had lower measures of salivary α-amylase than subjects without tinnitus ($M = 87.81$, $Sd = 48.04$). **(B)** Salivary cortisol and **(C)** salivary neopterin yielded no statistical significant difference for the effect of time. *Indicates significant difference.

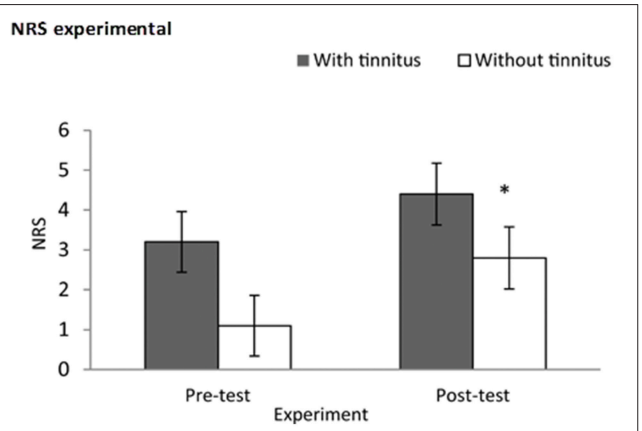

FIGURE 4 | Experimental validation using a numeric rating scale. Subjects had increases stress scores post-test in comparison to baseline; this was characterized by subjects with tinnitus ($M = 4.40$, $Sd = 2.50$) exhibiting higher stress scores than subjects without tinnitus ($M = 2.80$, $Sd = 2.39$). *Indicates significant difference.

Sensitivity Analysis

A Mann–Whitney test revealed a significant effect for AUC of sAA. Subjects with tinnitus ($M = 2510.20$, $Sd = 1566.34$, $Md = 2548.12$) had lower scores than subjects without tinnitus ($M = 5369.55$, $Sd = 2975.72$, $Md = 4317.75$), $U = 19$, $p = 0.01$, $r = -0.52$. No significant effects were obtained for AUC of salivary cortisol, AUC of salivary neopterin.

Significant correlations were obtained between AUC of salivary cortisol and AUC of salivary neopterin ($r_s = 0.53$, $p = 0.01$). Between AUC of sAA and respectively AUC of salivary cortisol ($r_s = 0.19$, $p = 0.40$) and AUC of salivary neopterin ($r_s = -0.22$, $p = 0.35$) no significant correlation could be obtained.

A logistic regression including AUC of sAA, AUC of salivary cortisol and AUC of salivary neopterin as independent variables and group (subjects with tinnitus vs. subjects without tinnitus) as dependent variable was conducted. This analysis revealed an overall effect, $c^2 = 11.87$, $p = 0.008$, Nagelkerke $R^2 = 0.62$. A closer look indicated that AUC of sAA was a good predictor ($W = 4.26$, $p = 0.04$, $\beta = -2.50$) indicating that 84.2% of the subjects could be correctly classified. The β-value further indicated that the lower the individual score is on AUC of sAA the higher the probability is that a subject has tinnitus. Neither AUC of salivary cortisol nor AUC of salivary neopterin reached significance. In addition, a Spearman's correlation revealed a significant effect between PSS and respectively, AUC of sAA ($r_s = -0.65$, $p = 0.002$), α-amylase over cortisol ($r_s = -0.68$, $p = 0.001$), cortisol over α-amylase ($r_s = 0.65$, $p = 0.002$), α-amylase over neopterin ($r_s = -0.62$, $p = 0.003$), neopterin over α-amylase ($r_s = 0.57$, $p = 0.008$), and cortisol over neopterin ($r_s = 0.62$, $p = 0.003$), but not neopterin over cortisol ($r_s = 0.22$, $p = 0.35$). The correlations suggest that the higher the stress score, the lower the individual score on AUC of sAA, α-amylase over cortisol, or α-amylase over neopterin, indicating a higher probability that a subject has stress and vice versa. The higher an individual score is on cortisol over α-amylase, neopterin over α-amylase, and cortisol over neopterin, the higher the likelihood is that a subject has chronic stress or vice versa (as suggested by the positive correlation). No significant correlations were obtained for AUC of salivary cortisol ($r_s = 0.11$, $p = 0.63$), and AUC of salivary neopterin ($r_s = 0.24$, $p = 0.29$). For an overview, see **Figure 5**.

DISCUSSION

This is a preliminary study to examine the feasibility of utilizing stress related markers in tinnitus. The data from this preliminary study will help us pursue a larger investigation on the feasibility of using salivary stress related responses in tinnitus. To our knowledge, this is the first study with regard to the examination of sAA, salivary cortisol, and salivary neopterin, simultaneously, in male subjects with tinnitus. The results of this preliminary study show that sAA levels were lower in the tinnitus group in comparison to subjects without tinnitus, suggesting impaired sympathetic activity in the tinnitus group. Furthermore, sAA remained stable throughout the stress experiment (i.e., acute stress); further confirming impaired sympathetic activity in the tinnitus group. The results of this preliminary study found no correlations between sAA and salivary cortisol and salivary neopterin, respectively, highlighting the distinctiveness of sAA as a stress-related marker. Furthermore, sAA ratio was found to be more strongly associated with measures of stress and depression

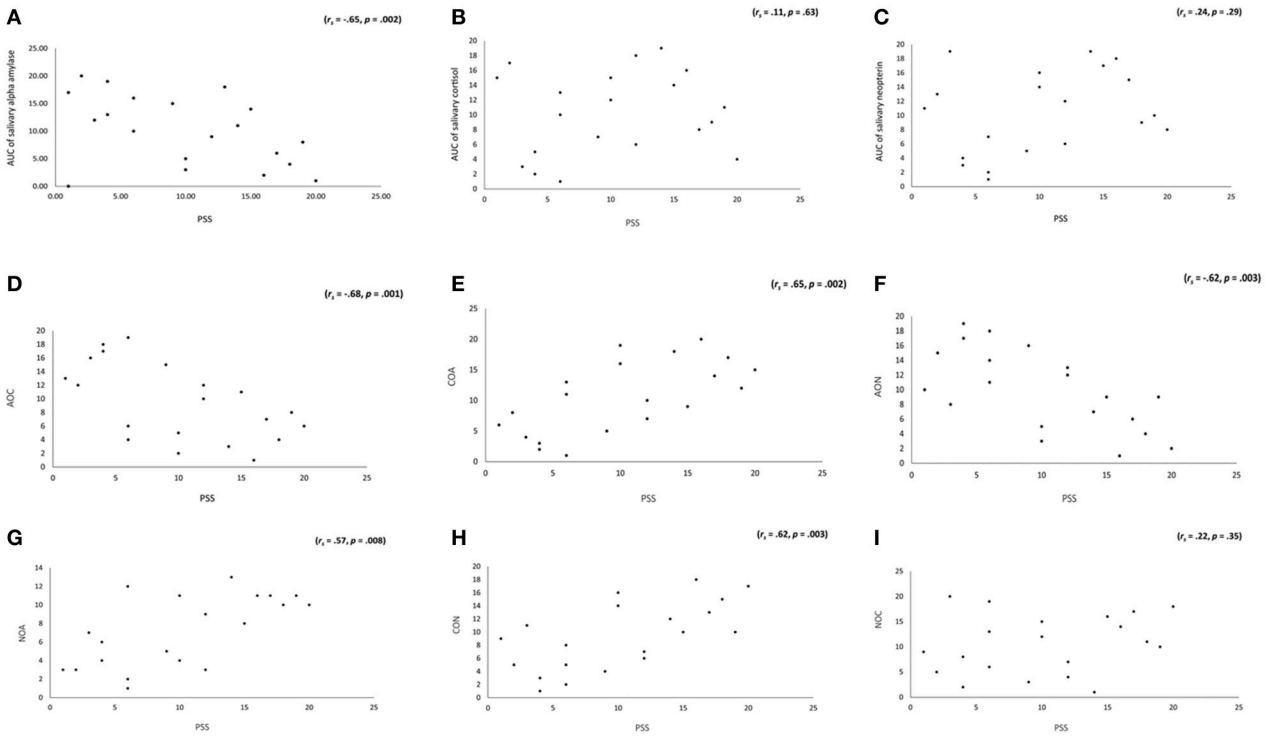

FIGURE 5 | (A) Significant correlations was obtained between AUC of salivary α-amylase, but not with **(B)** AUC salivary cortisol, or **(C)** AUC neopterin.Spearman's Rho were significant for **(D)** AOC, **(E)** COA, **(F)** AON, **(G)** NOA, and **(H)** CON, but not for **(I)** NOC. This suggests that the higher the scores of the PSS the lower the individual scores of AUC for salivary α-amylase, salivary cortisol, and salivary neopterin, and the higher the likelihood that subjects has chronic stress.

and is regarded as an index of deregulation in the stress system (Ali and Pruessner, 2012). In addition, this preliminary data found a strong effect observed for sAA in relation to baseline, stress test, follow-up and AUC, indicating that the expected value of sAA response decreases as stress increases, particularly for the tinnitus group.

Salivary α-amylase, Cortisol, and Neopterin

An increasing amount of research is supporting the proposal of a dynamic relationship between tinnitus and stress (Vanneste et al., 2010; De Ridder et al., 2011). Stress induces biological changes, such as alterations in stress-related markers. sAA was suggested as a valuable index for the assessment of pain (Shirasaki et al., 2007; Ahmadi-Motamayel et al., 2013; Ferrara et al., 2013; Liu et al., 2013), psychological stress (Chatterton et al., 1997) and physical stress (Chatterton et al., 1996). Thus, because of the symptomatic similarities between tinnitus, stress, and pain, it may be expected that a common pathophysiology may underlie these conditions. There are reports of lower sAA in chronically stressed patients with mood depressive disorders (Cubala and Landowski, 2014) and personality disorders (Nater et al., 2010). It is known that chronic activation of this stress system can lead to changes in functioning (i.e., allostatic load), leading to impaired or inadequate responses to subsequent challenges (Chrousos, 2009). This can be confirmed by our data. During a stress test, no changes could be obtained in the tinnitus group; in the healthy controls, immediate changes were observed. Previous research suggests that patients habituated to stress are indicated by a decrease or decline of sAA concentrations (Schumacher et al., 2013, 2014). Interestingly, previous research in tinnitus has revealed that highly distressed tinnitus patients show increased activity in specific brain areas (i.e., insula, dorsal, and subgenual anterior cingulate cortex; Vanneste et al., 2010, 2014) that are known to be mediated by the SNS (Vanneste and De Ridder, 2013).

With regard to salivary cortisol, no effect was found in relation to stress between the two groups at baseline. However, a significant positive relationship was obtained for salivary cortisol after correcting for salivary neopterin with stress independent of whether the subject had tinnitus or not. Our finding is in line with findings of a recent report showing no differences in responses of the HPA axis, as measured by salivary cortisol after exposure to stressful events (McKune et al., 2014). This could be indicative of the effect the type of stress (i.e., acute vs. chronic) has on the activation of the HPA axis. Taking into consideration that salivary cortisol is influenced by circadian rhythm (Lac, 2001), our finding can be considered a reflection of cortisol systematic influence by the circadian rhythm, with concentrations being higher during the day and lower in the evening.

A key characteristic of HPA functioning that has been frequently observed is the habituation after repeated exposure to an initially stressful event (i.e., tinnitus) (Wust et al., 2005).

Hebert and Lupien observed that basal levels of salivary cortisol are chronically changed in tinnitus patients (Hebert et al., 2004); however, they also report a blunted cortisol response to acute stress in patients who had experienced tinnitus for longer duration (Hebert and Lupien, 2007). Studies document that a habituation of adrenocortical responses does not necessarily occur (Al'Absi et al., 1997). It seems that characteristics of the stressor are important mediators determining the development of habituation, including intensity (Marti et al., 2001) and frequency (Ma and Lightman, 1998) of stress. Habituation to prolonged exposure to stressful events could possibly explain why tinnitus patients do not report any changes after the stress test. The lack of cortisol differences is in line with the findings of several other reports documenting no differences in endocrine responses to chronic stress (Hamilos et al., 1998; Scott and Dinan, 1998; Gaab et al., 2001).

In addition, while sAA levels were lower in the subjects with tinnitus, no changes could be obtained between subjects with tinnitus and healthy controls throughout the experiment for salivary cortisol. These findings further suggest dissociation between the two stress systems (endocrine vs. autonomic), and further point to a specific nature of the SNS-HPA axis relationship. Recent research already suggested an asymmetry between the HPA axis and SNS (Nater and Rohleder, 2009). The factors responsible for this asymmetry are largely unknown. One possible explanation could be that when it comes to habituation, these two systems operate at different rates. In support of this idea, evidence has been provided that in experimental animals, repeated stress exposure leads to a decreased response over time in the SNS with sustained HPA axis response (Britton et al., 1992). Whatever the causes, it has been suggested that asymmetry between these two biological systems may have unhealthy consequences (Bauer et al., 2002) and might contribute to pathogenesis development and/or maintenance (Monteleone et al., 2011).

Research has suggested that neopterin production might be linked with immune pathogenesis (Zhang et al., 2009; Chittiprol et al., 2010). It is known that neopterin is a significant and reliable indicator for the endogenous formation of IFN- γ (Fuchs et al., 2009). A prolonged elevation of IFN-γ was suggested to induce hyperexcitability neurons, which may lead to amplify pain perceptions, a process referred to as central sensitization (Vikman et al., 2007). Interestingly, tinnitus has also been considered a central sensitization phenomenon that results from an increase in responsivity to neural activity (Zenner, 2006; Zenner et al., 2006). Overall, numerous studies have shown an association between increase in immune system function due to infections (Fuchs et al., 1988, 1993b; Avanzas et al., 2004; Breinekova et al., 2007; Chittiprol et al., 2010; Euteneuer et al., 2012) and deregulation of endocrine and autonomic systems. However, we were unable to make such an assumption because in this study subjects were excluded if they had infectious or inflammatory diseases. On the other hand, our results do support variability in salivary neopterin that seems to be independent of fluctuation in sAA and salivary cortisol, suggesting that there also an asymmetry with neopterin. However, we are not aware of any former study that reports on the exact relationship between salivary measures of neopterin and respectively sAA or salivary cortisol; therefore, further examination of this assumption is necessary.

Feasibility of Using Salivary Stress-Related Biomarkers

sAA levels in this study showed statistically significant lower values in the subjects with tinnitus compared to subjects without tinnitus and sAA levels were correlated with stress. After correction for salivary cortisol and neopterin levels, the effects remained significant. As such, these results suggest a promising role of sAA as a possible biological stress marker in tinnitus. Given that in subjects without tinnitus, sAA was able to measure acute stress, it suggests that subjects with tinnitus had an impaired or inadequate functioning of the SNS.

LIMITATIONS

Although the present study has yielded significant findings in regard to the use of stress-related markers—specifically with regard to salivary α amylase—its design is not without limitations. Thus, a number of caveats need to be noted regarding the present work. First, this is a preliminary study, and thus it was primarily limited by its small sample size and statistical need to run multiple comparisons. Second, ideally including subjects of both sexes and increasing the collection range to longer time period would expand the sample size and further aid in generalizing the findings. Third, the small number of subjects may have led to an underestimation of the differences in salivary markers. Fourth, due to time constraints only a portion of the TSST test was administered, and although the portion administered is proven to induce stress, administering the entire TSST would have probably led to a larger increase in stress. Finally, although there was no significant rise in salivary cortisol secretions after the stressful event, subjective reports indicated that the stress-induced task used was effective enough to induce stress as measured by salivary α amylase.

CONCLUSION

Although stress can be a versatile event, reliable biological indicators of stress reactions have been shown to be valuable markers in both psycho physiological research and clinical practice. As such, alteration in the psychological state of a patient with tinnitus can alter biological indictors of stress reactions as measured by salivary stress related biomarkers. With this in mind, stress related markers have been emerged as potential non-invasive techniques with collection methods that can be administrated by health and non-health professionals. As a final point, it is important to note that even if salivary measures of stress do not map well due to their sensitivity, they may yet predictive of diseases and well-being. Future research relating the validity of using salivary stress related measures is needed to better determine their clinical relevance. Particularly, studies should further confirm whether sAA is a sensitive maker of stress and how it is associated with other

mediators of the allostatic load network, such as pro- and anti-inflammatory cytokines (e.g., interleukin-6, tumor necrosis factor-alpha).

AUTHOR CONTRIBUTIONS

OA collected and analyzed the data, prepared and wrote the manuscript, as well as secured the funding. DT monitored data collection, advised on data analyses, advised on manuscript perpetration. SV data analyses, manuscript perpetration, and manuscript writing.

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REFERENCES

Ahmadi-Motamayel, F., Shahriari, S., Goodarzi, M. T., Moghimbeigi, A., Jazaeri, M., and Babaei, P. (2013). The relationship between the level of salivary alpha amylase activity and pain severity in patients with symptomatic irreversible pulpitis. *Restor. Dent. Endod.* 38, 141–145. doi: 10.5395/rde.2013.38.3.141

Al'Absi, M., Bongard, S., Buchanan, T., Pincomb, G. A., Licinio, J., and Lovallo, W. R. (1997). Cardiovascular and neuroendocrine adjustment to public speaking and mental arithmetic stressors. *Psychophysiology* 34, 266–275. doi: 10.1111/j.1469-8986.1997.tb02397.x

Ali, N., and Pruessner, J. C. (2012). The salivary alpha amylase over cortisol ratio as a marker to assess dysregulations of the stress systems. *Physiol. Behav.* 106, 65–72. doi: 10.1016/j.physbeh.2011.10.003

Avanzas, P., Arroyo-Espliguero, R., Cosin-Sales, J., Quiles, J., Zouridakis, E., and Kaski, J. C. (2004). Prognostic value of neopterin levels in treated patients with hypertension and chest pain but without obstructive coronary artery disease. *Am. J. Cardiol.* 93, 627–629. doi: 10.1016/j.amjcard.2003.11.035

Bauer, A. M., Quas, J. A., and Boyce, W. T. (2002). Associations between physiological reactivity and children's behavior: advantages of a multisystem approach. *J. Dev. Behav. Pediatr.* 23, 102–113. doi: 10.1097/00004703-200204000-00007

Breinekova, K., Svoboda, M., Smutna, M., and Vorlova, L. (2007). Markers of acute stress in pigs. *Physiol. Res.* 56, 323–329.

Britton, K. T., Segal, D. S., Kuczenski, R., and Hauger, R. (1992). Dissociation between *in vivo* hippocampal norepinephrine response and behavioral/neuroendocrine responses to noise stress in rats. *Brain Res.* 574, 125–130. doi: 10.1016/0006-8993(92)90808-M

Carnuta, M., Crisan, L. G., Vulturar, R., Opre, A., and Miu, A. C. (2015). Emotional non-acceptance links early life stress and blunted cortisol reactivity to social threat. *Psychoneuroendocrinology* 51, 176–187. doi: 10.1016/j.psyneuen.2014.09.026

Chatterton, R. T. Jr., Vogelsong, K. M., Lu, Y. C., and Hudgens, G. A. (1997). Hormonal responses to psychological stress in men preparing for skydiving. *J. Clin. Endocrinol. Metab.* 82, 2503–2509. doi: 10.1210/jc.82.8.2503

Chatterton, R. T., Vogelsong, K. M., Lu, Y. C., Ellman, A. B., and Hudgens, G. A. (1996). Salivary alpha-amylase as a measure of endogenous adrenergic activity. *Clin. Physiol.* 16, 433–448. doi: 10.1111/j.1475-097X.1996.tb00731.x

Chittiprol, S., Venkatasubramanian, G., Neelakantachar, N., Babu, S. V., Reddy, N. A., Gangadhar, B. N., et al. (2010). Oxidative stress and neopterin abnormalities in schizophrenia: a longitudinal study. *J. Psychiatr. Res.* 44, 310–313. doi: 10.1016/j.jpsychires.2009.09.002

Chrousos, G. P. (2000). The role of stress and the hypothalamic-pituitary-adrenal axis in the pathogenesis of the metabolic syndrome: neuro-endocrine and target tissue-related causes. *Int. J. Obes. Relat. Metab. Disord.* 24(Suppl. 2), S50–S55. doi: 10.1038/sj.ijo.0801278

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nat. Rev. Endocrinol.* 5, 374–381. doi: 10.1038/nrendo.2009.106

Chrousos, G. P., and Gold, P. W. (1992). The concepts of stress and stress system disorders. Overview of physical and behavioral homeostasis. *JAMA* 267, 1244–1252. doi: 10.1001/jama.1992.03480090092034

Cohen, M., and Khalaila, R. (2014). Saliva pH as a biomarker of exam stress and a predictor of exam performance. *J. Psychosom. Res.* 77, 420–425. doi: 10.1016/j.jpsychores.2014.07.003

Cohen, S., Tyrrell, D. A., and Smith, A. P. (1991). Psychological stress and susceptibility to the common cold. *N. Engl. J. Med.* 325, 606–612. doi: 10.1056/NEJM199108293250903

Cubala, W. J., and Landowski, J. (2014). Low baseline salivary alpha-amylase in drug-naive patients with short-illness-duration first episode major depressive disorder. *J. Affect. Disord.* 157, 14–17. doi: 10.1016/j.jad.2013.12.043

De Ridder, D., Vanneste, S., and Congedo, M. (2011). The distressed brain: a group blind source separation analysis on tinnitus. *PLoS ONE* 6:e24273. doi: 10.1371/journal.pone.0024273

Euteneuer, F., Schwarz, M. J., Hennings, A., Riemer, S., Stapf, T., Rief, W., et al. (2012). Psychobiological aspects of somatization syndromes: contributions of inflammatory cytokines and neopterin. *Psychiatry Res.* 195, 60–65. doi: 10.1016/j.psychres.2011.07.032

Ferrara, P., Bottaro, G., Angeletti, S., Gatto, A., Vitelli, O., Battaglia, D., et al. (2013). Salivary alpha-amylase: a new non-invasive biomarker for assessment of pain perception in epileptic children. *Acta Neurol. Belg.* 113, 279–283. doi: 10.1007/s13760-013-0180-z

Folmer, R., and Griest, S. (2000). Tinnitus and insomnia. *Am. J. Otolaryngol.* 21, 287–293. doi: 10.1053/ajot.2000.9871

Folmer, R., Griest, S., Meikle, M., and Martin, W. (1999). Tunnitus severity, loudness, and depression. *Otolaryngol. Head Neck Surg.* 121, 48–51. doi: 10.1016/S0194-5998(99)70123-3

Fuchs, D., Avanzas, P., Arroyo-Espliguero, R., Jenny, M., Consuegra-Sanchez, L., and Kaski, J. C. (2009). The role of neopterin in atherogenesis and cardiovascular risk assessment. *Curr. Med. Chem.* 16, 4644–4653. doi: 10.2174/092986709789878247

Fuchs, D., Hausen, A., Reibnegger, G., Werner, E. R., Dierich, M. P., and Wachter, H. (1988). Neopterin as a marker in HIV infection. *Clin. Chem.* 34, 466–467.

Fuchs, D., Samsonov, M., Weiss, G., Reibnegger, G., Nasonov, E. L., and Wachter, H. (1993a). The clinical significance of neopterin in human diseases. *Ter. Arkh.* 65, 80–87.

Fuchs, D., Weiss, G., and Wachter, H. (1993b). Neopterin, biochemistry and clinical use as a marker for cellular immune reactions. *Int. Arch. Allergy Immunol.* 101, 1–6. doi: 10.1159/000236491

Gaab, J., Huester, D., Peisen, R., Engert, V., Schad, T., Schuermeyer, T., et al. (2001). Hypothalamus-pituitary-adrenal axis reactivity in chronic fatigue syndrome and health under psychological, physiological and pharmacological stimulation. *Psychosom. Med.* 63, 188–188.

Halford, J., and Anderson, S. (1991). Anxiety and depression in Tinnitus sufferers. *J. Psychosomatic Disord. 35,* 383–390. doi: 10.1016/0022-3999(91)90033-K

Hamilos, D. L., Nutter, D., Gershtenson, J., Redmond, D. P., Di Clementi, J. D., Jones, J. F., et al. (1998). Core body temperature is normal in chronic fatigue syndrome. *Biol. Psychiatry* 43, 293–302. doi: 10.1016/S0006-3223(97)83214-3

Hebert, S., and Lupien, S. J. (2007). The sound of stress: blunted cortisol reactivity to psychosocial stress in tinnitus sufferers. *Neurosci. Lett.* 411, 138–142. doi: 10.1016/j.neulet.2006.10.028

Hebert, S., Paiement, P., and Lupien, S. J. (2004). A physiological correlate for the intolerance to both internal and external sounds. *Hear. Res.* 190, 1–9. doi: 10.1016/S0378-5955(04)00021-8

Het, S., Vocks, S., Wolf, J. M., Hammelstein, P., Herpertz, S., and Wolf, O. T. (2015). Blunted neuroendocrine stress reactivity in young women with eating disorders. *J. Psychosom. Res.* 78, 260–267. doi: 10.1016/j.jpsychores.2014.11.001

Hinton, D. E., Chhean, D., Pich, V., Hofmann, S. G., and Barlow, D. H. (2006). Tinnitus among Cambodian refugees: relationship to PTSD severity. *J. Trauma. Stress* 19, 541–546. doi: 10.1002/jts.20138

Jastreboff, P. J., and Hazell, J. W. (1993). A neurophysiological approach to tinnitus: clinical implications. *Br. J. Audiol.* 27, 7–17. doi: 10.3109/03005369309077884

Kim, H. J., Lee, H. J., An, S. Y., Sim, S., Park, B. Choi, H. G., et al. (2015). Analysis of the prevalence and associated risk factors of tinnitus in adults. *PLoS ONE* 10:e0127578. doi: 10.1371/journal.pone.0127578

Kirschbaum, C., Pirke, K. M., and Hellhammer, D. H. (1993). The "Trier Social Stress Test"–a tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology* 28, 76–81. doi: 10.1159/000119004

Kudielka, B. M., and Wust, S. (2010). Human models in acute and chronic stress: assessing determinants of individual hypothalamus-pituitary-adrenal axis activity and reactivity. *Stress* 13, 1–14. doi: 10.3109/10253890902874913

Lac, G. (2001). Saliva assays in clinical and research biology. *Pathol. Biol.* 49, 660–667. doi: 10.1016/S0369-8114(01)00228-0

Lindsay, A., Lewis, J., Scarrott, C., Draper, N., and Gieseg, S. P. (2015). Changes in acute biochemical markers of inflammatory and structural stress in rugby union. *J. Sports Sci.* 33, 882–891. doi: 10.1080/02640414.2014.971047

Liu, H., Dong, W. Y., Wang, J. B., Wang, T., Hu, P. Wei, S. F., et al. (2013). Association between salivary alpha-amylase activity and pain relief scale scores in cancer patients with bone metastases treated with radiotherapy. *Chin. Med. J.* 126, 4444–4447. doi: 10.3760/cma.j.issn.0366-6999.20130654

Lupis, S. B., Lerman, M., and Wolf, J. M. (2014). Anger responses to psychosocial stress predict heart rate and cortisol stress responses in men but not women. *Psychoneuroendocrinology* 49, 84–95. doi: 10.1016/j.psyneuen.2014.07.004

Ma, X. M., and Lightman, S. L. (1998). The arginine vasopressin and corticotrophin-releasing hormone gene transcription responses to varied frequencies of repeated stress in rats. *J. Physiol. 510(Pt* 2), 605–614. doi: 10.1111/j.1469-7793.1998.605bk.x

Marti, O., Garcia, A., Vellès, A., Harbuz, M. S., and Armario, A. (2001). Evidence that a single exposure to aversive stimuli triggers long-lasting effects in the hypothalamus-pituitary-adrenal axis that consolidate with time. *Eur. J. Neurosci.* 13, 129–136. doi: 10.1111/j.1460-9568.2001.01355.x

McKune, A. J., Bach, C. W., Semple, S. J., and Dyer, B. J. (2014). Salivary cortisol and alpha-amylase responses to repeated bouts of downhill running. *Am. J. Hum. Biol.* 26, 850–855. doi: 10.1002/ajhb.22605

Monteleone, P., Scognamiglio, P., Canestrelli, B., Serino, I., Monteleone, A. M., and Maj, M. (2011). Asymmetry of salivary cortisol and alpha-amylase responses to psychosocial stress in anorexia nervosa but not in bulimia nervosa. *Psychol. Med.* 41, 1963–1969. doi: 10.1017/S0033291711000092

Moynihan, J. A. (2003). Mechanisms of stress-induced modulation of immunity. *Brain Behav. Immun.* 17(Suppl. 1), S11–S16. doi: 10.1016/S0889-1591(02)00060-0

Nater, U. M., Bohus, M., Abbruzzese, E., Ditzen, B., Gaab, J., Kleindienst, N., et al. (2010). Increased psychological and attenuated cortisol and alpha-amylase responses to acute psychosocial stress in female patients with borderline personality disorder. *Psychoneuroendocrinology* 35, 1565–1572. doi: 10.1016/j.psyneuen.2010.06.002

Nater, U. M., and Rohleder, N. (2009). Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. *Psychoneuroendocrinology* 34, 486–496. doi: 10.1016/j.psyneuen.2009.01.014

Nondahl, D. M., Cruickshanks, K. J., Huang, G. H., Klein, B. E., Klein, R., Tweed, T. S., et al. (2011). Tinnitus and its risk factors in the Beaver Dam offspring study. *Int. J. Audiol.* 50, 313–320. doi: 10.3109/14992027.2010.551220

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., and Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* 28, 916–931. doi: 10.1016/S0306-4530(02)00108-7

Schumacher, S., Gaudlitz, K., Plag, J., Miller, R., Kirschbaum, C., Fehm, L., et al. (2014). Who is stressed? *A pilot study of salivary cortisol and alpha-amylase concentrations in agoraphobic patients and their novice therapists undergoing in vivo exposure. Psychoneuroendocrinology* 49, 280–289. doi: 10.1016/j.psyneuen.2014.07.016

Schumacher, S., Kirschbaum, C., Fydrich, T., and Strohle, A. (2013). Is salivary alpha-amylase an indicator of autonomic nervous system dysregulations in mental disorders?–a review of preliminary findings and the interactions with cortisol. *Psychoneuroendocrinology* 38, 729–743. doi: 10.1016/j.psyneuen.2013.02.003

Scott, L. V., and Dinan, T., G. (1998). Urinary free cortisol excretion in chronic fatigue syndrome, major depression and in healthy volunteers. *J. Affect. Disord.* 47, 49–54. doi: 10.1016/S0165-0327(97)00101-8

Shargorodsky, J., Curhan, G., and Farwell, W. (2010). Prevalence and characteristics of tinnitus among US adults. *Am. J. Med.* 123, 711–718. doi: 10.1016/j.amjmed.2010.02.015

Shirasaki, S., Fujii, H., Takahashi, M., Sato, T., Ebina, M., Noto, Y., et al. (2007). Correlation between salivary alpha-amylase activity and pain scale in patients with chronic pain. *Reg. Anesth. Pain Med.* 32, 120–123. doi: 10.1097/00115550-200703000-00005

Tsigos, C., and Chrousos, G. P. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *J. Psychosom. Res.* 53, 865–871. doi: 10.1016/S0022-3999(02)00429-4

van West, D., and Maes, M. (2001). Neuroendocrine and immune aspects of fibromyalgia. *Bio. Drugs* 15, 521–531. doi: 10.2165/00063030-200115080-00004

Vanneste, S., Congedo, M., and De Ridder, D. (2014). Pinpointing a highly specific pathological functional connection that turns phantom sound into distress. *Cereb. Cortex* 24, 2268–2282. doi: 10.1093/cercor/bht068

Vanneste, S., and De Ridder, D. (2013). Brain areas controlling heart rate variability in tinnitus and tinnitus-related distress. *PLoS ONE* 8:e59728. doi: 10.1371/journal.pone.0059728

Vanneste, S., Plazier, M., der Loo, E., de Heyning, P. V., Congedo, M., and De Ridder, D. (2010). The neural correlates of tinnitus-related distress. *Neuroimage* 52, 470–480. doi: 10.1016/j.neuroimage.2010.04.029

Vikman, K. S., Duggan, A. W., and Siddall, P. J. (2007). Interferon-gamma induced disruption of GABAergic inhibition in the spinal dorsal horn *in vivo*. *Pain* 133, 18–28. doi: 10.1016/j.pain.2007.02.010

Widner, B., Laich, A., Sperner-Unterweger, B., Ledochowski, M., and Fuchs, D. (2002). Neopterin production, tryptophan degradation, and mental depression–what is the link? *Brain Behav. Immun.* 16, 590–595. doi: 10.1016/S0889-1591(02)00006-5

Wust, S., Federenko, I. S., van Rossum, E. F., Koper, J. W., and Hellhammer, D. H. (2005). Habituation of cortisol responses to repeated psychosocial stress-further characterization and impact of genetic factors. *Psychoneuroendocrinology* 30, 199–211. doi: 10.1016/j.psyneuen.2004.07.002

Zenner, H. P. (2006). Tinnitus sensitization: a neurophysiological pathway of chronic complex tinnitus. *Otolaryngol. Pol.* 60, 485–489. doi: 10.1097/01.mao.0000231604.64079.77

Zenner, H. P., Pfister, M., and Birbaumer, N. (2006). Tinnitus sensitization: sensory and psychophysiological aspects of a new pathway of acquired centralization of chronic tinnitus. *Otol. Neurotol.* 27, 1054–1063. doi: 10.1097/01.mao.0000231604.64079.77

Zhang, X. Y., Zhou, D. F., Qi, L. Y., Chen, S., Cao, L. Y. C., Kosten, T. R., et al. (2009). Superoxide dismutase and cytokines in chronic patients with schizophrenia: association with psychopathology and response to antipsychotics. *Psychopharmacology* 204, 177–184. doi: 10.1007/s00213-008-1447-6

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frontiers in Human Neuroscience

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Impact of Multiple Factors on the Degree of Tinnitus Distress

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Objective: The primary cause of subjective tinnitus is a dysfunction of the auditory system; however, the degree of distress tinnitus causes depends largely on the psychological status of the patient. Our goal was to attempt to associate the grade of tinnitus-related distress with the psychological distress, physical, or psychological discomfort patients experienced, as well as potentially relevant social parameters, through a simultaneous analysis of these factors.

Methods: We determined the level of tinnitus-related distress in 531 tinnitus patients using the German version of the tinnitus questionnaire (TQ). In addition, we used the Perceived Stress Questionnaire (PSQ); General Depression Scale Allgemeine Depression Skala (ADS), Berlin Mood Questionnaire (BSF); somatic symptoms inventory (BI), and SF-8 health survey as well as general information collected through a medical history.

Results: The TQ score significantly correlated with a score obtained using PSQ, ADS, BSF, BI, and SF-8 alongside psychosocial factors such as age, gender, and marital status. The level of hearing loss and the auditory properties of the specific tinnitus combined with perceived stress and the degree of depressive mood and somatic discomfort of a patient were identified as medium-strong predictors of chronic tinnitus. Social factors such as gender, age, or marital status also had an impact on the degree of tinnitus distress. The results that were obtained were implemented in a specific cortical distress network model.

Conclusions: Using a large representative sample of patients with chronic tinnitus permitted a simultaneous statistical measurement of psychometric and audiological parameters in predicting tinnitus distress. We demonstrate that single factors can be distinguished in a manner that explains their causative association and influence on the induction of tinnitus-related distress.

Keywords: the grade of tinnitus-related distress, the psychological distress, physical or psychological discomfort, social parameters, multi-level analyses

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INTRODUCTION

Multi-Dimensional Causes of Tinnitus

Tinnitus distress is a multidimensional phenomenon that can be associated with problems such as difficulties with concentration, insomnia, or negative thinking which can amplify it in vicious cycles (Zirke et al., 2013; McKenna et al., 2014). Complex relationships between tinnitus, other stressors, and social factors such as education, age, and gender

have been reported in the literature (Nondahl et al., 2012; Seydel et al., 2013). Furthermore, tinnitus distress seems to be more commonly reported by patients from lower social classes. Tinnitus percept results from an imbalance between excitatory and inhibitory functions in the auditory pathway (Eggermont and Roberts, 2012). Cortical mental hyperactivity can exacerbate tinnitus distress and turn it into a chronic condition. Sub-cortical structures that participate in the emotional processing of auditory signals may contribute to negative interpretations of the noise and can amplify it in response to stress, anxiety, or other factors (Leaver et al., 2011). Tinnitus can also be worsened by various orthopedic conditions (Biesinger et al., 2008).

Influence of Psychological and Somatic Complaints

A patient's level of awareness of tinnitus depends on multiple sound-processing mechanisms (Hesse, 2008) and is considered a result of selective attention combined with an absence of habituation (Hallam et al., 2004). From a psychological point of view, reducing negative interpretations of the noise and changing certain types of behavior will generally reduce emotional distress while increasing habituation, to render the tinnitus less intrusive (Wallhausser-Franke et al., 2012; McKenna et al., 2014). The neurophysiological model of tinnitus assumes that it originates in the inner ear, followed by detection to the subcortex and further steps of processing, perception, and evaluation to various cortical areas (Jastreboff, 1999). A current model views specific thalamic nuclei as filters or gates where decisions about the conscious processing of tinnitus are made (Rauschecker et al., 2010). While these psychological and neurophysiological models appear similar, they emphasize different conscious and unconscious processes in attempting to explain the experience of tinnitus distress and the treatments they propose, reflecting differences in their philosophical underpinnings (Hallam et al., 2004).

Whatever the mechanism that generates tinnitus distress, it is widely thought to involve a link between overly negative thoughts and emotions and auditory stimuli. Patients recite a common litany of despair, persecution, hopelessness, a loss of enjoyment in life, a desire for peace and quiet, and often beliefs that others do not understand (Wilson and Henry, 1998). Tinnitus distress often leads to negative thinking about the disease itself and its role in a patient's life (Henry and Wilson, 1995; Andersson and Westin, 2008). In advanced stages, tinnitus distress amplifies negative thoughts about the disease itself, starting a cycle that can lead to an even greater emotional decline. At some point 65% of tinnitus patients are diagnosed with depression, anxiety, or another mental condition (Hiller and Goebel, 1992; Andersson and Kaldo, 2004; Zirke et al., 2013).

Recently these links were highlighted in a report on the association between catastrophic thinking, high subjective judgments of the loudness of the tinnitus, poorer coping, the appearance of symptoms of depression, and more frequent medical visits (Weise et al., 2013). Catastrophic interpretations of tinnitus were also associated with fear (Cima et al., 2011; Pattyn et al., 2016). Several other studies have suggested a link between a poor emotional state and increased tinnitus distress (Halford and Anderson, 1991; Zoger et al., 2006; Wallhausser-Franke et al., 2012; Milerova et al., 2013). The overall process appears to include an increase in stress arousal, culminating in selective attention, and more monitoring of tinnitus, which in turn increases its detection and creates a vicious cycle (McKenna et al., 2014). This is consistent with observations that catastrophic interpretations lead to fear, increased attention devoted to the tinnitus, and a decrease in quality of life (Cima et al., 2011). Another result is that patients change their behavior in ways that are intended to reduce the threat, but in fact perpetuate it or increase negative thinking even more (Hesser and Andersson, 2009; Kleinstauber et al., 2013).

Such observations have strengthened the notion that links between limbic and auditory areas of the brain play a significant role in processing tinnitus and generating accompanying distress. The perception of tinnitus must follow from some neurophysiological process that sensitizes cognitive areas (Zenner, 2003). This model suggests that neuronal plasticity lowers normal perceptual thresholds and makes tinnitus audible, and the neural network responds by making subject, emotional, and somatic/motoric functions hyper-reactive. In the diathesis-stress model and similar frameworks, tinnitus is regarded as a stressor and may be aggravated by the elevated levels of stress associated with a vulnerable constitution (Andersson and McKenna, 1998; Kroner-Herwig et al., 2006).

Comorbidity

Many polygenic diseases with high rates of comorbidity are considered to incorporate a significant stress component (Chrousos, 2009). The influence of stress on the auditory system has been detected on molecular and cellular levels (Ma et al., 1995; Horner, 2003; Mazurek et al., 2010a, 2012; Kraus and Canlon, 2012). Stress can arise from a number of sources, including an individual's general disposition or psychological coping mechanisms. The severity of tinnitus has been related to the personality traits of perfectionism (Andersson et al., 2005) and anxiety sensitivity (Andersson and Kaldo, 2004; Hesser and Andersson, 2009). Optimism, as might be expected, is negatively associated with tinnitus distress (Vollmann et al., 2013).

Establishing a causal relationship between such personality variables and tinnitus distress is challenging. The appearance of traits of anxiety shortly after the onset of tinnitus has been used as a predictor that the distress will be greater 6 months later (Langenbach et al., 2005) suggesting that people who are already emotionally strained are more likely to be distressed by tinnitus. An additional relationship has been found between distress, quality of life, and Type D personalities (marked by a generally sad and gloomy view of life and social inhibition) (Bartels et al., 2010a,b). The influence of this personality type on the severity of distress was partly mediated by anxiety and depression (also possibly reactions to tinnitus). Cognitive variables such as dysfunctional thoughts (Lee et al., 2004), particularly catastrophization could also influence this relationship (Weise et al., 2013), as could tinnitus-specific perceptions of illness (Vollmann et al., 2013). Dysfunctional coping strategies are related to an increase in tinnitus distress (Budd and Pugh, 1996; Scott and Lindberg, 2000). An increase

in suspicions about external control reinforces the psychiatric symptoms associated with tinnitus (Budd et al., 1998).

The presence of a formal psychological disorder is another source of stress for the person. While psychiatric disturbance may be a reaction to tinnitus, it has also been proposed as a trigger for tinnitus (Henry et al., 2005). In 64% of cases, the onset of tinnitus is preceded by other mental disorders (Goebel and Floezinger, 2008). Studies point to a link between problematic tinnitus and depression (Wilson et al., 1991; Goebel and Floezinger, 2008), connecting the severity of tinnitus to that of depression or anxiety (Budd and Pugh, 1996; Andersson et al., 2005; Zoger et al., 2006). Accordingly, an improvement in depressive symptoms has been associated with a reduction of tinnitus distress (Folmer, 2002).

The literature offers the overall impression of a diathesis-stress interaction in which the diathesis is a patient's vulnerability to distress, and the stress is the level of tinnitus, an idea supported by Andersson and McKenna (1998); Kroner-Herwig et al. (2006). Patients who find tinnitus highly distressing often exhibit additional somatic conditions or comorbidities including autoimmune diseases, cardiovascular, endocrine, or metabolic diseases (Stobik et al., 2003). Increased tension of the head and neck muscles and other functional impairments have been described in tinnitus patients (Rubinstein et al., 1990; Peroz, 2003). These factors have raised clinical interest in a classification of tinnitus distress that takes into account not only the degree of tinnitus, hearing loss, and the audiological characteristics of the conditions but also psychosocial variables and comorbid factors (i.e., a biopsychosocial model) (Seydel et al., 2010).

Prior studies have linked single mental and physical factors to tinnitus distress; in the present study we carry out *simultaneous analyses* of its association with many factors, hoping to develop a broader view. The literature shows that stress, anxiety or depression contribute to tinnitus-related distress; here, we wanted to know if this distress is affected by *individual but networked conditions* that can be assessed by the subscales of psychometric instruments. Using a large data set, we also aimed to determine *where* such conditions overlap with tinnitus, as well as ways in which tinnitus-induced distress *differs* from the experience of other types of stress and emotions. Finally, we used quantitative methods to assign weights to the factors in terms of their relative contributions to tinnitus impairment.

METHODS

Patients

Five hundred and thirty one patients with chronic tinnitus were recruited to this study, which was carried out between January 2008 and March 2010. The patients originated from a routine flow of individuals consecutively admitted to day ward of Tinnitus Center for treatment. The study sample consisted of 251 (47%) men and 280 (53%) women with a mean age of 49 years (SD 13.29 + Min 16 Max 59). All patients were informed of the purpose for which the data was being collected and gave their consent. This study was approved by the local Ethics Committee.

Audiometry

Pure tone audiometry was performed on both ears of each patient to determine the degree and nature of any hearing loss. A discomfort threshold was used to determine the possible presence of hyperacusis; (data not included in the evaluation). We used tinnitus matching (frequency and loudness) to detect and provide an audiometric description of each patient's tinnitus.

Psychometric Evaluation

The study employed the self-reporting psychometric instruments shown in **Table 1**, chosen on the basis of clinical experience and representative cross-reference data from the Department of Psychosomatic Medicine, Charité, Universitätsmedizin Berlin (scores obtained from tinnitus patients are compared with those of patients with psychosomatic disorders in Zirke et al., 2013 and Devine et al., 2016).

- The degree of tinnitus distress was measured using the German version of tinnitus questionnaire TQ (Goebel and Hiller, 1994). The subscales include emotional and cognitive stress, intrusiveness of tinnitus, hearing problems, sleep disorders, and somatic symptoms associated with tinnitus.
- The Perceived Stress Questionnaire (Fliege et al., 2005) registers a subject's level and perception of stress (tension, worry, joy).
- Depressive symptoms were measured using the validated German version of the Center for Epidemiologic Studies Depression Scale, abbreviated here as ADS—Allgemeine Depression Skala (Radloff, 1991).
- Additional measurements were made of "anxious depression," "annoyance," and "positive mood" with the Berlin mood questionnaire (BSF) (Hoerhold and Klapp, 1993).
- Subjects' quality of life and mental and physical functions were assessed with the Short Form Health Survey (SF-36) (Morfeld et al., 2005).
- The Somatic Symptoms Inventory was used to characterize somatic symptoms considered independent of tinnitus.

Computational support included the use of a personal digital assistant (PDA) and data analysis, permitting physicians to provide subjects with an immediate interpretation of the results of their survey (Rose et al., 1999).

Statistical Evaluation

The analysis was performed with SPSS Statistics (version 15). First, the data were examined to determine the relationship between psychometric and audiological data and tinnitus distress (total tinnitus distress-score from TQ and its corresponding subscales). Correlations were calculated to provide initial values for the strength of each connection, followed by multiple regressions to examine cause and effect relationships. The dependent variable was TQ score whereas independent variables were the scores of remaining instruments and audiometric values. In addition to the psychometric tests, social data (age, sex, marital status, education, employment), and audiometric parameters such as hearing loss, tinnitus loudness, and frequency were included. This produced multiple regression model, which achieved a high level of significance, with an overall regression

TABLE 1 | Psychometric tests used during the study.

Questionnaire	Item example	Measured domain
Tinnitus questionnaire by Goebel and Hiller (TQ, 51 items)	**Emotional stress:** to be worried sick	Tinnitus distress
	Cognitive stress: thoughts on prognosis	
	Intrusiveness: distraction of tinnitus	
	Hearing problems: distortion of voices	
	Sleep disorders: falling asleep	
	Somatic symptoms: severe headache	
Questionnaire to tinnitus localization and quality (TLQ, 10 Items)	**Localization:** left, **quality**: rustle	Tinnitus
Visual analog scales (VAS, 3 items)	**Loudness of tinnitus**	Tinnitus
	Frequency of perception: Affected by tinnitus	
Perceived stress questionnaire (PSQ; 20 items)	**General requirements:** They feel under deadline pressure	Stress amount
	Tension: You feel tense	Stress perception
	Worries: Your problems seem to pile up in front of you	
	Joy: You are a light hearted	
General depression scale (ADS-L; 20 Items)	**Depressive affect:** self-devaluation	Depression
	Somatic symptoms: drive	
	Interpersonal experiences: Rejection by others	
	Positive affect: zest for life	
Berlin mood questionnaire (BSF; 30 Items)	**Anxious depression:** I feel worried	Mood
	Anger: I feel aggressive	
	Elation: I feel solved	
	Engagement: I feel included	
Somatic symptoms inventory (BI; 24 Items)	**Fatigue:** fatigue	Somatic symptoms
	Stomach complaints: bloating	
	Limb pain: neck pain	
	Heart disease: shortness of breath	
Short form—8 health survey (SF8; 8 Items)	How much have you physical health or emotional problems in the past 4 weeks, limiting your normal contacts with family members or friends?	Quality of life
Total: 175 items		

coefficient $R = 0.993$ (ANOVA: $F = 24.753$, $p < 0.000$). Due to limited capacity of SPSS version 15, the predictor importance was computed with a stochastically defined, randomly chosen by software subset of the sample ($N = 140$). In addition, multiple regression model was performed separately for the subset of 140 patients chosen for predictor importance computing. That model has also achieved high level of significance with regression coefficient $R = 0.890$ (ANOVA: $F = 4.896$, $p < 0.000$). All correlations that were significant for the whole sample remained significant in the subset and no new correlations were identified, indicative of sample homogeneity.

RESULTS

Based on the tinnitus distress score TQ, 400 patients (75%) were classified as having a compensated, moderate tinnitus response (TQ-score < 46), while 131 patients (25%) exhibited decompensated, severe tinnitus reactions (TQ score>, 47). The first interview recorded stress, depressive symptoms, disease-related fears, patients' medication and drug use, alongside audiometric measurements, and a medical history.

Dependence of the Degree of Tinnitus Distress (TQ) on Somatic Factors

Hearing

The audiometric examination revealed a moderate hearing loss of 23.53 dB/HL for the left ear and 22.96 dB/HL for the right ear in the study population.

Hearing Loss

The correlation between hearing loss and tinnitus distress (TQ-score) was significant both for the total value of the TQ and the subscales "hearing problems" and "intrusiveness of tinnitus" (**Table 3**).

Multiple regressions (linear model, all variables) found no significant association between hearing loss, specific

TABLE 2 | Patients' description.

	Number of patients	Mean value	Standard deviation	Minimum	Maximum
Age	531	48.88	13.29	16	79
Mean total score TQ	531	34.73	16.38	2	79
TQ emotional	531	9.53	5.49	0	23
TQ cognitive	531	6.13	3.90	0	16
TQ intrusiveness	531	9.51	3.55	0	16
TQ acoustic	531	4.70	3.55	0	14
TQ sleep	531	3.07	2.57	0	8
TQ somatic symptoms	531	1.79	1.74	0	6
PSQ worries	531	36.13	22.20	0	100
PSQ tension	531	50.83	22.88	0	100
PSQ joy	531	52.24	22.61	0	100
PSQ demands	531	44.91	24.17	0	100
Mean score ADSL	531	16.11	11.03	0	53
Tinnitus frequency right (Hz)	279	5058.17	2758.00	125	10,000
Tinnitus frequency left (Hz)	292	5380.14	2776.07	125	10,000
Tinnitus loudness right (dB)	276	36.09	21.17	-4	100
Tinnitus loudness left (dB)	290	37.42	20.97	2	93
Mean hearing loss right (dB)	531	23.00	16.73	1.9	130.0
Mean hearing loss left (dB)	530	23.28	15.01	0.6	86.4

characteristics of tinnitus and the level of distress it caused. A tendency for a significant negative regression was found between hearing loss on the right and TQ-total value ($t = -1.793$, $p = 0.075$).

Tinnitus Loudness

The loudness of tinnitus (dB HL, separated for unilateral and bilateral tinnitus), but not its frequency (**Table 2**), correlated with the scores of the TQ (Spearman correlation). Significant correlations were found between tinnitus loudness and the subscales "hearing problems" and "intrusiveness" and, with lower significance, for the total value of the TQ (**Table 3**). In the regression model, neither the loudness nor frequency of the tinnitus (respectively right/left) achieved significance.

Somatic Symptoms Inventory (*BI*)

A significant correlation was found between somatic symptoms measured by Somatic Symptoms Inventory, all subscales of TQ, and the total value (**Table 3**).

The regression analysis revealed a significant regression of fatigue with tinnitus distress (TQ total) with$t = 2.038$ ($p = 0.043$).

Dependence of Tinnitus Distress (from TQ) on Psychological Burden

Stress (PSQ) Related to Tinnitus Distress (TQ)

The level of perceived stress was significantly associated with tinnitus distress (TQ-scores) and correlated with the following PSQ subscales: demands, worry, tension, and joy (**Table 3**).

The regression analysis revealed no significant correlation in the overall model.

Depression Score (ADS) Correlates with Tinnitus Distress (TQ)

The total score for the Depression-Scale, which mainly focuses on cognitive aspects of depression such as a tendency to worry and negative thought circuits, correlated to the total TQ score as well as with its all separate subscales at a high level of significance (**Table 3**).

The multiple regression model revealed a significant regression with $t = 2.98$ ($p = 0.003$).

Mood Scores (BSF) are Related to Tinnitus Distress (TQ)

Symptoms of anxiety and depression, which were evaluated with the BSF, correlated significantly with the total TQ score and all its separate subscales, as was the case for "annoyance." The positive moods, commitment and joy, had significant negative correlations with the total value and all subscales (**Table 3**).

Multiple regression found a significant relationship for anxious depression and total TQ with $t = -2.20$ ($p = 0.029$).

Dependence of Tinnitus Distress (TQ) on Social Stress

Quality of Life (SF-8) is Significantly Affected by Tinnitus Distress (TQ)

A significant negative correlation was found between physical and mental health as measured by the SF-8 and the total TQ score as well as all TQ subscales (**Table 3**). In the regression model, no significant correlations were found.

TABLE 3 | Results of correlation analysis in the study population ($n = 531$).

		Tinnitus distress total score	Emotional and cognitive stress	Hearing problems	Somatic symptoms	Intrusiveness	Sleep problems
1. HEARING							
Hearing loss right	r_S	0.173*	0.090	0.247*	0.088	0.197*	−0.006
Hearing loss left	r_S	0.235**	0.135*	0.384**	0.095	0.269*	0.064
2. TINNITUS							
Tinnitus loudness right	r_S	0.176*	0.092	0.247*	0.083	0.227**	0.094
Tinnitus loudness left	r_S	0.111*	0.027	0.239**	0.041	0.158*	0.039
Tinnitus frequency right	r_S	0.008	−0.003	0.010	0.351**	0.426**	0.385**
Tinnitus frequency left	r_S	−0.029	−0.018	−0.054	−0.065	−0.022	0.022
3. SOMATIC SYMPTOMS INVENTORY BI							
Fatigue	r_S	0.535**	0.498**	0.359**	0.397**	0.424**	0.325**
Stomach	r_S	0.403**	0.368**	0.359**	0.313**	0.283**	0.239**
Limbs	r_S	0.478**	0.384**	0.403**	0.538**	0.373**	0.273**
Heart	r_S	0.401**	0.358**	0.320**	0.330**	0.303**	0.225**
BI_total score	r_S	0.588**	0.520**	0.449*	0.513**	0.452**	0.345**
4. PSQ							
Worries	r_S	0.444**	0.472**	0.254**	0.226*	0.342**	0.208**
Joy	r_S	−0.388**	−0.376**	−0.296**	−0.269**	−0.306**	−0.151*
Tension	r_S	0.485**	0.442**	0.320**	0.328**	0.449**	0.270**
General requirements	r_S	0.216**	0.196*	0.189*	0.162*	0.173*	0.083
PSQ_ total score	r_S	0.463**	0.449**	0.322**	0.298**	0.382**	0.214**
5. GENERAL DEPRESSION SCALE AND BERLIN MOOD QUESTIONNAIRE BSF, ADS							
Engagement	r_S	0.335**	−0.306**	−0.258**	0.239**	−0.269**	−0.178*
Joy	r_S	−0.409**	−0.396**	−0.249**	−0.272**	−0.347**	−0.217**
Annoyance	r_S	0.512**	0.518**	0.306**	0.311**	0.396**	0.275**
Anxious depression.	r_S	0.586**	0.637**	0.283**	0.344**	0.426**	0.302**
ADS_ total score	r_S	0.579**	0.572**	0.349**	0.351**	0.426**	0.385**
6. SHORT FORM – 8 HEALTH SURVEY SF 8							
Physical health	r_S	−0.446**	0.417**	0.323**	−0.270**	−0.363**	−0.258**
Mental health	r_S	−0.455**	−0.464**	0.295**	−0.249**	−0.339**	−0.240**
SF8_ total score	r_S	−0.388**	−0.370**	−0.315**	−0.201*	−0.299**	−0.199*

r_S, correlation coefficient.
**, significance $p < 0.05$.*
***, significance $p < 0.01$.*

Age, Gender, and Marital Status are Related to Tinnitus Distress (TQ)

Patients who lived alone had a higher hearing loss on both sides and greater hearing problems than those living with a partner, as indicated by scores of the respective subscales (TQ). A similar trend was found for tinnitus loudness, judged higher by people living alone. The regression analysis showed a significant positive correlation with age $t = 2.23$ ($p = 0.027$).

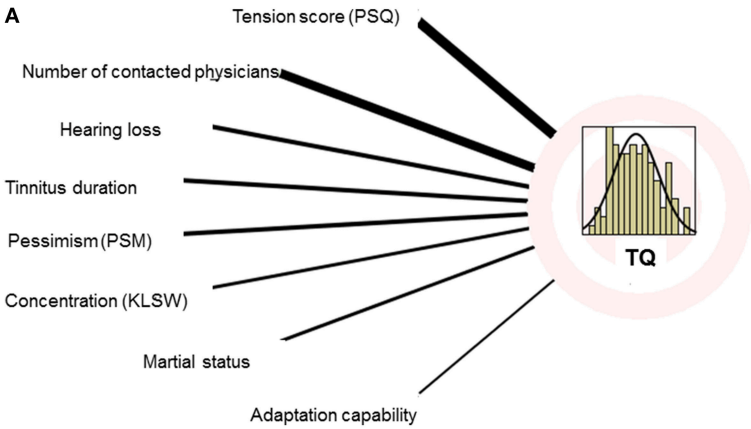
Weighting of Each Component in Relation to Tinnitus Distress (TQ) in the Regression Analysis

The multiple regression analysis [evaluating the effects of tinnitus distress (TQ) for other psychometric data] showed that depression and stress scores had particularly high predictor values (**Figures 1**, **2**).

The analysis of social variables revealed significant correlations with age, education, employment, and the number of physicians a patient had consulted. Additionally, a correlation, between hearing loss and tinnitus distress was found.

Summary of Results

- There was a clear association between physical symptoms and tinnitus distress TQ, particularly seen in correlations with the TQ subscales "hearing problems" and "somatic symptoms," and to some degree with "emotional stress."
- Quality of life and tinnitus distress are negatively correlated, as indicated by the negative influence of the subscales "emotional stress," "somatic symptoms," and "hearing problems" on mental health.
- Tinnitus distress (TQ total score) correlated significantly with perceived stress and (depressive) mood. For perceived stress (PSQ), the TQ subscales "emotional stress" and "hearing problems" proved to be especially significant. The subscales "emotional stress," "sleep problems" and, to a lesser



B

Source	Significance level (*p*)	Predictor importance
Tension score (PSQ)	0.000	0.434
Number of contacted physicians	0.000	0.216
Hearing loss	0.025	0.079
Tinnitus duration	0.039	0.066
Pessimism (PSM)	0.046	0.062
Concentration (KLSW)	0.054	0.058
Martial status	0.072	0.050
Adaptation capability	0.129	0.036

FIGURE 1 | (A) Regression analysis graphical model [overall model: $R = 0.993$ (ANOVA: $F = 322.97$, $p < 0.000$)] calculated with subset of the data ($n = 140$). **(B)** Computed predictor importance and significance.

degree, "tinnitus intrusiveness" significantly correlated with the depression grade.

DISCUSSION

The aim of our study was to simultaneously measure associations between tinnitus distress and other mental and physical conditions in a single group of patients. Previous studies have linked distress to a number of individual factors, but their relative contributions to the outcome of tinnitus distress have not been ranked in a single model. Our efforts found a number of correlations between measurements of tinnitus distress (TQ) and physical symptoms, the quality of life, perceived stress, and depressive symptoms.

Psychometric methods have been established as a means of expressing tinnitus distress. A number of studies have concluded that psychoacoustic features of tinnitus, such as loudness, cannot be used to predict a patient's degree of distress (Henry and Meikle, 2000; Hiller and Goebel, 2007). Here we demonstrate that acoustic variables such as the loudness and frequency of tinnitus correlate only marginally with the overall tinnitus-related burden experienced by patients. Tinnitus loudness could only be correlated with the TQ subscales "hearing problems" and "intrusiveness of tinnitus." The louder the tinnitus, the more penetrating is its perception. However, our study revealed no correlation was found between tinnitus frequency and the TQ total score.

Past studies have reported some correlations between auditory processing and patient mood. A representative study recently conducted in Sweden concluded that the strongest predictor for the *occurrence* of tinnitus was hearing loss. A patient's subjective *experience* of tinnitus, however, was more dependent on the presence of a depressive co-morbidity (Hebert et al., 2012; Kraus and Canlon, 2012).

In earlier work (Mazurek et al., 2010b), we determined that tinnitus-induced distress is positively correlated with hearing loss, and the present study corroborates this finding.

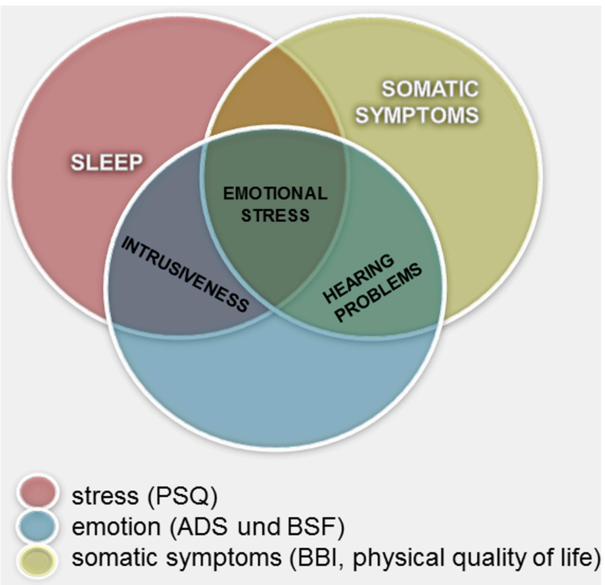


FIGURE 2 | Measurements of constructs related to tinnitus distress: graphical summary of the overlapping and not covering areas.

We also found that age has a significant impact on tinnitus distress. This confirms findings from single-factor association studies that have linked distress to hearing problems and sleep disorders, which also increase with age. Similar findings from other recent studies demonstrate that older patients find tinnitus more stressful (Schlee et al., 2011a). Previous work by our group has shown that age influences various aspects of tinnitus distress; starting at the age of 60, patients report more distress (Seydel et al., 2013).

Here our computing of predictor importance identifies only two factors that account for a significant amount of the variance of tinnitus distress: the side on which patients experience tinnitus, and the overall status of their hearing.

No significant correlations were found based on age or gender.

We have shown that tinnitus distress is closely related to more general types of stress, a negative mood, and hearing loss. Increasing age is generally accompanied by poorer hearing and other somatic symptoms, which then amplify tinnitus distress. Age-related changes in tinnitus have been reported in the literature (see a review Henry et al., 2005) and in detail by our group (Seydel et al., 2013, 2015). The correlations that emerge, however, are multidimensional. Causal relationships seem to be indicated for gender, the duration of tinnitus, hearing impairment, age, and psychosocial variables (such as employment, education level, and use of the health system).

An analysis of the influence of marital status of tinnitus patients revealed a trend toward greater hearing loss in people who live alone. They perceive tinnitus louder than patients who live with a partner. Somatic symptoms and general physical health (quality of life-SF 8) are co-morbidities whose effects vary significantly in association with hearing, and partly in relation to age and sex. A decline in hearing had a significant influence on other symptoms captured in the somatic symptoms inventory (BI). As people age, they generally report a decline in aspects of the quality of life linked to physical health. For tinnitus patients, the loss of hearing likely has an additive effect on these negative perceptions.

A review of associations that have been made between tinnitus and subjective tinnitus distress in the literature yielded high correlations with "somatic problems" and "intrusiveness." This association seems to depend on a patient's perception of tinnitus and its additive effects on other somatic problems, some of which may have a physiological basis. Liotti and Mayberg have proposed that a negative mood or depressive emotions, which chiefly originate in the limbic system, can lead to down-regulations of activity in the inferior parietal cortex and dorsolateral prefrontal cortex (Liotti and Mayberg, 2001). These regions include the auditory cortex, where acoustic stimuli are processed. The effects may extend to a wider network involved in attention and spatial orientation (Sturm et al., 2004; Schonwiesner et al., 2007; McKenna et al., 2014). By modulating processes and brain areas involved in attention, negative moods may also influence activity in the auditory or somatosensory areas of the brain.

Previous studies have reported high correlations between the total score for tinnitus distress (as determined by the -TQ) with stress and stress perception (Olderog et al., 2004), with depression and negative mood (Andersson and Kaldo, 2004), and even with physical complaints and perceptions of a decline in quality of life (Cima et al., 2011). Bi-directional interference has been observed between tinnitus perception and stress, pain, sleep, and fatigue Hebert et al. (2012). Langguth (2011) suggest that there are similarities in the pathophysiology of depression and tinnitus, especially in regard to associated distress factors (Langguth, 2011). Here we confirm a strong correlation between tinnitus distress and other forms of stress and depression. However, the regression analysis offers a multi-dimensional view of these relationships, suggesting that other forms of somatic stress and depression contribute to total tinnitus distress (TQ) in distinct ways. The stress scales (PSQ) showed correlations to all subscales of TQ; correlation coefficients were particularly high for emotional stress, intrusiveness and tinnitus-induced hearing problems. Depression, on the other hand, was predominantly correlated with the TQ-subscales "emotional stress," "intrusiveness," and "sleep."

So far these studies have exposed a number of factors that play some role in the development of tinnitus and the ways patients cope, but they have not turned up a specific association that correlates with a high-distress response reliably enough to serve as a predictor. This will come as no surprise to clinicians, who are familiar with the diverse symptoms and responses of patients. What has been missing is a more complex model that integrates these findings, reveals combinations of factors that influence the facets of tinnitus, and assigns weights to components that reflect their contributions to various processes.

Our approach involved a large cohort of tinnitus patients, from whom we simultaneously captured a wide range of psychological and somatic parameters. We used computational methods to detect associations between high tinnitus distress and various combinations of psychological, biological, and lifestyle factors. The analysis revealed a network of associations linking aspects of tinnitus distress to other aspects of patients' lives and health. In some cases we could assess the contributions of each component and distinguish crucial factors from weak contributors.

We anticipated that our combined approach would reveal new, more precise associations between subscales of tinnitus distress and diverse aspects of patients' lives; these, in turn, could provide new insights into causal mechanisms that linked them. Finding a strong correlation between tinnitus perception and depression and negative moods, for example, is evidence of a connection between acoustic perception and the regulation of emotions, thus implicating specific regions of the brain and possibly suggesting something about their coordination.

The Global Brain Model of Tinnitus proposes a scenario in which acoustic input to the brain falls, which can be caused by various types of damage to the auditory system (Schlee et al., 2011b). This leads to a change in the central auditory system, where inhibitory mechanisms usually dampen the strength of acoustic signals; now those mechanisms become less active, resulting in more excitation of cortical areas.

The activity of auditory areas is modulated by a fronto-parietal-cingular network, where higher activity is associated with high levels of patient distress. The particularly important structures in this network are the dorsolateral prefrontal cortex, the orbitofrontal cortex, the anterior cingulum, and the precuneus/posterior cingulate. De Ridder et al. (2011) describes a network of ACC, amygdala, and anterior insula (tested by EEG data) and claims that it is responsible for tinnitus distress. This network is also activated in pain-associated distress (Moisset and Bouhassira, 2007). Recent epidemiological studies suggest that similar or related symptoms might account for the relationship between tinnitus distress and depression, but they may also have common etiologies (Hebert et al., 2012). These ideas are interesting in light of the observation that either depression or a high general level of stress leads to a more pronounced

perception of tinnitus symptoms by patients (Bartels et al., 2010b).

The diverse models that have been proposed for tinnitus distress may indicate that it might be reached through several routes that depend on different mechanisms. Clinical experience would tend to support this view, given the diversity of patients affected by tinnitus and their subjective experiences of it. Ultimately, hypotheses and models of tinnitus mechanisms must be validated in patient studies, and ideally they should be designed with an eye to clinical benefits and new, effective types of treatment.

Further studies will be necessary to clarify how different processing mechanisms interact to produce the complex symptoms of tinnitus. At least three types of components are involved: somatic (generators, influenced by a range of somatosensory stimuli), psychological (emotional "depressive" processing, attention, stress), and social. The unique features of tinnitus might trigger a unique "Distress Network"; this might explain the increased level of stress and other physical and psychological symptoms observed in patients with chronic decompensated tinnitus (De Ridder et al., 2011). Helping patients become habituated to tinnitus and find healthy ways to compensate will probably require therapies that address and reduce combinations of symptoms related to stress and depression.

AUTHOR CONTRIBUTIONS

PB: study conception and design; data acuisition; analysis and interpretation of data; drafting of manuscript. AS: analysis and interpretation of data; drafting of manuscript. MR: analysis and interpretation of data. LM: critical revision; drafting of manuscript. HO: critical revision. BM: study design; data acuisition; analysis and interpretation of data.

REFERENCES

Andersson, G., Airikka, M., Buhrman, M., and Kaldo, V. (2005). Dimensions of perfectionism and tinnitus distress. *Psychol. Health Med.* 10, 78–87. doi: 10.1080/13548500512331315389

Andersson, G., and Kaldo, V. (2004). Internet-based cognitive behavioral therapy for tinnitus. *J. Clin. Psychol.* 60, 171–178. doi: 10.1002/jclp.10243

Andersson, G., and McKenna, L. (1998). Tinnitus masking and depression. *Audiology* 37, 174–182. doi: 10.3109/00206099809072971

Andersson, G., and Westin, V. (2008). Understanding tinnitus distress: introducing the concepts of moderators and mediators. *Int. J. Audiol.* 47(Suppl. 2), S106–S111. doi: 10.1080/14992020802301670

Bartels, H., Middel, B., Pedersen, S. S., Staal, M. J., and Albers, F. W. (2010a). The distressed (Type D) personality is independently associated with tinnitus: a case-control study. *Psychosomatics* 51, 29–38. doi: 10.1176/appi.psy.51.1.29

Bartels, H., Pedersen, S. S., Van Der Laan, B. F., Staal, M. J., Albers, F. W., and Middel, B. (2010b). The impact of Type D personality on health-related quality of life in tinnitus patients is mainly mediated by anxiety and depression. *Otol. Neurotol.* 31, 11–18. doi: 10.1097/MAO.0b013e3181bc3dd1

Biesinger, E., Reisshauer, A., and Mazurek, B. (2008). [The role of the cervical spine and the craniomandibular system in the pathogenesis of tinnitus. Somatosensory tinnitus]. *HNO* 56, 673–677. doi: 10.1007/s00106-008-1721-2

Budd, R. J., Oles, G., and Hughes, I. C. (1998). The relationship between coping style and burden in the carers of relatives with schizophrenia. *Acta Psychiatr. Scand.* 98, 304–309. doi: 10.1111/j.1600-0447.1998.tb10088.x

Budd, R. J., and Pugh, R. (1996). Tinnitus coping style and its relationship to tinnitus severity and emotional distress. *J. Psychosom. Res.* 41, 327–335. doi: 10.1016/S0022-3999(96)00171-7

Chrousos, G. P. (2009). Stress and disorders of the stress system. *Nat. Rev. Endocrinol.* 5, 374–381. doi: 10.1038/nrendo.2009.106

Cima, R. F., Crombez, G., and Vlaeyen, J. W. (2011). Catastrophizing and fear of tinnitus predict quality of life in patients with chronic tinnitus. *Ear Hear.* 32, 634–641. doi: 10.1097/AUD.0b013e31821106dd

De Ridder, D., Elgoyhen, A. B., Romo, R., and Langguth, B. (2011). Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8075–8080. doi: 10.1073/pnas.1018466108

Devine, J., Fliege, H., Kocalevent, R., Mierke, A., Klapp, B. F., and Rose, M. (2016). Evaluation of Computerized Adaptive Tests (CATs) for longitudinal monitoring of depression, anxiety, and stress reactions. *J. Affect. Disord.* 190, 846–853. doi: 10.1016/j.jad.2014.10.063

Eggermont, J. J., and Roberts, L. E. (2012). The neuroscience of tinnitus: understanding abnormal and normal auditory perception. *Front. Syst. Neurosci.* 6:53. doi: 10.3389/fnsys.2012.00053

Fliege, H., Rose, M., Arck, P., Walter, O. B., Kocalevent, R. D., Weber, C., et al. (2005). The Perceived Stress Questionnaire (PSQ) reconsidered: validation and reference values from different clinical and healthy adult samples. *Psychosom. Med.* 67, 78–88. doi: 10.1097/01.psy.0000151491.80178.78

Folmer, R. L. (2002). Long-term reductions in tinnitus severity. *BMC Ear Nose Throat Disord.* 2:3. doi: 10.1186/1472-6815-2-3

Goebel, G., and Floezinger, U. (2008). Pilot study to evaluate psychiatric co-morbidity in tinnitus patients with and without hyperacusis. *Audiol. Med.* 6, 78–84. doi: 10.1080/16513860801959100

Goebel, G., and Hiller, W. (1994). [The tinnitus questionnaire. A standard instrument for grading the degree of tinnitus. Results of a multicenter study with the tinnitus questionnaire]. *HNO* 42, 166–172.

Halford, J. B., and Anderson, S. D. (1991). Anxiety and depression in tinnitus sufferers. *J. Psychosom. Res.* 35, 383–390. doi: 10.1016/0022-3999(91)90033-K

Hallam, R. S., McKenna, L., and Shurlock, L. (2004). Tinnitus impairs cognitive efficiency. *Int. J. Audiol.* 43, 218–226. doi: 10.1080/14992020400050030

Hebert, S., Canlon, B., Hasson, D., Magnusson Hanson, L. L., Westerlund, H., and Theorell, T. (2012). Tinnitus severity is reduced with reduction of depressive mood–a prospective population study in Sweden. *PLoS ONE* 7:e37733. doi: 10.1371/journal.pone.0037733

Henry, J. A., Dennis, K. C., and Schechter, M. A. (2005). General review of tinnitus: prevalence, mechanisms, effects, and management. *J. Speech Lang. Hear. Res.* 48, 1204–1235. doi: 10.1044/1092-4388(2005/084)

Henry, J. A., and Meikle, M. B. (2000). Psychoacoustic measures of tinnitus. *J. Am. Acad. Audiol.* 11, 138–155.

Henry, J. L., and Wilson, P. H. (1995). Coping with tinnitus: two studies of psychological and audiological characteristics of patients with high and low tinnitus-related distress. *Int. Tinnitus J.* 1, 85–92.

Hesse, G. (2008). [Neurootologic and psychosomatic habituation therapy. Treatment approaches in chronic tinnitus]. *HNO* 56, 686–693. doi: 10.1007/s00106-008-1723-0

Hesser, H., and Andersson, G. (2009). The role of anxiety sensitivity and behavioral avoidance in tinnitus disability. *Int. J. Audiol.* 48, 295–299. doi: 10.1080/14992020802635325

Hiller, W., and Goebel, G. (1992). A psychometric study of complaints in chronic tinnitus. *J. Psychosom. Res.* 36, 337–348. doi: 10.1016/0022-3999(92)90070-I

Hiller, W., and Goebel, G. (2007). When tinnitus loudness and annoyance are discrepant: audiological characteristics and psychological profile. *Audiol. Neurootol.* 12, 391–400. doi: 10.1159/000106482

Hoerhold, M., and Klapp, B. (1993). Testing the invariance and hierarchy of a multidimensional model of mood by means of repeated measurement with student and patient samples. *Z. Med. Psychol.* 2, 27–35.

Horner, K. C. (2003). The emotional ear in stress. *Neurosci. Biobehav. Rev.* 27, 437–446. doi: 10.1016/S0149-7634(03)00071-X

Jastreboff, P. J. (1999). Tinnitus retraining therapy. *Br. J. Audiol.* 33, 68–70.

Kleinstauber, M., Jasper, K., Schweda, I., Hiller, W., Andersson, G., and Weise, C. (2013). The role of fear-avoidance cognitions and behaviors in patients with chronic tinnitus. *Cogn. Behav. Ther.* 42, 84–99. doi: 10.1080/16506073.2012.717301

Kraus, K. S., and Canlon, B. (2012). Neuronal connectivity and interactions between the auditory and limbic systems. Effects of noise and tinnitus. *Hear. Res.* 288, 34–46. doi: 10.1016/j.heares.2012.02.009

Kroner-Herwig, B., Zachriat, C., and Weigand, D. (2006). Do patient characteristics predict outcome in the outpatient treatment of chronic tinnitus? *Psychosoc. Med.* 3:Doc07.

Langenbach, M., Olderog, M., Michel, O., Albus, C., and Kohle, K. (2005). Psychosocial and personality predictors of tinnitus-related distress. *Gen. Hosp. Psychiatry* 27, 73–77. doi: 10.1016/j.genhosppsych.2004.08.008

Langguth, B. (2011). A review of tinnitus symptoms beyond 'ringing in the ears': a call to action. *Curr. Med. Res. Opin.* 27, 1635–1643. doi: 10.1185/03007995.2011.595781

Leaver, A. M., Renier, L., Chevillet, M. A., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2011). Dysregulation of limbic and auditory networks in tinnitus. *Neuron* 69, 33–43. doi: 10.1016/j.neuron.2010.12.002

Lee, S. Y., Kim, J. H., Hong, S. H., and Lee, D. S. (2004). Roles of cognitive characteristics in tinnitus patients. *J. Korean Med. Sci.* 19, 864–869. doi: 10.3346/jkms.2004.19.6.864

Liotti, M., and Mayberg, H. S. (2001). The role of functional neuroimaging in the neuropsychology of depression. *J. Clin. Exp. Neuropsychol.* 23, 121–136. doi: 10.1076/jcen.23.1.121.1223

Ma, Y. L., Gerhardt, K. J., Curtis, L. M., Rybak, L. P., Whitworth, C., and Rarey, K. E. (1995). Combined effects of adrenalectomy and noise exposure on compound action potentials, endocochlear potentials and endolymphatic potassium concentrations. *Hear. Res.* 91, 79–86. doi: 10.1016/0378-5955(95)00172-7

Mazurek, B., Haupt, H., Joachim, R., Klapp, B. F., Stover, T., and Szczepek, A. J. (2010a). Stress induces transient auditory hypersensitivity in rats. *Hear. Res.* 259, 55–63. doi: 10.1016/j.heares.2009.10.006

Mazurek, B., Haupt, H., Klapp, B. F., Szczepek, A. J., and Olze, H. (2012). Exposure of Wistar rats to 24-h psycho-social stress alters gene expression in the inferior colliculus. *Neurosci. Lett.* 527, 40–45. doi: 10.1016/j.neulet.2012.08.019

Mazurek, B., Olze, H., Haupt, H., and Szczepek, A. J. (2010b). The more the worse: the grade of noise-induced hearing loss associates with the severity of tinnitus. *Int. J. Environ. Res. Public Health* 7, 3071–3079. doi: 10.3390/ijerph7083071

McKenna, L., Handscomb, L., Hoare, D. J., and Hall, D. A. (2014). A scientific cognitive-behavioral model of tinnitus: novel conceptualizations of tinnitus distress. *Front. Neurol.* 5:196. doi: 10.3389/fneur.2014.00196

Milerova, J., Anders, M., Dvorak, T., Sand, P. G., Koniger, S., and Langguth, B. (2013). The influence of psychological factors on tinnitus severity. *Gen. Hosp. Psychiatry* 35, 412–416. doi: 10.1016/j.genhosppsych.2013.02.008

Moisset, X., and Bouhassira, D. (2007). Brain imaging of neuropathic pain. *Neuroimage* 37(Suppl. 1), S80–S88. doi: 10.1016/j.neuroimage.2007.03.054

Morfeld, M., Bullinger, M., Nantke, J., and Brahler, E. (2005). [The version 2.0 of the SF-36 health survey: results of a population-representative study]. *Soz. Praventivmed.* 50, 292–300. doi: 10.1007/s00038-005-4090-6

Nondahl, D. M., Cruickshanks, K. J., Huang, G. H., Klein, B. E., Klein, R., Tweed, T. S., et al. (2012). Generational differences in the reporting of tinnitus. *Ear Hear.* 33, 640–644. doi: 10.1097/AUD.0b013e31825069e8

Olderog, M., Langenbach, M., Michel, O., Brusis, T., and Kohle, K. (2004). [Predictors and mechanisms of tinnitus distress - a longitudinal analysis]. *Laryngorhinootologie* 83, 5–13. doi: 10.1055/s-2004-814235

Pattyn, T., Van Den Eede, F., Vanneste, S., Cassiers, L., Veltman, D. J., Van De Heyning, P., et al. (2016). Tinnitus and anxiety disorders: a review. *Hear. Res.* 333, 255–265. doi: 10.1016/j.heares.2015.08.014

Peroz, I. (2003). [Dysfunctions of the stomatognathic system in tinnitus patients compared to controls]. *HNO* 51, 544–549. doi: 10.1007/s00106-002-0750-5

Radloff, L. S. (1991). The use of the Center for Epidemiologic Studies Depression Scale in adolescents and young adults. *J. Youth Adolesc.* 20, 149–166. doi: 10.1007/BF01537606

Rauschecker, J. P., Leaver, A. M., and Muhlau, M. (2010). Tuning out the noise: limbic-auditory interactions in tinnitus. *Neuron* 66, 819–826. doi: 10.1016/j.neuron.2010.04.032

Rose, M., Hess, V., Horhold, M., Brahler, E., and Klapp, B. F. (1999). [Mobile computer-assisted psychometric diagnosis. Economic advantages and results on test stability]. *Psychother. Psychosom. Med. Psychol.* 49, 202–207.

Rubinstein, B., Axelsson, A., and Carlsson, G. E. (1990). Prevalence of signs and symptoms of craniomandibular disorders in tinnitus patients. *J. Craniomandib. Disord.* 4, 186–192.

Schlee, W., Kleinjung, T., Hiller, W., Goebel, G., Kolassa, I. T., and Langguth, B. (2011a). Does tinnitus distress depend on age of onset? *PLoS ONE* 6:e27379. doi: 10.1371/journal.pone.0027379

Schlee, W., Lorenz, I., Hartmann, T., Müller, N., Schulz, H., and Weisz, N. (2011b). "A global brain model of tinnitus," in *Textbook of Tinnitus,* eds A. R. Møller, B. Langguth, D. De Ridder, and T. Kleinjung (New York, NY: Springer New York), 161–169.

Schonwiesner, M., Novitski, N., Pakarinen, S., Carlson, S., Tervaniemi, M., and Naatanen, R. (2007). Heschl's gyrus, posterior superior temporal gyrus, and mid-ventrolateral prefrontal cortex have different roles in the detection of acoustic changes. *J. Neurophysiol.* 97, 2075–2082. doi: 10.1152/jn.01083.2006

Scott, B., and Lindberg, P. (2000). Psychological profile and somatic complaints between help-seeking and non-help-seeking tinnitus subjects. *Psychosomatics* 41, 347–352. doi: 10.1176/appi.psy.41.4.347

Seydel, C., Haupt, H., Olze, H., Szczepek, A. J., and Mazurek, B. (2013). Gender and chronic tinnitus: differences in tinnitus-related distress depend on age and duration of tinnitus. *Ear Hear.* 34, 661–672. doi: 10.1097/AUD.0b013e31828149f2

Seydel, C., Haupt, H., Szczepek, A. J., Hartmann, A., Rose, M., and Mazurek, B. (2015). Three years later: report on the state of well-being of patients with chronic tinnitus who underwent modified tinnitus retraining therapy. *Audiol. Neurootol.* 20, 26–38. doi: 10.1159/000363728

Seydel, C., Haupt, H., Szczepek, A. J., Klapp, B. F., and Mazurek, B. (2010). Long-term improvement in tinnitus after modified tinnitus retraining therapy enhanced by a variety of psychological approaches. *Audiol. Neurootol.* 15, 69–80. doi: 10.1159/000231632

Stobik, C., Weber, R. K., Munte, T. F., and Frommer, J. (2003). [Psychosomatic stress factors in compensated and decompensated tinnitus]. *Psychother. Psychosom. Med. Psychol.* 53, 344–352. doi: 10.1055/s-2003-40947

Sturm, W., Longoni, F., Fimm, B., Dietrich, T., Weis, S., Kemna, S., et al. (2004). Network for auditory intrinsic alertness: a PET study. *Neuropsychologia* 42, 563–568. doi: 10.1016/j.neuropsychologia.2003.11.004

Vollmann, M., Scharloo, M., Langguth, B., Kalkouskaya, N., and Salewski, C. (2013). Illness representations as mediators of the relationship between dispositional optimism and depression in patients with chronic tinnitus: a cross-sectional study. *Psychol. Health* 29, 81–93. doi: 10.1080/08870446.2013.828294

Wallhausser-Franke, E., Brade, J., Balkenhol, T., D'amelio, R., Seegmuller, A., and Delb, W. (2012). Tinnitus: distinguishing between subjectively perceived loudness and tinnitus-related distress. *PLoS ONE* 7:e34583. doi: 10.1371/annotation/96f457f9-3f48-4f88-a7f0-1d5e6067e7a5

Weise, C., Hesser, H., Andersson, G., Nyenhuis, N., Zastrutzki, S., Kroner-Herwig, B., et al. (2013). The role of catastrophizing in recent onset tinnitus: its nature and association with tinnitus distress and medical utilization. *Int. J. Audiol.* 52, 177–188. doi: 10.3109/14992027.2012.752111

Wilson, P. H., Henry, J., Bowen, M., and Haralambous, G. (1991). Tinnitus reaction questionnaire: psychometric properties of a measure of distress associated with tinnitus. *J. Speech Hear. Res.* 34, 197–201. doi: 10.1044/jshr.3401.197

Wilson, P. H., and Henry, J. L. (1998). Tinnitus cognitions questionnaire: development and psychometric properties of a measure of dysfunctional cognitions associated with tinnitus. *Int. Tinnitus J.* 4, 23–30.

Zenner, H. P. (2003). [Cognitive tinnitus desensitization: evidence-based and guideline-adherent habituation therapy for chronic tinnitus sensitization]. *HNO* 51, 687–689. doi: 10.1007/s00106-003-0939-2

Zirke, N., Seydel, C., Szczepek, A. J., Olze, H., Haupt, H., and Mazurek, B. (2013). Psychological comorbidity in patients with chronic tinnitus: analysis and comparison with chronic pain, asthma or atopic dermatitis patients. *Qual. Life Res.* 22, 263–272. doi: 10.1007/s11136-012-0156-0

Zoger, S., Svedlund, J., and Holgers, K. M. (2006). Relationship between tinnitus severity and psychiatric disorders. *Psychosomatics* 47, 282–288. doi: 10.1176/appi.psy.47.4.282

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Cognitive Mechanisms in Chronic Tinnitus: Psychological Markers of a Failure to Switch Attention

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The cognitive mechanisms underpinning chronic tinnitus (CT; phantom auditory perceptions) are underexplored but may reflect a failure to switch attention away from a tinnitus sound. Here, we investigated a range of components that influence the ability to switch attention, including cognitive control, inhibition, working memory and mood, on the presence and severity of CT. Our participants with tinnitus showed significant impairments in cognitive control and inhibition as well as lower levels of emotional well-being, compared to healthy-hearing participants. Moreover, the subjective cognitive complaints of tinnitus participants correlated with their emotional well-being whereas complaints in healthy participants correlated with objective cognitive functioning. Combined, cognitive control and depressive symptoms correctly classified 67% of participants. These results demonstrate the core role of cognition in CT. They also provide the foundations for a neurocognitive account of the maintenance of tinnitus, involving impaired interactions between the neurocognitive networks underpinning attention-switching and mood.

Keywords: tinnitus, cognition, attention, depression, salience, neurocognitive networks

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INTRODUCTION

Chronic tinnitus (CT), commonly described as 'ringing in the ears,' can have a broad impact on an individual, affecting cognition, mood, social functioning, and general well-being. The overall prevalence of tinnitus varies across studies, ranging from 5 to 43% depending on the definition and criteria used (McCormack et al., 2016), with CT typically occurring in 10–15% of the general population (Henry et al., 2005). Despite its significant impact, there remains no cure or agreement on the mechanisms underpinning the maintenance of CT.

Tinnitus is often conceptualized as the result of a failure in the process of habituation (Hallam et al., 1984). Habituation is the ability to suppress information that is not primary to our goals, or indicative of a threat to our well-being. This process is important for facilitating our ability to control our cognitive resources and switch toward information that is of primary concern at a given moment in time. Since our cognitive capacity is limited, cognitive control of attention plays an important role in prioritizing access to relevant resources (Engle and Kane, 2004; Unsworth et al., 2014). This is achieved by modulating or 'switching' attention toward more salient or relevant stimuli and inhibiting stimuli less salient or relevant to task goals (Gazzaley and Nobre, 2012).

A Neurocognitive Account of Attention-Switching

Cognitive control of attention is a core aspect of our executive functions, and is thought to involve three neural networks: the cognitive control network (CCN), the salience network (SN), and the affective network (AN; **Table 1**). Cognitive control of attention can be conceptualized as arising from the flexible balance of interactions between self-directed (the CCN) and sensory-directed (the SN) neural networks to determine the information that crosses an individual's awareness threshold for further processing (Corbetta and Shulman, 2002; Menon and Uddin, 2010; Cocchi et al., 2013). This may be modulated by the emotional associations of a given stimulus (AN), resulting in increased monitoring of emotional information through increased SN–AN connectivity, or reduced top–down regulation from the CCN (Ochsner and Gross, 2005). In other words, the emotions associated with stimuli are important to consider, particularly with regard to engagement of the SN due to its high connectivity with the CCN and limbic system structures and proposed role in facilitating network de/activation (Seeley et al., 2007; Craig, 2009; Bonnelle et al., 2012).

The ability to switch our attention may be influenced by (1) our ability to control our cognitive resources, drawing on processes of inhibitory control and working memory to support flexible cognitive processing of incoming stimuli, and (2) our ability to down regulate our emotions in response to these stimuli. Specifically, proficient cognitive control enables effective attention-switching by utilizing working memory resources to maintain awareness of relevant information, and inhibitory control to resist distraction from irrelevant stimuli or thoughts (Diamond, 2013). Proficient emotion regulation influences our ability to switch attention by limiting the potential intrusion of goal-irrelevant emotionally salient information via top–down modulation of the emotions associated with a given stimulus (Ochsner and Gross, 2005; Buhle et al., 2014). In the case of tinnitus, a failure to switch attention resulting from impaired cognitive control and poor emotional down regulation may lead to ongoing engagement with the tinnitus sound, thus maintaining awareness of the sound (**Figure 1**).

TABLE 1 | Neural networks proposed to underpin attention-switching.

Network	Function	Core structures
CCN	Goal directed orientation of cognitive, attention, and memory resources	Prefrontal cortex, intraparietal sulcus[1]
SN	Identification of relevant sensory inputs and facilitation of further processing	Dorsal anterior cingulate cortex, insula[2]
AN	Regulates the experience of emotions	Cingulate cortex, prefrontal cortex, amygdala, nucleus accumbens[3]

CCN, cognitive control network; SN, salience network; AN, affective network; [1]Corbetta and Shulman (2002), [2]Menon and Uddin (2010), [3]Ochsner and Gross (2005).

Cognitive Functioning in Chronic Tinnitus

The literature on unipolar depression has identified CCN dysfunction, notably hypoactivation, as potentially underpinning symptoms of rumination, poor attention-switching, and emotion dysregulation (Rayner et al., 2016). Behavioral indicators of these symptoms include (1) biased information processing and poor disengagement from negative stimuli, and (2) difficulties with cognitive flexibility that can be detected when assessing attention, working memory, and inhibitory control (Gotlib and Joormann, 2010). People with CT have shown impaired performance on tasks assessing these behavioral markers of CCN dysfunction when compared to people without CT (Andersson et al., 2007; Stevens et al., 2007; Pierce et al., 2012).

In particular, highly distressed individuals with tinnitus have reported greater personal salience of emotive tinnitus sentences than low-distressed and tinnitus-free individuals, which was associated with increased activation of hubs of the CCN and SN, including the insula, prefrontal cortex, and cingulate cortex (Golm et al., 2013). In addition, research investigating the cognitive functioning of people with and without tinnitus in groups matched for emotional well-being (anxiety and depressive symptoms) show impaired performance of the tinnitus group on tasks relying on cognitive control processes (Heeren et al., 2014; Jackson et al., 2014). Such findings suggest CT is associated with cognitive deficits, independent of any emotional effects that may impair cognitive function.

A recent systematic review of nine studies of working memory and attention abilities in people with tinnitus investigated subcomponents of attention, such as sustained, alerting, selective and executive attention. This showed preliminary evidence for impaired executive attention, including inhibition and switching, whereas, findings for other subcomponents were mixed (Mohamad et al., 2016). Supporting this, people with CT subjectively report a greater number of attention and memory failures in everyday life compared to hearing impaired and healthy-hearing individuals (Hallam et al., 2004; Alam et al., 2012).

These results support the hypothesis that there may be an overarching failure of cognitive control processes in people with CT, reflected in a reduced ability to switch attention away from the tinnitus sound to achieve efficient cognitive performance. This, in part, may be due to the heightened salience of the tinnitus, and its association with negative emotions, particularly anxiety (Pattyn et al., 2016) and depression (Langguth et al., 2011). Stated another way, an imbalance in the regulation of the interactions between the CCN, SN, and AN may contribute to the maintenance of tinnitus by continued assignment of attentional resources and emotional salience to the tinnitus sound (**Figure 1**). As such, furthering our understanding of cognitive control, and how this relates to the emotional well-being and subjective cognitive experiences of individuals with CT represents an important next step in understanding the role of cognition in the maintenance of CT.

The aim of the current study was to investigate cognitive markers of the ability to switch attention in people with CT to determine the role of impaired cognitive control and emotional down regulation in the maintenance of tinnitus. We

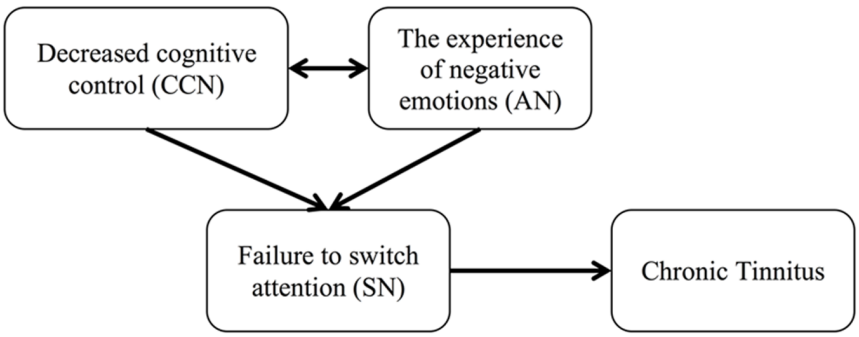

FIGURE 1 | Flow diagram of the proposed relationship between attention-switching and chronic tinnitus (CT), resulting from decreased cognitive control and emotional down regulation.

hypothesized that (1) participants with CT would have slower reaction times on a cognitive control task compared to healthy-hearing participants, reflecting less efficient CCN functioning; (2) participants with CT would report poorer emotional well-being than healthy-hearing participants, reflecting the emotional impact and salience of the tinnitus; (3) both poor cognitive control and emotional well-being would predict the experience of tinnitus, reflecting their dual contribution to the maintenance of CT; and (4) subjective report of cognitive difficulties would be associated with objective cognitive task performance.

MATERIALS AND METHODS

Participants

We recruited 26 people with self-identified CT who formed the CT group, and 29 healthy-hearing controls (HC) between 18 and 60 years using advertisements in local online newsletters and on noticeboards. Using G*Power (v3.1.9.2), a minimum total sample size of 42 was estimated to detect a moderate effect size ($f = 0.39$, derived from Heeren et al., 2014) in a repeated measures ANOVA, with power of 0.80 and $\alpha = 0.05$. Inclusion criteria for the CT group were (1) the experience of tinnitus for ≥3 months, and (2) experiencing the tinnitus as constantly (always) present (Hoffman and Reed, 2004). For the HC group, individuals were screened for tinnitus symptoms and hearing impairments, resulting in the exclusion of three individuals. The study was approved by the Human Research Ethics Committee at The University of Melbourne, and all participants gave written informed consent in accordance with the Declaration of Helsinki. There were no significant differences between the two groups for age, gender or education level (all $p > 0.10$). Demographic characteristics of the two groups are summarized in **Table 2**.

We used the World Health Organisation's definition of hearing impairment to determine the degree of hearing health for all participants for whom an audiogram was available (CT $n = 25$, HC $n = 26$; **Figure 2**; Concha-Barrientos et al., 2004). For each participant we calculated average hearing thresholds (dB) across four main frequencies (500, 1000, 2000, 4000 Hz) for each ear. The resulting average hearing threshold for the better ear was then classified using the World Health Organisation's hearing impairment grading system to classify the potential impact of hearing loss on daily life. This scale ranges from no impairment (≤25 dB) to profound impairment (≥81 dB; Concha-Barrientos et al., 2004). We found no significant difference between the two groups with regard to hearing impairment [$\chi^2(2, n = 51)$, 3.32, $p = 0.19$] and the average hearing threshold for each frequency was <25 dB in both groups, suggesting normal hearing ability at the group-level at each frequency. However, Mann–Whitney U-test comparing the groups at each frequency indicated that hearing thresholds in the CT group were significantly lower than the HC group at 2000 Hz ($U = 212.50$, $p = 0.03$) and 4000 Hz ($U = 155.50$, $p < 0.001$). Thus, we examined the data individually and found that all HC participants had normal hearing, while three participants in the CT group (12%) had impaired hearing (one slight impairment, two moderate impairment). Since removal of these three participants from the main analyses of the study did not change the significance of the results, all participants were retained for sample completeness and to reflect the heterogeneity of the presentation of CT. The tinnitus characteristics of the CT group are summarized in **Table 3**.

TABLE 2 | Participant characteristics.

	Chronic tinnitus group ($n = 26$)	Healthy control group ($n = 26$)
Mean age, years (*SD*)	40.31 (14.67)	34.15 (11.55)
Gender	42% female	54% female
Education level	77% tertiary	92% tertiary

Cognitive Tasks

The cognitive control and inhibition tasks were programmed using Presentation software (Neurobehavioral Systems Inc, 2014) and delivered on a 13″ laptop screen, with responses made using a wireless mouse. The stimuli were capital letters in white Tahoma 24 point font on a black background, presented centrally on the screen. Participants were tested in a sound proof booth, and sat at a desk at a comfortable viewing distance and angle from the screen.

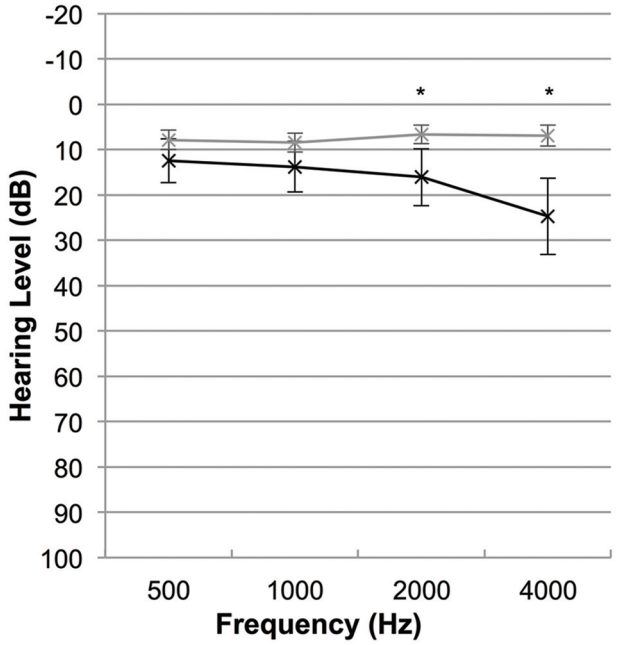

FIGURE 2 | Mean audiograms for the chronic tinnitus (CT; black line) and healthy control (HC; gray line) groups (*$p < 0.05$). 25 dB is considered the impairment threshold.

TABLE 3 | Tinnitus characteristics ($n = 26$).

Mean years with tinnitus (SD)	13.50 (14.08)
Mean tinnitus awareness, range 0–100 (SD)	39.35 (26.45)
Mean tinnitus annoyance, range 0–100 (SD)	17.17 (21.57)
Mean tinnitus loudness, range 0–100 (SD)	41.92 (22.18)
Tinnitus laterality	
Left ear	2 (8%)
Both ears, worse in left	2 (8%)
Both ears/inside the head	14 (53%)
Both ears, worse in right	5 (19%)
Right ear	3 (12%)
Onset	
Sudden	7 (27%)
Gradual	18 (69%)
Unknown	1 (4%)
Believed cause	
Known[a]	17 (65%)
Unknown	9 (35%)

[a] *65% of individuals reported known causes of their tinnitus including stress (25%), music (25%), medical causes (20%), loud sound blast (10%), prolonged noise exposure (10%), head injury (5%), and hearing changes (5%).*

Cognitive Control

We utilized an established cognitive control task also known to activate the CCN in neuroimaging work; the '*n*-back' task (Owen et al., 2005; Niendam et al., 2012). We measured cognitive control in conditions of low and high cognitive load as reaction times on this task are sensitive to manipulations of cognitive load (Braver et al., 1997; Jaeggi et al., 2007). One of eight capital letters ("C", "G", "H", "K", "P", "Q", "T", or "W") was shown on the laptop screen in a pseudorandom order for 500 ms, followed by a blank screen for 2500 ms. Participants were asked to respond by clicking the mouse whenever a target letter was shown. In the low cognitive load condition (0-back), the target was a specific letter that participants were instructed to respond to at the start of each block (e.g., "C"). In the high cognitive load condition (2-back), participants were instructed to respond to the target which occurred when a letter was the same as the one seen two previously. Each block lasted approximately 1 min and comprised 20 letters, of which 30% were targets. Each participant completed one run of the task, comprising three blocks of the 0-back and three blocks of the 2-back, presented in random order. Before each block, participants were reminded of the instructions, and could take a break if required. We also used a short 10-letter practice block to train participants on the task, for which 60% of targets had to be correctly identified in both the 0-back and 2-back conditions prior to commencing the experimental run.

Inhibitory Control

We used the gold-standard Stop-Signal Task to assess the inhibitory control of attention given this contributes to performance on the n-back task (Logan et al., 1997; Avila and Parcet, 2001). Participants were required to respond as quickly as possible to the letters 'A' and 'Z' when they appeared on the laptop screen using the left ('A') and right ('Z') mouse buttons respectively. The letters were preceded by a fixation cross (500 ms) and displayed for 1000 ms. Participants were instructed to inhibit their response if a letter 'X' was also shown on the screen (the 'stop signal'). The 'stop signal' was presented in red font for 150 ms, with a delay between the onset of the primary letter ('A' or 'Z') and the stop signal of 250 ms. This delay varied by ±50 ms based on the participant's stopping accuracy (with a minimum delay of 50 ms and a maximum of 500 ms). There was a 1000 ms break between each trial, with 25% stop trials randomly presented over a total of 160 trials (80 for each letter), presented in random order. A rest break was provided half-way through the task. Before completing the task, participants were required to achieve a minimum of 50% correct responses on the practice trials, with a minimum of 10 practice trials shown.

Working Memory

We used the digit-span subtest of the Wechsler Adult Intelligence Scale – Fourth edition (Wechsler, 2008) to assess working memory given its contribution to cognitive control task performance. For this test, participants were asked to repeat back strings of numbers that gradually increased in length. All three subtests of the digit-span test were administered according to the test manual. In the first subtest, Digit Span Forward, participants repeated the numbers in the same order. In the second subtest, Digit Span Backward, participants were asked to recall the numbers in the backward order. Finally in the third subtest, Digit Span Sequencing, participants recalled the numbers in ascending order (from low to high). Each correct response is awarded one point, with a maximum of 16 points for each subtest. The total score for the three subtests formed the overall digit-span score. All raw scores on this test were converted to

age-scaled scores, estimated using the norms in the WAIS-IV manual (Wechsler, 2008).

Emotional Well-being Measures

We used established questionnaires with well-documented psychometric properties as indicated by their internal consistency scores (α) to assess anxiety-proneness and depressive symptoms. The trait subscale of the State Trait Anxiety Inventory (STAI; Spielberger et al., 1983) was used to assess anxiety-proneness ($\alpha = 0.91$) and depressive symptoms were assessed using the Beck Depression Inventory (BDI-II, $\alpha = 0.93$; Beck et al., 1996).

Procedure

After providing informed consent, participants underwent a hearing test and provided sociodemographic information. The CT group also provided a history of their tinnitus experiences using the Tinnitus Case Sample History Questionnaire (Langguth et al., 2007), and we assessed the subjective impact of their tinnitus on daily life using the Tinnitus Handicap Inventory (THI; Newman et al., 1996). All participants also completed the Cognitive Failures Questionnaire (CFQ) to assess the subjective experience of memory and attention failures (Broadbent et al., 1982). Participants then completed the working memory and inhibition tasks, presented in counter-balanced order, after which they completed the cognitive control task in conditions of silence and in the presence of a repetitive background noise (control condition), with the order counterbalanced. Participants finished with the emotional well-being questionnaires.

Data Analysis

The Statistical Package for the Social Sciences Version 22 (SPSS) was used for all analyses. We tested the data for assumptions of parametric testing and identified extreme outliers, for which we applied a 90% winsorisation (<1% of data; Field, 2008). Where the assumptions of parametric tests were not upheld, more conservative non-parametric tests and log transformations for reaction time data were used to confirm the results. As no differences between parametric and non-parametric test outcomes were observed, the results of parametric tests on the raw data are reported here. We calculated effect size estimates for all statistical tests using eta squared (η^2) for analysis of variance models (ANOVA) and Cohen's (*d)* for independent sample *t*-test (Fritz et al., 2012).

To test hypothesis 1, that the CT group would have slower mean reaction times on the cognitive control task compared to the HC group, we performed an ANOVA on the reaction time data, with group as the between-subjects factor and cognitive load (0-back, 2-back) as the within-subjects factor. For the inhibitory control task, individual performance was first estimated using the integration method to calculate each participant's mean stop signal reaction time (SSRT; Verbruggen et al., 2013). We then compared the average performance of the CT and HC groups using an independent samples *t*-test. For the working memory task, we compared the Digit Span scaled scores for the CT and HC groups using independent samples *t*-test for the total test score and for each subtest. As inhibitory control and working memory are component skills of the n-back task, we performed secondary level analyses to adjust for these on the cognitive control task using analysis of covariance (ANCOVA) models.

To test hypothesis 2, that the CT group would report poorer emotional well-being than the HC group, we performed independent samples *t*-test on the group mean scores of the BDI-II and STAI-T measures. As mood and anxiety can influence cognitive performance, we also performed secondary level analyses to adjust for these on the cognitive control task, again using ANCOVA models.

To test hypothesis 3, that poor cognitive control and emotional well-being would independently predict the experience of tinnitus (i.e., group membership) we conducted a stepwise discriminant function analysis (DFA) with $\alpha = 0.05$ as the criterion for variables entering the analysis. We identified relevant cognitive and emotional well-being variables from those showing significant group differences in the analyses described above, and then examined their effectiveness in differentiating people with and without CT.

Finally, to test hypothesis 4, that subjective report of cognitive difficulties would be associated with objective cognitive task performance, we used one-tailed Pearson correlations (*r*) to determine if measures of objective cognitive ability and emotional well-being were significantly correlated with participant scores on the CFQ.

RESULTS

Decreased Cognitive Control in Chronic Tinnitus

In support of our first hypothesis there was a significant main effect of group, indicating that individuals with tinnitus responded more slowly on the 2-back and 0-back conditions of the cognitive control task compared to the HC group in silence [$F(1,50) = 7.23$, $p = 0.010$, $\eta^2 = 0.13$; see **Figure 3**]. As expected, there was also a main effect of cognitive load [$F(1,50) = 92.33$, $p < 0.001$, $\eta^2 = 0.64$], with faster reaction times in the 0-back condition ($M = 440.12$, $SE = 8.79$, 95%CI 422.46, 457.77) compared to the 2-back condition ($M = 602.53$, $SE = 19.21$, 95%CI 563.95, 641.11). There was no interaction between cognitive load and group [$F(1,50) = 1.95$, $p = 0.169$, $\eta^2 = 0.01$].

In addition to group differences on the cognitive control task, the CT group were also significantly slower on the inhibitory control task ($M = 242.01$, $SD = 47.25$) than the HC group [$M = 217.31$, $SD = 35.80$, $t(50) = 2.13$, $p = 0.039$, $d = 0.59$]. In contrast, there were no significant working memory differences between the CT ($M = 11.23$, $SD = 3.14$) and HC groups [$M = 11.65$, $SD = 3.17$, $t(50) = -0.48$, $p > 0.250$, $d = 0.13$] on the Digit Span total or subtest scores (all $p > 0.250$).

When we adjusted cognitive control scores for performance on the inhibitory control task by entering SSRT as a covariate, the main effect for group remained, although exhibited a smaller effect size and marginal significance [$F(1,49) = 3.96$, $p = 0.052$, $\eta^2 = 0.07$]. This suggests cognitive control task performance partially reflects inhibitory control performance. When we adjusted scores for working memory ability by entering

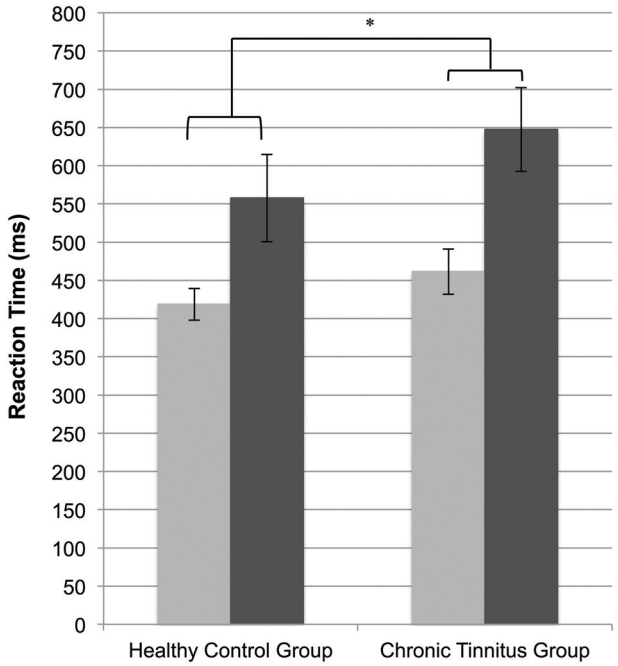

FIGURE 3 | Mean reaction times for the chronic tinnitus (CT) and healthy control (HC) groups on the 0-back (light gray) and 2-back (dark gray) versions of the cognitive control task (*$p < 0.05$).

the Digit Span total test score as a covariate, the main effect for group remained significant and showed a similar effect size [$F(1,49) = 6.85$, $p = 0.012$, $\eta^2 = 0.12$]. While the groups did not differ significantly for demographic or hearing abilities, these factors could influence cognitive control and as such we assessed each factor as a potential covariate to determine the robustness of our findings. In support of our results, the main effect of group remained significant after accounting for age [$F(1,49) = 4.33$, $p = 0.043$, $\eta^2 = 0.07$], hearing ability [$F(1,48) = 7.32$, $p = 0.009$, $\eta^2 = 0.13$], gender [$F(1,49) = 6.64$, $p = 0.013$, $\eta^2 = 0.12$], and education [$F(1,49) = 6.26$, $p = 0.016$, $\eta^2 = 0.12$].

We also assessed if reduced cognitive control was associated with CT factors, including (a) the subjective loudness of the tinnitus sound, (b) the perceived impact of tinnitus on daily life, and (c) any perceived worsening of the tinnitus during task performance. Spearman correlations indicated that neither subjective loudness nor tinnitus handicap were associated with performance of any of the cognitive tasks (all $p > 0.10$). Similarly, independent *t*-test showed no significant differences for participants who experienced a worsening of their tinnitus ($n = 12$) compared to no change ($n = 14$) whilst performing the tasks (all $p > 0.10$).

Finally, we assessed whether our main effects could be replicated in the presence of background sound and found a significant main effect for group [$F(1,50) = 6.51$, $p = 0.014$, $\eta^2 = 0.12$] and cognitive load [$F(1,50) = 83.74$, $p < 0.001$, $\eta^2 = 0.63$], as well as an interaction effect where reaction times increased more for the CT group between the 0- and 2-back conditions [$F(1,50) = 4.23$, $p = 0.045$, $\eta^2 = 0.08$]. To distinguish whether these results were due to the presence of the tinnitus sound *per se* or a failure to switch attention away from the sound in the CT group, we compared the CT group's task performance in silence (tinnitus sound present) to the HC group's performance in the presence of the repetitive background sound. Here, we found a larger interaction effect [$F(1,50) = 5.74$, $p = 0.020$, $\eta^2 = 0.10$] indicating that when a noise source is present (tinnitus or background sound), there is a greater performance cost for the CT group as cognitive load increases (**Figure 4**). Consistent with the earlier analyses, we also observed main effects for group [$F(1,50) = 13.38$, $p = 0.001$, $\eta^2 = 0.21$] and cognitive load [$F(1,50) = 85.27$, $p < 0.001$, $\eta^2 = 0.63$].

Poor Emotional Well-being in Chronic Tinnitus

In support of our second hypothesis, the CT group reported significantly more symptoms of depression ($M = 11.79$, $SD = 10.03$) than the HC group ($M = 6.38$, $SD = 5.78$); $t(39.96) = 2.38$, $p = 0.022$, $d = 0.68$. In the CT group, 43% of people reported depressive symptoms in the mild-severe range (23% mild, 12% moderate, 8% severe) compared to 12% of the HC group (8% mild, 4% moderate, 0% severe). One tinnitus participant had a current diagnosis of clinical depression and was identified as a statistical outlier, however, removal of this participant did not change the significance of any of the described effects, and hence she was retained for sample

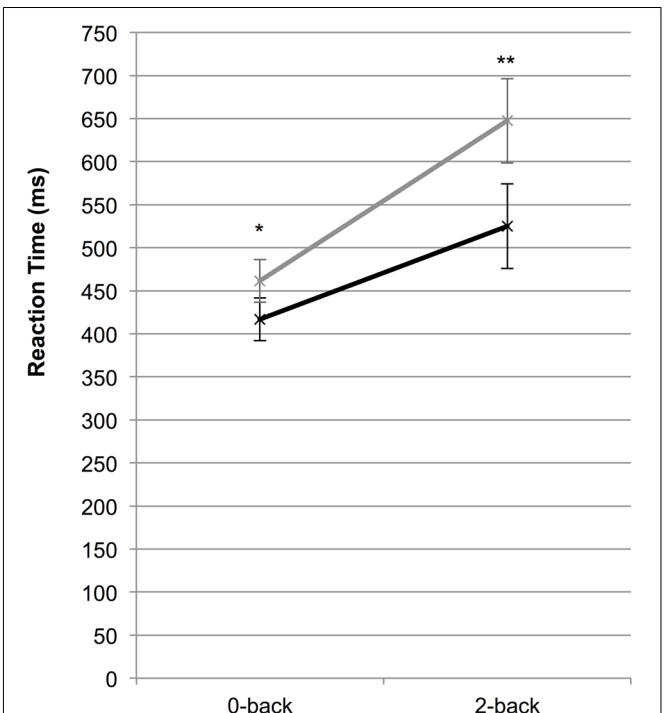

FIGURE 4 | Mean reaction time for the chronic tinnitus group (CT; gray line) in silence (i.e., tinnitus sound present) and the healthy control group (HC; black line) in the presence of a repetitive background sound while performing the cognitive control task (*$p < 0.05$, **$p < 0.001$).

completeness. No differences were observed between groups for the trait of anxiety-proneness $t(50) = 1.35$, $p = 0.182$, $d = 0.37$. We also assessed the influence of emotional well-being on performance of the cognitive control task, first by entering BDI-II scores as a covariate in the analysis. This indicated that the main effect of group remained significant with a similar effect size [$F(1,49) = 7.54$, $p = 0.008$, $\eta^2 = 0.13$]. Similarly, when we entered anxiety-proneness as a covariate the groups remained significantly different, again with similar effect size [$F(1,49) = 8.50$, $p = 0.005$, $\eta^2 = 0.14$].

Predictors of Chronic Tinnitus

For factors that showed group differences (cognitive control, inhibitory control, and depressive symptoms), we conducted a DFA to identify those factors that were most significant in discriminating between people with CT and healthy controls. Assumption checks for the stepwise DFA showed moderately strong correlations between the 0-back and 2-back conditions of the cognitive control task for both groups [r_s(CT) = 0.40, $p = 0.022$; r_s(HC) = 0.40, $p = 0.025$]. As such, to address the assumption of multicollinearity we removed the 0-back condition from the DFA in favor of the more cognitively demanding 2-back task. In this model, 2-back performance was identified as the core discriminating factor of people with and without CT, Wilk's $\lambda = 0.90$, $F(1,50) = 5.47$, $p = 0.023$, with the addition of depressive symptoms in the second step further improving the model, Wilk's $\lambda = 0.81$, $\chi^2(2) = 10.12$, $p = 0.006$. Combined, these two factors correctly classified 67% of cases and showed good sensitivity for participants with CT (65%), indicating that poor cognitive control and greater depressive symptoms were predictive of CT group membership. There was also good specificity (69%) for the HC group, suggesting low depressive symptoms and more effective cognitive control were consistent features of this group. Inhibitory control did not significantly contribute to the model.

To explore the data for effects relating to tinnitus severity, we conducted Spearman correlation (r_s) analyses between severity of tinnitus impact, cognitive control performance, and depressive symptoms. Since severity of the impact is considered a separate factor from the constant presence of the sound in the 'vicious cycle' of CT (Jastreboff et al., 1996), this analysis aimed to identify if either factor relating to its presence, also related to its perceived severity. We found that the degree of tinnitus impact was selectively associated with depressive symptoms ($r_s = 0.42$, $p = 0.033$), but not with cognitive control ($r_s = -0.32$, $p = 0.112$) despite both factors playing a role in correctly classifying people with and without CT. Combined these findings suggest ongoing awareness of the tinnitus sound may relate to reduced cognitive control, whereas depressive symptoms may determine the severity of its impact on an individual's daily life.

Correlates of Subjective Cognitive Complaints

The CT group reported significantly more subjective cognitive complaints ($M = 43.07$, $SD = 12.00$) than the HC group ($M = 34.35$, $SD = 14.51$); $t(50) = 2.36$, $p = 0.022$, $d = 0.66$, consistent with their objective performance on the cognitive control and inhibition tasks. Interestingly, we found differences in the pattern of associations between the CT and HC groups. The HC group showed an association between objective cognitive performance and subjective cognitive complaints, with significant correlations between scores on the CFQ and 2-back performance ($r = 0.38$, $p = 0.028$), between CFQ and SSRT scores ($r = 0.57$, $p = 0.001$), but no significant associations between CFQ scores and depressive symptoms or anxiety-proneness. In contrast, the CT group showed no significant associations between objective cognitive performance and subjective cognitive complaints. Rather, CFQ scores were associated with anxiety-proneness ($r = 0.69$, $p < 0.001$) and with depressive symptoms ($r = 0.55$, $p = 0.002$).

DISCUSSION

The present study addresses the role of attention-switching in CT by investigating two key aspects of attention-switching hypothesized to show impairments in this population, namely cognitive control and the experience of negative emotions. Consistent with our first hypothesis, we demonstrated slower reaction times in cognitive and inhibitory control in people with CT, whereas working memory function was similar to healthy controls. Consistent with our second hypothesis, we also found poorer emotional well-being in people with CT reflected by elevated depressive symptoms, whereas trait anxiety (anxiety-proneness) was similar to healthy controls. Third, we demonstrated a core role of reduced cognitive control in accurately classifying people with CT, while depressive symptoms were associated with its perceived severity. Taken together, these findings suggest that reduced control of the ability to switch attention may constitute a cognitive mechanism that maintains the awareness and severity of the tinnitus sound.

Cognitive Control and Tinnitus Awareness

Our results indicate that people with CT are less proficient at performing a cognitive control task compared to people with healthy-hearing, an effect which remained after adjusting for (1) performance on tasks assessing the component skills of inhibitory control and working memory, and (2) emotional well-being, including depressive symptoms and anxiety-proneness. Importantly, all participants were able to accurately perform and execute the cognitive tasks (*n*-back, stop-signal, and digit-span) and the groups were similar in age and hearing ability, two factors thought to influence cognitive abilities (Baltes and Lindenberger, 1997). Therefore, given the similarities in demographic variables between the groups, and the robustness of the effect after accounting for other cognitive, emotional and behavioral factors that could influence the results, it appears likely that the observed impairment in cognitive control is primarily related to the presence of CT.

An additional investigation of task performance in the presence of repetitive background sound provided further support for a failure to switch attention underpinning the

observed impairment in cognitive control. In particular, replication of poorer performance of the CT group on the cognitive control task compared to the HC group in the presence of background noise suggests that the results were not due to the presence of the constant tinnitus sound itself, but rather a failure in attention-switching in people with CT. Specifically, there was a greater performance cost for people with CT as cognitive load increased when both groups were exposed to potential auditory distractors, suggesting that impaired top–down regulation of less relevant sensory information may underpin maintained awareness of the tinnitus sound. Consistent with this interpretation, we found no evidence that cognitive performance was influenced by factors relating to the tinnitus sound itself, including its volume, impact, or perceived worsening during task performance.

Since we found no overall differences in hearing ability between the groups, nor any influence of hearing ability on cognitive performance, and our main findings did not change with removal of participants with hearing impairment in the CT group, we feel it is unlikely that hearing ability accounts for the present results. However, given previous research has shown an association between uncorrected hearing impairment and cognitive functioning, particularly in older adults (Wayne and Johnsrude, 2015), the inclusion of individuals with hearing impairments may represent a limitation of our study. Future research directly investigating the relationships between cognition, age, and hearing ability may help to clarify the potential influence of these factors on the experience of CT, including the point at which hearing loss may pose a significant risk to cognition.

The similarity between groups for working memory may reflect this task's reliance on performance accuracy over processing speed, the latter being crucial to the performance of the cognitive control and inhibition tasks. This suggests that tasks relying on information processing speed may provide more sensitive measures of attention-switching in people with CT than measures of accuracy alone, potentially accounting for discrepant findings in previous research (Mohamad et al., 2016).

Psychological Salience and Tinnitus Severity

We have shown that there is a subjective emotional cost associated with the severity of tinnitus. Depressive symptoms not only predicted the presence of tinnitus but were also correlated with its severity, suggesting they play a specific role in determining the psychosocial impact of CT. This is consistent with recent findings of a core role of depressive symptoms in maintaining the awareness and impact of CT (Trevis et al., 2016). Depressive symptoms were also correlated with perceived cognitive difficulties in everyday life in the CT group, which may reflect the presence of a negative cognitive bias, which is a characteristic feature of depression (Rayner et al., 2016). These findings emphasize the importance of considering the psychological well-being of people with CT with regard to both effective treatment, and furthering our understanding of how cognition and emotion regulation, particularly with regard to low mood, can interact to maintain tinnitus awareness and severity. In addition, assessing the cognitive functioning of people with CT with or without depression would help delineate the contributions of mood and cognitive control to the presence and severity of CT.

Chronic Tinnitus and Attention-Switching

The present results indicate that both cognitive control and emotional regulation, two processes proposed to influence the ability to switch attention, are impaired in people with CT. Importantly, our results also suggest these processes serve different functions that are consistent with research on chronic pain, a condition considered to have similar underlying mechanisms to CT (Moller, 2007). In particular, our results suggest that depressive symptoms, cognitive control, and to some degree inhibitory control, may relate to the ongoing perception of the tinnitus sound, while emotion regulation, specifically of depressive symptoms, may relate to the perceived severity of its impact. Of note, symptoms of depression appear to be involved in maintaining both the awareness and severity of CT, which may reflect distinct roles for the cognitive and affective features of depression in specific aspects of tinnitus maintenance. For example, cognitive depressive symptoms (associated with CCN dysfunction) may be involved in the ongoing awareness of the sound while affective or somatic depressive symptoms (associated with AMN/AN dysfunction) may increase the severity and psychological salience of the tinnitus sound. In the chronic pain literature, research suggests that attention factors selectively modulate the awareness of pain, while mood selectively modulates the unpleasantness of the pain (Bushnell et al., 2013). Impaired cognitive control has also been implicated in anxiety (Derryberry and Reed, 2002) and depression (Fales et al., 2008), with both psychological conditions having a high incidence rate in CT populations (Zirke et al., 2013). As such, we propose that the ongoing awareness and severity of CT is underpinned by a failure of top–down cognitive resources, specifically cognitive and emotional control, resulting in a reduced ability to switch attention away from the tinnitus sound.

A Neurocognitive Approach to Chronic Tinnitus

A strength of our study involved the use of the *n*-back task, which is well-established in the cognitive neuroimaging literature to activate the CCN. In light of this, our findings suggest that people with CT may show hypoactivation of the CCN, decreasing top–down inhibition of the tinnitus sound. This, in turn, would increase the psychological salience of the sound (SN), which, in combination with reduced top–down inhibition of the AN by the CCN, may facilitate negative thinking about the sound (e.g., rumination) and emotion dysregulation (e.g., low mood).

In addition, the CCN is antithetical to the autobiographical memory network (AMN), also known as the 'resting state' or 'default mode' network, which is associated with introspection and rumination (Sheline et al., 2009; Rayner et al., 2016). To

engage with the outside world through goal-directed behaviors we need to reduce self-focused thinking by suppressing the AMN, allowing the CCN to direct our cognitive resources and actions (Rayner et al., 2016). In CT, hypoactivation of the CCN may be related to a hyperactive, internally focused AMN, as suggested by resting-state connectivity data showing a highly connected auditory-limbic resting state network in people with tinnitus compared to people without tinnitus (Maudoux et al., 2012).

As illustrated in **Figure 5**, this neurocognitive account conceives tinnitus as a functional imbalance in the interaction of neurocognitive networks. Importantly, it distinguishes the neural networks associated with maintained awareness of the tinnitus sound, underpinned by a hypoactive CCN and hyperactive AMN, which facilitates ongoing attention toward a salient tinnitus sound. It also distinguishes the severity of the impact of tinnitus, underpinned by dysfunction of the AN, AMN, and SN, facilitating negative, internally focused processing of the sound. Considered this way, CT may reflect a fundamental failure of the CCN to inhibit the tinnitus percept when it is emotionally salient to an individual.

In support of the neurocognitive model, auditory processing of aversive sounds has shown reliable activation in the affective and SNs, particularly in the amygdala which is a core AN hub with strong connections to auditory processing (Zald and Pardo, 2002; Kumar et al., 2012). In addition, rapid habituation of the amygdala to aversive auditory stimuli has been proposed to be mediated by the SN (Büchel et al., 1998). There is also evidence of top–down control of auditory processing and associated neuroplasticity of auditory regions via CCN and SN hubs, including pre-frontal and parietal cortices, insula and the anterior cingulate cortex (Tzourio et al., 1997; Westerhausen et al., 2010). As such, there are established links between the functioning and plasticity of auditory pathways and the neurocognitive networks associated with cognitive control, emotion regulation and the salience of incoming sensory information (Westerhausen et al., 2010; Moore, 2013; Moreno and Bidelman, 2014). In light of this, the application of neuroimaging techniques to assess the integrity of these networks in people with and without CT would be beneficial to further assess the potential role of neurocognitive mechanisms in CT.

These network interactions are exemplified in a pilot real-time fMRI auditory control training study which found that conscious suppression of auditory cortex activation was associated with greater activation of the CCN and deactivation of the antithetical AMN, supporting the proposed role for these large-scale neurocognitive networks in the maintenance of CT (Haller et al., 2009). Consistent with this, a range of auditory-based activities have been shown to be protective of the psychological impact of CT, including auditory training therapies, real-time fMRI training of auditory control, and music training (Flor et al., 2004; Haller et al., 2009; Schneider et al., 2009). In particular, music training may be effective in alleviating tinnitus symptoms due to its effectiveness in improving (1) emotion regulation through its effect on the AN (Moore, 2013), and (2) cognitive functions via 'far transfer' effects on attention and working memory processes, which are mediated by CCN functions (Strait et al., 2010; Argstatter et al., 2012; Moreno and Bidelman, 2014). Investigating the possibility of training attention abilities through music or auditory control tasks may be a potential mechanism to attenuate the awareness and severity of CT worthy of further research.

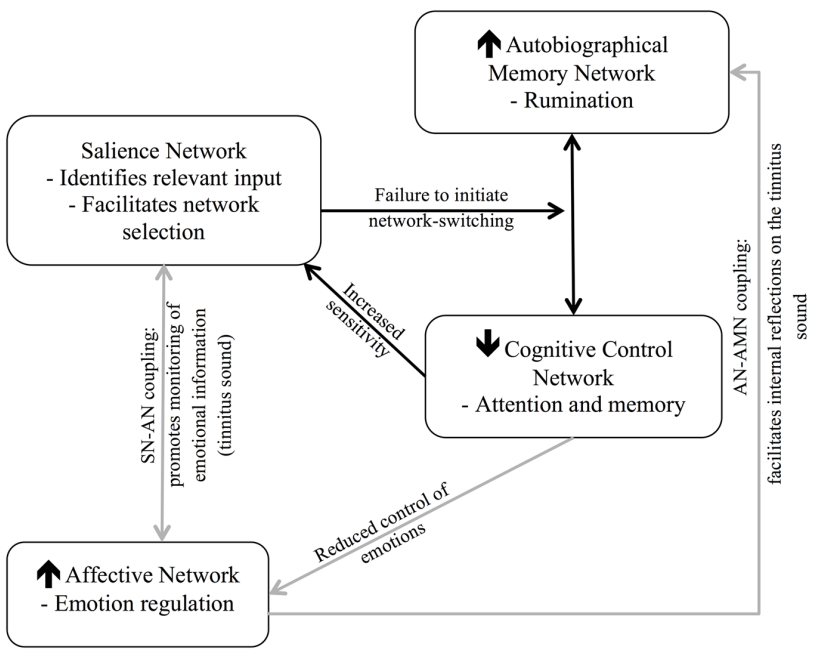

FIGURE 5 | Neurocognitive networks proposed to maintain tinnitus awareness (black arrows) and tinnitus severity (gray arrows).

Furthermore, psychological treatments aimed at strengthening goal-directed attentional control, such as mindfulness and cognitive behavior therapy (CBT) may benefit people with CT by increasing the proficiency of the CCN and improving its regulation of associated networks (SN, AN). Both CBT and mindfulness have been shown to be effective in lessening the impact of CT and improving psychosocial well-being (Philippot et al., 2011; Cima et al., 2012, 2014; Kreuzer et al., 2012). Specifically, evidence of neuroplasticity in regions of the neurocognitive networks proposed to underpin the impact and presence of CT have been found following CBT or mindfulness interventions (Frewen et al., 2008; Chiesa and Serretti, 2009; Brewer et al., 2011). In addition, the functioning of core hubs in these networks, including the dorsal anterior cingulate cortex and regions of the prefrontal cortex have been found to predict the effectiveness of CBT. This suggests that examining the functioning of the CCN and SN as part of 'treatment readiness' prior to commencing CBT may help to improve prognosis, engagement, and potential treatment benefits (Klumpp et al., 2014).

CONCLUSION

Our findings suggest that people with CT are less proficient in switching attention away from the tinnitus sound compared to people with healthy hearing. This highlights a core role of cognition, particularly cognitive control, in maintaining awareness of the tinnitus, as well as reduced emotional down regulation, with depressive symptoms associated with the severity of the tinnitus. Importantly, cognitive control and depressive symptoms provide targets for future treatment studies aimed at reducing awareness of the tinnitus sound and improving the well-being of people with CT. Finally, our results provide a foundation for establishing a new neurocognitive account of CT. This account suggests that a functional imbalance of specific large-scale neural networks associated with processes of attention-switching and emotion regulation may underpin chronic awareness of the tinnitus sound and the severity of its impact.

AUTHOR CONTRIBUTIONS

All authors contributed to the study concept. KT collected the data and completed the data analysis and interpretation under the supervision of SW. KT and SW wrote the manuscript, and NM provided critical revisions. All authors approved the final version of the manuscript for submission.

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REFERENCES

Alam, N., Katarkar, A., Shah, P., Jalvi, R., Jain, A., and Shah, M. (2012). Audiological, psychological and cognitive characteristics of tinnitus sufferers. *Indian J. Otol.* 18, 20–25. doi: 10.4103/0971-7749.98288

Andersson, G., Kyrre Svalastog, O., Kaldo, V., and Sarkohi, A. (2007). Future thinking in tinnitus patients. *J. Psychosom. Res.* 63, 191–194. doi: 10.1016/j.jpsychores.2007.02.012

Argstatter, H., Grapp, M., Hutter, E., Plinkert, P., and Bolay, H. V. (2012). Long-term effects of the äúheidelberg model of music therapy äù in patients with chronic tinnitus. *Int. J. Clin. Exp. Med.* 5, 273–288.

Avila, C., and Parcet, M. A. (2001). Personality and inhibitory deficits in the stop-signal task: the mediating role of Gray's anxiety and impulsivity. *Pers. Individ. Dif.* 31, 975–986. doi: 10.1016/S0191-8869(00)00199-9

Baltes, P. B., and Lindenberger, U. (1997). Emergence of a powerful connection between sensory and cognitive functions across the adult life span: a new window to the study of cognitive aging? *Psychol. Aging* 12, 12–21. doi: 10.1037/0882-7974.12.1.12

Beck, A. T., Steer, R. A., and Brown, G. K. (1996). *Beck Depression Inventory-II*. San Antonio, TX: The Psychological Corporation.

Bonnelle, V., Ham, T. E., Leech, R., Kinnunen, K. M., Mehta, M. A., Greenwood, R. J., et al. (2012). Salience network integrity predicts default mode network function after traumatic brain injury. *Proc. Natl. Acad. Sci. U. S. A.* 109, 4690–4695. doi: 10.1073/pnas.1113455109

Braver, T. S., Cohen, J. D., Nystrom, L. E., Jonides, J., Smith, E. E., and Noll, D. C. (1997). A parametric study of prefrontal cortex involvement in human working memory. *NeuroImage* 5, 49–62. doi: 10.1006/nimg.1996.0247

Brewer, J. A., Worhunsky, P. D., Gray, J. R., Tang, Y.-Y., Weber, J., and Kober, H. (2011). Meditation experience is associated with differences in default mode network activity and connectivity. *Proc. Natl. Acad. Sci. U. S. A.* 108, 20254–20259. doi: 10.1073/pnas.1112029108/-/DCSupplemental

Broadbent, D. E., Cooper, P. F., FitzGerald, P., and Parkes, K. R. (1982). The cognitive failures questionnaire (CFQ) and its correlates. *Br. J. Clin. Psychol.* 21, 1–16. doi: 10.1111/j.2044-8260.1982.tb01421.x

Büchel, C., Morris, J., Dolan, R. J., and Friston, K. J. (1998). Brain systems mediating aversive conditioning: an event-related fMRI study. *Neuron* 20, 947–957. doi: 10.1016/S0896-6273(00)80476-6

Buhle, J. T., Silvers, J. A., Wager, T. D., Lopez, R., Onyemekwu, C., Kober, H., et al. (2014). Cognitive reappraisal of emotion: a meta-analysis of human neuroimaging studies. *Cereb. Cortex* 24, 2981–2990. doi: 10.1093/cercor/bht154

Bushnell, M. C., Čeko, M., and Low, L. A. (2013). Cognitive and emotional control of pain and its disruption in chronic pain. *Nat. Rev. Neurosci.* 14, 502–511. doi: 10.1038/nrn3516

Chiesa, A., and Serretti, A. (2009). A systematic review of neurobiological and clinical features of mindfulness meditations. *Psychol. Med.* 40, 1239–1252. doi: 10.1017/S0033291709991747

Cima, R. F., Maes, I. H., Joore, M. A., Scheyen, D. J., Ei Refaie, A., Baguley, D. M., et al. (2012). Specialised treatment based on cognitive behaviour therapy versus usual care for tinnitus: a randomised controlled trial. *Lancet* 379, 1951–1959. doi: 10.1016/S0140-6736(12)60469-3

Cima, R. F. F., Andersson, G., Schmidt, C. J., and Henry, J. A. (2014). Cognitive-behavioral treatments for tinnitus: a review of the literature. *J. Am. Acad. Audiol.* 25, 29–61. doi: 10.3766/jaaa.25.1.4

Cocchi, L., Zalesky, A., Fornito, A., and Mattingley, J. B. (2013). Dynamic cooperation and competition between brain systems during cognitive control. *Trends Cogn. Sci.* 17, 493–501. doi: 10.1016/j.tics.2013.08.006

Concha-Barrientos, M., Campbell-Lendrum, D., and Steenland, K. (2004). *Occupational Noise: Assessing the Burden of Bisease from Work-related Hearing Impairment at National and Local Levels. WHO. Environmental Burden of Disease Series, No. 9*. Geneva: World Health Organization.

Corbetta, M., and Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nat. Rev. Neurosci.* 3, 215–229. doi: 10.1038/nrn755

Craig, A. D. (2009). How do you feel—now? the anterior insula and human awareness. *Nat. Rev. Neurosci* 10, 59–70.

Derryberry, D., and Reed, M. A. (2002). Anxiety-related attentional biases and their regulation by attentional control. *J. Abnorm. Psychol.* 111, 225–236. doi: 10.1037//0021-843X.111.2.225

Diamond, A. (2013). Executive Functions. *Annu. Rev. Psychol.* 64, 135–168. doi: 10.1146/annurev-psych-113011-143750

Engle, R. W., and Kane, M. J. (2004). Executive attention, working memory capacity, and a two-factor theory of cognitive control. *Psychol. Learn. Motiv.* 44, 145–199. doi: 10.1016/S0079-7421(03)44005-X

Fales, C. L., Barch, D. M., Rundle, M. M., Mintun, M. A., Snyder, A. Z., Cohen, J. D., et al. (2008). Altered emotional interference processing in affective and cognitive-control brain circuitry in major depression. *Biol. Psychiatry* 63, 377–384. doi: 10.1016/j.biopsych.2007.06.012

Field, A. (2008). *Discovering Statistics Using SPSS*. Thousand Oaks, CA: SAGE Publications.

Flor, H., Hoffmann, D., Struve, M., and Diesch, E. (2004). Auditory discrimination training for the treatment of tinnitus. *Appl. psychophysiol. Biofeedback* 29, 113–120.

Frewen, P. A., Dozios, D. J. A., and Lanius, R. (2008). Neuroimaging studies of psychological interventions for mood and anxiety disorders: empirical and methodological review. *Clin. Psychol. Rev.* 28, 228–246. doi: 10.1016/j.cpr.2007.05.002

Fritz, C. O., Morris, P. E., and Richler, J. J. (2012). Effect size estimates: current use, calculations, and interpretation. *J. Exp. Psychol. Gen.* 141, 2–18. doi: 10.1037/a0024338

Gazzaley, A., and Nobre, A. C. (2012). Top-down modulation: bridging selective attention and working memory. *Trends Cogn. Sci.* 16, 128–134. doi: 10.1016/j.tics.2011.11.014

Golm, D., Schmidt-Samoa, C., Dechent, P., and Kröner-Herwig, B. (2013). Neural correlates of tinnitus related distress: an fMRI-study. *Hear. Res.* 295, 87–99. doi: 10.1016/j.heares.2012.03.003

Gotlib, I. H., and Joormann, J. (2010). Cognition and depression: current status and future directions. *Annu. Rev. Clin. Psychol.* 6, 285–312. doi: 10.1146/annurev.clinpsy.121208.131305

Hallam, R. S., McKenna, L., and Shurlock, L. (2004). Tinnitus impairs cognitive efficiency. *Int. J. Audiol.* 43, 218–226. doi: 10.1080/14992020400050030

Hallam, R. S., Rachman, S., and Hinchcliffe, R. (1984). "Psychological aspects of tinnitus," in *Contributions to Medical Psychology* Vol. 3, ed. S. Rachman, (Oxford: Pergamon Press), 31–53.

Haller, S., Birbaumer, N., and Veit, R. (2009). Real-time fMRI feedback training may improve chronic tinnitus. *Eur. Radiol.* 20, 696–703. doi: 10.1007/s00330-009-1595-z

Heeren, A., Maurage, P., Perrot, H., De Volder, A., Renier, L., Araneda, R., et al. (2014). Tinnitus specifically alters the top-down executive control sub-component of attention: evidence from the attention network task. *Behav. Brain Res.* 269, 147–154. doi: 10.1016/j.bbr.2014.04.043

Henry, J. A., Dennis, K. C., and Schechter, M. A. (2005). General review of tinnitus: prevalence, mechanisms, effects, and management. *J. Speech Lang. Hear. Res.* 48, 1204–1235. doi: 10.1044/1092-4388(2005/084)

Hoffman, H. J., and Reed, G. W. (2004). "Epidemiology of tinnitus," in *Tinnitus Theory and Management*, ed. J. B. Snow Jr. (Lewiston, ME: BC Decker Inc.), 16–41.

Jackson, J. G., Coyne, I. J., and Clough, P. J. (2014). A preliminary investigation of potential cognitive performance decrements in non-help-seeking tinnitus sufferers. *Int. J. Audiol.* 53, 88–93. doi: 10.3109/14992027.2013.846481

Jaeggi, S. M., Buschkuehl, M., Etienne, A., Ozdoba, C., Perrig, W. J., and Nirkko, A. C. (2007). On how high performers keep cool brains in situations of cognitive overload. *Cogn. Affect. Behav. Neurosci.* 7, 75–89. doi: 10.3758/CABN.7.2.75

Jastreboff, P. J., Gray, W. C., and Gold, S. L. (1996). Neurophysiological approach to tinnitus patients. *Am. J. Otol.* 17, 236–240.

Klumpp, H., Fitzgerald, D. A., Angstadt, M., Post, D., and Phan, K. L. (2014). Neural response during attentional control and emotion processing predicts improvement after cognitive behavioral therapy in generalized social anxiety disorder. *Psychol. Med.* 44, 3109–3121. doi: 10.1017/S0033291714000567

Kreuzer, P. M., Goetz, M., Holl, M., Schecklmann, M., Landgrebe, M., Staudinger, S., et al. (2012). Mindfulness-and body-psychotherapy-based group treatment of chronic tinnitus: a randomized controlled pilot study. *BMC Complement. Altern. Med.* 12:235. doi: 10.1186/1472-6882-12-235

Kumar, S., Kriegsteinvon, K., Friston, K., and Griffiths, T. D. (2012). Features versus feelings: dissociable representations of the acoustic features and valence of aversive sounds. *J. Neurosci.* 32, 14184–14192. doi: 10.1523/JNEUROSCI.1759-12.2012

Langguth, B., Goodey, R., Azevedo, A., Bjorne, A., Cacace, A., Crocetti, A., et al. (2007). "Consensus for tinnitus patient assessment and treatment outcome measurement: Tinnitus research initiative meeting, regensburg, july 2006," in *Progress in Brain Research Progress in Brain Research*, eds B. Langguth, G. Hajak, T. Kleinjung, A. Cacace, and A. R. Moller (Amsterdam: Elsevier), 525–536. doi: 10.1016/S0079-6123(07)66050-6

Langguth, B., Landgrebe, M., Kleinjung, T., Sand, G. P., and Hajak, G. (2011). Tinnitus and depression. *World J. Biol. Psychiatry* 12, 489–500. doi: 10.3109/15622975.2011.575178

Logan, G. D., Schachar, R. J., and Tannock, R. (1997). Impulsivity and inhibitory control. *Psychol. Sci.* 8, 60–64. doi: 10.1111/j.1467-9280.1997.tb00545.x

Maudoux, A., Lefebvre, P., Cabay, J.-E., Demertzi, A., Vanhaudenhuyse, A., Laureys, S., et al. (2012). Auditory resting-state network connectivity in tinnitus: a functional mri study. *PLoS ONE* 7:e36222. doi: 10.1371/journal.pone.0036222.t004

McCormack, A., Edmondson-Jones, M., Somerset, S., and Hall, D. (2016). A systematic review of the reporting of tinnitus prevalence and severity. *Hear. Res.* 337, 70–79. doi: 10.1016/j.heares.2016.05.009

Menon, V., and Uddin, L. Q. (2010). Saliency, switching, attention and control: a network model of insula function. *Brain Struct. Funct.* 214, 655–667. doi: 10.1007/s00429-010-0262-0

Mohamad, N., Hoare, D. J., and Hall, D. A. (2016). The consequences of tinnitus and tinnitus severity on cognition: a review of the behavioural evidence. *Hear. Res.* 332, 199–209. doi: 10.1016/j.heares.2015.10.001

Moller, A. R. (2007). Tinnitus and pain. *Prog. Brain Res.* 166, 47–53. doi: 10.1016/S0079-6123(07)66004-X

Moore, K. S. (2013). A systematic review on the neural effects of music on emotion regulation: implications for music therapy practice. *J. Music Ther.* 50, 198–242. doi: 10.1093/jmt/50.3.198

Moreno, S., and Bidelman, G. M. (2014). Examining neural plasticity and cognitive benefit through the unique lens of musical training. *Hear. Res.* 308, 84–97. doi: 10.1016/j.heares.2013.09.012

Neurobehavioral Systems Inc (2014). Presentation®, Version 17. Berkeley, CA. Available at: www.neurobs.com

Newman, C. W., Jacobson, G. P., and Spitzer, J. B. (1996). Development of the tinnitus handicap inventory. *Arch. Otolaryngol. Head Neck Surg.* 122, 143–148. doi: 10.1001/archotol.1996.01890140029007

Niendam, T. A., Laird, A. R., Ray, K. L., Dean, Y. M., Glahn, D. C., and Carter, C. S. (2012). Meta-analytic evidence for a superordinate cognitive control network subserving diverse executive functions. *Cogn. Affec. Behav. Neurosci.* 12, 241–268. doi: 10.3758/s13415-011-0083-5

Ochsner, K. N., and Gross, J. J. (2005). The cognitive control of emotion. *Trends Cogn. Sci.* 9, 242–249. doi: 10.1016/j.tics.2005.03.010

Owen, A. M., McMillan, K. M., Laird, A. R., and Bullmore, E. (2005). N-back working memory paradigm: a meta-analysis of normative functional neuroimaging studies. *Hum. Brain Mapp.* 25, 46–59. doi: 10.1002/hbm.20131

Pattyn, T., Van Den Eede, F., Vanneste, S., Cassiers, L., Veltman, D. J., Van de Heyning, P., et al. (2016). Tinnitus and anxiety disorders: a review. *Hear. Res.* 333, 255–265. doi: 10.1016/j.heares.2015.08.014

Philippot, P., Nef, F., Clauw, L., Romrée, M., and Segal, Z. (2011). A randomized controlled trial of mindfulness-based cognitive therapy for treating tinnitus. *Clin. Psychol. Psychother.* 19, 411–419. doi: 10.1002/cpp.756

Pierce, K. J., Kallogjeri, D., Piccirillo, J. F., Garcia, K. S., Nicklaus, J. E., and Burton, H. (2012). Effects of severe bothersome tinnitus on cognitive function measured with standardized tests. *J. Clin. Exp. Neuropsychol.* 34, 126–134. doi: 10.1080/13803395.2011.623120

Rayner, G., Jackson, G., and Wilson, S. (2016). Cognition-related brain networks underpin the symptoms of unipolar depression: evidence from a systematic review. *Neurosci. Biobehav. Rev.* 61, 53–65. doi: 10.1016/j.neubiorev.2015.09.022

Schneider, P., Andermann, M., Wengenroth, M., Goebel, R., Flor, H., Rupp, A., et al. (2009). Reduced volume of Heschl's gyrus in tinnitus. *NeuroImage* 45, 927–939. doi: 10.1016/j.neuroimage.2008.12.045

Seeley, W. W., Menon, V., Schatzberg, A. F., Keller, J., Glover, G. H., Kenna, H., et al. (2007). Dissociable intrinsic connectivity networks for salience processing and executive control. *J. Neurosci.* 27, 2349–2356. doi: 10.1523/JNEUROSCI.5587-06.2007

Sheline, Y. I., Barch, D. M., Price, J. L., Rundle, M. M., Vaishnavi, S. N., Snyder, A. Z., et al. (2009). The default mode network and self-referential processes in depression. *Proc. Natl. Acad. Sci. U. S. A.* 106, 1942–1947.

Spielberger, C. D., Gorsuch, R., Lushene, R. E., Vagg, P. R., and Jacobs, G. A. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.

Stevens, C., Walker, G., Boyer, M., and Gallagher, M. (2007). Severe tinnitus and its effect on selective and divided attention. *Int. J. Audiol.* 46, 208–216. doi: 10.1080/14992020601102329

Strait, D. L., Kraus, N., Parbery-Clark, A., and Ashley, R. (2010). Musical experience shapes top-down auditory mechanisms: evidence from masking and auditory attention performance. *Hear. Res.* 261, 22–29. doi: 10.1016/j.heares.2009.12.021

Trevis, K. J., McLachlan, N. M., and Wilson, S. J. (2016). Psychological mediators of chronic tinnitus: the critical role of depression. *J. Affect. Disord.* 204, 234–240. doi: 10.1016/j.jad.2016.06.055

Tzourio, N., Massioui, El, F., Crivello, F., Joliot, M., Renault, B., et al. (1997). Functional anatomy of human auditory attention studied with PET. *NeuroImage* 5, 63–77. doi: 10.1006/nimg.1996.0252

Unsworth, N., Fukuda, K., Awh, E., and Vogel, E. K. (2014). Working memory and fluid intelligence: capacity, attention control, and secondary memory retrieval. *Cognit. Psychol.* 71, 1–26. doi: 10.1016/j.cogpsych.2014.01.003

Verbruggen, F., Chambers, C. D., and Logan, G. D. (2013). Fictitious inhibitory differences how skewness and slowing distort the estimation of stopping latencies. *Psychol. Sci.* 24, 352–362. doi: 10.1177/0956797612457390

Wayne, R. V., and Johnsrude, I. S. (2015). A review of causal mechanisms underlying the link between age-related hearing loss and cognitive decline. *Ageing Res. Rev.* 23, 154–166. doi: 10.1016/j.arr.2015.06.002

Wechsler, D. (2008). *Wechsler Adult Intelligence Scale, 4th Edn*. San Antonio, TX: Psychological Corporation.

Westerhausen, R., Moosmann, M., Alho, K., Belsby, S. O., Hämäläinen, H., Medvedev, S., et al. (2010). Identification of attention and cognitive control networks in a parametric auditory fMRI study. *Neuropsychologia* 48, 2075–2081. doi: 10.1016/j.neuropsychologia.2010.03.028

Zald, D. H., and Pardo, J. V. (2002). The neural correlates of aversive auditory stimulation. *NeuroImage* 16, 746–753. doi: 10.1006/nimg.2002.1115

Zirke, N., Seydel, C., Arsoy, D., Klapp, B. F., Haupt, H., Szczepek, A. J., et al. (2013). Analysis of mental disorders in tinnitus patients performed with composite international diagnostic interview. *Qual. Life Res.* 22, 2095–2104. doi: 10.1007/s11136-012-0338-9

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Alexithymia Is Associated with Tinnitus Severity

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Objective: Alexithymia is considered to be a personality trait with a tendency to express psychological distress in somatic rather than emotional form and, therefore, may play a vital role in somatization. Although, such a propensity can be found in patients suffering from tinnitus, the relationship between alexithymic characteristics and the subjective experience of tinnitus severity remains yet unclear. Our aim was to evaluate which alexithymic characteristics are linked to the subjective experience of tinnitus symptomatology.

Methods: We evaluated tinnitus severity (Tinnitus Handicap Inventory, THI), alexithymia (20-item Toronto Alexithymia Scale, TAS-20), and depression (Beck Depression Inventory, BDI) in 207 outpatients with tinnitus. Correlation analyses and multiple regression analyses were calculated in order to investigate the relationship between alexithymic characteristics, tinnitus severity, and depression.

Results: Highly significant positive correlations were found between THI total score and TAS-20 total score as well as BDI score. Regarding the TAS-20 subscales, multiple regression analyses showed that only the TAS-20 subscale "difficulty in identifying feelings" (DIF) and the BDI significantly predicted the subjective experience of tinnitus severity. Regarding the THI subscales, only higher scores of the THI subscale "functional" demonstrated an independent moderate association with higher scores for DIF.

Conclusion: We found an independent association between the subjective experience of tinnitus severity and alexithymic characteristics, particularly with regard to limitations in the fields of mental, social, and physical functioning because of tinnitus and the difficulty of identifying feelings facet of alexithymia. These findings are conducive to a better understanding of affect regulation that may be important for the psychological adaptation of patients suffering from tinnitus.

Keywords: tinnitus, alexithymia, Tinnitus Handicap Inventory, Toronto Alexithymia Scale, depressive symptoms

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INTRODUCTION

Tinnitus is defined as the auditory perception of sound without any corresponding external sound stimulation and occurs in 10–19% of persons in industrialized societies, of which one in five will require medical attention (1–3). It is not completely understood why some persons adapt to their tinnitus symptoms and why others do not (4, 5), but many authors suggest that psychological factors

have a notable influence on the subjective experience of tinnitus (6, 7). Langguth et al. proved the importance of anxiety and depression as indicators of experiencing of tinnitus severity by using the Tinnitus Handicap Inventory (THI) (8, 9). Furthermore, a substantial association has been described between tinnitus severity and depression as well as a positive effect of antidepressive treatment on tinnitus severity (10). Moreover, Hiller et al. demonstrated that tinnitus occurred more often in patients with somatization or hypochondriacal disorder and stated that tinnitus may be a somatoform symptom with a possible comorbidity of these different conditions (11). Numerous studies support these suggestions by illustrating similar patterns of subjective loudness and of pitch of tinnitus in patients with great annoyance and in those without annoyance of tinnitus (12–14). A further well-described aspect is the association between tinnitus and reduced quality of life assessed by a standard test procedure (15–17) as well as the association between the greater emotional distress due to tinnitus and the attention that is paid to tinnitus (14).

One condition that may complicate the adaption to emotional distress and lead to a maladaptive coping behavior is alexithymia, which was introduced by Nemiah and Sifneos about 40 years ago based on the clinical observations on patients with psychosomatic disorders (18, 19). Alexithymia is a multifacet personality trait characterized by a reduced ability in identifying and describing one's feelings, a reduced ability in distinguishing own feelings from bodily sensations, an externally oriented style of thinking, and a restricted imaginal process (20). Alexithymia is associated with increased individual distress (21), reduced health-related quality of life (22), and reduced empathic brain responses (23). Alexithymic people are prone to express psychological distress in somatic rather than emotional form (24), which is considered a triggering factor for psychiatric and behavioral problems such as somatization (24, 25). Congruously, it was found that alexithymia was more prevalent in people with somatoform disorders than in healthy controls (26). These findings are supported by other studies where important factors of alexithymia like difficulties in identifying and describing feelings were related to a greater amount of severe dizziness symptoms (27). Although originally associated with psychosomatic diseases, many studies also already demonstrated a higher prevalence of alexithymia in different psychiatric disorders like panic disorder (28), eating disorders (29, 30), alcohol dependence (31), posttraumatic stress disorders (32), and personality disorders (33, 34) as well as in somatic diseases like inflammatory bowel disease (35), recurrent severe asthma (36), or essential hypertension (37).

Despite numerous publications on alexithymia and somatic symptoms, there are hardly any studies that deal with the associations among alexithymia and tinnitus. As far as we know, only one study exists and has not revealed any correlation between alexithymia and tinnitus severity in a community sample of elderly people aged between 70 and 85 years (38).

However, due to the assumption of the somatoform symptom quality of tinnitus and the mentioned finding that alexithymic characteristics are more prevalent in somatic symptom reporting, our aim was to investigate the relationship between alexithymia and the subjective experience of tinnitus severity in individuals with tinnitus. Furthermore, we wanted to examine which alexithymic characteristics are linked to the subjective experience of tinnitus symptomatology, because they might play an important role for the psychological adaptation of patients suffering from tinnitus.

MATERIALS AND METHODS

Participants

The study was authorized by the ethics committee of the canton of Zurich. Two hundred eighty patients referred to the tinnitus outpatient service at University Hospital Zurich and seen between December 2012 and May 2014 were asked to participate in the study (16). The patients' medical histories were assessed prior to data recording and all subjects suffering from acute or chronic somatic diseases that could be causing the symptomatology were excluded as well as subjects with chronic psychiatric diseases. All participants gave their written electronic consent before starting to answer the questionnaires online. In case of participants' questions or uncertainties, a trained medical student provided help in completing the questionnaires. The final sample comprised 207 patients who filled out the questionnaires completely, spoke fluent German, and reported to have had tinnitus for at least 1 month in order to exclude people with temporary symptoms and focus on people with post-acute and chronic tinnitus.

Measures

To evaluate tinnitus severity, the validated German version of the THI was used, which represents the most standardized tinnitus handicap measuring tool in the literature with excellent internal consistency (Cronbach's alpha = 0.93) (39, 40). The THI is a self-reported measure consisting of 25 questions grouped into three subscales: functional (11 questions measuring the functional aspects of tinnitus such as mental, social, and physical functioning), emotional (9 questions reflecting affective responses to tinnitus), and catastrophic (5 questions representing catastrophic responses to tinnitus, which include depression and sleep disturbance) (41, 42). Every of the 25 items can be scored with 0 ("no"), 2 ("sometimes"), or 4 ("yes") points. The total score can be calculated in a range from 0 to 100 and can be subdivided into different grades of subjective experience of tinnitus severity: light (0–16), mild (18–36), moderate (38–56), severe (58–76), and catastrophic handicap (78–100) (43). Furthermore, scores can be calculated for the three subscales: functional (maximum score = 44), emotional (maximum score = 36), and catastrophic (maximum score = 20) (44).

In order to assess alexithymia, the 20-item Toronto Alexithymia Scale (TAS-20) (German version) was administered to the participants (45, 46). The TAS-20, the most commonly used measure of alexithymia, is a valid and reliable 20-item self-report questionnaire with a total score from 0 to 100 and consists of three subscales, measuring the difficulty in identifying feelings (DIF), the difficulty in describing feelings (DDF), and the externally oriented thinking (EOT) (45, 47). There is evidence that the TAS-20 is a reliable and valid measure of alexithymia in normal and clinical adult samples (Cronbach's alpha = 0.81) (48).

The severity of depression was assessed by the German version of the Beck Depression Inventory (BDI) that consists of 21 items including clinical symptoms of depression with a total score from 0 to 63, with higher scores reflecting higher levels of depression. A total score of 0–10 corresponds with no or minimal depression, of 11–17 with a mild or moderate depression, of 18–63 with a clinical relevant depression. The German version of the questionnaire has shown good psychometric properties (49, 50).

Statistical Analysis

Descriptive statistics for different measures were calculated for all participants. Data were checked for normal distribution before further statistical analysis. Associations between THI total scores and TAS-20 total scores with subscale scores as well as BDI total scores, and age were tested by using Pearson correlations (two-sided). Afterward, stepwise multiple regression analyses were performed in order to investigate the independent relationship between scores of the TAS-20 with subscales and THI. All statistical calculations were performed using the statistical software package SPSS™/Version 22.0 for Windows (SPSS Inc., Chicago, IL, USA). The significance level was set at $p \leq 0.05$.

RESULTS

Seventy-three out of the 207 patients who completed our questionnaires were females (35.3%). Mean age was 46.7 years (SD = 13.9). The average duration of tinnitus was 66.1 months (SD = 92.5). Mean scores on subjective tinnitus severity, alexithymia, and depression ($n = 207$) are presented in **Table 1**.

Patients showed, on average, moderate levels of tinnitus severity with a mean THI total score of 44.4 (SD = 23.3). In detail, 26 patients (12.6%) of the sample reported a slight handicap, 60 patients (29.0%) reported a mild handicap, 58 patients (28.0%) reported a moderate handicap, 45 patients (21.7%) reported a severe handicap, and 18 patients (8.7%) reported a catastrophic handicap. These findings are similar to results from other studies (51, 52).

The mean value of the TAS-20 total score in our sample was 44.0 (SD = 10.8), which is slightly higher as compared to the mean score of 39.9 (SD = 8.4) in a representative reference sample of the German population ($n = 306$) (53). Using the TAS-20 cut-off score ≥ 61 (54, 55), 19 patients (9.2%) could be classified as alexithymic, which is consistent with prevalence rates of alexithymia in the German general population (56).

In terms of depression severity, patients showed a mean BDI sum-score of 9.3 (SD = 6.9), which indicates none or minimal depression (57). According to the BDI manual, 133 patients (64.3%) were classified as not depressed, 50 patients (24.2%) were classified as mildly to moderately depressed, and 24 patients (11.6%) were classified as clinically relevant depressed.

Table 2 gives an overview of the Pearson correlations between THI total scores and TAS-20 total scores with subscale scores as well as BDI total scores and age. Highly significant correlations were found between THI total score and BDI score as well as TAS-20 total score and two subscales, measuring the DIF and the DDF (all $p < 0.01$; see **Table 2**). The third TAS-20 subscale, measuring EOT, and also age did not correlate with THI total score.

A stepwise multiple regression analysis including all TAS-20 subscales and the BDI score was performed in order to asses for independent relationships between these variables and THI total score (as dependent variable). It was found that only BDI (Beta = 0.64, adjusted $R^2 = 0.49$, $p < 0.01$) and the DIF-subscale (Beta = 0.12, adjusted $R^2 = 0.50$, $p < 0.05$) significantly predicted subjective level of tinnitus severity measured by THI.

In order to further estimate the association between tinnitus severity and DIF, a second stepwise multiple regression analysis was calculated with the THI subscales as independent variables and the TAS-20 DIF subscale as dependent variable. The THI total score was not used in conjunction with the THI subscales to exclude redundancy of the data analyses. According to these findings, only higher scores of the THI subscale "functional" demonstrated an independent association with higher scores for difficulty identifying feelings (Beta = 0.45, adjusted $R^2 = 0.20$, $p < 0.01$).

DISCUSSION

Our findings establish the existence of a moderate relationship between the subjective experience of tinnitus severity and alexithymic deficits in emotion regulation. More specifically, we found a positive correlation between the functional subscale of the THI, which reflects limitations tinnitus causes in the mental, occupational, social, and physical areas, and the TAS-20 dimension for difficulty identifying feelings. To the best of our

TABLE 1 | Mean scores on the Tinnitus Handicap Inventory (THI) with subscales, the Toronto Alexithymia Scale (TAS-20) with subscales, and the Beck Depression Inventory (BDI); $n = 207$.

Variable	Mean	SD
THI total	44.4	23.3
THI functional	20.6	11.5
THI emotional	13.4	8.5
THI catastrophic	10.4	4.9
TAS-20 total	44.0	10.8
TAS-20 difficulty in identifying feelings	14.1	5.4
TAS-20 difficulty in describing feelings	11.0	3.5
TAS-20 externally oriented thinking	18.9	4.4
BDI	9.3	6.9

TABLE 2 | Pearson correlation coefficients between Tinnitus Handicap Inventory (THI), the Toronto Alexithymia Scale (TAS-20) with subscales, the Beck Depression Inventory (BDI), and age; $n = 207$, $^{**}p < 0.01$.

	TAS-20 total	TAS-20 difficulty in identifying feelings	TAS-20 difficulty in describing feelings	TAS-20 externally oriented thinking	BDI	Age
THI total	0.33**	0.46**	0.28**	0.02	0.70**	−0.09
THI functional	0.33**	0.45**	0.28**	0.04	0.68**	−0.07
THI emotional	0.29**	0.42**	0.26**	−0.02	0.64**	−0.12
THI catastrophic	0.30**	0.41**	0.26**	0.03	0.64**	−0.05

knowledge, only one previous study has evaluated the association between tinnitus and alexithymia, but in a community sample of elderly people. In contrast to our findings, Salonen et al. did not find any correlation between TAS-20 scores and tinnitus severity (38). The discrepancy to our results may be explained by the fact that Salonen et al. did not use a standardized instrument to measure tinnitus severity, which was only classified by indicating one of the three groups: no tinnitus, tinnitus without annoyance, and tinnitus with annoyance (38).

Some limitations should be taken into consideration when discussing the results. First, data were collected by self-report questionnaires even though alexithymic patients may have difficulty in adequately assessing their emotional deficits (58). Second, the cross-sectional design of our study precluded any causal interpretation of the relationship between tinnitus severity and alexithymia. We are also quite aware of the fact that the significant association between DIF and THI total score was small in terms of the overall variance explained. Thus, DIF may play a role in the subjective experience of tinnitus severity, but this is not completely confirmed by the actual study. Furthermore, the relation between tinnitus severity and depressive symptoms, which was reported similarly in other studies (43, 59) might be ascribed to a content overlap between the used self-report questionnaires (52). Also further studies are needed to understand the relationship between alexithymic characteristics and the experience of tinnitus severity, which are focused on patients with chronic tinnitus, i.e., tinnitus symptoms for at least 6 months, in order to avoid including patients who are still under posttraumatic distress.

Despite the mentioned limitations our findings suggest that people with difficulties in identifying feelings may tend to experience greater limitations in social, daily, and reading activities involving concentration, auditory acuity, attention, and rest due to tinnitus (as measured by the functional subscale of THI), which is in accordance with De Gucht and Heiser (26). They reported similar outcomes in their review of the empirical literature on somatization and alexithymia: DIF demonstrated the strongest association with the number of symptoms reported, even stronger than the association with general alexithymia. Our results were also consistent with previous investigation that showed that the difficulties identifying feelings factor of the TAS-20 was particularly effective in predicting somatization (60).

Taken together, the disturbances in DIF and the subjective experience of tinnitus severity should be considered in future studies for more precise understanding of this association, preferably with the additional application of observer rated or interview-based methods for measuring alexithymia as the Toronto Structured Interview for Alexithymia (61, 62). Our results benefit a better understanding of emotion regulation difficulties in patients suffering from tinnitus. Furthermore, if replicated, they may have important clinical implications: because people with difficulties in identifying feelings are characterized by using escape-avoidance strategies, individualized psychotherapeutic interventions might potentially benefit these patients. Further research may also point to the fact that not just alexithymic characteristics can predict the experience of tinnitus severity, but a more general impairment in awareness and regulation of mental states (63–65), for example, an impaired self-reflection as found in different psychiatric disorders (66).

ETHICS STATEMENT

The study was authorized by the ethics committee of the canton of Zurich, Switzerland. All participants gave their written (electronic) informed consent in accordance with the Declaration of Helsinki.

AUTHOR CONTRIBUTIONS

JW managed data collection, analyzed and interpreted the collected data, and wrote the first draft for the article. TK initiated the collaborative project, conceptualized and designed the project, collected and monitored data collection, and revised the article. MK, NP, and MM collected data, monitored data collection, and interpreted data, and critically revised the draft paper. MR contributed to the concept and design, interpreted data, and critically revised the draft paper. SW initiated the collaborative project, conceptualized and designed the project, designed data collection tools, collected and monitored data collection, interpreted the data, and revised the article. All authors read and approved the final manuscript.

REFERENCES

1. Henry JA, Schechter MA, Loovis CL, Zaugg TL, Kaelin C, Montero M. Clinical management of tinnitus using a "progressive intervention" approach. *J Rehabil Res Dev* (2005) 42(4 Suppl 2):95–116. doi:10.1682/JRRD.2005.01.0005
2. Michikawa T, Nishiwaki Y, Kikuchi Y, Saito H, Mizutari K, Okamoto M, et al. Prevalence and factors associated with tinnitus: a community-based study of Japanese elders. *J Epidemiol* (2010) 20(4):271–6. doi:10.2188/jea.JE20090121
3. Probst T, Pryss RC, Langguth B, Spiliopoulou M, Landgrebe M, Vesala M, et al. Outpatient tinnitus clinic, self-help web platform, or mobile application to recruit tinnitus study samples? *Front Aging Neurosci* (2017) 9:113. doi:10.3389/fnagi.2017.00113
4. Scott B, Lindberg P. Psychological profile and somatic complaints between help-seeking and non-help-seeking tinnitus subjects. *Psychosomatics* (2000) 41(4):347–52. doi:10.1176/appi.psy.41.4.347
5. Durai M, Searchfield G. Anxiety and depression, personality traits relevant to tinnitus: a scoping review. *Int J Audiol* (2016) 55(11):605–15. doi:10.1080/14992027.2016.1198966
6. Langguth B, Landgrebe M, Kleinjung T, Sand GP, Hajak G. Tinnitus and depression. *World J Biol Psychiatry* (2011) 12(7):489–500. doi:10.3109/15622975.2011.575178
7. Milerova J, Anders M, Dvorak T, Sand PG, Koniger S, Langguth B. The influence of psychological factors on tinnitus severity. *Gen Hosp Psychiatry* (2013) 35(4):412–6. doi:10.1016/j.genhosppsych.2013.02.008
8. Langguth B, Kleinjung T, Fischer B, Hajak G, Eichhammer P, Sand PG. Tinnitus severity, depression, and the big five personality traits. *Prog Brain Res* (2007) 166:221–5. doi:10.1016/S0079-6123(07)66020-8
9. Pinto PC, Marcelos CM, Mezzasalma MA, Osterne FJ, de Melo Tavares de Lima MA, Nardi AE. Tinnitus and its association with psychiatric disorders: systematic review. *J Laryngol Otol* (2014) 128(8):660–4. doi:10.1017/S0022215114001030

10. Folmer RL, Griest SE, Meikle MB, Martin WH. Tinnitus severity, loudness, and depression. *Otolaryngol Head Neck Surg* (1999) 121(1):48–51. doi:10.1016/S0194-5998(99)70123-3
11. Hiller W, Janca A, Burke KC. Association between tinnitus and somatoform disorders. *J Psychosom Res* (1997) 43(6):613–24. doi:10.1016/S0022-3999(97)00188-8
12. Dobie RA, Sakai CS, Sullivan MD, Katon WJ, Russo J. Antidepressant treatment of tinnitus patients: report of a randomized clinical trial and clinical prediction of benefit. *Am J Otol* (1993) 14(1):18–23.
13. Figueiredo RR, Rates MA, Azevedo AA, Oliveira PM, Navarro PB. Correlation analysis of hearing thresholds, validated questionnaires and psychoacoustic measurements in tinnitus patients. *Braz J Otorhinolaryngol* (2010) 76(4):522–6. doi:10.1590/S1808-86942010000400018
14. Newman CW, Wharton JA, Jacobson GP. Self-focused and somatic attention in patients with tinnitus. *J Am Acad Audiol* (1997) 8(3):143–9.
15. Nondahl DM, Cruickshanks KJ, Dalton DS, Klein BE, Klein R, Schubert CR, et al. The impact of tinnitus on quality of life in older adults. *J Am Acad Audiol* (2007) 18(3):257–66. doi:10.3766/jaaa.18.3.7
16. Weidt S, Delsignore A, Meyer M, Rufer M, Peter N, Drabe N, et al. Which tinnitus-related characteristics affect current health-related quality of life and depression? A cross-sectional cohort study. *Psychiatry Res* (2016) 237:114–21. doi:10.1016/j.psychres.2016.01.065
17. Krog NH, Engdahl B, Tambs K. The association between tinnitus and mental health in a general population sample: results from the HUNT Study. *J Psychosom Res* (2010) 69(3):289–98. doi:10.1016/j.jpsychores.2010.03.008
18. Nemiah JC, Sifneos PE. Psychosomatic illness: a problem in communication. *Psychother Psychosom* (1970) 18(1):154–60. doi:10.1159/000286074
19. Sifneos PE. The prevalence of 'alexithymic' characteristics in psychosomatic patients. *Psychother Psychosom* (1973) 22(2):255–62. doi:10.1159/000286529
20. Taylor GJ, Bagby RM. The alexithymia personality dimension In: Widige TA, editor. *The Oxford Handbook of Personality Disorders.* New York, NY: Oxford University Press (2012) p. 648–73.
21. Humphreys TP, Wood LM, Parker JD. Alexithymia and satisfaction in intimate relationships. *Pers Individ Dif* (2009) 46(1):43–7. doi:10.1016/j.paid.2008.09.002
22. Leenen K, Rufer M, Moergeli H, Grabe H-J, Jenewein J, Nuñez DG, et al. Alexithyme Patientenmerkmale und Lebensqualität–Eine Querschnittsstudie an 79 ambulanten Patienten mit Angststörungen. *Z Psychiatr Psychol Psychother* (2015) 61:17–26. doi:10.1024/1661-4747/a000136
23. Bird G, Silani G, Brindley R, White S, Frith U, Singer T. Empathic brain responses in insula are modulated by levels of alexithymia but not autism. *Brain* (2010) 133(Pt 5):1515–25. doi:10.1093/brain/awq060
24. Lundh LG, Simonsson-Sarnecki M. Alexithymia, emotion, and somatic complaints. *J Pers* (2001) 69(3):483–510. doi:10.1111/1467-6494.00153
25. Taylor GJ, Bagby RM, Parker JDA. *Disorders of Affect Regulation: Alexithymia in Medical and Psychiatric Illness*. Cambridge; New York: Cambridge University Press (1997). xxii,359 p.
26. De Gucht V, Heiser W. Alexithymia and somatisation: quantitative review of the literature. *J Psychosom Res* (2003) 54(5):425–34. doi:10.1016/S0022-3999(02)00467-1
27. von Rimscha S, Moergeli H, Weidt S, Straumann D, Hegemann S, Rufer M. Alexithymia and health-related quality of life in patients with dizziness. *Psychopathology* (2013) 46(6):377–83. doi:10.1159/000345357
28. Parker JD, Taylor GJ, Bagby RM, Acklin MW. Alexithymia in panic disorder and simple phobia: a comparative study. *Am J Psychiatry* (1993) 150(7):1105–7. doi:10.1176/ajp.150.7.1105
29. Corcos M, Guilbaud O, Speranza M, Paterniti S, Loas G, Stephan P, et al. Alexithymia and depression in eating disorders. *Psychiatry Res* (2000) 93(3):263–6. doi:10.1016/S0165-1781(00)00109-8
30. Taylor GJ, Parker JD, Bagby RM, Bourke MP. Relationships between alexithymia and psychological characteristics associated with eating disorders. *J Psychosom Res* (1996) 41(6):561–8. doi:10.1016/S0022-3999(96)00224-3
31. Loas G, Otmani O, Lecercle C, Jouvent R. Relationships between the emotional and cognitive components of alexithymia and dependency in alcoholics. *Psychiatry Res* (2000) 96(1):63–74. doi:10.1016/S0165-1781(00)00189-X
32. Zeitlin SB, McNally RJ, Cassiday KL. Alexithymia in victims of sexual assault: an effect of repeated traumatization? *Am J Psychiatry* (1993) 150(4):661–3. doi:10.1176/ajp.150.4.661
33. Joyce AS, Fujiwara E, Cristall M, Ruddy C, Ogrodniczuk JS. Clinical correlates of alexithymia among patients with personality disorder. *Psychother Res* (2013) 23(6):690–704. doi:10.1080/10503307.2013.803628
34. Nicolo G, Semerari A, Lysaker PH, Dimaggio G, Conti L, D'Angerio S, et al. Alexithymia in personality disorders: correlations with symptoms and interpersonal functioning. *Psychiatry Res* (2011) 190(1):37–42. doi:10.1016/j.psychres.2010.07.046
35. Porcelli P, Zaka S, Leoci C, Centonze S, Taylor GJ. Alexithymia in inflammatory bowel disease. A case-control study. *Psychother Psychosom* (1995) 64(1):49–53. doi:10.1159/000288990
36. Serrano J, Plaza V, Sureda B, de Pablo J, Picado C, Bardagi S, et al. Alexithymia: a relevant psychological variable in near-fatal asthma. *Eur Respir J* (2006) 28(2):296–302. doi:10.1183/09031936.06.00008105
37. Jula A, Salminen JK, Saarijarvi S. Alexithymia: a facet of essential hypertension. *Hypertension* (1999) 33(4):1057–61. doi:10.1161/01.HYP.33.4.1057
38. Salonen J, Johansson R, Joukamaa M. Alexithymia, depression and tinnitus in elderly people. *Gen Hosp Psychiatry* (2007) 29(5):431–5. doi:10.1016/j.genhosppsych.2007.05.002
39. Kleinjung T, Fischer B, Langguth B, Sand PG, Hajak G, Dvorakova J, et al. Validation of the German-version Tinnitus Handicap Inventory (THI). *Psychiat Prax* (2007) 34:S140–2. doi:10.1055/s-2006-940218
40. Newman CW, Jacobson GP, Spitzer JB. Development of the Tinnitus Handicap Inventory. *Arch Otolaryngol Head Neck Surg* (1996) 122(2):143–8. doi:10.1001/archotol.1996.01890140029007
41. Newman CW, Sandridge SA, Jacobson GP. Psychometric adequacy of the Tinnitus Handicap Inventory (THI) for evaluating treatment outcome. *J Am Acad Audiol* (1998) 9(2):153–60.
42. Wrzosek M, Szymiec E, Klemens W, Kotylo P, Schlee W, Modrzynska M, et al. Polish translation and validation of the Tinnitus Handicap Inventory and the Tinnitus Functional Index. *Front Psychol* (2016) 7:1871. doi:10.3389/fpsyg.2016.01871
43. Zeman F, Koller M, Langguth B, Landgrebe M, Tinnitus Research Initiative Database Study Group. Which tinnitus-related aspects are relevant for quality of life and depression: results from a large international multicentre sample. *Health Qual Life Outcomes* (2014) 12:7. doi:10.1186/1477-7525-12-7
44. Rejali D, Sivakumar A, Balaji N. *Ginkgo biloba* does not benefit patients with tinnitus: a randomized placebo-controlled double-blind trial and meta-analysis of randomized trials. *Clin Otolaryngol Allied Sci* (2004) 29(3):226–31. doi:10.1111/j.1365-2273.2004.00814.x
45. Bach M, Bach D, de Zwaan M, Serim M, Bohmer F. [Validation of the German version of the 20-item Toronto Alexithymia Scale in normal persons and psychiatric patients]. *Psychother Psychosom Med Psychol* (1996) 46(1):23–8.
46. Bagby RM, Parker JD, Taylor GJ. The twenty-item Toronto Alexithymia Scale – I. Item selection and cross-validation of the factor structure. *J Psychosom Res* (1994) 38(1):23–32. doi:10.1016/0022-3999(94)90005-1
47. Taylor GJ, Bagby RM, Parker JD. The 20-Item Toronto Alexithymia Scale. IV. Reliability and factorial validity in different languages and cultures. *J Psychosom Res* (2003) 55(3):277–83. doi:10.1016/S0022-3999(02)00601-3
48. Popp K, Schafer R, Schneider C, Brahler E, Decker O, Hardt J, et al. [Factor structure and reliability of the Toronto Alexithymia Scale (TAS-20) in the German population]. *Psychother Psychosom Med Psychol* (2008) 58(5):208–14. doi:10.1055/s-2007-986196
49. Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry* (1961) 4:561–71. doi:10.1001/archpsyc.1961.01710120031004
50. Hautzinger M. [The Beck Depression Inventory in clinical practice]. *Nervenarzt* (1991) 62(11):689–96.
51. Andersson G, Freijd A, Baguley DM, Idrizbegovic E. Tinnitus distress, anxiety, depression, and hearing problems among cochlear implant patients with tinnitus. *J Am Acad Audiol* (2009) 20(5):315–9. doi:10.3766/jaaa.20.5.5
52. Ooms E, Meganck R, Vanheule S, Vinck B, Watelet JB, Dhooge I. Tinnitus severity and the relation to depressive symptoms: a critical study. *Otolaryngol Head Neck Surg* (2011) 145(2):276–81. doi:10.1177/0194599811403381

53. Koch AS, Kleiman A, Wegener I, Zur B, Imbierowicz K, Geiser F, et al. Factorial structure of the 20-item Toronto Alexithymia Scale in a large sample of somatoform patients. *Psychiatry Res* (2015) 225(3):355–63. doi:10.1016/j.psychres.2014.12.013
54. Parker JD, Michael Bagby R, Taylor GJ, Endler NS, Schmitz P. Factorial validity of the 20-item Toronto Alexithymia Scale. *Eur J Personality* (1993) 7(4):221–32. doi:10.1002/per.2410070403
55. Parker JD, Taylor GJ, Bagby RM. Alexithymia and the recognition of facial expressions of emotion. *Psychother Psychosom* (1993) 59(3–4):197–202. doi:10.1159/000288664
56. Franz M, Popp K, Schaefer R, Sitte W, Schneider C, Hardt J, et al. Alexithymia in the German general population. *Soc Psychiatry Psychiatr Epidemiol* (2008) 43(1):54–62. doi:10.1007/s00127-007-0265-1
57. Beck AT, Steer RA, Carbin MG. Psychometric properties of the Beck Depression Inventory: twenty-five years of evaluation. *Clin Psychol Rev* (1988) 8(1):77–100. doi:10.1016/0272-7358(88)90050-5
58. Lumley MA, Neely LC, Burger AJ. The assessment of alexithymia in medical settings: implications for understanding and treating health problems. *J Pers Assess* (2007) 89(3):230–46. doi:10.1080/00223890701629698
59. Crocetti A, Forti S, Ambrosetti U, Bo LD. Questionnaires to evaluate anxiety and depressive levels in tinnitus patients. *Otolaryngol Head Neck Surg* (2009) 140(3):403–5. doi:10.1016/j.otohns.2008.11.036
60. Grabe HJ, Spitzer C, Freyberger HJ. Alexithymia and personality in relation to dimensions of psychopathology. *Am J Psychiatry* (2004) 161(7):1299–301. doi:10.1176/appi.ajp.161.7.1299
61. Bagby RM, Taylor GJ, Parker JD, Dickens SE. The development of the Toronto Structured Interview for Alexithymia: item selection, factor structure, reliability and concurrent validity. *Psychother Psychosom* (2006) 75(1):25–39. doi:10.1159/000089224
62. Grabe HJ, Lobel S, Dittrich D, Bagby RM, Taylor GJ, Quilty LC, et al. The German version of the toronto structured interview for alexithymia: factor structure, reliability, and concurrent validity in a psychiatric patient sample. *Compr Psychiatry* (2009) 50(5):424–30. doi:10.1016/j.comppsych.2008.11.008
63. Lysaker PH, Gumley A, Luedtke B, Buck KD, Ringer JM, Olesek K, et al. Social cognition and metacognition in schizophrenia: evidence of their independence and linkage with outcomes. *Acta Psychiatr Scand* (2013) 127(3):239–47. doi:10.1111/acps.12012
64. Semerari A, Carcione A, Dimaggio G, Falcone M, Nicolo G, Procacci M, et al. How to evaluate metacognitive functioning in psychotherapy? The metacognition assessment scale and its applications. *Clin Psychol Psychot* (2003) 10(4):238–61. doi:10.1002/cpp.362
65. Velotti P, Garofalo C, Petrocchi C, Cavallo F, Popolo R, Dimaggio G. Alexithymia, emotion dysregulation, impulsivity and aggression: a multiple mediation model. *Psychiatry Res* (2016) 237:296–303. doi:10.1016/j.psychres.2016.01.025
66. Dimaggio G, Vanheule S, Lysaker PH, Carcione A, Nicolo G. Impaired self-reflection in psychiatric disorders among adults: a proposal for the existence of a network of semi independent functions. *Conscious Cogn* (2009) 18(3):653–64. doi:10.1016/j.concog.2009.06.003

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Transition from Acute to Chronic Tinnitus: Predictors for the Development of Chronic Distressing Tinnitus

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Background: Acute tinnitus and its transition to chronic tinnitus are poorly investigated, and factors associated with amelioration *versus* exacerbation are largely unknown. Aims of this study were to identify early predictors for the future development of tinnitus severity.

Method: Patients with tinnitus of no longer than 4 weeks presenting at an otolaryngologist filled out questionnaires at inclusion (T1), as well as 3 (T3), and 6 months (T4) after tinnitus onset. 6 weeks after onset, an interview was conducted over the phone (T2). An audiogram was taken at T1, perceived tinnitus loudness, and tinnitus-related distress were assessed separately and repeatedly together with oversensitivity to external sounds and the levels of depression and anxiety. Furthermore, coping strategies with illness were recorded.

Results: Complete remission until T4 was observed in 11% of the 47 participants, while voiced complaints at onset were stable in the majority. In the subgroup with a relevant level of depression at T1, tinnitus-related distress worsened in 30% until T4. For unilateral tinnitus, perceived loudness in the chronic condition correlated strongly with hearing loss at 2 kHz on the tinnitus ear, while a similar correlation was not found for tinnitus located to both ears or within the head.

Conclusion: Results suggest early manifestation of tinnitus complaints, and stress the importance of screening all patients presenting with acute tinnitus for levels of depression and tinnitus-related distress. Furthermore, hearing levels should be monitored, and use of hearing aids should be considered to reduce tinnitus loudness after having ascertained that sound sensitivity is within normal range.

Keywords: recent-onset tinnitus, prospective study, acute-chronic transition, hearing impairment, depression, anxiety, coping with illness

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INTRODUCTION

Subjective tinnitus is an acoustic perception which is not caused by an external sound source, but by aberrant activation within the auditory system (1). This type of tinnitus is rather common, and because of its subjective nature, characteristics of the tinnitus are mostly derived from patients' reports. Tinnitus is usually associated with hearing loss (HL) as detected by pure tone audiometry,

but the perceived severity bears only weak to moderate relations with hearing thresholds and other psycho-acoustically determined features of the tinnitus, while high tinnitus-related distress is often associated with poor mental well-being (2–4). As tinnitus may severely impair life quality of affected individuals, it is crucial to identify factors that are predictive for the development of disabling tinnitus before it becomes a chronic condition.

There exists a multitude of cross-sectional studies on factors associated with chronic tinnitus (e.g., this Frontiers Topic), whereas only few studies have attempted to assess participants with acute tinnitus (5–7) including a study that investigated tinnitus patients one year after their first visit to a clinic if they entered the clinic within 6 months of tinnitus onset (8). Therefore, time course as well as mechanisms involved in the transition from the acute to the chronic condition are unknown, and factors predisposing for the development of a chronic, disabling, or decompensated tinnitus are mostly inferred from retrospective reports. Furthermore, studies on acute tinnitus usually were concerned with tinnitus following sudden HL (6, 7) or acute acoustic trauma (9), which appear to be distinct as indicated by high remission rates which may reach 70% (10). High remission rates concomitant with an incidence of HL are likely related to partial recovery of hearing function in the first weeks after a temporary threshold shift (11), but not all patients can relate tinnitus onset to worsening of their hearing. Therefore, remission rates may be lower and it may not be advantageous to wait for spontaneous remission but take action early on to prevent the development of a chronic disabling tinnitus.

Across many cross-sectional studies on chronic tinnitus, psychiatric comorbidity in particular depression is the most frequent condition for those with severe tinnitus (3, 4, 12, 13). Therefore, it has been suggested that pre-existent psychiatric comorbidity may foster decompensation (5), a notion that is corroborated by the few studies with early interventions, which suggest that psychological interventions may prevent the development of disabling tinnitus to some extent (5, 14–16). In contrast, tinnitus related to acute worsening of hearing thresholds may be susceptible to pharmacological interventions (17, 18). However, effectiveness of all these interventions is challenged because of unknown remission rates.

We set out to investigate the development of acute tinnitus within the 6 months after its first appearance, i.e., during transition from the acute to the chronic condition (19). As tinnitus is a phenomenon associated with the ears, patients experiencing it for the first time are likely to seek help by an otolaryngologist, at least in a country where the general health system allows them to do so without extra cost. Therefore, we aimed to investigate the history of tinnitus in patients seen by otolaryngologists within 4 weeks after tinnitus onset. Concerned with these patients the otolaryngologist has to decide on the type of treatment that is suitable for the particular patient knowing that some may lose their tinnitus and that most will not bother with it in the long run, while a low percentage will develop a distressing tinnitus that severely affects their quality of life. Because early interventions may prevent the acute symptoms to become a chronic disabling condition (5, 14–18), it is crucial to find out early which patients are at risk to develop a disabling chronic tinnitus, and which interventions may be suitable for the individual patient. To our knowledge, the present study represents the first attempt to assess in detail the characteristics of this tinnitus population including spontaneous recovery.

Factors to be investigated in relation to tinnitus were derived from the literature on chronic tinnitus and from our experience with acute tinnitus patients (5, 14). Particular focus on the instruments used was their applicability by otolaryngologists. In addition to pure tone audiometry, self-report measures on subjectively perceived tinnitus loudness and tinnitus-related distress were used in conjunction with screening instruments for the levels of depression and anxiety and a questionnaire on coping with illness.

Aims of the study are to

(1) describe characteristics of acute tinnitus patients that seek medical help from otolaryngologists.
(2) describe the time course of transition from the acute to the chronic condition.
(3) find out whether a high level of depression at tinnitus onset promotes development of a decompensated chronic tinnitus, and whether individuals at risk can be identified early on.
(4) identify maladaptive ways of coping with tinnitus,
(5) describe factors associated with spontaneous remission.

Preliminary data based on 28 participants have been published already (20).

MATERIALS AND METHODS

Procedure

Between 6/2013 and 4/2016, all first-time presenters with tinnitus at the Ear, Nose and Throat Clinic of the University Medical Centre Mannheim and at 4 participating ENT-practices in the region (see acknowledgements) were asked if they were willing to participate in the study. All willing to participate and meeting inclusion criteria were informed about aims and time required for the study. Inclusion criteria were first time tinnitus of no longer than 4 weeks, age above 18, and sufficient command of German. Exclusion criteria were neurological diseases and simultaneous psychological therapies. Participants were informed that in case they were developing distressing tinnitus, they would be assisted to contact a specialized outpatient care for tinnitus patients at the Central Institute of Mental Health, Mannheim. After completion of the study, those who wished a feedback were informed about their level of tinnitus-related distress. Study design and procedures were approved by the Ethics Committee II of Heidelberg University at the Medical Faculty Mannheim.

After giving written informed consent, the audiogram that was routinely taken was archived with the study documents. In addition, study participants filled out a comprehensive paper and pencil questionnaire with the instruments outlined below, and they gave the exact date when they first noticed their tinnitus (T1). Questionnaires were collected at a regular basis by the authors. A telephone interview was conducted 6 weeks after the recorded date of tinnitus onset (T2). During this assessment participants

could ask tinnitus-related questions. Hence, this interview can be seen as additional counseling which was received by each of the participants. At T2, the interviewer remembered each participant that two more questionnaires were going to be sent within 3 (T3) and 6 (T4) months after tinnitus onset. Questionnaires were accompanied by pre-stamped return envelopes. Patients who had not answered within 2 weeks after sending these questionnaires were contacted by phone, and asked to return the filled-in questionnaire.

Instruments

At T1, demographic information was collected, and participants were asked about date and circumstances of tinnitus onset. At all assessments, they answered questions regarding tinnitus laterality and character, aggravation, or alleviation in relation to first appearance and previous treatments. In addition, the self-report measures described below were administered.

Audiometry

At T1, a pure tone audiogram was taken separately for each ear according to general procedures in otolaryngology practices in Germany for the standard frequencies between 0.25 and 8 kHz. When no response to stimulation was evoked, the measurement was considered to be impossible, and for calculation, a value of 100 dB was used.

Outcome-Variables on Tinnitus Severity

Since clinically there appear to be significant differences between patients who focus on sensory aspects of the tinnitus and those who focus on functional disability and handicap (4, 21), we used separate measures for those two aspects. Tinnitus loudness as perceived by the participant (T-NRS) was recorded at T1, T3, and T4 on a Numeric Rating Scale [NRS (21)] with the anchors "heard only during silence" (0) and "louder than all other sounds" (10).

Tinnitus-related distress was assessed with the 12-item Tinnitus Questionnaire [Mini-TQ12 (22)]. According to Zeman et al. (23), the Mini-TQ12 shows satisfying psychometric results and is sensitive to changes in tinnitus severity. For the Mini-TQ12, each of 12 items may be answered with "true," "partially true," and "not true," scored as 2, 1, and 0, respectively. Tinnitus-related distress is the sum of points given to the 12 items and ranges from 0 (no distress) to 24 (maximal distress). According to Hiller and Goebel (22), 4 grades are discerned with grade 1 (0–7) representing no or mild distress and grade 2 (8–12) representing moderate distress. The range of 0–12 is also classified as compensated tinnitus, while decompensated tinnitus includes grades 3 (distressing tinnitus: 13–18), and 4 (severely distressing tinnitus: 19–24). In order to prevent a potential aggravation when asking for specific tinnitus-related consequences on life at T1, the Mini-TQ12 was used at T2, i.e., 6 weeks after tinnitus onset, for the first time and then again at T3 and T4. During the telephone interview at T2, the twelve Mini-TQ12 items were read to the participant together with the response options. Questions were repeated as needed.

As a second instrument to address tinnitus-related distress, we employed the Sheehan Disability Scale (SDS, 24) at T1, T3, and T4. In contrast to the Mini-TQ12, the SDS circumvents the potential risk of tinnitus aggravation. It assesses functional impairment in work/school (SDS1), social (SDS2), and family life (SDS3) on three 10 point visual analog scales that present numeric and verbal descriptive anchors in addition. There is no cutoff but scores of ≥5 on any of the scales; and high scores in general are associated with significant functional impairment. A SDS total sum score was not calculated, because not all participants worked and therefore not all reported a SDS1 score. The SDS was validated and shown to be sensitive to treatment-related changes in a variety of conditions (24–27) but has not been used in relation to tinnitus before.

Outcome Predictors

Outcome predictors were chosen according to the literature on chronic tinnitus and according to our experience with acute and chronic tinnitus patients.

Depression and Anxiety

Depression and anxiety are common comorbidities of disabling tinnitus (3, 4). Levels were assessed at T1, T3, and T4, with the freely available PHQ9 scale for depression and the GAD7 scale for generalized anxiety disorder (GAD) [(28), German (29)]. Both scales have been validated in primary care populations [PHQ9 (30); GAD7 (31)], there exist normative data for Germany (29, 32), and the scales have been used in tinnitus studies (4, 33). GAD is the most frequent anxiety disorder in primary care, and the GAD7 is sensitive also to other common forms of anxiety (29). Subjects were asked how often, during the past 2 weeks, they have been bothered, and response options for both scales were "not at all," "several days," "more than half the days," "nearly every day" scored as 0, 1, 2, and 3, respectively. PHQ9 scores range from 0 to 24, and GAD7 scores range from 0 to 21. In both scales, lower score points indicate favorable conditions, while sum scores ≥10 indicate potential problems in these fields, and scores of 15 or above in the PHQ9 scale are classified as Major Depression.

Coping with Illness

The way people cope with their tinnitus was shown to be related to adjustment to the tinnitus (34), and it may be improved by psychological interventions (5, 14, 35). Coping strategies with illness were assessed with the German-language Freiburg Questionnaire of Coping with Illness (FKV: 36) at T1, T3, and T4. This 35-item questionnaire covers the following coping strategies in 5 subscales: depressive coping, active problem-oriented coping, distraction and self-affirmation, religiousness and search for meaning, and trivialization and wishful thinking. Answers are scored on 5-point Likert scales with higher scores indicating a higher intensity of coping in a particular domain. Mean scores in subscales are used for analysis. The questionnaire has good internal consistency with a Cronbach's α of 0.68 and 0.77 for single scales (36) and has been used in relation to tinnitus (37).

Oversensitivity to External Sounds

As tinnitus is often associated with diminished sound-level tolerance or hyperacusis (38), oversensitivity to external sound was assessed on a 0–10 NRS (Hyper-NRS) with the extremes "normal" (0) and "extremely sensitive" (10) at T1, T3, and T4.

Statistics

Descriptive statistics, and tests for statistical significance were performed with SPSS version 24 (SPSS/IBM, Chicago, IL, USA). Changes of the tinnitus variables, sound sensitivity, and mental health factors during the study were monitored, and differences were tested for statistical significance with the Global Linear Model for repeated measures and Bonferroni corrected *post Hoc* tests in the case of normal distribution, or with non-parametric Friedman and *post hoc* Wilcoxon tests if the variables were not normally distributed. A p value below 0.05 was considered to be statistical significant, and a p below 0.01 was considered as statistically highly significant. Bivariate correlations were performed between predictor variables at T1 and outcome variables at T4. Since this was an exploratory study, bivariate p-values of correlations are presented along with an indication which p-levels achieve the Bonferroni-corrected significance level. Because 54 bivariate correlations were performed, the Bonferroni-corrected p-value to reach statistical significance was 0.0009. Step-wise regression analyses were performed separately for each of the 5 outcome variables. Predictor variables with significant correlations, or correlations reaching a Pearson correlation coefficient above 0.300 and an uncorrected p-value below 0.05 with an outcome variable were included in the final step-wise regression analysis for this outcome.

Participant Characteristics

Fifty-seven participants were included in the study, three were excluded because the time interval between tinnitus onset and T1 was longer than 4 weeks, and one participant was excluded for taking part in a psychotherapeutical tinnitus intervention in a tinnitus clinic during the study interval. Six participants dropped out, one obviously after his tinnitus was not present at T2 anymore and the others without notice, leaving 47 (82.5%) with complete data that contributed to the results. Their sociodemographic data are given in **Table 1**. Most lived in a long-term partnership, education standard was high and no one used a hearing aid.

TABLE 1 | Sociodemographic factors at T1.

	Persisting tinnitus ($N = 41$)	Remitting ($N = 5$) or fluctuating ($N = 1$) tinnitus
Sex (m/f)	53.2%/46.8%	83.3%/16.7%
Age in years [mean (SD)]	41.4 (15.6)	41.0 (14.8)
Range	21–79	23–61
Cohabitation		
Single	8.5%	
Cohabitating	91.5%	100%
Highest educational achievement		
None	2.1%	–
Basic	8.5%	33.3%
Middle	19.1%	–
University entrance	27.6%	33.3%
University degree	42.6%	33.3%
Occupational activities		
Working	74.5%	83.3%
Scholastic	10.6%	16.7%
Working in own household	6.4%	
Retired	6.4%	
Inability to work	4.3%	
Not working because of tinnitus	0%	
Other chronic health conditions		
No/yes/no statement	68.3%/29.3%/2.4%	66.7%/33.3%

TABLE 2 | Comparison of subgroups with persisting and remitting tinnitus.

Assessment at T1/T2	Persisting tinnitus ($N = 41$)	Remitting tinnitus ($N = 6$)
T-NRS	3.95 ± 1.66	3.17 ± 1.72
Mini-TQ12	8.32 ± 5.76	No tinnitus
Sheehan Disability Scale (SDS)1	3.82 ± 2.43	2.00 ± 2.0
SDS2	3.38 ± 2.15	2.00 ± 1.5
SDS3	3.60 ± 2.23	2.00 ± 2.10
Hyper-NRS	4.66 ± 2.77	3.00 ± 1.50
PHQ9	7.24 ± 4.76	3.83 ± 1.60
GAD7	5.66 ± 3.85	3.00 ± 2.50
Audiogram mean right ear	17.74 ± 9.65	15.77 ± 4.25
Audiogram mean left ear	19.51 ± 9.44	14.13 ± 2.22

Means and SDs are shown.

RESULTS

Potential underlying causes of the tinnitus were unknown to most participants. Tinnitus onset was associated with a reduction of hearing ability that was noticeable to the patient in 24%. Some presented with vertigo which was not the major complaint, however, and no one was diagnosed with Meniere's. Two participants had an ear infection. Of note is that most of the study participants did not have a very loud or very distressing tinnitus when seeking medical advice (**Table 2**).

Tinnitus Therapies during the Study

Therapies were prescribed by the ENT-physician and were independent of study participation except for psychological/psychotherapeutic therapies that let to exclusion. Of the six participants with remitting tinnitus, one took an antibiotic for middle ear infections, another two received corticosteroid infusions, and one took medication enhancing blood flow, while two did not undergo a tinnitus-specific therapy.

Of the 41 participants with persisting tinnitus, 23 underwent a tinnitus-specific therapy until T2, and an additional 2 participants had received a tinnitus-specific therapy until T4. Fourteen received corticosteroids *via* infusions or orally, 9 took a Gingko preparation, 4 were prescribed Arlevert or Dusodril and one received an antibiotic for middle ear infections. Two underwent acupuncture and 2 received physical therapy. No one was prescribed a hearing aid. Nine participants used several therapies in combination. Tinnitus onset appeared suddenly more often in the group that underwent a tinnitus-specific therapy (84%) as compared to those without (56%). Averages of T-NRS, SDS1-3, PHQ9, and GAD7 scores at T1 were higher in the group that underwent a tinnitus-specific therapy. Group differences and most importantly group differences in the reductions of tinnitus complaints between T1 and T4 did not reach statistical significance, however. Therefore, data of both groups were pooled.

Tinnitus Remission

Complete remission of the tinnitus until T4 occurred in 5 study participants, and tinnitus was absent at T2 and T3 in another one but returned until T4. One presented with a middle ear infection at T1 that was treated successfully with an antibiotic and the tinnitus had vanished until T2. Two reported sudden HL, they were treated with steroid infusions, their hearing recovered and the tinnitus was gone before T2. Reasons for remission in the others are unknown as well as for the fluctuation of tinnitus in another one for whom the tinnitus had reappeared at T4. Further characteristics of the group with tinnitus remission are shown in **Tables 1**–**3** and in **Figure 1**. Although average age was similar to the group with persisting tinnitus, maximum age was lower, tinnitus onset was more often associated with a noticeable HL, and a higher percentage localized their tinnitus to one ear as opposed to both ears or within the head (**Table 3**). Whereas perceived tinnitus loudness was similar to the group with persisting tinnitus, tinnitus-related distress and oversensitivity to sound were lower (**Table 2**), coinciding with personal statements regarding changes in tinnitus loudness and distress between onset and T1. No one with remitting tinnitus reported an increase in tinnitus loudness or distress. Most notable were also the lower levels of depression and anxiety (**Table 2**).

TABLE 3 | Comparison of tinnitus characteristics between groups with persisting and remitting tinnitus.

Tinnitus characteristics at T1	Persisting tinnitus ($N = 41$)	Remitting ($N = 5$) or fluctuating ($N = 1$) tinnitus
Days between onset and T1		
Mean ± SD	14.9 ± 8.5	7.7 ± 10.7
Range	1–28	2–29
Sudden onset	30 (73.2%)	3 (50%)
Subtle onset	11 (26.3%)	3 (50%)
Onset		
Associated with hearing loss (HL)	7 (17.1%)	3 (50%)
Not associated with HL	34 (82.9%)	3 (50%)
Permanent	28 (68.3%)	4 (66.7%)
Intermittent	13 (31.7%)	2 (33.3%)
Localization		
Right ear	10 (24.4%)	4 (66.7%)
Left ear	16 (39.0%)	1 (16.7%)
Both ears	10 (24.4%)	1 (16.7%)
Within head	5 (12.2%)	1 (16.7%)[a]
Change in perceived loudness between onset and T1		
None	28 (68.3%)	2 (33.3%)
Less loud	4 (9.8%)	4 (66.7%)
Louder	7 (17.1%)	0
No statement	2 (4.9%)	
Change in tinnitus-related distress between onset and T1		
None	19 (46.3%)	2 (33.3%)
Less distressing	3 (7.3%)	1 (16.7%)
More distressing	11 (26.8%)	
No statement	8 (19.5%)	3 (50%)

Differences between these groups exist for localization of the tinnitus, a higher frequency of sudden onset, a higher percentage with no change or increasing loudness and distress between onset and T1.

[a]*Same participant that experienced tinnitus on both ears.*

As data on tinnitus variables was not available for all time points for participants with remitting tinnitus, the following analyses on the progression on tinnitus loudness and distress was performed with the remaining 41 with persisting tinnitus until T4.

Acute Condition

For 73% with persisting tinnitus, the tinnitus had started suddenly whereas it began gradually in the remaining 27%, and 68% reported permanent tinnitus (**Figure 2**). Tinnitus onset coincided with notable HL in 17%, 26 localized tinnitus to either left (16) or right ear (10), whereas 15 heard it on both ears (10) or within the head (5). The tinnitus sound was characterized as whistling (49%), hissing (10%), complex (34%), or other (7%). Between onset and T1, tinnitus loudness had augmented in 17% and lessened in 10%, while tinnitus-related distress had worsened in 27% and lessened in 7%. T-NRS at T1 was 5 or less in 76%, and SDS scores were 5 or below in all scales in 63% of the study participants, while 15% reported a score above 5 in all scales. At T2, 30% presented with a decompensated tinnitus indicated by a sum score of 13 or above in the Mini-TQ12.

Hearing Loss

Audiograms were available from 39 participants. In 9 thresholds did not exceed 20 dB in any of the measured frequencies, 20 had mild to moderate HL exceeding 20 dB, 9 presented with severe HL exceeding 50 dB in at least one frequency on one ear, and one presented with single sided deafness. In unilateral tinnitus, HL was more pronounced at the tinnitus ear, and HL differences were smaller for frequencies below 2 kHz but larger for frequencies above 6 kHz as compared to bilateral tinnitus (**Figure 1**).

Change of Tinnitus Complaints during Study Period

Almost 90% of the study participants developed a chronic tinnitus. At each assessment, participants were asked whether their tinnitus had changed since onset, and until T4 about half experienced stable loudness and distress, while increases were reported by 7 and 15%, respectively (**Table 4**). Localization and sound quality remained essentially constant, but time of awareness lessened considerably from 68% with permanent tinnitus at T1 to 29% at T4.

In contrast, average T-NRS did not change significantly during the study period (mean ± SD: T1: 3.95 ± 1.66/T4: 3.44 ± 1.98), and therefore, initial reports at least of those who retain their tinnitus are likely to reflect perceived loudness in the chronic state. There exists no estimate for a significant clinical change for the NRS-loudness estimate, but one can assume that a change of 3 points or 27% on the 0–10 NRS represents a noticeable change. Applying this criterion, two participants experienced significant worsening of tinnitus loudness between T1 and T4, while it lessened significantly in 6 and remained stable in a majority of 80.5%. In contrast, subjective reports (**Table 4**) indicated 3/18 with noticeably increased/decreased loudness. Thus 12 participants thought that their tinnitus loudness had decreased since tinnitus onset despite indicating a reduction of less than 3 on the T-NRS scale, and one experienced an increase

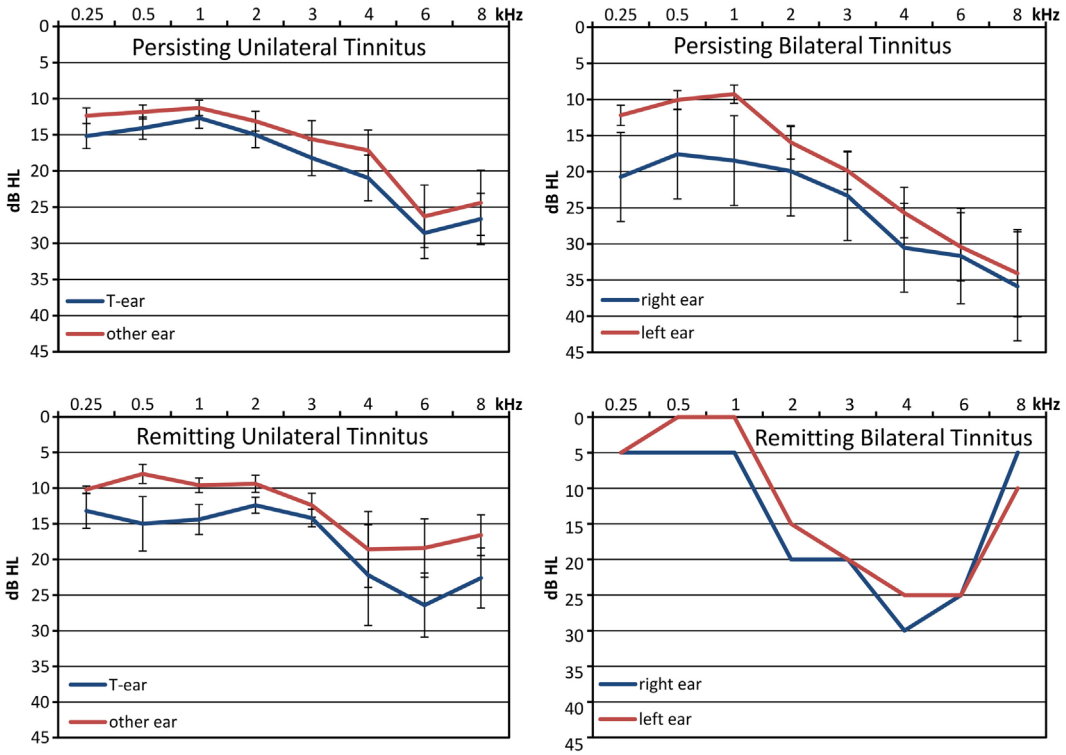

FIGURE 1 | Pure Tone Audiograms for groups with persisting and remitting, unilateral and bilateral tinnitus: shown are means with respective standard errors. Unilateral tinnitus: tinnitus heard from one ear, bilateral tinnitus: tinnitus heard on both ears or within the head. An audiogram was not available from one individual with persisting unilateral tinnitus and from another one with persisting bilateral tinnitus.

in tinnitus loudness despite a difference of less than 3 points on the T-NRS scale. Therefore, a change of 3 points appears to be a rather conservative measure for a relevant change of tinnitus loudness.

Reduction of tinnitus-related distress was statistically significant with both assessment instruments, the SDS and the Mini-TQ12. Between T1 and T4, SDS1 reduced from 3.82 ± 2.43 to 2.69 ± 2.56 ($\chi^2 = 11.642$; $p = 0.003^{**}$); SDS2 reduced from 3.38 ± 2.15 to 2.29 ± 2.45 ($\chi^2 = 16.487$; $p = 0.004^{**}$), and SDS3 reduced from 3.60 ± 2.23 to 2.37 ± 2.46 ($\chi^2 = 13.795$; $p = 0.001^{**}$). Average scores in the Mini-TQ12 were 8.32 ± 5.76 at T2 and 6.80 ± 5.65 at T4 ($F = 4.425$; $p = 0.015^{*}$). *Posthoc* tests with Bonferroni correction revealed that for the 3 SDS scales reductions between T1 and T3 and between T1 and T4 were highly significant, and for the Mini-TQ12 a significant decrease was present between T2 and T4. Average reductions between first and last assessment were small, however (SDS1: 1.2 ± 1.92; SDS2: 1.05 ± 2.10; SDS3: 1.20 ± 2.09, Mini-TQ12: 1.63 ± 3.55). As for the T-NRS scale, a reduction of 3 points in the SDS scales may be seen as a clinically relevant difference. According to this criterion, tinnitus complaints remained stable in 80% (SDS1), 73% (SDS2), and 75% (SDS3), while reduction of complaints between T1 and T4 was reported by 20%/25%/23% and worsening was reported by one individual for each of the SDS2 and SDS3 scales. According to the literature, a reduction of at least 5 points or 21% on the 0–24 point Mini-TQ12 may be seen as a criterion for a relevant decrease on this 24 point scale (20, 39). According to this criterion, the Mini-TQ12 score worsened significantly in 10% and ameliorated in 20%, while responses to the question whether tinnitus-related distress had changed since onset were improvement for 37%, and worsening for 15% (**Table 4**).

All instruments indicate, that tinnitus-related distress is stable for the majority within a short time interval following onset, and although average reductions were statistically significant, clinically relevant changes were seen only in few individuals. Regarding a detection of relevant changes, assessments with the SDS scales were the most conservative measure. Also, a 5 point increase in the Mini-TQ12 appears to be a rather conservative criterion for a relevant change, but it constituted a more sensitive instrument for the detection of change in tinnitus-related distress than the SDS scales, and measures derived from this questionnaire can be compared across studies.

Predictors of Chronic Tinnitus Severity

In order to find potential predictors for chronic tinnitus-related distress at T4, bivariate correlations were calculated for all predictor variables at T1 with the outcome variables at T4. For unilateral tinnitus, HL at 2 kHz on the tinnitus ear showed a strong correlation with T-NRS at T4 ($r = 0.626$; $p = 0.001$), while this was

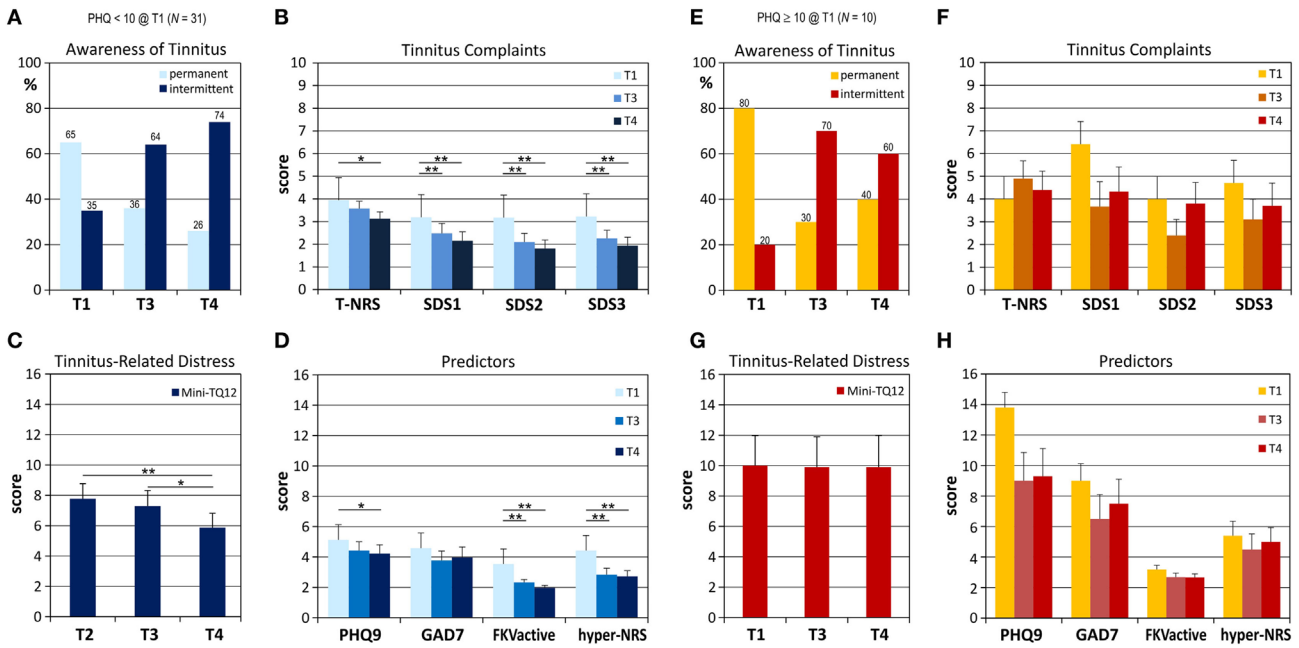

FIGURE 2 | Development of tinnitus complaints and variables that were identified as predictors for future tinnitus intensity for the group with low to moderate levels of depression [**(A–D)**, blue columns], and the group with high levels of depression, [**(E–H)**, orange-red bars]. Means and respective SEs are shown. **(A,E)** Awareness of the tinnitus, **(B,F)** perceived tinnitus loudness (T-NRS), and distress as assessed by the Sheehan scales (SDS), and **(C,G)** with the Mini-TQ12. **(D,H)** Predictor variables are levels of depression (PHQ9) and anxiety (GAD7), active coping with illness (FKVactive) and oversensitivity to sound (hyper-NRS). Values for all tinnitus measures dropped in the group with low PHQ9 scores at T1 with all reductions reaching statistical significance, while there was no significant decrease for these factors in the group with high depression levels. Also, scores of all predictor variables dropped significantly in the former while any reduction observed in the latter group did not reach statistical significance between assessments. $^{*}p < 0.05$; $^{**}p < 0.01$.

TABLE 4 | Response to question whether tinnitus had changed since onset.

		Change since onset			
		T1	**T2**	**T3**	**T4**
Perceived Loudness	None	28 (68.3%)	17 (41.5%)	20 (48.8%)	20 (48.8%)
	More	7 (17.1%)	3 (7.3%)	3 (7.3%)	3 (7.3%)
	Less	4 (9.8%)	19 (46.3%)	18 (43.9%)	18 (43.9%)
	No statement	2 (4.9%)	2 (4.9%)	–	–
Perceived distress	None	19 (46.3%)	15 (36.6%)	23 (56.1%)	20 (48.8%)
	More	11 (26.8%)	4 (9.8%)	4 (9.8%)	6 (14.6%)
	Less	3 (7.3%)	19 (46.3%)	11 (26.8%)	15 (36.6%)
	No statement	8 (19.5%)	3 (7.3%)	3 (7.3%)	–
Change in hearing if tinnitus onset was associated with hearing impairment	None		21 (51.2%)	12 (29.3%)	10 (24.4%)
	Better		7 (17.1%)	4 (9.8%)	2 (4.9%)
	Worse		2 (4.9%)	–	1 (2.4%)
	No statement		11 (26.8%)	25 (61%)	28 (68.3%)

not evident for the whole sample if HL was averaged across ears for those with bilateral tinnitus or tinnitus localized to the head. Bivariate correlations did not reach the Bonferroni-corrected p-level for significance ($p = 0.0009$) except for the correlations between the SDS scales at T1 and T4, and for the Mini-TQ12 at T2 and T4, (**Table 5**). These findings indicated that tinnitus complaints shortly after onset are potential predictors for the severity of chronic complaints. Furthermore, PHQ9, GAD7, hyper-NRS, FKVactive, and FKVdistraction at T1 exhibited correlation levels with outcome variables at T4 (**Table 5**) that were strong enough to be considered for the regression analysis.

Separate stepwise regression analyses for each of the outcome variables and the predictors with sufficiently strong correlations showed that the level of complaints at T1 in each scale was a good predictor for scores in that scale at T4. Mini-TQ12 scores at T4 were explained to 70% by the factors Mini-TQ12 at T2 and PHQ9 at T1. For Tinnitus loudness, the factors T-NRS, FKVactive, and PHQ9 all at T1 explained 50% of the variance. Scores in the

TABLE 5 | Pearson correlation coefficients (*r*) for bivariate correlations between predictor variables at T1 and outcome variables at T4 are shown together with *p*-values.

Predictors	Outcomes				
	T4-T-NRS	**T4-SDS1**	**T4-SDS2**	**T4-SDS3**	**T4-Mini-TQ12**
Same variable at T1 or T2, respectively					
r	**0.488**	**0.730**	**0.595**	**0.610**	**0.807**
p	**0.001**	**<0.0001***	**<0.0001***	**<0.0001***	**<0.0001***
T1 hyper-NRS					
r	0.252	**0.425**	**0.324**	**0.331**	**0.355**
p	0.112	**0.010**	**0.039**	**0.035**	**0.023**
T1 PHQ9					
r	**0.342**	**0.396**	**0.371**	**0.347**	**0.459**
p	**0.029**	**0.017**	**0.017**	**0.026**	**0.003**
T1 GAD7					
r	**0.366**	**0.352**	**0.366**	**0.360**	**0.466**
p	**0.019**	**0.035**	**0.019**	**0.021**	**0.002**
T1 FKVdepressive					
r	0.051	0.047	−0.18	0.044	0.221
p	0.757	0.787	0.913	0.786	0.170
T1 FKVactive					
r	**0.424**	**0.397**	**0.407**	**0.424**	**0.378**
p	**0.006**	**0.018**	**0.009***	**0.006**	**0.016**
T1 FKVdistraction					
r	**0.390**	**0.381**	0.306	**0.321**	0.231
p	**0.013**	**0.024**	0.055	**0.043**	0.152
T1 FKV search for meaning					
r	0.104	0.177	−0.030	0.041	0.066
p	0.521	0.309	0.852	0.801	0.687
T1 FKV trivialization					
r	0.203	0.074	0.037	0.043	0.212
p	0.208	0.673	0.821	0.793	0.189

*As this was an exploratory study, variables with a p-value below 0.05 and a correlation coefficient r above 0.300 (bold letters) were included in the step-wise regression analysis for the respective outcome variable. The Bonferroni-corrected p-value indicating statistical significance was *p = 0.0009.*

TABLE 6 | Results of step-wise regression analysis.

Predictor at T1	Outcome at T4				
	T-NRS	**SDS1**	**SDS2**	**SDS3**	**Mini-TQ12**
Explained variance	50%	53%	35%	49%	70%
R^2/adj R^2	0.497/0.455	0.527/0.509	0.354/0.337	0.485/0.457	0.695/0.678
F	11.841	30.026	20.819	21.483	42.111
Same variable at T1/T2					
Stand. $beta_{in}$	0.444	0.726	0.595	0.540	0.734
p	0.001**	<0.001**	<0.001**	<0.001**	<0.001**
T1 PHQ9					
Stand. $beta_{in}$	0.283				0.218
p	0.024*				0.030*
T1 FKVactive					
Stand. $beta_{in}$	0.376			0.350	
p	0.003**			0.007**	

*Stepwise linear regression analysis was performed separately for each of the outcome variables. Variables included in the analysis were those with sufficient bivariate correlations of r > 0.300 and a p < 0.05 with the respective outcome in the bivariate analyses as shown in **Table 5**. For the regression analysis significant predictors (*p < 0.05; **p < 0.01) together with the standardized beta values are shown for each of the outcome variables.*

SDS1 scale at T4 were explained to 53% the SDS1 score at T1, whereas the SDS2 score at T4 was explained by 35% by the SDS2 score at T1, and the SDS3 score at T4 was explained by 49% by the SDS3 score and the FKVactive score at T1 (**Table 6**). Thus, tinnitus-related distress can be foreseen in the acute state, and best predictors were the Mini-TQ12 and the PHQ9.

Groups with High versus Low Depression Scores

One of our initial hypotheses was that a high level of depression at tinnitus onset promotes development of a decompensated chronic tinnitus. We therefore compared the group with low level of depression, i.e., a PHQ9 score below 10 at T1 ($N = 31$) and the group with conspicuous levels of depression at T1 ($N = 10$), and found that a high level of depression around tinnitus onset allows a distinction between problematic and unproblematic tinnitus at T4. Tinnitus symptoms were lower and reduced significantly during the study interval in the group with low depression scores at T1, while they remained unchanged for the group with PHQ9 scores of 10 or above (**Figure 2**). In addition, GAD7 scores and hypersensitivity to external sounds was more pronounced in the latter group, and members of the group with high depression scores were more likely to undergo a tinnitus-specific therapy during the study interval (70%) compared to the group with an inconspicuous PHQ9 score (58%).

DISCUSSION

Aim of the present study was to describe the transition from acute to chronic tinnitus, identify factors associated with remission of the tinnitus and to identify predictors for the development of chronic disabling tinnitus. To our knowledge, this is the first study that aims to investigate individuals with a first episode of tinnitus who seek medical help from private practices of otolaryngologists. Former studies investigated tinnitus sufferers that were hospitalized for the treatment of sudden HL (6, 7), took part in a psychological therapy for acute tinnitus (14), or army personal developing tinnitus shortly after exposure to high noise levels (9).

Study Sample

The characteristics of our study sample suggest that patients seeking help from otolaryngologists for acute tinnitus are not necessarily the ones with high tinnitus-related distress or loud tinnitus as indicated by the rather moderate averages of these measures in comparison to those assessed with the same instruments in a large number of subjects with chronic tinnitus (4: T-NRS: 6.0 ± 2.5; Mini-TQ12: 10.4 ± 6.4). Averages are comparable, however, to those found by earlier studies with acute tinnitus patients (5, 6, 17). The authors of one study (17) assume that tinnitus questionnaires may underestimate tinnitus-related distress in acute patients because they were developed for the chronic condition. Alternatives which are not mutually exclusive are that patients with high tinnitus-related distress consult other specialists, or that tinnitus-related distress increases over time. Another observation was that not many patients consult an otolaryngologist early after tinnitus onset. This is in line with a former report, stating that the majority seeks medical help only years after tinnitus onset [(40); Lockwood et al. (41)], and with the results of a recent review on the literature (12). Of note is also that average scores of depression and anxiety are similar to those found with the same instruments for a large sample with chronic tinnitus [(4): PHQ9: 7.1 ± 5.5; GAD7: 6.0 ± 4.8] which is considerably higher than in the German normative samples [(32): PHQ9: 2.9 ± 3.5; 29: GAD7: 3.0 ± 3.4].

In the present sample, average age is 41.4 ± 15.6 years in the group with persisting tinnitus and 41.0 ± 14.8 years in the group with remitting tinnitus. This is lower than in a sample with tinnitus following sudden HL [(6): 47.3 years, range 19–78], but comparable to the average of a large group with chronic tinnitus who retrospectively reported age at tinnitus onset to be 42.4 ± 13.5 years (42). In that report, study participants with later tinnitus onset experienced a more distressing tinnitus right from the beginning (42). Whereas in a preliminary analysis of the present study with the data of 28 participants (20), we also found a significant correlation of moderate strength between age at tinnitus onset and tinnitus-related distress, we could not confirm this finding for the Mini-TQ12, the instrument used by Schlee et al. (42), in this larger sample, but found a stronger correlation between age at onset and tinnitus-related distress as assessed by the SDS2 and SDS3 scales at T4. In addition, we found strong correlations between age at onset and HL at frequencies of 3 kHz and above (r: 0.384–0.718). While prevalence of HL increases with age, it may go undetected by the affected individuals (43) and it is not known whether the sample investigated by Schlee et al. (42) had hearing levels within the normal range at tinnitus onset. HL increases the risk for developing tinnitus, and tinnitus-related distress may be augmented by the distress the participant experiences because of the accompanying HL (42).

Tinnitus Onset, Remission, and Transition to Chronic Condition

Remission rate was about 11% in the current study, it occurred more often in individuals who developed tinnitus concomitant with a noticeable HL, and remission usually occurred during the first weeks after onset, resembling the time course of recovery of hearing after incidences of acute HL (7, 11). The majority of our sample did not experience worsening of hearing concomitant with tinnitus onset, but this percentage is higher in the group with complete remission. Higher remission rates are commonly reported in studies with tinnitus in response to acute auditory insults (9), likely because of recovery after acute acoustic insult (11). Furthermore, hearing recovery appears to be age- and frequency dependent, as it is more complete in younger individuals and at frequencies below and above 4 kHz (44). As acute temporary threshold shifts are known to recover to some extent resulting in a less severe permanent threshold shift (11), tinnitus remission is more likely if its appearance is associated with acute worsening of hearing level. In animal models, threshold shifts of up to about 50 dB immediately after a single-noise exposure may recover completely, and recovery has been reported for periods extending up to 3 weeks. But even with complete recovery of hearing threshold large numbers of synapses between hair cells and primary afferent neurons may be lost resulting in hearing deficits that are not necessarily detected by audiometry (11).

Recovery in hearing seems to precede decreases in tinnitus loudness or tinnitus remission (45), and complete remission sets in earlier, i.e., by day 7, and benefits a significantly higher share of patients with mild-moderate HL as compared to those with more

profound HL in whom remission was observed in a period of up to 30 days. Also, hearing impairment and tinnitus in response to an ear infection tend to recover completely (46). Interestingly, the ones with remitting tinnitus in our sample indicated at T1 that loudness or annoyance of their tinnitus had decreased since onset suggesting a gradual recovery of hearing thresholds and reduction of tinnitus severity soon after tinnitus onset. Mühlmeier et al. (7) report that tinnitus remission lagged complete hearing recovery, and that complete hearing recovery and complete tinnitus remission were not independent of each other confirming the association between both factors. Taken together, data suggest that remission can be expected with substantial recovery of hearing threshold, for instance after incidences of sudden HL or acoustic trauma while remission is not to be expected if tinnitus onset was not associated with a recent incidence of HL.

Most pronounced was the reduction of tinnitus awareness between T1 and T3. It did not necessarily result in a less distressing tinnitus, however. 59% retained the level of tinnitus-related distress experienced at T2 until T4, while some habituated as indicated by lower distress 6 months after onset. About 10% reported significant worsening of tinnitus-related distress during the study. Hence, tinnitus-related distress reported in the acute condition is likely to be representative for the chronic condition. This finding is corroborated by the results of the regression analysis. Similarly, perceived loudness of the tinnitus was stable between T1 and T4 in most of the study participants, which is no surprise since for most tinnitus onset was not related to worsening of the hearing. Taken together, tinnitus remission occurred early, but the tinnitus remained in 90%, and tinnitus complaints did not change in the majority. Therefore, initial complaints about high tinnitus-related distress and perceived loudness require immediate therapeutic action.

The time course of transition from the acute to the chronic condition is not entirely clear. Whereas, some define duration of up to 3 months as subacute and a tinnitus lasting at least 6 months as being chronic (12), others assume the chronic condition is reached already after 3 months (19). Assessments in the present study were chosen to investigate these time intervals and together with studies on spontaneous remission after sudden HL (7) indicate that chronification of the tinnitus is taking place during the first days or weeks after onset. This is nicely shown by an interventional study (18) reporting that adults with unilateral tinnitus who are treated with intratympanic dexamethasone or saline both together with oral gingko biloba, the latter thought to be a placebo treatment, reduced awareness of the tinnitus within a month of tinnitus onset, and also led to reductions of tinnitus loudness and annoyance within the following 4 weeks irrespective of the treatment. In contrast, Holgers et al. (8) report that between the first consultation which took place during the first 6 months after tinnitus onset and the endpoint of the study one year later, tinnitus symptoms decreased in 75% of the study participants, whereas the rest had symptoms that did not decrease over time.

Hearing Loss As a Predictive Factor

Hearing loss has been identified as the most relevant etiologic factor and as the probable trigger for tinnitus, although non-auditory factors may ameliorate or worsen the tinnitus (47). In the present sample 25% of those with unilateral tinnitus and 20% with bilateral tinnitus did not have an overt HL in the standard audiogram indicated by thresholds not exceeding 20 dB at any frequency. For those with unilateral tinnitus, thresholds in the affected ear were worse for the tinnitus ear which is in line with findings for patients with idiopathic sensorineural HL (48). Noteworthy, in our sample is the high correlation between HL at 2 kHz and future tinnitus loudness which has not been described before. In accordance, Mühlmeier et al. (7) report that tinnitus loudness 3 months after tinnitus onset was statistically significantly correlated with the level of PTA both at baseline and at 3 months.

Reasons why a tinnitus was heard despite normal hearing thresholds might be due to HL in frequencies above 8 kHz as seen in a recent study in patients suffering from acute tinnitus (49). Furthermore, tinnitus in normal hearing subjects may be due to cochlear pathologies undetected by the audiogram (11), or to a less well functioning efferent auditory system (50).

Depression and Anxiety As Predictive Factors

Variables with notable correlations with future tinnitus severity were the levels of depression and anxiety at T1. In the group with high levels of depression at T1, tinnitus loudness and distress did not diminish with time, but even augmented in most of those fulfilling the criterion for Major Depression. High levels of depression have been associated with chronic disabling tinnitus (4). Holgers et al. (8) report that above all symptoms of depression and anxiety together with HL and physical immobility are predictors for severity of the tinnitus, and high depression levels were not observed in any one of their patients that showed tinnitus remission.

In our sample, we find significant reductions of tinnitus-related distress, loudness, and the level of depressive symptoms only in the group with low levels of depression at T1, while neither factor reduced during the study interval if PHQ9 scores at T1 indicated conspicuous problems with depression. In addition, we found a significant correlation between tinnitus-related distress at onset and chronic tinnitus-related distress. This supports the notion, that high levels of tinnitus-related distress in particular in combination with high levels of depressive symptoms are indicators for the potential development of incapacitating tinnitus. Therefore, it is indicated to assess tinnitus-related distress in acute tinnitus patients, and this should always be accompanied by screening of the level of depression. As an additional measure, the level of anxiety could be assessed, as levels of depression and anxiety show strong correlations and it was found that during transition, for instance in the course of chronification, levels of anxiety show a stronger correlation with tinnitus symptoms than indicators of depression [(8, 17, 51)—this Frontiers Topic].

Data indicate the importance of interventions already during the acute phase of tinnitus, as previously suggested (14). Effects of such interventions should be more pronounced in acute tinnitus associated with high levels of depression. Therefore, we

consider early psychological/psychotherapeutic intervention to be important to prevent the development of a decompensated chronic tinnitus that severely impairs life quality of affected individuals.

Active Coping with Tinnitus Is Maladaptive

Results of the regression analysis indicate that active coping is maladaptive in regard to tinnitus. Active coping with the tinnitus reduces significantly in the group with an inconspicuous level of depression at T1, while no reduction is found in the group with high levels of depression. It therefore appears that those with psychological problems tend to adhere to this strategy.

Limitations

Some limitations of the study should be named. As in the other few longitudinal studies, the number of participants is low. Furthermore, because measures on tinnitus-loudness and distress are not available on a large-scale for the acute condition, it cannot be decided, whether our sample is representative for the acute tinnitus population, but these measures may rather serve as a start to describe acute tinnitus patients.

Only few individuals appear to consult an otolaryngologist shortly after noticing the appearance of a tinnitus (40), and nowadays acute tinnitus patients may better be contacted via internet sources as suggested by a recent publication [(52), this Frontiers Topic]. An alternative which is not mutually exclusive is that perceived tinnitus loudness and tinnitus-related distress are mild at first but may increase over the life span. An increase of tinnitus loudness is likely because with advancing age most suffer from presbyacusis, and it has been shown that tinnitus loudness rather increases with age. An increase of tinnitus-related distress is not self-explicable as many of those with long-existing tinnitus report adjustment to the tinnitus and experience a decrease of tinnitus-related distress (4). It may be possible, however, that tinnitus-related distress increases for instance in individuals with high levels of depression or anxiety.

Because perceived tinnitus loudness and tinnitus-related distress were both low to moderate at T1/T2 reductions of tinnitus loudness and distress may not have been detected. We used the GAD7 for screening anxiety as it is a short freely available and validated instrument for screening GAD and was reported to perform almost as well for detecting other types of common anxiety disorders (29). A very recent publication of the same group (53) suggests that the first four items discriminate better than the last three items with respect to latent anxiety, and therefore suggest to use a 2item GAD version (GAD2) which will be even more time-economic. Nonetheless, we decided to report the sum scores of the GAD7 as they have been reported for a normative sample (29) and in former tinnitus studies (4, 33) but suggest to monitor the literature regarding further developments.

CONCLUSION

(1) Patients that present with acute tinnitus at otolaryngology practices are not a homogenous group in which psychological aspects are important to predict future progression of the tinnitus. Therefore, mental health should be assessed early on, for instance with the readily available PHQ9 and GAD7 questionnaires used in this study.
(2) If levels of depression or anxiety are elevated, patients should be referred to specialists treating these conditions.
(3) Results support early manifestation of tinnitus-related distress, and that development of disabling tinnitus is fostered by poor mental health.

Therefore, we suggest to screen depression symptoms and tinnitus-related distress of all tinnitus patients presenting with acute or subacute tinnitus, and to refer them to specialist evaluation and therapy if indicated. Furthermore, we suggest to consider provision with hearing prostheses, although it has to be assured first that a potentially existing oversensitivity to sounds, has reversed when using hearing aids.

ETHICS STATEMENT

Study design and procedures were approved by the Ethics Committee II of Heidelberg University at the Medical Faculty Mannheim (2013-541N-MA).

AUTHOR CONTRIBUTIONS

EW-F designed the study, collected part of the data, analyzed the data, and wrote the manuscript. RD interpretation of data, AG collected and analyzed part of the data, WD designed the study, JS critical review, KH critical review, IR recruitment and interpretation of data.

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REFERENCES

1. Eggermont JJ, Roberts LE. The neuroscience of tinnitus: understanding abnormal and normal auditory perception. *Front Syst Neurosci* (2012) 6:53. doi:10.3389/fnsys.2012.00053
2. Hiller W, Goebel G. Factors influencing tinnitus loudness and annoyance. *Arch Otolaryngol Head Neck Surg* (2006) 132:1323–30. doi:10.1001/archotol.132.12.1323
3. Langguth B, Kreuzer PM, Kleinjung T, De Ridder D. Tinnitus: causes and clinical management. *Lancet Neurol* (2013) 12:920–30. doi:10.1016/S1474-4422(13)70160-1

4. Wallhäusser-Franke E, Brade J, Balkenhol T, D'Amelio R, Seegmüller A, Delb W. Tinnitus: distinguishing between subjectively perceived loudness and tinnitus-related distress. *PLoS One* (2012) 7:e34583. doi:10.1371/journal.pone.0034583
5. D'Amelio R, Archonti C, Scholz S, Falkai P, Plinkert PK, Delb W. Psychological distress associated with acute tinnitus. *HNO* (2004) 52:599–603. doi:10.1007/s00106-003-0944-5
6. Langenbach M, Olderog M, Michel O, Albus C, Köhle K. Psychosocial and personality predictors of tinnitus-related distress. *Gen Hosp Psychiatry* (2005) 27:73–7. doi:10.1016/j.genhosppsych.2004.08.008
7. Mühlmeier G, Baguley D, Cox T, Suckfüll M, Meyer T. Characteristics and spontaneous recovery of tinnitus related to idiopathic sudden sensorineural hearing loss. *Otol Neurotol* (2016) 37:634–41. doi:10.1097/MAO.0000000000001081
8. Holgers KM, Zöger S, Svedlund K. Predictive factors for development of severe tinnitus suffering – further characterisation. *Int J Audiol* (2005) 44:584–92. doi:10.1080/14992020500190235
9. Bonfort G, Billot D, Trendel D, Salf E, Lindas P, Barberot JP. Acute acoustic trauma, a retrospective analysis about 225 military cases. *Rev Laryngol Otol Rhinol (Bord)* (2014) 135:25–31.
10. Weinaug P. Spontaneous remission in sudden deafness. *HNO* (1984) 32:346–51.
11. Ryan AF, Kujawa SG, Hammill T, Le Prell C, Kil J. Temporary and permanent noise-induced threshold shifts: a review of basic and clinical observations. *Otol Neurotol* (2016) 37:e271–5. doi:10.1097/MAO.0000000000001071
12. Altissimi G, Salviati M, Turchetta R, Orlando MP, Greco A, De Vincentiis M, et al. When alarm bells ring: emergency tinnitus. *Eur Rev Med Pharmacol Sci* (2016) 20:2955–73.
13. Dobie RA. Depression and tinnitus. *Otolaryngol Clin North Am* (2004) 36:383–8. doi:10.1016/S0030-6665(02)00168-8
14. Delb W, D'Amelio R, Boisten CJ, Plinkert PK. Evaluation of the tinnitus retraining therapy as combined with a cognitive behavioral group therapy. *HNO* (2002) 50:997–1004. doi:10.1007/s00106-002-0645-5
15. Gerhards F, Brehmer D. Distraction and relaxation training in acute tinnitus: effects of a complement to otorhinolaryngological treatment. *HNO* (2010) 58:488–96. doi:10.1007/s00106-009-2019-8
16. Nyenhuis N, Zastrutzki S, Weise C, Jäger B, Kröner-Herwig B. The efficacy of minimal contact interventions for acute tinnitus: a randomised controlled study. *Cogn Behav Ther* (2013) 42:127–38. doi:10.1080/16506073.2012.655305
17. Schildt A, Tönnies S, Böttcher S. Inpatient infusion treatment for acute tinnitus with and without adjuvant psychotherapeutic intervention. A comparison of psychological effectivenes. *HNO* (2006) 54:781–91. doi:10.1007/s00106-006-1447-y
18. Shim HJ, Lee ES, An YH, Kim DH. Comparison of long-term outcome of intratympanic dexamethasone therapy between acute noise-induced tinnitus and acute idiopathic tinnitus. *J Int Adv Otol* (2017) 13:53–60. doi:10.5152/iao.2016.2632
19. Zenner HP, Delb W, Kröner-Herwig B, Jäger B, Peroz I, Hesse G, et al. On the interdisciplinary S3 guidelines for the treatment of chronic idiopathic tinnitus. *HNO* (2015) 63:419–27. doi:10.1007/s00106-015-0011-z
20. Wallhäusser-Franke E, Repik I, Delb W, Glauner A, Hörmann K. Long-term development of acute tinnitus. *Laryngorhinootologie* (2015) 94:759–69. doi:10.1055/s-0035-1550039
21. Meikle MB, Stewart BJ, Griest SE, Henry JA. Tinnitus outcomes assessment. *Trends Amplif* (2008) 12:223–35. doi:10.1177/1084713808319943
22. Hiller W, Goebel G. Rapid assessment of tinnitus-related psychological distress using the mini-TQ. *Int J Audiol* (2004) 43:600–4. doi:10.1080/14992020400050077
23. Zeman F, Koller M, Schecklmann M, Langguth B, Landgrebe M. Tinnitus assessment by means of standardized self-report questionnaires: psychometric properties of the tinnitus questionnaire (TQ), the tinnitus handicap inventory (THI), and their short versions in an international and multi-lingual sample. *Health Qual Life Outcomes* (2012) 10:128. doi:10.1186/1477-7525-10-128
24. Sheehan KH, Sheehan DV. Assessing treatment effects in clinical trials with the Discan metric of the Sheehan Disability Scale. *Int Clin Psychopharmacol* (2008) 23:70–83. doi:10.1097/YIC.0b013e3282f2b4d6
25. Arbuckle R, Frye MA, Brecher M, Paulsson B, Rajagopalan K, Palmer S, et al. The psychometric validation of the Sheehan Disability Scale (SDS) in patients with bipolar disorder. *Psychiatry Res* (2009) 165:163–74. doi:10.1016/j.psychres.2007.11.018
26. Coles T, Coon C, DeMuro C, McLeod L, Gnanasakthy A. Psychometric evaluation of the Sheehan Disability Scale in adult patients with attention-deficit/hyperactivity disorder. *Neuropsychiatr Dis Treat* (2014) 10:887–95. doi:10.2147/NDT.S55220
27. Luciano JV, Bertsch J, Salvador-Carulla L, Tomás JM, Fernández A, Pinto-Meza A, et al. Factor structure, internal consistency and construct validity of the Sheehan Disability Scale in a Spanish primary care sample. *J Eval Clin Pract* (2010) 16:895–901. doi:10.1111/j.1365-2753.2009.01211.x
28. Kroenke K, Spitzer RL, Williams JB, Löwe B. The patient health questionnaire somatic, anxiety, and depressive symptom scales: a systematic review. *Gen Hosp Psychiatry* (2010) 32:345–59. doi:10.1016/j.genhosppsych.2010.03.006
29. Löwe B, Spitzer RL, Williams JBW, Mussell M, Schellberg D, Kroenke K. Depression, anxiety and somatization in primary care: syndrome overlap and functional impairment. *Gen Hosp Psychiatry* (2008) 30:191–9. doi:10.1016/j.genhosppsych.2008.01.001
30. Kroenke K, Spitzer RL, Williams JB. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med* (2001) 16:606–13. doi:10.1046/j.1525-1497.2001.016009606.x
31. Spitzer RL, Kroenke K, Williams JB. Validation and utility of a self-report version of PRIME-MD: the PHQ primary care study. *JAMA* (1999) 282:1737–44. doi:10.1001/jama.282.18.1737
32. Kocalevent RD, Hinz A, Brähler E. Standardization of the depression screener patient health questionnaire (PHQ-9) in the general population. *Gen Hosp Psychiatry* (2013) 35:551–5. doi:10.1016/j.genhosppsych.2013.04.006
33. Brüggemann P, Szczepek AJ, Klee K, Gräbel S, Mazurek B, Olze H. In patients undergoing cochlear implantation, psychological burden affects tinnitus and the overall outcome of auditory rehabilitation. *Front Hum Neurosci* (2017) 11:226. doi:10.3389/fnhum.2017.00226
34. Budd RJ, Pugh R. Tinnitus coping style and its relationship to tinnitus severity and emotional distress. *J Psychosom Res* (1996) 641:327–35. doi:10.1016/S0022-3999(96)00171-7
35. Henry JA, Thielman EJ, Zaugg TL, Kaelin C, Schmidt CJ, Griest S, et al. Randomized controlled trial in clinical settings to evaluate effectiveness of coping skills education used with progressive tinnitus management. *J Speech Lang Hear Res* (2017) 60:1378–97. doi:10.1044/2016_JSLHR-H-16-0126
36. Muthny FA. *Freiburger Fragebogen zur Krankheitsverarbeitung (FKV) Manual*. Weinheim, Germany: Beltz Test (1989).
37. Konzag TA, Rübler D, Bloching M, Bandemer-Greulich U, Fikentscher E, Frommer J. Counselling versus a self-help manual for tinnitus outpatients: a comparison of effectiveness. *HNO* (2006) 54:599–604. doi:10.1007/s00106-005-1350-y
38. Schecklmann M, Landgrebe M, Langguth B; TRI Database Study Group. Phenotypic characteristics of hyperacusis in tinnitus. *PLoS One* (2014) 9(1):e86944. doi:10.1371/journal.pone.0086944.e86944
39. Hall D. Interpreting treatment-related changes using the tinnitus questionnaire in Argstatter H, Grapp M, Plinkert PK, Bolay HV. Heidelberg neuromusic therapy for chronic-tonal tinnitus – treatment outline and psychometric evaluation. Int Tinnitus J (2012) 17:31-41. *Int Tinnitus J* (2017) 20:73–5. doi:10.5935/0946-5448.20160014
40. Bhatt JM, Lin HW, Bhattacharyya N. Prevalence, severity, exposures and treatment patterns of tinnitus in the United States. *JAMA Otolaryngol Head Neck Surg* (2016) 142:959–65. doi:10.1001/jamaoto.2016.1700
41. Henry JA, Dennis KC, Schechter MA. General review of tinnitus: prevalence, mechanisms, effects, and management. *J Speech Lang Hear Res* (2005) 48:1204–35. doi:10.1044/1092-4388(2005/084)
42. Schlee W, Kleinjung T, Hiller W, Goebel G, Kolassa IT, Langguth B. Does tinnitus distress depend on age of onset? *PLoS One* (2011) 6(11):e27379. doi:10.1371/journal.pone.0027379
43. Moser S, Luxenberger W, Freidl W. Perception of hearing problems in the older population. *HNO* (2017) 65:671–9. doi:10.1007/s00106-017-0334-z
44. Harada H, Ichikawa D, Imamura A. Course of hearing recovery according to frequency in patients with acute acoustic sensorineural hearing loss. *Int Tinnitus J* (2008) 14:83–8.
45. Ishida IM, Sugiura M, Teranishi M, Katayama N, Nakashima T. Otoacoustic emissions, ear fullness and tinnitus in the recovery course of sudden deafness. *Auris Nasus Larynx* (2008) 35:41–6. doi:10.1016/j.anl.2007.04.003

46. Park JH, Park SJ, Kim YH, Park MH. Sensorineural hearing loss: a complication of acute otitis media in adults. *Eur Arch Otorhinolaryngol* (2014) 271:1879–84. doi:10.1007/s00405-013-2675-x
47. Langguth B, Landgrebe M, Schlee W, Schecklmann M, Vielsmeier V, Steffens T, et al. Different patterns of hearing loss among tinnitus patients: a latent class analysis of a large sample. *Front Neurol* (2017) 20:46. doi:10.3389/fneur.2017.00046
48. Lee HY, Choi MS, Chang DS, Kim AY, Cho CS. Acute-onset tinnitus is associated with contralateral hearing in sudden deafness. *Audiol Neurootol* (2015) 20(6):370–5. doi:10.1159/000438919
49. Sakata T, Ueno T, Takase H, Shiraishi K, Nakagawa T. Acute idiopathic sensorineural hearing impairment at frequency exceeding 8 kHz. *Acta Otolaryngol* (2010) 130:1141–6. doi:10.3109/00016481003664793
50. Riga M, Papadas T, Werner JA, Dalchow CV. A clinical study of the efferent auditory system in patients with normal hearing who have acute tinnitus. *Otol Neurotol* (2007) 28:185–90. doi:10.1097/MAO.0b013e31802e2a14
51. Servais JJ, Hörmann K, Wallhäusser-Franke E. Unilateral cochlear implantation reduces tinnitus loudness in bimodal hearing: a prospective study. *Front Neurol* (2017) 8:60. doi:10.3389/fneur.2017.00060
52. Probst T, Pryss RC, Langguth B, Spiliopoulou M, Landgrebe M, Vesala M, et al. Outpatient tinnitus clinic, self-help web platform, or mobile application to recruit tinnitus study samples? *Front Aging Neurosci* (2017) 21:113. doi:10.3389/fnagi.2017.00113
53. Jordan P, Shedden-Mora MC, LoÈwe B. Psychometric analysis of the generalized anxiety disorder scale (GAD-7) in primary care using modern item response theory. *PLoS One* (2017) 12:e0182162. doi:10.1371/journal.pone.0182162

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Tinnitus Patients with Comorbid Headaches: The Influence of Headache Type and Laterality on Tinnitus Characteristics

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Background: Both clinical experience and clinical studies suggest a relationship between tinnitus and headache. Here, we aimed to investigate the influence of comorbid headache type and headache laterality on tinnitus characteristics.

Method: The Tinnitus Research Initiative database was screened for patients of the Tinnitus Center of the University Regensburg who reported comorbid headaches. These patients were contacted to complete additional validated questionnaires. Based on these data, patients were categorized according to headache type and headache laterality, and their clinical characteristics were compared with tinnitus patients, who did not report comorbid headaches.

Results: Data from 193 patients with tinnitus and comorbid headaches were compared with those from 765 tinnitus patients without comorbid headaches. Tinnitus patients with comorbid headache have higher scores in tinnitus questionnaires, a lower quality of life and more frequently comorbidities such as painful sensation to loud sounds, vertigo, pain (neck, temporomandibular, and general), and depressive symptoms when compared with tinnitus patients without headaches. Both headache laterality and headache type interact with the degree of comorbidity with higher impairment in patients with left-sided and bilateral headaches as well as in patients with migraine or cluster headache.

Conclusion: The observed increased impairment in tinnitus patients with comorbid headache can be explained as an additive effect of both disorders on health-related quality of life. The more frequent occurrence of further comorbidities suggests a generally increased amplification of sensory signals in a subset of tinnitus patients with comorbid headaches.

Keywords: migraine, cluster headache, trigeminus, phantom sound, laterality, comorbidity

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INTRODUCTION

Tinnitus, the perception of sound in the absence of a corresponding sound source, is a frequent disorder. In some forms of tinnitus, there is an internal sound source like sounds from abnormal blood flow because of vascular anomalies or palatal myoclonus. These forms are defined as objective tinnitus. In contrast, in subjective tinnitus, there exist neither external nor internal sound sources.

Subjective tinnitus can vary in its perceptual characteristics (loudness, pitch, number of tones, tonal or noise-like, pulsatile vs. non-pulsatile), its laterality (unilateral, bilateral, in the head), its maskability, its etiology, and its comorbidities. Accordingly, it has been assumed that there exist many different forms of tinnitus that also may differ in their pathophysiology. The identification of relevant criteria for subtyping different forms represents a major challenge in tinnitus research (1, 2). Specific comorbidities such as hyperacusis (3) or temporomandibular joint (TMJ) disorders (4) have turned out to represent potentially relevant criteria for subtyping. As an example patients with tinnitus and comorbid TMJ disorders were younger, more frequently female and suffered from less hearing loss (4), indicating that this group represents a clinically relevant tinnitus subtype.

Moreover, comorbidities such as hyperacousis (3), hearing loss (5), insomnia (6, 7), depression (8, 9), and pain syndromes (10) play a major role for tinnitus-related impairment in quality of life. Tinnitus-related health burden can be measured by specific validated tinnitus questionnaires (TQs) like the TQ (11) or the tinnitus handicap inventory (12), but also by numeric rating scales (13).

In the previous studies, an association between tinnitus and headaches has been demonstrated (14–16). These studies indicate that between 26 and 47% of patients with tinnitus also suffer from headache. Particularly frequent among tinnitus patients are unilateral headache syndromes (16). Since unilateral headaches and unilateral tinnitus symptoms occur in the majority of cases on the same side and headache and tinnitus are interacting over time, alterations in the trigeminal nerve activity have been proposed as a potentially relevant overlapping pathophysiological factor (16). Based on this reasoning, one may assume that headache as a comorbidity may represent a relevant factor for subtyping of tinnitus.

In order to investigate comorbid headache as potential criterion for tinnitus subtyping, we retrospectively analyzed clinical data from patients who presented at the multidisciplinary Tinnitus Center at the University of Regensburg. Patients who reported the existence of headaches in the Tinnitus Sample Case History Questionnaire (TSCHQ) (17) and who completed an additional headache questionnaire (18) were compared in their clinical and demographic characteristics with those patients who had tinnitus without headaches. In detail, we investigated whether tinnitus patients with specific forms or laterality of headache differ in demographic or other clinical characteristics from those tinnitus patients without headaches.

MATERIALS AND METHODS

Sample

The analysis was based on datasets of all patients, who presented at the multidisciplinary Tinnitus Center of the University of Regensburg between 2003 and 2011 and whose data were included in the Tinnitus Research Initiative database (19) ($n = 1{,}817$). All patients who reported the existence of headaches in the TSCHQ [answer "yes" to the question "Do you suffer from headaches?" (17)] ($n = 489$) were contacted by mail and asked to complete additional questionnaires (16). In these additional questions, patients were asked about the laterality of tinnitus and headache (Is your headache on one or predominantly one-sided? Is your tinnitus on one or predominantly one-sided?) and about the relationship between tinnitus and headache (time of onset of tinnitus and headache, respectively, and interaction between tinnitus and headache intensity). Completed headache and TQs were obtained from 193 patients, and this sample was compared with patients from the database who answered "no" to the question "Do you suffer from headaches?" in the TSCHQ ($n = 765$). Please note that three patients did not indicate the site of the headaches and were not included for the statistical analyses of headache laterality. Informed consent was obtained from all patients participating in the study. The study was approved by the Ethic committee of the University of Regensburg (11-101-0286), and all data were pseudonymized for analysis.

To exclude sample bias, we compared the patients with completed questionnaires with the group of patients with headache who did not respond to the mail ($n = 235$). Both groups did not differ significantly with respect to age at tinnitus onset ($t = 0.805$; df = 395; $p = 0.421$), gender ($\chi^2 = 0.566$; df = 1; $p = 0.452$), tinnitus duration ($t = 0.693$; df = 395; $p = 0.489$), tinnitus distress as indicated by TQ ($t = 0.114$; df = 405; $p = 0.909$) and tinnitus handicap inventory ($t = 0.072$; df = 413; $p = 0.943$), and mean hearing threshold ($t = 0.513$; df = 304; $p = 0.608$).

Assessment of Headaches and Tinnitus Severity

For the classification of headaches, the headache questionnaire by Fritsche et al. (18) was used, which was developed and validated according to the 2nd version of the classification criteria of the International Headache Society. The questionnaire enables to differentiate migraine, tension headache, cluster headache, combination of migraine and tension headache, and combination of tension- and cluster headache and non-classifiable headache.

Clinical and demographic information was obtained from available data of the investigated patients in the TRI database (19). Available data included the TSCHQ (17), the TQ (11), the Tinnitus Handicap Inventory (20, 21), various numeric tinnitus rating scales (13), the Beck depression inventory (BDI) (22), and the WHO Quality of Life Questionnaire (23).

Statistical Analysis

The relationship between the different demographic and clinical characteristics of tinnitus and the existence of comorbid headache, its laterality and its type was analyzed by using chi-square tests for categorical clinical variables and with analyses of variance (ANOVAs) for metric clinical variables. *Post hoc* tests were done for significant effects using least significant difference tests for ANOVAs and adjusted residuals ($z > 1.96$ was indicated as significant) for the chi-square tests and are indicated in **Tables 1** and **2**.

All analyses were performed with SPSS (Statistical Package for Social Studies, Version 19; SPSS Inc., USA). All tests were performed as two-sided tests, and the level of significance was set at 0.001 to correct for the fact that 56 comparisons were performed.

TABLE 1 | Influence of headache laterality on sample characteristics.

	Headache left	Headache right	Headache bilateral	No headache	Statistics
N	49	43	98	765	n.a.
Age at tinnitus onset (years)	44.8 ± 15.3 (*n* = 47)	42.4 ± 11.9 (*n* = 41)	41.6 ± 13.3 (*n* = 89)	44.0 ± 14.3 (*n* = 715)	$F = 0.937$; df = 3,888; $p = 0.422$
Duration of tinnitus (months)	89.9 ± 85.3 (*n* = 47)	100.7 ± 119.2 (*n* = 41)	97.5 ± 118.6 (*n* = 89)	100.7 ± 106.4 (*n* = 714)	$F = 0.165$; df = 3,887; $p = 0.920$
Gender (female/male)	**22/27**[a]	**22/21**[a]	**31/67**	**208/557**[a]	**$\chi^2 = 17.413$; df = 3; $p < 0.001$**
Tinnitus questionnaire	**47.4 ± 19.7 (*n* = 46); >no**	**40.6 ± 18.3 (*n* = 40)**	**46.9 ± 16.8 (*n* = 96) >no**	**38.8 ± 17.2 (*n* = 736)**	**$F = 10.964$; df = 3,914; $p < 0.001$**
Tinnitus handicap inventory	**54.3 ± 23.8 (*n* = 46); >no**	**48.8 ± 24.2 (*n* = 42)**	**56.2 ± 21.9 (*n* = 97); >no**	**44.0 ± 22.3 (*n* = 737)**	**$F = 10.983$; df = 3,918; $p < 0.001$**
Beck depression inventory	**14.2 ± 9.8 (*n* = 44); >no**	**12.0 ± 9.1 (*n* = 42); >no**	**14.5 ± 8.8 (*n* = 96); >no**	**9.1 ± 7.4 (*n* = 731)**	**$F = 19.545$; df = 3,909; $p < 0.001$**
Numeric rating scale loudness	6.7 ± 2.5 (*n* = 44)	6.5 ± 1.8 (*n* = 40)	6.8 ± 2.1 (*n* = 98)	6.2 ± 2.2 (*n* = 726)	$F = 3.262$; df = 3,904; $p = 0.021$
Numeric rating scale discomfort	**7.6 ± 2.0 (*n* = 43); >no**	**7.0 ± 2.2 (*n* = 41)**	**7.6 ± 2.1 (*n* = 97); >no**	**6.6 ± 2.3 (*n* = 725)**	**$F = 7.241$; df = 3,902; $p < 0.001$**
Numeric rating scale annoyance	7.1 ± 2.4 (*n* = 43)	6.7 ± 2.7 (*n* = 40)	7.1 ± 2.2 (*n* = 98)	6.4 ± 2.4 (*n* = 728)	$F = 3.402$; df = 3,905; $p = 0.017$
Numeric rating scale unpleasentness	6.9 ± 2.3 (*n* = 44)	6.7 ± 2.6 (*n* = 40)	7.1 ± 2.2 (*n* = 98)	6.4 ± 2.4 (*n* = 728)	$F = 2.858$; df = 3,906; $p = 0.036$
Numeric rating scale ignorability	7.0 ± 2.6 (*n* = 44)	7.0 ± 2.8 (*n* = 41)	7.0 ± 2.4 (*n* = 98)	6.6 ± 2.7 (*n* = 728)	$F = 0.872$; df = 3,907; $p = 0.455$
WHO quality of live—physical health	**13.6 ± 2.9 (*n* = 33); <no**	**14.3 ± 3.0 (*n* = 27); <no**	**13.4 ± 3.0 (*n* = 66); <no**	**15.5 ± 2.6 (*n* = 515)**	**$F = 16.543$; df = 3,537; $p < 0.001$**
WHO quality of live—psychological factors	**13.0 ± 3.6 (*n* = 33); <no**	**14.0 ± 2.8 (*n* = 28)**	**13.3 ± 2.7 (*n* = 66); <no**	**14.7 ± 2.6 (*n* = 417)**	**$F = 8.243$; df = 3,540; $p < 0.001$**
WHO quality of live—social relationships	14.2 ± 3.7 (*n* = 33)	13.6 ± 3.7 (*n* = 27)	14.0 ± 2.6 (*n* = 66)	15.0 ± 3.1 (*n* = 417)	$F = 3.614$; df = 3,539; $p = 0.013$
WHO quality of live—environment	15.7 ± 2.4 (*n* = 33)	15.9 ± 2.0 (*n* = 28)	15.7 ± 2.0 (*n* = 65)	16.5 ± 2.1 (*n* = 418)	$F = 3.916$; df = 3,540; $p = 0.009$
Sensitivity to loud sounds	3.4 ± 1.2 (*n* = 47)	3.3 ± 1.2 (*n* = 40)	3.3 ± 1.2 (*n* = 96)	3.2 ± 1.2 (*n* = 744)	$F = 1.329$; df = 3.923; $p = 0.264$
Painful sensations by loud sounds (yes/no)	**28/14**	**26/13**	**68/23**[a]	**354/314**	**$\chi^2 = 19.053$; df = 3; $p < 0.001$**
Pulsatile tinnitus (no/yes with…/yes different from heartbeat)	33/7/7	28/7/5	80/10/6	605/72/64	$\chi^2 = 7.863$; df = 6; $p = 0.248$
Tinnitusquality (tonal/noise/cricket/other)	25/6/11/4	27/3/7/5	61/14/17/5	449/76/144/72	$\chi^2 = 5.686$; df = 9; $p = 0.771$
Influence by noise (yes/no)	31/12	32/5	63/21	493/158	$\chi^2 = 2.658$; df = 3; $p = 0.447$
Somatic modulation (yes/no)	24/24	17/25	42/55	258/486	$\chi^2 = 7.045$; df = 3; $p = 0.070$
Mean hearing threshold	26 ± 13 (*n* = 44)	19 ± 11 (*n* = 42)	18 ± 12 (*n* = 96)	23 ± 15 (*n* = 731)	$F = 3.890$; df = 3,682; $p = 0.009$
Side of worse hearing (left/left = right/right)	20/2/10	13/2/7	35/5/33	284/35/228	$\chi^2 = 2.914$; df = 6; $p = 0.820$
Vertigo (yes/no)	**28/19**[a]	**23/20**[a]	**52/43**[a]	**158/585**[a]	**$\chi^2 = 87.258$; df = 3; $p < 0.001$**
Temporomandibular joint complaints (yes/no)	**17/31**[a]	**11/31**	**29/68**[a]	**124/620**[a]	**$\chi^2 = 19.471$; df = 3; $p < 0.001$**
Neck pain (yes/no)	**42/7**[a]	**32/10**[a]	**69/29**[a]	**341/402**[a]	**$\chi^2 = 56.552$; df = 3; $p < 0.001$**
General pain (yes/no)	**25/22**[a]	**25/18**[a]	**58/39**[a]	**249/480**[a]	**$\chi^2 = 35.427$; df = 3; $p < 0.001$**
Current psychiatric treatment (yes/no)	**12/36**[a]	**8/32**	**26/70**[a]	**89/659**[a]	**$\chi^2 = 21.608$; df = 3; $p < 0.001$**

Bold printed lines indicate significant effects.

[a]*The number of cases in this cell is significant different from the number of expected cases under the assumption of independence of variables.*

< or > indicate significant post hoc contrasts.

Please note that high values in quality of life mean high quality of life and that high values in the other measures mean high burden.

TABLE 2 | Influence of headache type on sample characteristics.

	Non-classifiable headache	Migraine	Tension-type headache	Tension-type headache and migraine	Cluster headache	No headache	Statistics
N	63	86	25	11	8	765	n.a.
Age at tinnitus onset (years)	44.0 ± 14.2 (*n* = 59)	41.4 ± 12.8 (*n* = 81)	41.3 ± 12.5 (*n* = 24)	40.0 ± 17.7 (*n* = 8)	52.1 ± 12.1 (*n* = 7)	44.0 ± 14.2 (*n* = 715)	$F = 1.260$; df = 5,888; $p = 0.279$
Duration of tinnitus (months)	84.7 ± 107.3 (*n* = 59)	104.1 ± 114.1 (*n* = 81)	111.9 ± 119.5 (*n* = 24)	61.0 ± 82.3 (*n* = 8)	94.0 ± 95.9 (*n* = 7)	100.7 ± 106.4 (*n* = 714)	$F = 0.544$; d = 5,887; $p = 0.743$
Gender (female/male)	19/44	33/53	13/12[a]	6/5	5/3[a]	208/557[a] (*n* = 715)	$\chi^{2=}18.761$; df = 5; $p = 0.002$
Tinnitus questionnaire	**44.7 ± 18.2 (*n* = 60); >no**	**45.8 ± 18.4 (*n* = 81); >no**	**45.8 ± 17.7 (*n* = 24); >no**	**38.5 ± 14.7 (*n* = 11); <cluster**	**56.6 ± 15.4 (*n* = 8); >migraine + tension; >no**	**38.0 ± 17.2 (*n* = 736)**	***F* = 6.561; df = 5,914; *p* < 0.001**
Tinnitus handicap inventory	**52.0 ± 23.4 (*n* = 61); >no**	**54.9 ± 23.2 (*n* = 84); >no**	**55.5 ± 23.2 (*n* = 25); >no**	**43.9 ± 21.3 (*n* = 10)**	**61.9 ± 21.7 (*n* = 8); >no**	**44.0 ± 22.3 (*n* = 737)**	***F* = 6.379; df = 5,919; *p* < 0.001**
Beck depression inventory	**12.5 ± 8.4 (*n* = 59); >no**	**14.8 ± 9.2 (*n* = 83); >no**	**12.9 ± 10.3 (*n* = 25); >no**	**11.8 ± 8.5 (*n* = 10)**	**17.0 ± 10.4 (*n* = 8); >no**	**9.1 ± 7.4 (*n* = 731)**	***F* = 11.813; df = 5,910; *p* < 0.001**
Numeric rating scale loudness	6.5 ± 2.3 (*n* = 59)	6.8 ± 2.1 (*n* = 84)	7.0 ± 2.1 (*n* = 25)	7.0 ± 2.1 (*n* = 10)	6.6 ± 2.2 (*n* = 7)	6.2 ± 2.1 (*n* = 726)	$F = 1.973$; df = 5,905; $p = 0.080$
Numeric rating scale discomfort	**7.1 ± 2.2 (*n* = 60)**	**7.6 ± 2.1 (*n* = 83); >no**	**8.0 ± 1.8 (*n* = 24); >no**	**7.3 ± 2.3 (*n* = 10)**	**7.0 ± 1.6 (*n* = 7)**	**6.6 ± 2.3 (*n* = 725)**	***F* = 4.593; df = 5,903; *p* < 0.001**
Numeric rating scale annoyance	6.9 ± 2.4 (*n* = 59)	7.0 ± 2.3 (*n* = 83)	7.7 ± 2.2 (*n* = 25)	6.7 ± 2.5 (*n* = 10)	6.2 ± 2.2 (*n* = 7)	6.4 ± 2.4 (*n* = 728)	$F = 2.406$; df = 5,906; $p = 0.035$
Numeric rating scale unpleasentness	6.9 ± 2.2 (*n* = 59)	7.0 ± 2.4 (*n* = 84)	7.3 ± 2.5 (*n* = 25)	6.6 ± 2.4 (*n* = 10)	6.3 ± 2.2 (*n* = 7)	6.4 ± 2.4 (*n* = 728)	$F = 1.728$; df = 5,907; $p = 0.126$
Numeric rating scale ignorability	6.8 ± 2.3 (*n* = 60)	7.3 ± 2.7 (*n* = 84)	6.9 ± 2.7 (*n* = 25)	6.4 ± 2.6 (*n* = 10)	6.1 ± 2.2 (*n* = 7)	6.6 ± 2.7 (*n* = 728)	$F = 0.961$; df = 5,908; $p = 0.441$
WHO quality of live—physical health	**13.4 ± 3.3 (= 44); <no**	**13.5 ± 3.0 (*n* = 56); <no**	**14.8 ± 2.1 (*n* = 19)**	**15.7 ± 1.8 (*n* = 4)**	**12.8 ± 1.8 (*n* = 6); <no**	**15.5 ± 2.6 (*n* = 415)**	***F* = 10.764; df = 5,538; *p* < 0.001**
WHO quality of live—psychological factors	**13.5 ± 3.4 (*n* = 45); <no**	**13.2 ± 2.8 (*n* = 56); <no**	**13.8 ± 2.6 (*n* = 19)**	**15.8 ± 0.7 (*n* = 4); >no**	**12.3 ± 2.4 (*n* = 6); <tension + migraine; < no**	**14.7 ± 2.6 (*n* = 417)**	***F* = 5.556; df = 5,541; *p* < 0.001**
WHO quality of live—social relationships	14.3 ± 3.7 (*n* = 44)	13.8 ± 2.8 (*n* = 56)	14.4 ± 3.1 (*n* = 19)	14.7 ± 2.9 (*n* = 4)	11.6 ± 3.5 (*n* = 6)	15.0 ± 3.1 (*n* = 417)	$F = 2.940$; df = 5,540; $p = 0.012$
WHO quality of live—environment	15.9 ± 2.4 (*n* = 45)	15.5 ± 1.9 (*n* = 55)	16.6 ± 1.6 (*n* = 19)	15.1 ± 1.1 (*n* = 4)	14.4 ± 2.3 (*n* = 6)	16.5 ± 2.1 (*n* = 418)	$F = 3.635$; df = 5,541; $p = 0.003$
Sensitivity to loud sounds	3.1 ± 1.3 (*n* = 61)	3.5 ± 1.2 (*n* = 83)	3.2 ± 1.1 (*n* = 24)	3.1 ± 1.6 (*n* = 10)	4.0 ± 0.9 (*n* = 8)	3.2 ± 1.2 (*n* = 744)	$F = 1.929$; df = 5,924; $p = 0.087$
Painful sensations by loud sounds (yes/no)	**38/21**	**57/20[a]**	**15/6**	**9/1[a]**	**5/3**	**354/314[a]**	**$\chi^{2=}21.061$; df = 5; $p < 0.001$**
Pulsatile tinnitus (no/yes with…/yes different from heartbeat)	50/4/7	60/15/7	20/3/2	9/1/0	4/2/2	605/72/64	$\chi^{2=}13.263$; df = 10; $p = 0.209$
Tinnitusquality (tonal/noise/cricket/other)	40/11/8/4	52/9/16/6	14/4/5/1	6/0/3/1	2/0/3/3	449/76/144/72	$\chi^{2=}19.358$; df = 15; $p = 0.198$
Influence by noise (yes/no)	43/14	58/16	17/7	7/1	3/1	493/158	$\chi^{2=}1.193$; df = 5; $p = 0.946$
Somatic modulation (yes/no)	26/37	42/42	8/17	3/7	6/2	258/486	$\chi^{2=}13.751$; df = 5; $p = 0.017$
Mean hearing threshold	18.5 ± 12.3 (*n* = 47)	21.3 ± 13.4 (*n* = 55)	18.9 ± 10.5 (*n* = 16)	16.4 ± 6.7 (*n* = 8)	30.5 ± 9.1 (*n* = 7)	22.8 ± 14.5 (*n* = 555)	$F = 1.807$; df = 5,682; $p = 0.109$
Side of worse hearing (left/left = right/right)	25/2/107	28/6/20	8/0/8	3/1/4	5/0/1	284/35/228	$\chi^2 = 7.197$; df = 10; $p = 0.639$
Vertigo (yes/no)	**29/31[a]**	**52/33[a]**	**12/12[a]**	**5/6**	**6/2[a]**	**158/585[a]**	**$\chi^{2=}91.328$; df = 5; $p < 0.001$**

(*Continued*)

TABLE 2 | Continued

	Non-classifiable headache	Migraine	Tension-type headache	Tension-type headache and migraine	Cluster headache	No headache	Statistics
Temporomandibular joint complaints (yes/no)	**21/41**[a]	**25/59**[a]	**5/20**	**3/8**	**4/4**[a]	**124/620**[a]	$\chi^{2=}$**22.777; df = 5;** $p < 0.001$
Neck pain (yes/no)	**48/14**[a]	**62/24**[a]	**19/6**[a]	**9/2**[a]	**8/0**[a]	**341/402**[a]	$\chi^{2=}$**58.133; df = 5;** $p < 0.001$
General pain (yes/no)	**31/30**[a]	**52/34**[a]	**15/10**[a]	**7/3**[a]	**4/4**	**249/480**[a]	$\chi^{2=}$**36.522; df = 5;** $p < 0.001$
Current psychiatric treatment (yes/no)	**11/50**	**28/55**[a]	**4/21**	**1/9**	**2/6**	**89/659**[a]	$\chi^{2=}$**30.493; df = 5;** $p < 0.001$

Bold printed lines indicate significant effects.

[a]*The number of cases in this cell is significant different from the number of expected cases under the assumption of independence of variables.*

< or > indicate significant post hoc contrasts.

Please note that high values in quality of life mean high quality of life and that high values in the other measures mean high burden.

RESULTS

For evaluation of clinical and demographic characteristics of patients with tinnitus and headaches, patients with left-sided, right-sided, and bilateral headaches were compared with patients with tinnitus, but without headaches from the TRI database (see **Table 1**).

About 27% of the tinnitus patients in our database answered "yes" to the question "do you suffer from headaches." Even if comparisons with population-based studies are difficult, as results depend strongly on the method of assessment, a proportion of 27% is not indicative of a substantially altered prevalence of headaches among tinnitus patients.

There was no significant interaction between headache laterality and age at tinnitus onset or tinnitus duration, but a significant interaction between headache laterality and gender, with women suffering more frequently from unilateral headache.

Analyses of tinnitus distress, depressive symptoms, numeric ratings scales, and quality of life showed similar results for all of these measures with increased burden for the group of patients with bilateral and left-sided headaches in contrast to patients with no headaches. The group of patients with right-sided headache showed no difference to the group of patients without headaches or ranged in between the left-sided/bilateral groups and the group with no headaches. This pattern could be seen descriptively in all variables but falling below the significance threshold for TQ, tinnitus handicap inventory, BDI, the numeric rating scale discomfort, and the quality of life domains physical and psychological health. As an exploratory analysis, the TQ score (TQ of the database entry: $r = 0.321$; $n = 175$; $p < 0.001$; TQ by response to mail: $r = 0.356$; $n = 184$; $p < 0.001$) also correlated with the headache frequency (number of days with headache/month).

Painful sensations through loud sounds were more frequently reported by patients with bilateral headache in contrast to patients with unilateral or no headaches. There were no significant effects concerning sensitivity to loud sounds, pulsatile character, tonal versus noise-like character, the ability to mask tinnitus through other sounds, duration of tinnitus, and the ability to modulate tinnitus through neck or jaw movements. There was no significant relationship between headache laterality and hearing function (measured as mean hearing threshold across all frequencies and both sides) nor with the side of worse hearing.

Patients with all headache forms (bilateral, right-sided, and left-sided) reported more frequently comorbid vertigo, TMJ complaints (except right-sided headaches), neck pain as well as pain in general. Patients with headaches were also more frequently treated by psychiatrists (except right-sided headaches). In separate analyses, differences in tinnitus characteristics between patients with specific types of comorbid headaches and no headaches were analyzed. Detailed results are provided in **Table 2**.

The headache type had no significant influence on age at tinnitus onset nor on tinnitus duration. However, there was a non-significant trend toward an interaction between gender and headache type with women suffering more frequently from comorbid tension-type and cluster headache.

Tinnitus patients with comorbid migraine, tension-type headache, cluster headache, and non-classifiable headache had

all higher scores in the TQ, the THI, and the BDI when compared with tinnitus patients without comorbid headaches. Descriptively, highest scores were found for patients with comorbid cluster headache. Patients with combined tension-type headache and migraine did not differ from the other groups except for the TQ showing lower values in contrast to cluster headache.

In the different numeric rating scales, there were statistically significant differences for tinnitus discomfort (higher scores for patients with migraine and tension-type headaches in contrast to patients without headaches), whereas there were no differences for tinnitus severity, unpleasantness, and ability to ignore the tinnitus. The headache type had a significant influence on two domains of the WHOQoL (somatic, psychological) with impairment in quality of life in patients with comorbid unclassifiable headache, migraine, and cluster headaches in contrast to patients without headaches.

Headache type had no influence on the sensitivity to loud sounds, but on the induction of painful sensations by sounds, which was significantly more frequent in patients with migraine and in patients with combined migraine and tension-type headache.

There was no significant influence of headache type on the proportion of patients with hearing function, pulsatile tinnitus, tone or noise-like tinnitus, maskability of tinnitus by environmental sounds, or the ability to modulate tinnitus by somatic maneuvers.

Comorbid headaches had a significant influence on comorbid vertigo, neck pain, TMJ complaints, and pain in general. A higher prevalence of neck pain was found in all headache types and a higher prevalence of vertigo in all headache types apart from combined migraine and tension-type headache. TMJ complaints were more frequent in non-classifiable headache, migraine, and cluster headache, and general pain complaints were more frequent in all patients apart from cluster headache. Patients with comorbid migraine were also more frequently psychiatrically treated.

DISCUSSION

The main findings of this study are that tinnitus patients with comorbid headache have higher scores in TQs, a lower quality of life and more frequently comorbidities such as painful sensation to loud sounds, vertigo, neck pain, TMJ complaints, general pain, and depressive symptoms when compared with tinnitus patients without headaches. The higher impairment in quality of life in patients who suffer from both tinnitus and headache can be easily explained by a pure additive effect of both disorders on disease burden.

Both headache laterality and headache type interact with the degree of morbidity.

In detail, higher impairment is reported by patients with left-sided and bilateral headaches as well as by patients with migraine or cluster headache. We are aware that there is a certain interaction between headache type and headache laterality, which has to be considered in the interpretation of the results (see **Table 3**).

Our findings are in line with earlier studies, which demonstrated that tinnitus severity is higher in patients with comorbid headache (24) and correlates with headache frequency (25). A potential interaction between tinnitus and migraine (26–30) or other trigeminoautonomal headache syndromes (31, 32) has been described in many studies.

An important question is, whether the co-occurrence of tinnitus and headaches is pure co-incidence or whether there is a pathophysiological interaction. Several potential mechanisms for such a pathophysiological interaction have been proposed. First, increased excitability of the trigeminal system could link tinnitus and headache syndromes (16). Second, central sensitization in the context of migraine could provide an explanation for the development of tinnitus (28). Third, tinnitus could represent a symptom of vestibular migraine (33–35) or vestibular migraine could be related to a specific subtype of Meniere's disease (36). Fourth, migraine might cause pulsatile tinnitus by vascular alterations during Migraine attacks (30). Fifth, TMJ or neck pain could represent a common cause of comorbid headache and tinnitus. However, due to the cross-sectional design of our study, we can only describe symptom associations and cannot draw any firm conclusions about potential causal interactions between headaches and tinnitus.

In this study, patients with comorbid cluster headache demonstrated highest scores in TQs and most pronounced impairment in quality of life. Induction of painful sensations by loud sounds, general pain syndromes, and psychiatric treatment was most frequent in migraine patients. These findings do not necessarily indicate a specific pathophysiological interaction between headache syndromes and tinnitus, as an increased sensitivity to loud sounds is similar like vertigo a typical feature of migraine. The increased prevalence of psychiatric treatment can be easily explained by the well-known association of headache disorders with anxiety and depression (37). Patients who suffer from tinnitus and headache are more impaired in their quality of life and the higher scores in the TQs may purely reflect a higher health-related handicap, as many questions in the TQ and THI are not tinnitus specific. This fits with the finding that scores were most pronounced in patients with cluster headaches, which are known to be extremely debilitating. The higher comorbidities for pain syndromes, vertigo, sound-induced painful sensations,

TABLE 3 | Interaction between headache type and laterality.

	Non-classifiable headache	Migraine	Tension-type headache	Tension-type headache and migraine	Cluster headache
Headache left	14	24	2[a]	3	6[a]
Headache right	11	19	8	3	2
Headache bilateral	36	42	15	5	0[a]

Chi-square of independence indicates a significant association of headache type and laterality with decreased number of left-sided tension-type and bilateral cluster headache and increased frequency of left-sided cluster headache (χ^2=17.926; df = 8; p = 0.022).

[a]The number of cases in this cell is significant different from the number of expected cases under the assumption of independence of variables.

< or > indicate significant post hoc contrasts.

depressive syndromes, and the higher proportion of psychiatric treatment in this patient group could be explained by a generally increased amplification of sensory signals, which is, for example, encountered in patients with somatoform disorder.

Hints for specific interactions (e.g., a particular high proportion of vertigo in patients with migraine, which could point to vestibular migraine) could not be observed. However, our study cannot exclude that such specific interactions occur, as they can be missed by the statistical analysis, if they occur only in a small group of patients. However, our data also show hints against single additive effects. General pain is not increased in patients with cluster headaches and combination of migraine and tension-type headache did not result in higher burden as indicated by measures of tinnitus distress and discomfort, depressive syndrome, and quality of life.

In addition to headache type also headache laterality had an impact on patients' characteristics. Comorbid left-sided and bilateral headache had a particular impact on tinnitus severity, on quality of life, and on comorbid disorders such as vertigo, pain, depressive symptoms, and on the frequency of psychiatric treatment. Likewise for headache type, this pattern mainly reflects that patients with comorbid bilateral or left-sided headache are more severely impaired and more frequently suffer also from other somatic, somatoform, and psychiatric symptoms. An earlier analysis of the same sample revealed that left-sided headaches are also frequently associated with left-sided tinnitus and bilateral headaches more frequently with bilateral tinnitus (16). The finding of higher impairment of patients with left-sided symptoms is in line with the literature that shows a slight left-sided preponderance (55–60%) in somatoform disorders (38) and in somatoform pain (39). With respect to headaches, a relatively small study suggests that left-sided migraine is more frequently associated with psychiatric symptoms than right-sided migraine (40). Thus, among tinnitus patients with left-sided headaches, there might be a higher proportion of patients with a somatoform disorder, which can explain the higher impairment in this group. The same explanation may hold true for patients with bilateral headaches, as this group includes also all patients with a rather unspecific description of their headaches, and among patients with rather unspecific description of their headache syndrome a higher proportion of comorbid somatoform disorders is expected as well.

An alternative, but rather speculative explanation for our findings of higher impairment in patients with left-sided symptoms, is provided by a pilot study that investigated the relevance of laterality in able-bodied individuals desiring amputation of a limb (41). In most cases, these individuals desired amputation of a left-sided limb, the disorder was associated with elementary and complex somatosensory disturbances of the affected limb and the most frequent neurological comorbidity was migraine headache. Left-sidedness, limb specificity, and somatosensory disturbances of the affected limb were interpreted as hints for disturbed integration of multi-sensory information of the affected body parts into a coherent cerebral representation of the own body and suggestive of abnormal brain mechanisms in right frontoparietal cortex.

Tinnitus occurs also more frequently on the left side, is tonotopically specific, related to sensory disturbances (42, 43) and an incongruence between visual and auditory input (44), and might therefore be conceptualized as a symptom that compensates an otherwise incoherent cerebral representation of the acoustic environment in relation to the own body.

We are aware of methodological limitations of this study as all data come from one university center and may therefore not be representative. Moreover, data were solely collected by questionnaires and not verified by clinical examination. Thus, further research involving clinical evaluations will be needed to further explore the relationship between different forms of headaches and tinnitus. Nevertheless, our study revealed a greater impairment for tinnitus patients suffering from comorbid headaches and a hint for the occurrence of further comorbidities such as vertigo, pain syndromes, depression, and psychiatric disorders.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the ethics committee of the University Hospital Regensburg with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the ethics committee of the University Hospital Regensburg.

AUTHOR CONTRIBUTIONS

VH gathered the data and entered the data in a database. MS performed the statistical analysis. BL and MS drafted the manuscript. All the authors designed the study, interpreted the data, and approved the final version of the manuscript.

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REFERENCES

1. Elgoyhen AB, Langguth B, De Ridder D, Vanneste S. Tinnitus: perspectives from human neuroimaging. *Nat Rev Neurosci* (2015) 16(10):632–42. doi:10.1038/nrn4003
2. Langguth B, Kreuzer PM, Kleinjung T, De Ridder D. Tinnitus: causes and clinical management. *Lancet Neurol* (2013) 12(9):920–30. doi:10.1016/S1474-4422(13)70160-1
3. Schecklmann M, Landgrebe M, Langguth B. Phenotypic characteristics of hyperacusis in tinnitus. *PLoS One* (2014) 9(1):e86944. doi:10.1371/journal.pone.0086944
4. Vielsmeier V, Strutz J, Kleinjung T, Schecklmann M, Kreuzer PM, Landgrebe M, et al. Temporomandibular joint disorder complaints in tinnitus: further hints for a putative tinnitus subtype. *PLoS One* (2012) 7(6):e38887. doi:10.1371/journal.pone.0038887

5. Mazurek B, Olze H, Haupt H, Szczepek AJ. The more the worse: the grade of noise-induced hearing loss associates with the severity of tinnitus. *Int J Environ Res Public Health* (2010) 7(8):3071–9. doi:10.3390/ijerph7083071
6. Cronlein T, Langguth B, Geisler P, Hajak G. Tinnitus and insomnia. *Prog Brain Res* (2007) 166:227–33. doi:10.1016/S0079-6123(07)66021-X
7. Cronlein T, Langguth B, Pregler M, Kreuzer PM, Wetter TC, Schecklmann M. Insomnia in patients with chronic tinnitus: cognitive and emotional distress as moderator variables. *J Psychosom Res* (2016) 83:65–8. doi:10.1016/j.jpsychores.2016.03.001
8. Langguth B, Landgrebe M, Kleinjung T, Sand GP, Hajak G. Tinnitus and depression. *World J Biol Psychiatry* (2011) 12(7):489–500. doi:10.3109/15622975.2011.575178
9. Milerova J, Anders M, Dvorak T, Sand PG, Koniger S, Langguth B. The influence of psychological factors on tinnitus severity. *Gen Hosp Psychiatry* (2013) 35(4):412–6. doi:10.1016/j.genhosppsych.2013.02.008
10. Kreuzer PM, Landgrebe M, Vielsmeier V, Kleinjung T, De Ridder D, Langguth B. Trauma-associated tinnitus. *J Head Trauma Rehabil* (2014) 29(5):432–42. doi:10.1097/HTR.0b013e31829d3129
11. Hallam RS, Jakes SC, Hinchcliffe R. Cognitive variables in tinnitus annoyance. *Br J Clin Psychol* (1988) 27(Pt 3):213–22. doi:10.1111/j.2044-8260.1988.tb00778.x
12. Newman CW, Jacobson GP, Spitzer JB. Development of the tinnitus handicap inventory. *Arch Otolaryngol Head Neck Surg* (1996) 122(2):143–8. doi:10.1001/archotol.1996.01890140029007
13. Zeman F, Koller M, Langguth B, Landgrebe M. Which tinnitus-related aspects are relevant for quality of life and depression: results from a large international multicentre sample. *Health Qual Life Outcomes* (2014) 12:7. doi:10.1186/1477-7525-12-7
14. Rubinstein B, Axelsson A, Carlsson GE. Prevalence of signs and symptoms of craniomandibular disorders in tinnitus patients. *J Craniomandib Disord* (1990) 4(3):186–92.
15. Lainez MJ, Ponz A. Tinnitus with headaches. In: Moller ALB, De Riddeer D, Kleinjung T, editors. *Textbook of Tinnitus*. New York: Springer (2011). p. 487–90.
16. Langguth B, Hund V, Busch V, Jurgens TP, Lainez JM, Landgrebe M, et al. Tinnitus and headache. *Biomed Res Int* (2015) 2015:797416. doi:10.1155/2015/797416
17. Langguth B, Goodey R, Azevedo A, Bjorne A, Cacace A, Crocetti A, et al. Consensus for tinnitus patient assessment and treatment outcome measurement: Tinnitus Research Initiative meeting, Regensburg, July 2006. *Prog Brain Res* (2007) 166:525–36. doi:10.1016/S0079-6123(07)66050-6
18. Fritsche G, Hueppe M, Kukava M, Dzagnidze A, Schuerks M, Yoon MS, et al. Validation of a German language questionnaire for screening for migraine, tension-type headache, and trigeminal autonomic cephalgias. *Headache* (2007) 47(4):546–51. doi:10.1111/j.1526-4610.2007.00758.x
19. Landgrebe M, Zeman F, Koller M, Eberl Y, Mohr M, Reiter J, et al. The Tinnitus Research Initiative (TRI) database: a new approach for delineation of tinnitus subtypes and generation of predictors for treatment outcome. *BMC Med Inform Decis Mak* (2010) 10:42. doi:10.1186/1472-6947-10-42
20. Newman CW, Sandridge SA, Jacobson GP. Psychometric adequacy of the Tinnitus Handicap Inventory (THI) for evaluating treatment outcome. *J Am Acad Audiol* (1998) 9(2):153–60.
21. Kleinjung T, Fischer B, Langguth B, Sand PG, Hajak G, Dvorakova J, et al. [Validation of the German-Version Tinnitus Handicap Inventory (THI)]. *Psychiatr Prax* (2007) 34(S1):140–2. doi:10.1055/s-2006-940218
22. Beck AT, Steer RA. Internal consistencies of the original and revised Beck depression inventory. *J Clin Psychol* (1984) 40(6):1365–7. doi:10.1002/1097-4679(198411)40:6<1365::AID-JCLP2270400615>3.0.CO;2-D
23. The WHOQOL Group. Development of the World Health Organization WHOQOL-BREF quality of life assessment. *Psychol Med* (1998) 28(3):551–8. doi:10.1017/S0033291798006667
24. Lindberg P, Lyttkens L, Melin L, Scott B. Tinnitus – incidence and handicap. *Scand Audiol* (1984) 13(4):287–91. doi:10.3109/01050398409042138
25. Erlandsson SI, Hallberg LR, Axelsson A. Psychological and audiological correlates of perceived tinnitus severity. *Audiology* (1992) 31(3):168–79. doi:10.3109/00206099209072912
26. Sindhusake D, Golding M, Newall P, Rubin G, Jakobsen K, Mitchell P. Risk factors for tinnitus in a population of older adults: the blue mountains hearing study. *Ear Hear* (2003) 24(6):501–7. doi:10.1097/01.AUD.0000100204.08771.3D
27. Evans RW, Ishiyama G. Migraine with transient unilateral hearing loss and tinnitus. *Headache* (2009) 49(5):756–8. doi:10.1111/j.1526-4610.2008.01075.x
28. Volcy M, Sheftell FD, Tepper SJ, Rapoport AM, Bigal ME. Tinnitus in migraine: an allodynic symptom secondary to abnormal cortical functioning? *Headache* (2005) 45(8):1083–7. doi:10.1111/j.1526-4610.2005.05193_2.x
29. Guichard E, Montagni I, Tzourio C, Kurth T. Association between headaches and tinnitus in young adults: cross-sectional study. *Headache* (2016) 56(6):987–94. doi:10.1111/head.12845
30. Weinreich HM, Carey JP. Prevalence of pulsatile tinnitus among patients with migraine. *Otol Neurotol* (2016) 37(3):244–7. doi:10.1097/MAO.0000000000000968
31. Chan CC, Ghosh S. Red ear syndrome precipitated by a dietary trigger: a case report. *J Med Case Reports* (2014) 8:338. doi:10.1186/1752-1947-8-338
32. Kreuzer PM, Vielsmeier V, Poeppl TB, Langguth B. A case report on red ear syndrome with tinnitus successfully treated with transcranial random noise stimulation. *Pain Physician* (2017) 20(1):E199–205.
33. Chen J, Gong D, Cai S, Wu Z, Lin X, Ma X, et al. [Clinical characteristics of 100 vestibular migraine cases]. *Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi* (2016) 30(5):399–401.
34. Lopez-Escamez JA, Dlugaiczyk J, Jacobs J, Lempert T, Teggi R, von Brevern M, et al. Accompanying symptoms overlap during attacks in Menière's disease and vestibular migraine. *Front Neurol* (2014) 5:265. doi:10.3389/fneur.2014.00265
35. Eggers SD, Neff BA, Shepard NT, Staab JP. Comorbidities in vestibular migraine. *J Vestib Res* (2014) 24(5–6):387–95. doi:10.3233/VES-140525
36. Frejo L, Soto-Varela A, Santos-Perez S, Aran I, Batuecas-Caletrio A, Perez-Guillen V, et al. Clinical subgroups in bilateral meniere disease. *Front Neurol* (2016) 7:182. doi:10.3389/fneur.2016.00182
37. Peres MFP, Mercante JPP, Tobo PR, Kamei H, Bigal ME. Anxiety and depression symptoms and migraine: a symptom-based approach research. *J Headache Pain* (2017) 1(18):37. doi:10.1186/s10194-017-0742-1
38. Stone J, Sharpe M, Carson A, Lewis SC, Thomas B, Goldbeck R, et al. Are functional motor and sensory symptoms really more frequent on the left? A systematic review. *J Neurol Neurosurg Psychiatry* (2002) 73(5):578–81. doi:10.1136/jnnp.73.5.578
39. Min SK, Lee BO. Laterality in somatization. *Psychosom Med* (1997) 59(3):236–40. doi:10.1097/00006842-199705000-00005
40. Cologno D, Buzzi MG, Carlesimo GA, Cicinelli P, Costa A, Fadda L, et al. Psychiatric disorders and pain location in unilateral migraineurs. *J Headache Pain* (2005) 6(4):227–30. doi:10.1007/s10194-005-0192-z
41. Blanke O, Morgenthaler FD, Brugger P, Overney LS. Preliminary evidence for a fronto-parietal dysfunction in able-bodied participants with a desire for limb amputation. *J Neuropsychol* (2009) 3(Pt 2):181–200. doi:10.1348/174866408X318653
42. Norena A, Micheyl C, Chery-Croze S, Collet L. Psychoacoustic characterization of the tinnitus spectrum: implications for the underlying mechanisms of tinnitus. *Audiol Neurootol* (2002) 7(6):358–69. doi:10.1159/000066156
43. Schecklmann M, Vielsmeier V, Steffens T, Landgrebe M, Langguth B, Kleinjung T. Relationship between audiometric slope and tinnitus pitch in tinnitus patients: insights into the mechanisms of tinnitus generation. *PLoS One* (2012) 7(4):e34878. doi:10.1371/journal.pone.0034878
44. De Ridder D, Elgoyhen AB, Romo R, Langguth B. Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc Natl Acad Sci U S A* (2011) 108(20):8075–80. doi:10.1073/pnas.1018466108

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Cluster Analysis to Identify Possible Subgroups in Tinnitus Patients

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Introduction: In tinnitus treatment, there is a tendency to shift from a "one size fits all" to a more individual, patient-tailored approach. Insight in the heterogeneity of the tinnitus spectrum might improve the management of tinnitus patients in terms of choice of treatment and identification of patients with severe mental distress. The goal of this study was to identify subgroups in a large group of tinnitus patients.

Methods: Data were collected from patients with severe tinnitus complaints visiting our tertiary referral tinnitus care group at the University Medical Center Groningen. Patient-reported and physician-reported variables were collected during their visit to our clinic. Cluster analyses were used to characterize subgroups. For the selection of the right variables to enter in the cluster analysis, two approaches were used: (1) variable reduction with principle component analysis and (2) variable selection based on expert opinion.

Results: Various variables of 1,783 tinnitus patients were included in the analyses. Cluster analysis (1) included 976 patients and resulted in a four-cluster solution. The effect of external influences was the most discriminative between the groups, or clusters, of patients. The "silhouette measure" of the cluster outcome was low (0.2), indicating a "no substantial" cluster structure. Cluster analysis (2) included 761 patients and resulted in a three-cluster solution, comparable to the first analysis. Again, a "no substantial" cluster structure was found (0.2).

Conclusion: Two cluster analyses on a large database of tinnitus patients revealed that clusters of patients are mostly formed by a different response of external influences on their disease. However, both cluster outcomes based on this dataset showed a poor stability, suggesting that our tinnitus population comprises a continuum rather than a number of clearly defined subgroups.

Keywords: tinnitus, cluster analysis, subgroup identification, heterogeneity of tinnitus, principle component analysis

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INTRODUCTION

Tinnitus is a prevalent condition, estimated to affect 5–18% of the adult population (1), which may lead to severe impairment in quality of life. Although many trials on tinnitus therapies have been conducted, hardly ever a treatment effect is demonstrated. A potential explanation for the lack of effectivity of these treatments might be the underlying heterogeneity of the disease. Therefore,

consensus on the optimal treatment of tinnitus gradually shifts from a "one size fits all" approach to a more patient-tailored approach. Possibly, a particular group of patients would be more likely to respond to treatment, if a selection is made on etiology, tinnitus characteristics, or patient characteristics. It might be that in a specific subgroup of tinnitus patients a particular treatment is successful, while this treatment is not successful in another subgroup of tinnitus patients. Thus, insight in the heterogeneity of the tinnitus spectrum might improve the management of these patients.

Identification of tinnitus subgroups is also important with regard to concomitant mental distress. Hoekstra et al. demonstrated that patients who express certain characteristics (i.e., high percentage of experience of tinnitus during the day, self-reported depression or anxiety, and subjective experience of tinnitus loudness) are more at risk for a high tinnitus burden (2). This subgroup of patients with high tinnitus distress needs more extensive counseling and follow-up in order to prevent mental breakdown.

In an attempt to identify subgroups of tinnitus patients, cluster analysis was used in this study. Cluster analysis is a statistical technique that divides data into groups, or clusters, which are meaningful and/or useful. It is an explorative analysis that assigns patients to clusters based on certain characteristics, so that patients look very much alike within a cluster (high within-group homogeneity) and, at the same time, are very different from the other clusters (low between-group homogeneity) (3). In research, this cluster analysis method is not only used in medicine studies to identify groups of patients but also in marketing for finding customer segments for example.

In 2008, Tyler et al. performed a preliminary cluster analysis on 153 patients with tinnitus (4). The cluster analysis of Tyler et al. identified distinct cluster characteristics, which were described as: (1) "constant distressing tinnitus," (2) "varying tinnitus that is worse in noise," (3) "tinnitus patients who are copers and whose tinnitus is not influenced by somatic modulation," and (4) "tinnitus patients who are copers but whose tinnitus is worse in quiet environments." Tyler et al. did not report a statistic value to identify the degree to which patients are clustered in these groups.

In this paper, we report on an exploratory cluster analysis of patients from the tinnitus database of the University Medical Center Groningen ($n = 1{,}783$ patients). We initially attempted to replicate the cluster analysis reported by Tyler et al (4); however, this was not possible as many of the variables used in their analysis were not identical or not available in our database. Instead, we report on two further cluster analyses. In the first analysis, the choice of variables that were entered in the cluster analyses was fully guided by the statistical techniques. In the second analysis, the selection of variables was based on the expert opinions in our tinnitus clinic. The aim of this study was to identify subgroups of tinnitus using cluster analysis, based on a very large dataset of tinnitus patients.

MATERIALS AND METHODS

Tinnitus Population

This study was performed at the Otorhinolaryngology Department of the University Medical Center Groningen (The Netherlands), which has a specialized multidisciplinary care group for tinnitus patients since 2007. Patients with severe complaints of tinnitus can be referred to this care group for medical consultation and psychological support. Almost all patients who visit this care group have consulted an audiologist and/or otorhinolaryngologist earlier. However, these patients were referred to our specialized tertiary care group by these specialists, because of the severity and impact of the complaints. Consultation at our clinic consists of thorough evaluation by an otorhinolaryngologist, an audiologist, radiologist, a medical social worker, and/or a psychologist.

Variables

The variables that were available for this cluster analysis were demographic characteristics (e.g., sex and age), tinnitus characteristics (e.g., duration of tinnitus, onset, lateralization, pitch, variable loudness), factors of influence on patients tinnitus (e.g., influence of loud sounds, noisy environment, movement of head and neck), tinnitus and quality of life-related questionnaires [e.g., tinnitus handicap index (THI), visual analog scale (VAS), and hospital anxiety and depression scale (HADS)], and audiological characteristics [e.g., frequency matching, pure tone audiometry (PTAs), loudness matching of tinnitus]. Hearing loss was divided into categories based on the pure tone audiogram: (1) no or slight hearing loss (both ears thresholds <30 dB on PTA thresholds at 0.25–0.5–1–2–4–8 kHz), (2) asymmetrical hearing loss (≥30 dB difference between both ears on the mean PTA thresholds at 2–4–8 kHz), (3) bilateral high tone hearing loss (both ears thresholds ≥30 dB on PTA thresholds at 2–4–8 kHz), (4) bilateral severe hearing loss (PTA thresholds >30 dB on 0.25–0.5–1–2–4–8 kHz), and (5) others. The available variables are all listed in Table S1 in Supplementary Material. All patient-reported variables were completed by the patients in booklets during the visit at the tinnitus outpatient clinic. Physician reported data, such as audiological characteristics, were also reported in booklets by the physician. All these routinely collected data were anonymized and entered in a database. For the current analysis, these data were retrospectively analyzed. The collection of data was approved by the Institutional Reviewer Board of the UMCG. No full review was needed due to the retrospective nature of this study.

Selection of Variables for Cluster Analysis

All variables that were collected were entered in the database. However, not all of these variables could be entered in the cluster analysis. In cluster analysis, it is important to keep the sample size in mind when deciding how many variables to enter in the analysis. Formann recommends the number of variables (m) of 2^m = sample size (5). In our study, the sample size is $n = 1783$, implying that the number of variables should be 10 or 11. There are two ways to select appropriate variables for cluster analysis: (1) a statistical approach with the use of principal component analysis (PCA) and (2) selection of variables based on "expert opinion," i.e., variables that are presumed to be clinically relevant and thought to be discriminative in the total group. Both selection procedures were performed in this study, resulting in two different cluster analyses.

Variable Reduction by PCA

A PCA is a dimension reduction technique that condenses variables that are highly correlated into a set of factors, thereby removing overlap and redundancy. PCA with Varimax rotation was performed on all variables with missing values ≤20%. The PCA revealed several factors, and for each factor, the variable with the highest loading was selected for inclusion in the cluster analysis.

Variable Reduction by "Expert Panel"

After excluding variables with missing values >20%, variables were selected by a group of tinnitus care professionals and investigators [Minke J. C. van den Berge (otolaryngology resident, Ph.D. candidate in tinnitus research), Pim van Dijk (medical physicist, audiologist, involved in the tinnitus care group), and Emile de Kleine (medical physicist, audiologist, involved in the tinnitus care group)]. Based on clinical experience and knowledge, those variables were selected that were deemed important in discriminating subgroups of tinnitus.

Cluster Analysis

The "two-step" cluster analysis method was used as the analyses that contained both categorical and continuous variables (6). Continuous variables were standardized by default. For distance measures, the log-likelihood method was used, as both continuous and categorical variables were entered in the analysis. The number of clusters to be formed was not specified in advance. The "silhouette measure of cohesion and separation" is a measure for the overall goodness-of-fit of the cluster structure that was found. It ranges from −1 to 1 (<0.25: no substantial structure; 0.26–0.50: weak structure and could be artificial; 0.51–0.70: reasonable structure; 0.71–1.0: strong structure) (7).

Differences in characteristics between clusters were compared according to the cluster membership variable, using one-way ANOVA for continuous variables and Pearson Chi-square tests for categorical variables. SPSS version 23.0 (Chicago, IL, USA) was used for all tests. The significance level was set at $\alpha = 0.05$, and all tests were two tailed.

RESULTS

Subject Characteristics

For this study, data from 1,783 consecutive patients who visited the UMCG tinnitus clinic between July 2007 and June 2016 were collected. The baseline characteristics of this study population are shown in **Table 1**. Variables that had >20% missing values are not shown in this table. In this population, 39.3% were females and the mean age was 53.6 ± 13.5 years. Tinnitus was unilateral in 50.7% of the cases and bilateral or central in 48.2%. The mean THI in the total patient group was 42.5 ± 23.2.

Outcome of Cluster Analysis with Variables Selected by PCA

The PCA was performed to obtain eigen values for each factor. The Kaiser–Meyer–Olkin measure of sampling adequacy was

TABLE 1 | Demographic and tinnitus-related characteristic of included tinnitus patients (*n* = 1,783).

	Total *N*	
Demographic characteristics		
Mean age ± SD	1,783	53.6 ± 13.5
Female gender—no. (%)	1,783	701 (39.3)
Tinnitus characteristics		
Mean duration of tinnitus ± SD—in years	1,635	6.8 ± 8.7
Onset tinnitus—no. (%)	1,685	
Acute		813 (48.2)
Gradual		872 (51.8)
Lateralization of tinnitus—no. (%)	1,496	
Bilateral/central		738 (49.3)
Unilateral		758 (50.7)
Description of tinnitus—no. (%)	1,607	
Tonal		715 (44.5)
Noise		708 (44.1)
Other		184 (11.4)
Experience of tinnitus—no. (%)	1,645	
Continuous		1,512 (91.9)
Intervals		133 (8.1)
Pitch of tinnitus—no. (%)	1,620	
Low		79 (4.9)
Moderate		403 (24.9)
High		997 (61.5)
Other		141 (8.7)
Variable loudness of tinnitus—no. (%)	1,762	
Yes		1,271 (72.1)
No		491 (27.9)
Mean percentage of burden during awake time ± SD	1,671	74.7 ± 28.0
Preference for silence or noise—no. (%)	1,559	
Silence		697 (44.7)
Noisy environment		862 (55.3)
Highest burden at time of the day—no. (%)	1,522	
Waking up		131 (8.6)
Morning		38 (2.5)
Afternoon		34 (2.2)
Evening		282 (18.5)
Night		149 (9.8)
Other		888 (58.3)
Sound unpleasant—no. (%)	1,446	
Never		137 (9.5)
Seldom		192 (13.3)
Some times		590 (40.8)
Most of the time		357 (24.7)
Always		170 (11.8)
Factors of influence on tinnitus		
Influence of noisy background—no. (%)	1,551	
Tinnitus louder		212 (13.7)
No effect		753 (48.5)
Tinnitus less loud		586 (37.8)
Influence of loud sounds—no. (%)	1,539	
Tinnitus louder		693 (45.0)
No effect		523 (34.0)
Tinnitus less loud		323 (21.0)
Influence of movement of head and neck—no. (%)	1,559	
Tinnitus louder		479 (30.7)
No effect		995 (63.8)
Tinnitus less loud		85 (5.5)
Influence of nap in the afternoon—no. (%)	1,419	
Tinnitus louder		233 (16.4)
No effect		1,003 (70.7)
Tinnitus less loud		183 (12.9)

(*Continued*)

TABLE 1 | **Continued**

	Total *N*	
Influence of stress—no. (%)	1,508	
Tinnitus louder		937 (62.1)
No effect		552 (36.6)
Tinnitus less loud		19 (1.3)
Influence of sleep deprivation—no. (%)	1,491	
Tinnitus louder		847 (56.8)
No effect		629 (42.2)
Tinnitus less loud		15 (1.0)
Audiological characteristics		
Mean PTA over 1–2–4 kHz (mean ± SD)	1,764	29.7 ± 18.0
Difference in mean PTA over 1–2–4 kHz between both ears (mean ± SD)	1,764	11.5 ± 18.1
Frequency matching of tinnitus—no. (%)	1,469	
0–2,000 Hz		378 (24.0)
2,000–4,000 Hz		239 (15.5)
4,000–6,000 Hz		287 (18.6)
6,000–8,000 Hz		275 (17.9)
>8,000 Hz		290 (23.4)
Type of hearing loss—no. (%)	1,782	
No/slight hearing loss		989 (55.5)
Asymmetrical hearing loss		265 (14.9)
Bilateral high tone hearing loss		243 (13.6)
Bilateral severe hearing loss		246 (13.8)
Other		39 (2.2)
Tinnitus questionnaires		
VAS tinnitus loudness[a] (mean ± SD)	1,615	66.7 ± 20.9
VAS tinnitus annoyance[a] (mean ± SD)	1,641	69.1 ± 22.6
THI-score (mean ± SD)	1,505	42.5 ± 23.2
HADS-depression score ± SD	1,676	5.4 ± 4.3
Indication HADS-depression[b]—no. (%)	1,676	
No indication depression		1,321 (78.8)
Indication depression		355 (19.9)
HADS-anxiety score ± SD	1,690	6.9 ± 4.3
Indication HADS-anxiety[b]—no. (%)	1,690	
No indication anxiety		1,103 (65.3)
Indication anxiety		587 (34.7)

[a]*On visual analog scale (VAS) range from 0 to 100%.*
[b]*Range 0–21, indication for depression/anxiety with score >8.*
Range is 0–100 unless indicated otherwise.
dB, decibel; PTA, pure tone audiometry; THI, tinnitus handicap inventory; HADS, hospital anxiety and depression scale.

0.681 and the Bartlett's test of sphericity was significant [χ^2 (6,127) = 325, $p < 0.001$], both indicating an appropriate factor model. A total of eight factors were extracted (based on the eigenvalue >1 rule), which together explained 55% of the total variance. Variables with the highest loading on each factor were selected. Subsequently, these variables ($n = 8$) were entered in the cluster analysis. The clustering revealed a four-cluster solution. As the analysis excludes every case when there is any variable with a missing value (listwise exclusion), the analysis was based on $n = 976$ patients. The cluster outcome showed a "silhouette measure of cohesion and separation" of 0.20, indicating that it is a "no substantial" cluster solution (7). Characteristics of these four identified clusters are shown in **Table 2**. The variables in the table are ranked from most discriminative between groups (top of the table) to less discriminative (bottom of the table). All variables differed statistically significant between the four clusters, except for the variables "VAS tinnitus annoyance" and "frequency of the tinnitus" ($p = 0.925$ and $p = 0.478$, respectively).

Cluster 1 ($n = 293$) is characterized by the fact that tinnitus is not easily influenced: loud sounds, sleep deprivation, and nap in the afternoon have no effect on their tinnitus. These patients have a relatively high difference between hearing loss in the right and left ear. These patients have relatively low HADS-depression scores.

Cluster 2 ($n = 259$) is distinguished by a gradual onset of the tinnitus. Also in this group, tinnitus is easily negatively influenced, especially by loud sounds and sleep deprivation. Both make their tinnitus louder.

Cluster 3 ($n = 197$) is a group of patients who report that their tinnitus is less loud when they hear loud sounds. Sleep deprivation and a nap in the afternoon mostly have no effect on their tinnitus.

Cluster 4 ($n = 227$) is typically a group with tinnitus of acute onset. They report that their tinnitus is easily negatively influenced by loud sounds or sleep deprivation. They show relatively high HADS-depression scores.

Outcome of Cluster Analysis with Variables Selected by Expert Panel

For the alternative method of choosing variables for clustering, 11 variables were selected by a panel of experts in the field. The selected variables (see **Table 3**) were entered in the cluster analysis. The outcome was a three-cluster solution, with a "silhouette measure of cohesion and separation" of 0.20, again indicating a poor solution. Because of listwise exclusion as described earlier, this analysis was based on $n = 761$ patients. About 527 of these patients were also included in the first cluster analysis. Also in this table, variables are ranked according to their degree of discriminative value. All variables differed significantly between the clusters (all p-Values <0.001).

Cluster 1 ($n = 287$) is a group of patients whose tinnitus is not easily influenced: loud sounds, stress, or movement of head and neck have no effect on their tinnitus loudness. Patients prefer a noisy environment. Sounds are never to seldom experienced as uncomfortably loud. The tinnitus is mostly unilateral. Although most patients in this group have no or slight hearing loss, other types of hearing loss are present in this group as well. They are not very much bothered or depressed by their tinnitus, as the THI and HADS-depression scores are low.

Cluster 2 ($n = 247$) is a predominantly male group, whose tinnitus gets worse by stress, loud sounds, and movement of head and neck. These patients prefer to be in a noisy environment. Sometimes, sounds are experienced as uncomfortably loud. Most of the patients have no or slight hearing loss. Tinnitus is bilateral, and the loudness of the tinnitus is variable.

Cluster 3 ($n = 227$) is characterized by the fact that their tinnitus is easily negatively influenced: loud sounds and stress clearly make their tinnitus louder. These patients prefer a silent environment. Often, patients find sounds uncomfortably loud. Tinnitus is often bilateral with most patients having no or slight hearing loss or asymmetrical hearing loss. The loudness of the tinnitus is variable.

TABLE 2 | Characteristics of the four clusters identified by clustering with variable selection based on principal component analysis.

	Cluster 1 ($n = 293$)	Cluster 2 ($n = 259$)	Cluster 3 ($n = 197$)	Cluster 4 ($n = 227$)	p-Value
Influence of loud sound (%)					**<0.001**[a]
Tinnitus louder	37.5	54.4	0	68.7	
No effect	62.5	39.4	0	30.0	
Tinnitus less loud	0	6.2	100	1.3	
Influence of sleep deprivation (%)					**<0.001**[a]
Tinnitus louder	0	89.2	47.2	86.8	
No effect	100	10.8	49.7	11.5	
Tinnitus less loud	0	0.8	3.0	1.8	
Onset (%)					**<0.001**[a]
Acute	48.1	0	49.2	100	
Gradual	51.9	100	50.8	0	
Influence of nap afternoon (%)					**<0.001**[a]
Tinnitus louder	0	27.0	11.2	22.9	
No effect	100	56.8	84.3	52.4	
Tinnitus less loud	0	16.2	4.6	24.7	
Hospital anxiety and depression scale (mean)	4.6	5.6	5.5	6.0	**0.001**[b]
Difference in mean PTA ADS	13.1	10.8	7.9	11.9	**0.015**[b]
Frequency of tinnitus (%)					0.478[a]
0–2,000 Hz	23.2	23.2	21.8	25.1	
2,000–4,000 Hz	14.7	13.9	21.3	15.0	
4,000–6,000 Hz	18.8	20.5	20.3	15.0	
6,000–8,000 Hz	19.5	17.0	12.7	19.4	
>8,000 Hz	23.9	25.5	23.9	25.6	
Visual analog scale tinnitus annoyance (mean)	69.2	69.4	68.2	69.6	0.925[b]

[a]*Pearson Chi-square test.*
[b]*One-way ANOVA.*
ADS, both ears; PTA, pure tone audiometry.
Bold fonts represent a significant p-values ($p < 0.05$).

DISCUSSION

Key Results

In this study, we performed cluster analysis with the aim to identify subgroups in a population of tinnitus patients. Variable selection for cluster analysis was performed in two ways: by a strict methodological approach based on PCA, and by expert opinion. These analyses identified four- and three-patient clusters, where the clusters showed clearly different characteristics. However, the clustering solution in both analyses was not substantial, as indicated by a poor cluster solution quality.

Although both cluster analyses gave different outcomes, there were also interesting similarities. In both cluster solutions, the effects of "stress" and "loud sounds" on tinnitus have a relatively high discriminative value between groups. In each analysis, a group was revealed in which patients report that their tinnitus gets louder from loud sounds, and there was a group that reported that their tinnitus got less loud. In an earlier cluster analysis by Tyler et al., it is described that their found clusters differed by the effect of external factors on patients' tinnitus: some patients are easily and negatively influenced by external factors and, in others, this has no effect (4). On the contrary, Tyler et al. describe a group that is characterized by high scores on tinnitus questionnaires and the HADS depression and anxiety scale. However, this was not reflected in our cluster solutions.

In the cluster analysis based on variables selected by experts, there was a clear distinction between a group that preferred a silent environment for their tinnitus and another group that had a preference for a noisy environment. The fact that some patients with tinnitus prefer noise and others prefer silence has been described earlier (8). This is interesting, as one might speculate that the latter group may have a higher change of responding well to sound therapy than the other group.

When interpreting these results, it must be kept in mind that the "silhouette measure" of both analyses was only 0.2. This is lower than the critical boundary of 0.25, which implies that there was no substantial clustering in this patient cohort. A lack of clustering indicates that the transition from one cluster to another is relatively smooth, without clear-cut boundaries. As a comparison, consider a group of cities, where the coordinates of the cities would go into a cluster analysis. If one group of cities is clearly separated from another group by a stretch of open land, the silhouette value will be large (when viewed from a distance, the cities will have a distinct silhouette of their skyline). However, if there is no such open land between the clusters, the silhouette value is low, consistent with the absence of substantial clustering. In our patient cohort, there were clearly no distinct "open stretches of land" between the clusters, suggesting that patient form a continuum rather than a clear clustering. As discussed above, the cluster analysis of Tyler et al. identified clusters with characteristics that show some resemblance to the clusters reported here. Unfortunately, Tyler et al. do not report a silhouette value or other measure of clustering. Hence, at present, it is not possible to discuss the clustering strength in their cohort.

Cluster analysis has been upcoming in medical research. Recently, an interesting cluster analysis on bilateral Meniere disease was published to define clinical subgroups with potential

TABLE 3 | Characteristics of the four clusters identified by clustering with variables selected by expert opinion.

	Cluster 1 (n = 287)	Cluster 2 (n = 247)	Cluster 3 (n = 227)	p-Value
Influence of loud sound (%)				**<0.001**[a]
Tinnitus louder	16.0	27.9	96.9	
No effect	56.1	38.5	3.1	
Tinnitus less loud	27.9	33.6	0	
Influence of stress (%)				**<0.001**[a]
Tinnitus louder	24.4	87.4	78.4	
No effect	74.9	9.7	21.6	
Tinnitus less loud	0.7	2.8	0	
Preference for silence or noise (%)				**<0.001**[a]
Silence	28.2	25.5	89.9	
Noisy environment	71.8	74.5	10.1	
Are sounds uncomfortably loud? (%)				**<0.001**[a]
Never	18.8	6.9	0.4	
Seldom	28.9	13.4	3.1	
Sometimes	31.4	61.5	31.7	
Most of the time	14.6	13.4	43.2	
Always	6.3	4.9	21.6	
Lateralization of tinnitus (%)				**<0.001**[a]
Bilateral/central	31.0	84.2	38.3	
Unilateral	69.0	15.8	61.7	
Hearing loss category (%)				**<0.001**[a]
No hearing loss	51.9	72.5	44.9	
Asymmetrical hearing loss	15.7	2.4	35.2	
Bilateral high tone hearing loss	17.4	8.9	9.7	
Bilateral flat hearing loss	12.9	16.2	6.2	
Other	2.1	0	4.0	
Variable loudness (%)				**<0.001**[a]
No	49.8	15.0	18.1	
Yes	50.2	85.0	81.9	
Tinnitus handicap index-score (mean)	32.6	48.0	46.9	**<0.001**[b]
Influence of movement of head and neck (%)				**<0.001**[a]
Tinnitus louder	8.4	40.1	39.2	
No effect	84.3	55.5	55.9	
Tinnitus less loud	7.3	4.5	4.8	
Hospital anxiety and depression scale (mean)	4.2	5.9	5.4	**<0.001**[b]
Gender (%)				**<0.001**[a]
Male	59.9	74.1	55.9	
Female	40.1	25.9	44.1	

[a]*Pearson Chi-square test.*
[b]*One-way ANOVA.*
Bold fonts represent a significant p-values ($p < 0.05$).

similar etiologies. In this study, five clinical variants of bilateral Meniere disease were found based on six clinical variables and with a high silhouette measure of 0.8 (9). This study is not only beneficial to improve the selection of patients but also can explain the negative treatment effects of several treatment trials, as results can be biased by a heterogeneous patient group based on etiology (9). The difficulty in cluster analysis is that it is a type of analysis that is very sensitive to change of variables. The selection of variables is critical for the outcome of the cluster analysis (6). Generally, highly correlated variables should be avoided and it is important to select variables that can make a clear-cut differentiation between clusters (6). The systematic statistical approach of selecting variables using the highest factor loading on extracted factors by PCA is often used and has the advantage of choosing variables in a reproducible, transparent way. A downside of this technique is that the factor solution only explains a certain amount of variance and, therefore, much information is discarded. Eliminating factors with low loadings on the extracted factors has the same effect (10). This may lead to a reduced success of a subsequent cluster analysis. On the other hand, a disadvantage of selecting variables based on clinical knowledge or "gut feeling" is that it is less transparent. Also, unrecognized highly discriminating variables may remain undiscovered.

Strengths of the Study

For this study, a very large database of tinnitus patients was used with almost 1,800 patients. Even after exclusion of patients with missing values, $n = 976$ and $n = 761$ could be included in the cluster analyses. We expect that, if clear clustering would have existed with these variables, we would have been able to find it in these groups. There was an overlap of 527 patients who were included in both the first cluster outcome and the second cluster outcome. This is a substantial overlap, pointing out that it does not seem likely that the differences between both cluster analyses are caused by the differences in included patients.

Limitations

This study explored the patient cohort of a tertiary tinnitus referral center. Thus, the population described here consists of a group of tinnitus patients who were persistent in their search for treatments for their tinnitus. Our patient cohort may, therefore, be biased with a certain type of tinnitus patients. Potentially, a study including also less-persistent help seeking and non-help-seeking subjects would have identified a clearer clustering.

Although we had access to a large database of tinnitus patients ($n = 1{,}783$), over the years, there were changes in the variables that were collected because of changes made to the diagnostic protocol. Since the cluster analyses required a complete set of data for each patient, not all 1,783 patients could be included in the analysis, but 976 and 761.

Furthermore, it is debated internationally whether tinnitus is a disease or a symptom. One can look at it in both ways: when tinnitus is a result of an acoustic neuroma, then tinnitus can be a symptom. However, if we look at tinnitus as the result of defect on a cellular level of the auditory cortex, then tinnitus can be regarded as a disease. In most patients visiting our clinic, the etiology of the tinnitus is unclear. The fact is that these patients included in our dataset experience bothersome tinnitus. Within this group, we aimed to find subgroups such as patients with continuous central, loud tinnitus tend to have a high score on THI and VAS and find that their tinnitus gets worse in noisy environments. If we are able to find such patterns, may be we can adjust our treatment strategy to that (in this example, hearing aids might not be successful). Although the raised issue about tinnitus being a symptom or disease is important, we believe that this analysis looking for clusters of patients based on tinnitus characteristics transcends this issue.

Finally, the low silhouette value indicates that this patient cohort represents a heterogeneous group without clear clustering. Obviously, any cluster analysis outcome highly depends on the variables that we entered into the clustering algorithm. Our patient data consisted of mainly audiometry and questionnaire metrics. In these cluster analyses, tinnitus patients appear to represent a continuum rather than clearly defined subgroups, based on a low silhouette measure. However, it is possibly that other metrics (e.g., fMRI/EEG, genetic evaluation) are able to identify tinnitus subgroups. In other words, the lack of clustering in our analyses does not imply that clusters do not exist. However, if clusters exist, they cannot be identified with the variables that were considered here.

CONCLUSION

Two cluster analyses of a large patient cohort identified three and four groups of tinnitus patients, respectively. The clustering was not substantial, as a low silhouette measure of the cluster solutions was found. This indicates that in this particular cohort, tinnitus patients appear to represent rather a continuum than clearly defined subgroups. This finding may have consequences for future treatments: if clear subgroups would have been present, clearly distinct treatment might be developed in the future. However, for a continuum of patients, it may be necessary to use a number of treatments to find the optimum for each individual patient. Obviously, our conclusion is based on the set of variables that were at our disposal. Possibly, new future ways to characterize tinnitus patients may be able to find distinct subgroup in tinnitus patients.

AUTHOR CONTRIBUTIONS

Conception of study and design; writing of manuscript: MB and PD. Intellectual contribution to analyses: MB, PD, and EK. Critical revision of the manuscript: RF, RA, PD, and JD. Data acquisition: MB, PD, RA, RH, and EK.

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REFERENCES

1. Savage J, Waddell A. Tinnitus. *BMJ Clin Evid* (2014) 2014:0506.
2. Hoekstra CE, Wesdorp FM, van Zanten GA. Socio-demographic, health, and tinnitus related variables affecting tinnitus severity. *Ear Hear* (2014) 35(5):544–54. doi:10.1097/AUD.0000000000000045
3. Clatworthy J, Buick D, Hankins M, Weinman J, Horne R. The use and reporting of cluster analysis in health psychology: a review. *Br J Health Psychol* (2005) 10(Pt 3):329–58. doi:10.1348/135910705X25697
4. Tyler R, Coelho C, Tao P, Ji H, Noble W, Gehringer A, et al. Identifying tinnitus subgroups with cluster analysis. *Am J Audiol* (2008) 17(2):S176–84. doi:10.1044/1059-0889(2008/07-0044)
5. Formann AK. *Die Latent-Class-Analyse: Einführung in Theorie und Anwendung*. Weinheim, Germany: Beltz (1984). Available from: http://catalog.hathitrust.org/api/volumes/oclc/16851238.html
6. Mooi E, Sarstedt M. Cluster analysis. *A Concise Guide to Market Research*. Berlin, Heidelberg: Springer-Verlag (2011). p. 237–84.
7. Kaufman L, Rousseeuw PJ, editors. *Finding Groups in Data: An Introduction to Cluster Analysis*. Hoboken, NJ: John Wiley & Sons, Inc. (1990).
8. Stouffer JL, Tyler RS. Characterization of tinnitus by tinnitus patients. *J Speech Hear Disord* (1990) 55(3):439–53. doi:10.1044/jshd.5503.439
9. Frejo L, Soto-Varela A, Santos-Perez S, Aran I, Batuecas-Caletrio A, Perez-Guillen V, et al. Clinical subgroups in bilateral Meniere disease. *Front Neurol* (2016) 7:182. doi:10.3389/fneur.2016.00182
10. Dolnicar S, Grunn B. Challenging "factor-cluster-segmentation". *J Travel Res* (2008) 47:63–71. doi:10.1177/0047287508318910

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Neuroanatomical Alterations in Tinnitus Assessed with Magnetic Resonance Imaging

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Previous studies of anatomical changes associated with tinnitus have provided inconsistent results, with some showing significant cortical and subcortical changes, while others have found effects due to hearing loss, but not tinnitus. In this study, we examined changes in brain anatomy associated with tinnitus using anatomical scans from 128 participants with tinnitus and hearing loss, tinnitus with clinically normal hearing, and non-tinnitus controls with clinically normal hearing. The groups were matched for hearing loss, age and gender. We employed voxel- and surface-based morphometry (SBM) to investigate gray and white matter volume and thickness within regions-of-interest (ROI) that were based on the results of previous studies. The largest overall effects were found for age, gender, and hearing loss. With regard to tinnitus, analysis of ROI revealed numerous small increases and decreases in gray matter and thickness between tinnitus and non-tinnitus controls, in both cortical and subcortical structures. For whole brain analysis, the main tinnitus-related significant clusters were found outside sensory auditory structures. These include a decrease in cortical thickness for the tinnitus group compared to controls in the left superior frontal gyrus (SFG), and a decrease in cortical volume with hearing loss in left Heschl's gyrus (HG). For masked analysis, we found a decrease in gray matter volume in the right Heschle's gyrus for the tinnitus group compared to the controls. We found no changes in the subcallosal region as reported in some previous studies. Overall, while some of the morphological differences observed in this study are similar to previously published findings, others are entirely different or even contradict previous results. We highlight other discrepancies among previous results and the increasing need for a more precise subtyping of the condition.

Keywords: tinnitus, brain anatomy, auditory cortex, voxel-based morphometry, surface-based morphometry

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INTRODUCTION

Tinnitus, the perception of a phantom sound in the absence of an external source, is experienced chronically by approximately 5–15% of the population (Baguley et al., 2013). The onset of tinnitus is typically associated with aging or exposure to loud noise, cumulative or sudden, such that hair cell damage occurs with subsequent hearing impairment. Tinnitus can cause significant distress, causing problems such as depression, anxiety and sleep disorders (Henry et al., 2005).

The exact cause of tinnitus and its associated pathophysiology remains unknown. Although tinnitus is commonly initiated by damage to the peripheral auditory system, it is believed that the sound percept is generated and maintained in the brain. This has been confirmed by surgical interventions where the auditory nerve is bisected yet the perception of the tinnitus sound remains (House and Brackmann, 1981). Current consensus is that the initial cause of many forms of tinnitus is strongly related to cochlear damage and the resulting hearing loss, which may cause changes to neural coding properties (Seki and Eggermont, 2003).

Recently, both functional and structural imaging have been used to investigate changes in the brain associated with tinnitus. Functional studies based on changes in regional cerebral blood flow (using positron emission tomography; PET) have found regions of the brain associated with the perception of the tinnitus sound (Mirz et al., 1999, 2000a,b; Reyes et al., 2002). However, PET has limitations due to the required administration of radioactive tracer restricting longitudinal studies. Functional magnetic resonance imaging (fMRI) has also been used to measure changes in brain activity in people with tinnitus. This method has high spatial resolution, but significant acoustic noise is generated by the scanner during the imaging process that may have a confounding effect on the results (Melcher et al., 2000; Lanting et al., 2008, 2009). Moreover, fMRI is able to detect persistent changes in neural baseline activity that can be associated with chronic tinnitus. Magneto- and electroencephalography (MEG, EEG) have been used to examine differences in power spectra and to localize regions of the brain associated with tinnitus. However, these methods suffer from poor spatial resolution and results are contradictory (for review see Adjamian et al., 2009). All functional imaging methods have confounding issues that relate to task performance, and how well the participant is able to describe the changes they are experiencing.

Anatomical brain changes are believed to arise from plasticity and reorganization of the brain. Analysis of high resolution MRI anatomical data of the brain, thus complements functional imaging. Various studies have shown that functional changes are directly linked to structural changes in tinnitus (Mühlau et al., 2006; Schneider et al., 2009; Husain et al., 2011; Leaver et al., 2011, 2012; Mahoney et al., 2011; Aldhafeeri et al., 2012; Schecklmann et al., 2012; Boyen et al., 2013; Melcher et al., 2013). Adjamian et al. (2014) recently provided a comprehensive review of this literature.

Changes in structure, gray and white matter volumes and brain shape are indicative of differences in prolonged neuronal activity and connectivity between brain regions (Pfefferbaum et al., 1994; Good et al., 2001; Draganski et al., 2004; Maguire et al., 2006). We have previously reviewed several approaches to structural analysis that have been growing in popularity in recent years, including: voxel-based morphometry (VBM), surface-based morphometry (SBM), deformation-based morphometry (DBM), tensor-based morphometry (TBM) and diffusion tensor imaging (DTI) (Adjamian et al., 2014). VBM allows assessment using statistical metrics of voxel-wise changes in the gray matter volume of the neocortex between populations, or in any given population relative to a clinical measure. However, VBM has been criticized for being sensitive to image registration procedures that can yield spurious results (Bookstein, 2001). On the other hand, using the surface of the brain, SBM highlights the cortical folding of the brain and avoids the registration problems, to some extent, by investigating differences in the area, thickness of tissue or the curvature of the cortex between subjects (Winkler et al., 2010).

Current evidence regarding the tinnitus-related structural changes in the brain has produced a range of contradictory and varied results. Mühlau et al. (2006) were the first to show structural changes related to tinnitus using whole brain and region-of-interest (ROI) voxel-wise VBM analyses. This study showed a reduction in gray matter in subcallosal areas, such as the nucleus accumbens (NAc), and an increase in the medial geniculate nucleus (MGN). However, other studies have failed to replicate these results using largely similar methods (e.g., Landgrebe et al., 2009; Husain et al., 2011; Melcher et al., 2013). In line with Mühlau et al. (2006) findings, Rauschecker et al. (2010) suggested a gating model in which tinnitus results from a failure to inhibit noise, allowing unpleasant noise signals to reach the auditory cortex (AC). This model is based upon evidence from human neuroimaging and animal studies and involves cortical and subcortical regions consisting of the amygdala, the NAc, the ventromedial prefrontal cortex (vmPFC), and the reticular nucleus of the thalamus (Leaver et al., 2011, 2012; Seydell-Greenwald et al., 2014). This limbic corticostriatal pathway has been shown to play an important role in the suppression of unpleasant sounds. Consequently, abnormalities within these areas of the brain may lead to the perception of a tinnitus sound and the negative emotions associated with chronic tinnitus. As this model predicts tinnitus-related changes in the activity of specific structures, it can be evaluated using MRI-based morphological analysis techniques.

In a recent article, we identified various factors which may underlie the reported inconsistent findings (Adjamian et al., 2014). These include the heterogeneity of tinnitus characteristics such as its etiology, duration and lateralization. Moreover, in most studies, important parameters that may independently affect brain anatomy have not been adequately controlled for, such as age and hearing loss (Lee et al., 2007; Crippa et al., 2010). Another important factor may be the small size of participant groups, often due to recruitment difficulties, with sometimes as few as 11 tinnitus participants. Small sample sizes result in low statistical power, such that effects from one or two participants can dramatically change the overall outcomes. In addition to the low statistical power, many studies employ thresholds uncorrected for multiple comparisons, which increases the chance of false positives. Another possible reason for the inconsistent findings might be the masks used to specify ROIs in the analysis. Six groups have used the same masks as defined in Mühlau et al. (2006), but with varying results (Mühlau et al., 2006; Landgrebe et al., 2009; Husain et al., 2011; Leaver et al., 2011; Boyen et al., 2013; Melcher et al., 2013). Others have focused upon whole-brain analysis (Schecklmann et al., 2012, 2013), specific ROIs such as Heschl's gyri (Schneider et al., 2009)

and the inferior/superior/middle frontal gyri (Aldhafeeri et al., 2012), large ROIs such as the temporal lobe, upper brain stem and bilateral orbito-frontal cortices (Mahoney et al., 2011), or a range of brain regions such as the thalamus, caudate, putamen and globus pallidus (Leaver et al., 2012). This range of foci means that it is difficult to distinguish a consistent pattern across studies.

Given the previous inconsistent findings, we aimed to address some of these methodological issues using MRI data collected from a large cohort of tinnitus participants and matched controls at partner research centers in Nottingham. We tested the hypothesis that tinnitus is accompanied by changes in gray and white matter compared to non-tinnitus controls. More specifically, we aimed to detect structural changes using VBM and SBM, whilst controlling for variables such as tinnitus severity, hearing loss, and age. Based on the gating mechanism proposed by Rauschecker et al. (2010), we hypothesize that these changes in brain anatomy will be located in the MGN, the vmPFC, and the NAc. We use various ROI masks based on previous studies, including those by Mühlau et al. (2006) and Leaver et al. (2011) to examine areas beyond the subcallosal region. Finally, given that tinnitus is an ongoing sensation that has been shown to affect resting state activity (Husain and Schmidt, 2014), we also investigated anatomical changes in the areas constituting the default mode network (DMN), which is linked to resting-state activity (Greicius et al., 2003).

MATERIALS AND METHODS

Subject Recruitment

One hundred twenty eight participants (73 tinnitus and 55 controls) were recruited as part of other functional imaging studies at the Medical Research Council (MRC) Institute of Hearing Research (IHR; $n = 61$) and the National Institute for Health Research (NIHR) Nottingham Hearing Biomedical Research Unit (BRU; $n = 67$). The IHR cohort had been recruited as part of MEG studies to investigate oscillatory and evoked responses in tinnitus (Adjamian et al., 2012; Sereda et al., 2013). The BRU cohort was recruited for a study examining hearing aid benefits for tinnitus with functional MRI (Davies et al., 2014). The IHR study was approved by the National Health Service (NHS) East Midlands Nottingham local research ethics Committee 2, and sponsored by the MRC. The BRU study was approved by the North Nottinghamshire Research Ethics Committee, and sponsored by the NHS Nottingham University Hospital Trust.

Audiograms comprising frequencies from 0.25 to 12 kHz were acquired for each subject. Clinically normal hearing was based on the average of the pure-tone hearing threshold levels 250, 500, 1000, 2000 and 4000 Hz <20 dB Hearing Loss (HL; British Society of Audiology, 2011). Participants with tinnitus completed either the Tinnitus Handicap Inventory (THI; Newman et al., 1996; $N = 30$, the IHR cohort) or Tinnitus Handicap Questionnaire (THQ; Kuk et al., 1990; $N = 43$, the BRU cohort) to assess its severity. Because these questionnaire scores have the same range (0–100) and show high convergent validity (Fackrell et al., 2014), we applied a simple stratification to combine both sets of scores so that all tinnitus participants had a tinnitus severity score that fell within one of five categories: grade one, 0–16 (low); grade two, 17–36 (mild); grade three, 37–56 (moderate); grade four, 57–76 (severe); or, grade five, 77–100 (catastrophic). These boundaries were informed by a UK THI grading (McCombe et al., 2001).

Group Classifications

Three separate groups of participants were defined for statistical analysis. The aim of these groups is to isolate particular features so that we could maximize the statistical power to detect various potential effects.

Group 1—All Subjects

The first group consisted of all participants in the study, divided into two subgroups of tinnitus participants and non-tinnitus controls. This comparison maximized the statistical power available to detect possible changes related to tinnitus.

Group 2—Severe Tinnitus vs. Matched Controls

The second group consisted of participants with severe or catastrophic tinnitus and controls individually matched for age, gender and hearing loss. This comparison was made to assess the effect of highly intrusive tinnitus compared to a matched sample.

Group 3—Tinnitus With Clinically Normal Hearing vs. Matched Controls

The final group consisted of tinnitus participants with clinically normal hearing, again matched individually for age and gender to controls with clinically normal hearing. This comparison isolated the effect of tinnitus from the effect of hearing loss.

Information and number of participants pertaining to each of the subgroups are listed in **Table 1** and their mean audiograms are shown in **Figures 1A–C**. There were no significant differences in age, gender or hearing loss between the tinnitus participants and control participants in any of the three groups.

Data Acquisition

Subjects were scanned either on a 3-T or 1.5-T Philips scanner by means of a high resolution magnetization-prepared rapid gradient-echo (MPRAGE) acquisition (resolution = $1 \times 1 \times 1$ mm^3, repetition time (TR) = 8 s, echo time (TE) = 3.74 ms, field of view (FOV) = $256 \times 256 \times 160$ mm^3). One hundred twenty one participants were scanned on the 3 T scanner, and 7 (4 tinnitus and 3 non-tinnitus controls) on the 1.5 T, because, the 3 T scanner was unavailable.

VBM Data Processing

The data processing was performed using Statistical Parametric Mapping (SPM8[1]). Each participant's anatomical image was

[1]http://www.fil.ion.ucl.ac.uk/spm/software/spm8/

TABLE 1 | Demographic information and hearing status for each of the three groupings.

Group	Subgroup	*N*	Age (years)				Gender		PTA (dB HL)
			Mean	Std	Min	Max	Male	Female	Mean (both ears)
1 (All)	Tinnitus	73	58.38	12.41	24	80	43	30	28.62
	Controls	55	56.91	16.39	19	76	30	25	24.19
2 (Severe Tinnitus vs. matched Controls)	Tinnitus	16	55.06	15.53	24	80	8	8	21.51
	Controls	16	53.69	14.85	25	72	8	8	20.91
3 (Tinnitus with clinically normal hearing vs. matched controls)	Tinnitus	15	47.60	16.66	24	80	6	9	8.22
	Controls	15	50.20	17.25	20	71	6	9	8.62

segmented into gray matter, white matter, cerebrospinal fluid (CSF) and other tissues. For each group (described above), Diffeomorphic Anatomical Registration Through Exponentiated Lie algebra (DARTEL: Ashburner, 2007) was used to create a white matter and gray matter template using segmented images of participants from all of the groups to improve the registration to a common space. That is, each group had its own gray and white matter template to which both the tinnitus participants and controls were realigned. Whilst aligning the individual images to the template, the volume of white and gray matter was preserved (i.e., modulated images were computed).

The data were then resampled to 2-mm isotropic resolution, aligned with Montreal Neurological Institute (MNI) space and subsequently smoothed with an isotropic 10-mm full-width at half-maximum (FWHM) kernel. Finally, these images were set at

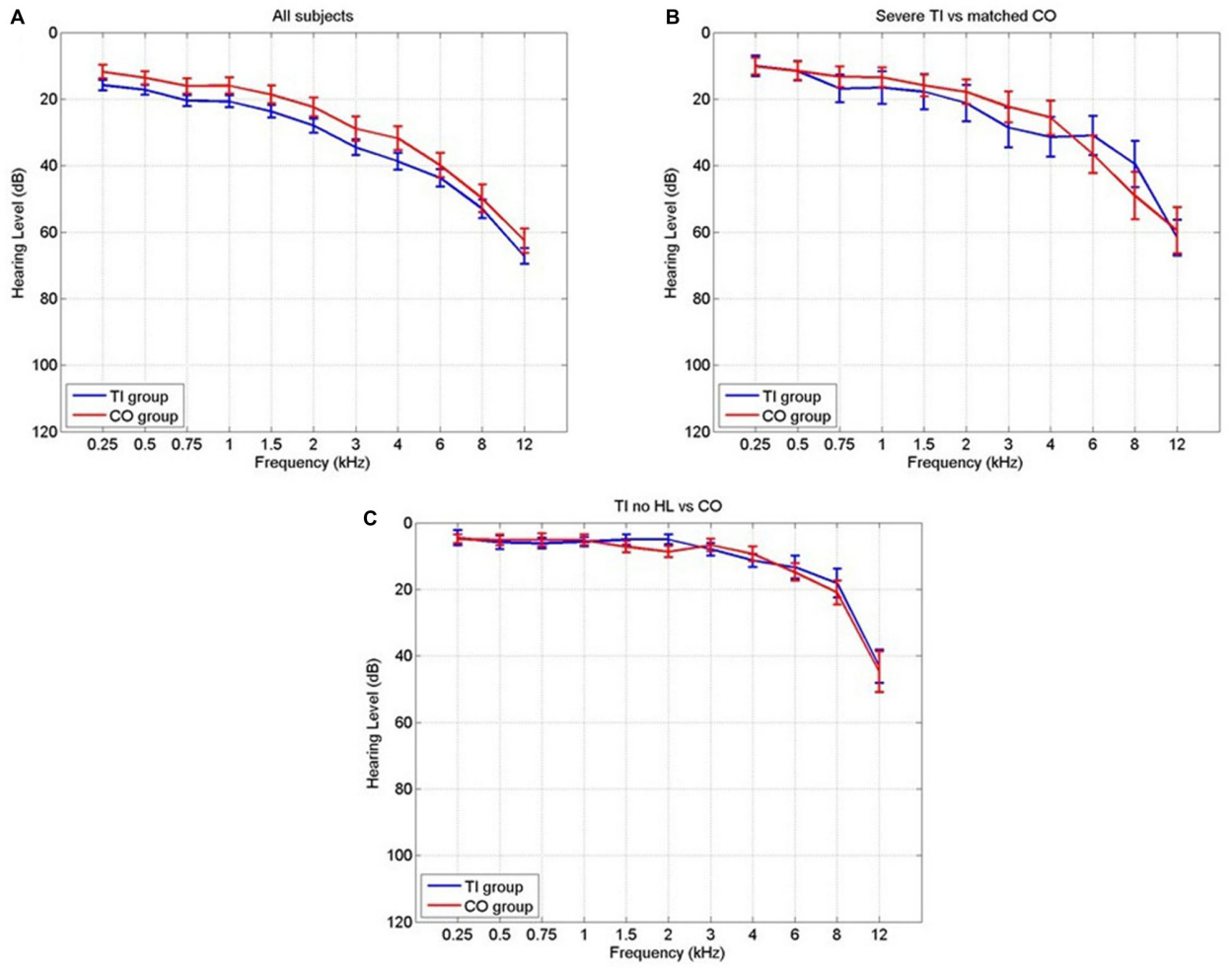

FIGURE 1 | The audiograms for each comparison group for the tinnitus participants (blue) and the controls (red) in (A) all subjects, (B) severe tinnitus and matched controls and (C) tinnitus with no hearing loss and matched controls.

TABLE 2 | The definitions of the masks used and how they were defined.

Mask	Brodmann areas	WFUpickatlas	MNI coordinates (*x, y, z*)	Volume (bilateral, cm^3)
Cochlear nucleus (CN)	–	–	±10, −38, −45	1.30
Superior olivary complex (SOC)	–	–	±13, −35, −41	1.30
Inferior colliculus (IC)	–	–	±6 , −33, −11	1.30
Medial geniculate nucleus (MGN)	–	–	±17, −24, −2	4.11
Heschl gyrus (HG)	41	–	–	13.45*
Auditory cortex (AC)	22, 41 and 42	–	–	74.74*
Superior temporal gyrus (STG)	–	Superior temporal gyrus	–	83.97
Nucleus accumbens (Na_C)	–	Nucleus accumbens left and right	–	4.25
Ventromedial prefrontal cortex (vmPFC)	10, 11, 12, 13, 14, 25 and 32	–	–	77.19
Default mode network (DMN)	–	Posterior cingulate, medial frontal gyrus and middle temporal gyrus	–	120.90

**Indicates volume after 2 mm dilation;—Indicates no information available.*

a threshold of 5% to compensate for edge voxels and the blurring effect caused by smoothing the data.

Statistical maps were produced using linear regression through an ANCOVA model with a group factor (tinnitus or control) and additional covariates for tinnitus severity grading (1–5); controls were all assigned a value equal to the average of the tinnitus subgroup in order for this regressor to be orthogonal to the group factor), left and right ear pure tone averages (PTA) over the tested frequencies to 8 kHz, age, and gender. The group factor and all four covariates were tested for statistical significance. In addition, for each subject, the whole brain gray matter and white matter volumes were used as an additional covariate in the respective ANCOVA models to compensate for whole brain volume differences between subjects. In an alternative model, the raw THI/THQ scores were used as a covariate instead of the tinnitus severity grade, but the results were equivalent and are therefore not shown here. All the statistical maps were family-wise error (FWE) corrected for multiple comparisons using Gaussian Field theory with a confidence threshold of 0.05. The statistical analysis was run on the whole brain as well as restricted to each of the masks defined below.

FreeSurfer Data Processing

Cortical surface reconstruction was performed using the standard FreeSurfer v 5.3.0[2] pipeline run on a Linux CentOS 6 platform. In brief, this includes removal of non-brain tissues, automated Talairach transformation, segmentation of the subcortical white matter and deep gray matter volumetric structures, intensity normalization, tessellation of the gray and white matter boundary, automated topological correction and surface deformation. The technical details are fully described in prior publications (Dale et al., 1999; Fischl et al., 1999a,b; Fischl and Dale, 2000).

Measures of each subject's cortical thickness, area and volume were computed. Each subject's cortical surfaces were morphed onto the standard inflated brain and the thickness, area and volume values were smoothed with a 10-mm FWHM Gaussian kernel. The same ANCOVA model as in the VBM analysis, with one group factor and four covariates, was fitted to each measure (one difference with the VBM analysis however is that we did not use mean/total thickness/area/volume as an additional covariate). Monte-Carlo simulations were run to correct for multiple comparisons at the cluster level (Hayasaka and Nichols, 2003), as implemented in Freesurfer (Hagler et al., 2006). For the simulations, the voxelwise (uncorrected) threshold was set to $p = 0.01$ and the clusterwise (corrected) threshold was set to $p = 0.05$. Statistical analysis was conducted on the whole surface as well as in each of the masks, defined below.

Masks

To complement the whole-brain analyses, 10 masks were defined to assess changes in particular regions of the brain that might be attributable to the tinnitus percept. These masks were chosen based on the regions specified by the gating mechanism proposed by Rauschecker et al. (2010), and those used by Mühlau et al. (2006) that had shown tinnitus-related changes in brain volume.

Detailed definition of each mask is given in **Table 2**. The masks based on Rauschecker's gating mechanism are shown in **Figure 2**. The WFUpickatlas in SPM8 was used to define the masks. The AC mask (Brodmann areas 22, 41 and 42) was dilated by 2 mm in all directions to accommodate small registration errors and individual variability issues. The NAc mask was defined from the WFUpickatlas, and the vmPFC consisted of Brodmann areas 10, 11, 12, 13, 14, 25 and 32. Heschl's gyrus (HG; Brodmann area 41) was also dilated by 2 mm to account for small misregistration effects. The DMN comprised the posterior cingulate cortex (pCC), medial frontal gyrus (mFC) and middle temporal gyrus (MTG). The superior temporal gyrus (STG) was selected as it relates to normal auditory functioning, but does not include all areas of the AC that lie on the supratemporal plane. The masks for the AC, HG and STG had overlapping regions. The vmPFC and DMN also showed overlap.

Four subcortical masks were defined using coordinates based on Mühlau's study, to determine if their results could be replicated with our larger cohort of participants. These masks are shown in **Figure 3**. Bilateral 5-mm radius spheres were defined for the Cochlear nucleus (CN), the Superior olivary complex (SOC), and the Inferior colliculus (IC). In addition to these, bilateral 8 mm radius spheres were defined in the MGN.

[2]http://surfer.nmr.mgh.harvard.edu/

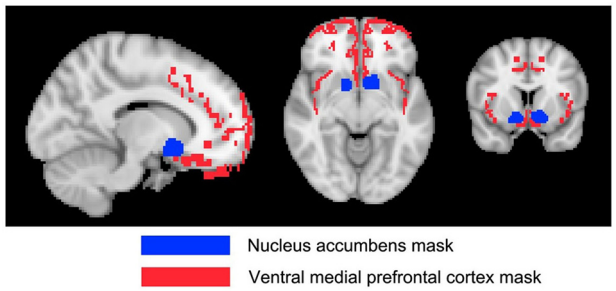

FIGURE 2 | The Rauschecker model brain regions [nucleus accumbens (NAc) and ventromedial prefrontal cortex (vmPFC)] that have been defined as a mask for further analysis.

All of the masks were used for the VBM analysis. Five of these masks were used for the SBM analysis (AC, DMN, HG, vmPFC and STG) as the other masks targeted subcortical structures. To create the surface masks, the volumetric masks were projected onto the standard inflated brain and manually corrected to remove non-contiguous vertices due to projection errors (corresponding to voxels contiguous in volumetric space but belonging to non-contiguous gyri).

ROI Analysis

For all of the masks and groups, ROI analyses were performed to compute the magnitude of differences between the tinnitus participants and controls. The various measures of interest (i.e., gray and white matter volume for VBM; surface thickness, area, and volume for SBM) were averaged (gray and white matter volume, surface cortical thickness) or summed (surface area and volume) across the voxels or vertices in a mask, and statistically compared between subgroups using *t*-tests. In Freesurfer, surface area/volume measures were normalized by the total surface area/volume of each hemisphere and all individual measures were averaged between the left and right surface masks.

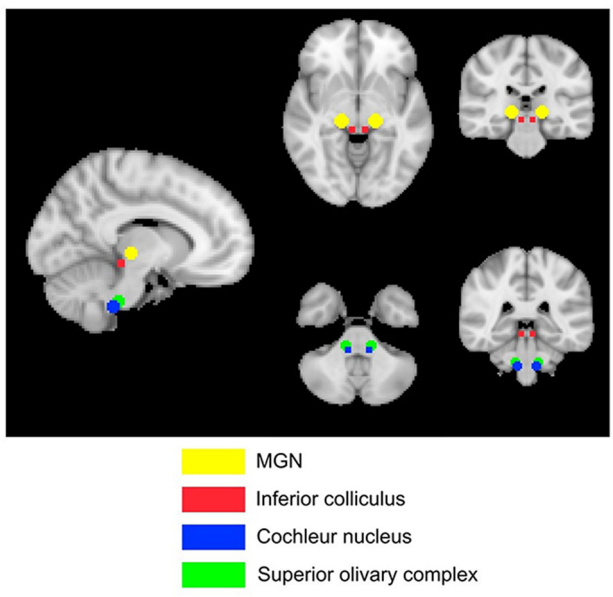

FIGURE 3 | The Mulhau masks that have previously shown anatomical changes relating to tinnitus.

RESULTS

Correlation Analysis

Pearson correlation coefficients were calculated between hearing loss PTA, age and tinnitus severity data to assess whether any covariates were interdependent.

The correlation between age and hearing loss across all subjects was significant ($r = 0.41$, $p = 10–6$; **Figure 4**). The correlations between tinnitus severity and hearing loss ($r = 0.08$, $p = 0.36$) or tinnitus severity and age ($r = -0.00$, $p = 0.98$) did not reach significance.

Morphometry

Overall, we found a number of clusters of significant effects in various masks and for different contrasts, varying in size. A summary of the significant effects of interest (Tinnitus vs. Controls and Tinnitus severity) is given in **Table 3** and they are described in detail in the following sections and shown in **Figures 6–11**. Full details of all findings, including significant effects of other covariates, are presented in the Supplementary Material Tables SI1–SI5.

Grouping 1—All subjects

Whole-head voxel/vertexwise analysis

In both the VBM and SBM analyses, by far the largest effects (in terms of extent of the significant clusters) were for the age and gender covariates. Regarding tinnitus, tinnitus severity, and hearing loss, only a few small clusters were found and only when using SBM.

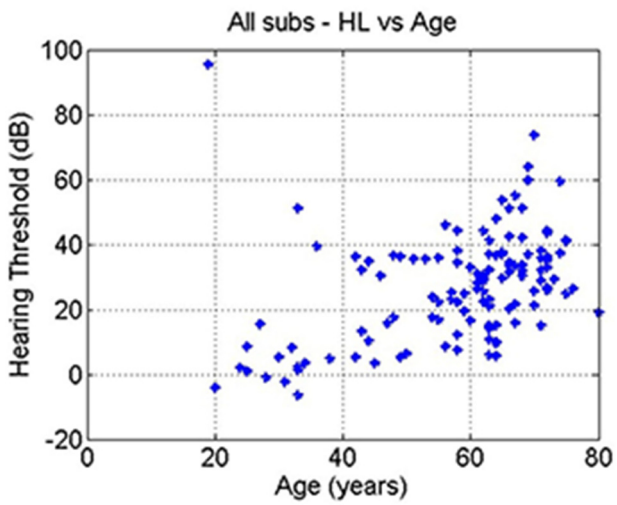

FIGURE 4 | The age of each subject plotted against their hearing threshold.

TABLE 3 | Summary of results.

	VBM		
	Gray matter		**White matter**
Grouping 1. (All TI vs. All controls)	Reduction for TI vs. CO in right HG (0.008 cm^3) and with increasing TI severityin right DMN (0.056 cm^3). ROI analysis: increase in SOC for TI vs. CO (5.5%)		Reduction in TI vs. CO in right MGN (0.50 cm^3)
Grouping 2. (Severe TI vs. Matched controls)	No Differences		Reduction in TI vs. CO in right MGN (0.19 cm^3)
Grouping 3. (TI with Clinically normal hearing vs. matched controls)	No Differences		Increase in TI vs. CO in left HG (0.008 cm^3); Increase with TI severity in left CN (0.008 cm^3)
	SBM		
	Thickness	**Area**	**Volume**
Grouping 1. (All TI vs. All controls)	Decrease for TI vs. CO in left AC/STG (4.51 cm^2), left superior frontal gyrus (5.04 cm^2), right STS (1.69 cm^2) and right HG (0.33 cm^2). Increase with TI severity in right middle Temporal gyrus (2.61 cm^2), and right rostro medial Frontal gyrus (2.02 cm^2). ROI analysis: 2.1% decrease for TI vs. CO in AC	Decrease with TI severity in right precuneus (12.5 cm^2) ROI analysis: increase for TI vs. CO in vmPFC (1.31%) and decrease in AC (−2.1%)	Decrease for TI vs. CO in right HG (0.27 cm^2) and with TI severity in right precuneus (8.35 cm^2) ROI analysis: decrease for TI vs. CO in Auditory cortex (−3.9%), HG (−4.5%) and STG (−2.4%)
Grouping 2. (Severe TI vs. Matched controls)	Decrease for TI vs. CO in left AC (0.85 cm^2).	No Differences	No Differences
Grouping 3. (TI with Clinically normal hearing vs. matched controls)	Decrease for TI vs. CO in AC/STG (1.17 cm^2) and right rostro-middle frontal gyrus (1.71 cm^2). Increase with tinnitus severity in left midtemporal gyrus (3.18 cm^2)	Decrease with TI severity in left HG/AC (3.18 cm^2), right superior parietal gyrus (5.80 cm^2) and right posterior cingulate (6.37 cm^2)	Decrease with TI severity in left HG/AC (2.50 cm^2)

*Results of VBM voxelwise analysis and SBM vertexwise analysis are reported as volume and surface area of significant clusters. ROI analysis results are reported in % change relative to the average/total ROI value averaged across TI and CO groups (TI, Tinnitus; CO, Control; MTG, Middle Temporal Gyrus; STS, Superior Temporal Sulcus. Other abbreviations as in **Table 1**).*

Whole brain VBM analysis (see Tables SI1,SI2) revealed many significant clusters in various parts of the brain, corresponding mainly to an increase in gray matter volume with age (totalling 10,234 voxels or 81.9 cm^3), and to a lesser extent a decrease in white matter volume with age (totalling 34.7 cm^3) and larger gray matter volume for males vs. female (totalling 13.9 cm^3). The largest of these clusters was an increase in gray matter volume with age in a 20 cm^3 volume near the boundaries of the ventricles (**Figure 5**). This cluster contains very little actual gray matter, being predominantly white matter and CSF. So the voxel grayscale is likely contaminated. As the ventricles are known to expand during the ageing process due to cortical atrophy, there is a likely explanation for this finding and so it is not discussed further. Other significant clusters were small in extent (<1 cm^3) and there were no significant effects of tinnitus, tinnitus severity or hearing loss.

In the whole brain SBM analysis (see Tables SI3–SI5), various clusters showed a decrease of cortical thickness, area and/or volume with age (total areas of the clusters were 558.5, 224.2 and 610.0 cm^2 respectively). There was an increase in cortical area and/or volume for males compared to females (total cluster areas were 1205.1 and 836.3 cm^2 respectively). Additionally, there were clusters of decreased cortical thickness for males in the left hemisphere (total area 7.9 cm^2). There were smaller significant clusters for tinnitus, tinnitus severity and hearing loss. These included a decrease in cortical thickness for the tinnitus group compared to controls in a 5.0 cm^2 area in the left superior frontal gyrus (SFG; **Figure 6A**), a decrease in cortical area and volume with tinnitus severity in an area of the right precuneus (over 12.5

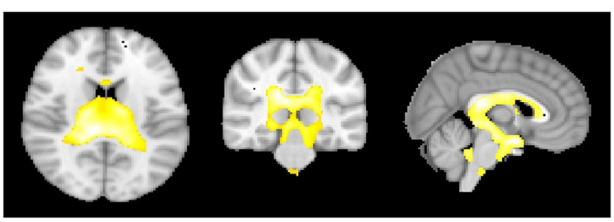

FIGURE 5 | The areas showing that as age increases there is a significant increase in gray matter volume at the cerebrospinal fluid (CSF) boundaries for the regression group of all subjects using age as the regressor of interest.

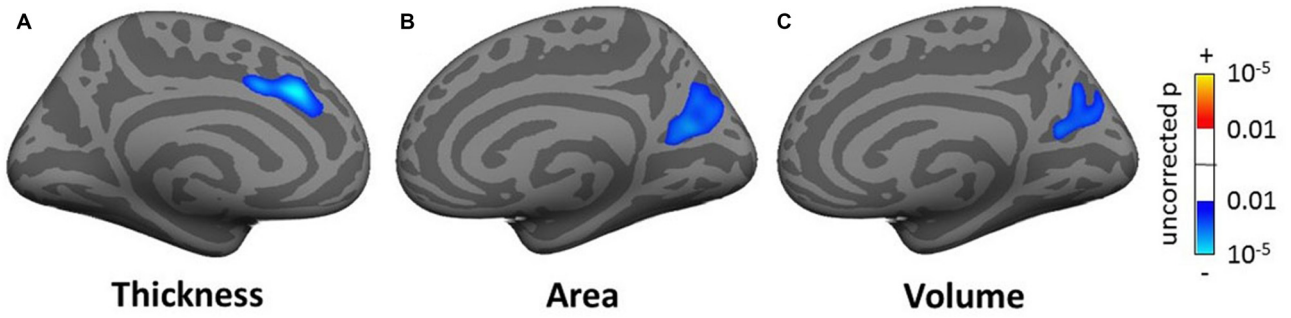

FIGURE 6 | Clusters showing a significant effect ($p < 0.05$ family wise error (FWE)-corrected) of tinnitus in the superior frontal gyrus (SFG; A) and of tinnitus severity in the pre-cuneus (B,C) in the whole-brain surface-based morphometry (SBM) analysis. Blue areas correspond to a negative effect (decrease in thickness for the tinnitus group vs. the control group and decrease in area and volume for increasing tinnitus severity).

and 8.4 cm^2 respectively; **Figures 6B,C**), a decrease in cortical volume with hearing loss in left HG (over 4.8 cm^2; not shown) and a decrease in cortical area and volume with hearing loss in the right fusiform gyrus (over 6.2 and 5.3 cm^2 respectively; not shown).

Masked voxel/vertexwise analysis

When restricting the VBM and SBM analyses to the various masks, the majority of clusters of significance were again found for the effects of age and gender and these effects were generally in the same direction as in the whole-head analysis. These clusters will not be detailed here (see Tables SI1–SI5). There were additional clusters of significance for tinnitus, tinnitus severity and hearing loss, which will be summarized below.

For the VBM masked analyses, we found a decrease in gray matter volume in the right HG (1 voxel or 0.008 cm^3; not shown) and in white matter volume for the tinnitus group compared to the control group in the right MGN (63 voxels or 0.50 cm^3; **Figure 7A**), a decrease in gray matter volume in the right DMN (0.056 cm^3) with increasing tinnitus severity (not shown). There were also two clusters of decreasing white matter volume with hearing loss in the left HG (found using AC, HG and STG masks) and left vmPFC. We also found one cluster of increasing white matter volume with hearing loss in the left NAc (all clusters <0.6 cm^3; not shown).

For the SBM masked analyses, we found several clusters of decreased cortical thickness or volume for the tinnitus group compared to controls. These included, two clusters of decreased thickness in the left AC (total cluster area 4.51 cm^2, **Figure 8A**, found using both the AC and STG masks), one cluster of decreased thickness in the bank of the right superior temporal sulcus (STS; 1.69 cm^2, **Figure 8B**, found using both the AC and STG masks), one cluster of decreased thickness and one of decreased volume in right HG (0.33 and 0.27 cm^2 respectively, **Figures 8C,D**) and one cluster of decreased thickness in the left SFG, identical to the one found in the whole brain analysis (**Figure 6A**). There were also two clusters of increasing cortical thickness with increasing tinnitus severity: one in the right MTG (2.61 cm^2, **Figure 8E**) and one in the right rostro-medial frontal gyrus (2.02 cm^2, **Figure 8F**).

ROI analysis

In the ROI analysis, we averaged the various measures across all voxels/vertices of each mask bilaterally and compared them between the tinnitus and control groups. This analysis showed additional significant effects, some of which were similar to the voxel/vertexwise effects. The only significant effect for the VBM ROI analysis was a 5.5% increase in gray matter volume for the tinnitus group in the SOC. For the SBM ROI analysis, we found a 2.1% decrease in cortical thickness in AC (similar to the vertex-wise effect shown in **Figures 8A,B**), a 1.3% increase in cortical area in vmPFC (similar to the vertexwise effect shown in **Figure 8F**) and decreases in cortical volume in AC, HG and STG (3.9%, 4.5% and 2.4% respectively). All effect sizes for the ROI analysis are reported in Tables SI6–SI10 and significant ones are summarized in **Table 3**).

Grouping 2—Severe Tinnitus vs. Matched Controls

Most significant clusters for this subgroup analysis were for the effect of age and gender and were in a direction similar to the ones described in the whole-head analysis with all subjects (grouping 1). These effects will not be described in detail here (see Tables SI1–SI5). There were a few significant clusters

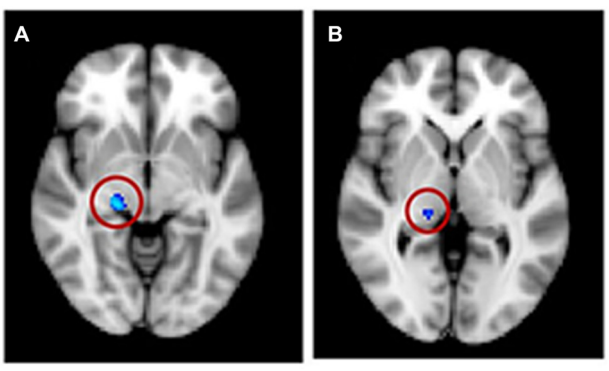

FIGURE 7 | Significant voxels (circled) found in the medial geniculate nucleus (MGN) for a reduction in white matter for tinnitus participants compared to controls for (A) regression group 1, all subjects and (B) regression group 2, severe tinnitus matched to controls.

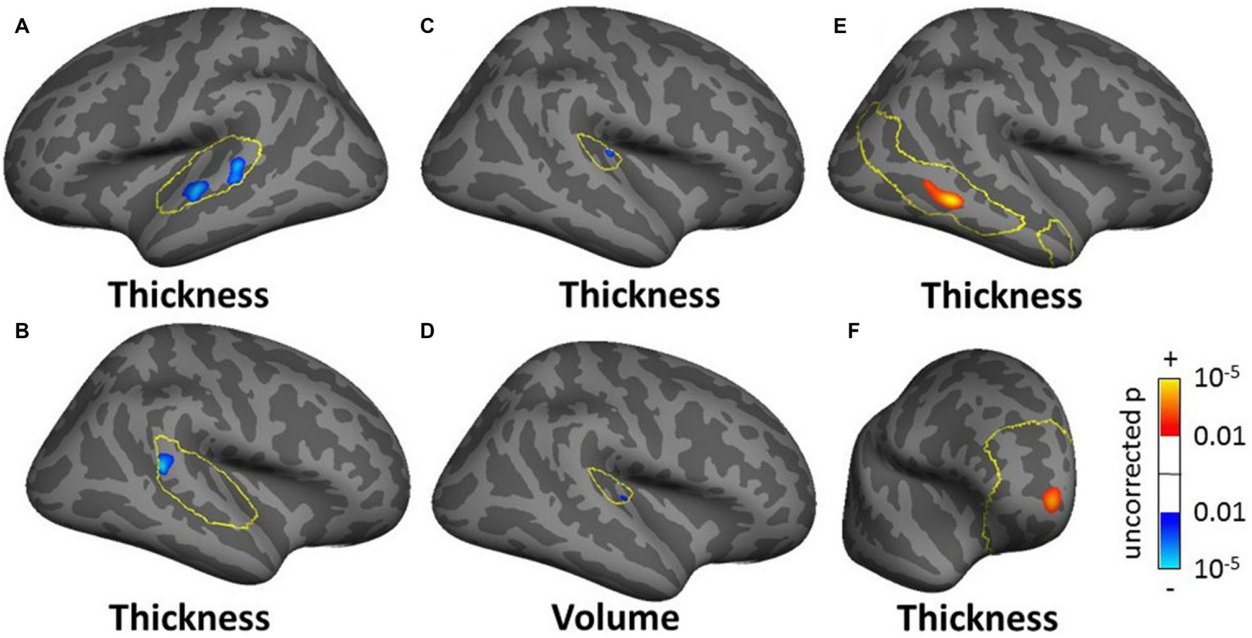

FIGURE 8 | Clusters showing a significant effect ($p < 0.05$ FWE-corrected) of tinnitus in left AC (A) right superior temporal sulcus (STS; B) right Heschl's gyrus (HG) (C,D) and of tinnitus severity in right middle temporal gyrus (MTG) and PFC (E,F) in the masked SBM analysis. The yellow outlines depict the masks used to restrict the vertexwise analysis (auditory cortex, AC for **A,B** HG for **C,D** default mode network (DMN) for **E**; PFC for **F)**. Blue areas correspond to a negative effect (decrease in thickness or volume for the tinnitus group vs. the control group) and the red areas to a positive effect (increasing thickness for increasing tinnitus severity).

showing an effect of tinnitus, tinnitus severity or hearing loss (described in the following section), and none in the whole-head analysis.

The masked voxelwise VBM analysis showed a 0.19 cm^3 cluster of decreased white matter volume in the right MGN for the severe tinnitus subgroup compared to matched controls (**Figure 7B**; similar to the effect described above for all subjects and **Figure 7A**) and two clusters of decreasing gray matter volume with increasing hearing loss in the left IC (0.02 cm^3; not shown) and the MGN bilaterally (1.22 cm^3; not shown). The masked vertexwise SBM analysis showed a cluster of decreased cortical thickness for the severe tinnitus subgroup compared to matched controls at the junction of HG and the STG in the left hemisphere (0.85 cm^2; **Figure 9**). There was also a cluster of increasing cortical thickness with increasing hearing loss in the right inferior parietal gyrus (1.47 cm^2, not shown).

The ROI-averaged analysis showed no significant effect of tinnitus for either the VBM or SBM analyses.

Grouping 3—Tinnitus With Clinically Normal Hearing vs. Matched Controls

As for grouping 2, most significant clusters were for the effect of age or gender. There were however a number of clusters of significance for the effect of tinnitus, tinnitus severity and hearing loss, including a few in the whole-head analyses.

The whole-brain SBM analysis showed two clusters of decreasing cortical area with increasing tinnitus severity in the right superior parietal gyrus and pCC (total areas of clusters 12.17 cm^2, **Figure 10**) and one cluster of increasing cortical volume with hearing loss in the right inferior parietal gyrus (4.55 cm^2, not shown).

The masked VBM analysis showed a few small clusters of significance: one voxel in left HG and another in left CN showing an increase in white matter volume for the normal-hearing tinnitus group compared to matched controls, a 0.12 cm^3 cluster

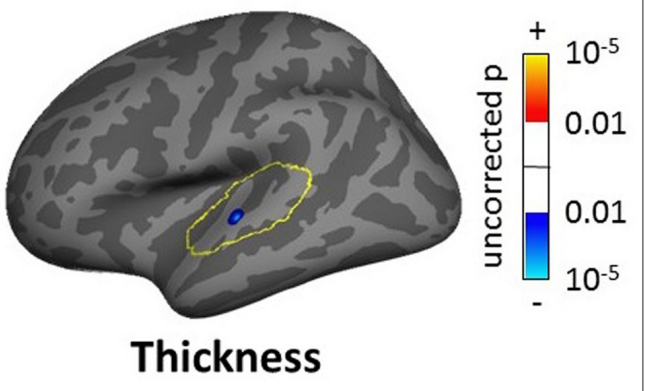

FIGURE 9 | Cluster showing a significant effect ($p < 0.05$ FWE-corrected) of tinnitus in left AC in the masked SBM analysis for the severe tinnitus grouping. The yellow outlines depict the AC masks used to restrict the vertexwise analysis. The blue area corresponds to a negative effect (decrease in thickness for the severe tinnitus group vs. matched controls).

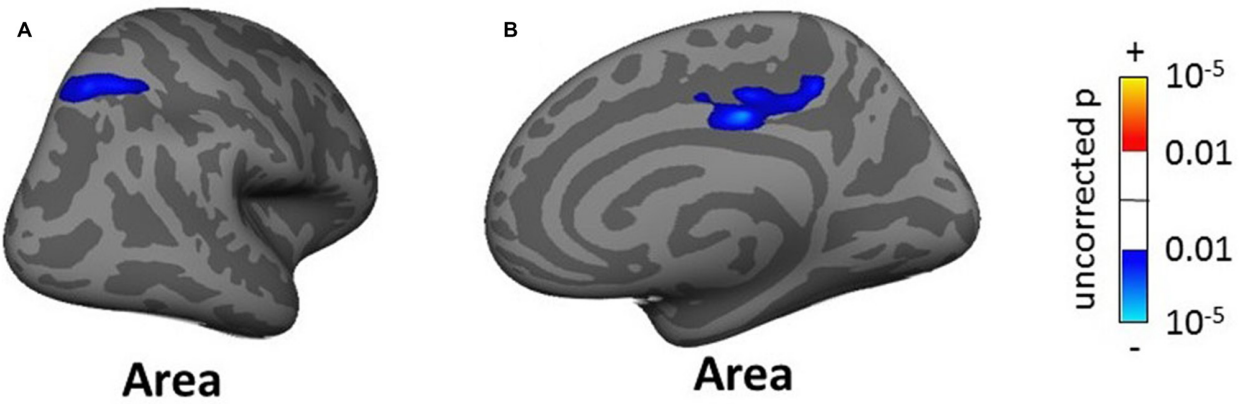

FIGURE 10 | Clusters showing a significant effect ($p < 0.05$ FWE-corrected) of tinnitus severity in right superior parietal gyrus (A) and right posterior cingulate cortex (B) in the whole-brain SBM analyses. Blue areas correspond to a negative effect (decrease in cortical area with increasing tinnitus severity).

in IC bilaterally showing increasing gray matter volume with hearing loss and two voxels (0.016 cm^3) in the right AC (found using both the AC and STG masks) showing increasing white matter volume with hearing loss (not shown).

The masked SBM analysis showed several clusters of significance for the effect of tinnitus and tinnitus severity: two clusters of decreased cortical thickness for the normal-hearing tinnitus group vs. matched controls in the left STG (1.17 cm^2, **Figure 11A**, found both using the STG and AC masks) and in the right rostro-middle frontal gyrus (1.71 cm^2, **Figure 11B**), one cluster of decreasing cortical area/volume with increasing tinnitus severity in left HG (3.18 cm^2, **Figures 11C,D** found using the AC, HG and STG masks) and one cluster of increasing cortical thickness with increasing tinnitus severity in the left MTG (3.18 cm^2, **Figure 11E**). There was also one cluster of increasing cortical thickness with hearing loss in the right rostro-middle frontal gyrus (1.81 cm^2, not shown).

The ROI-averaged analysis showed no significant effect of tinnitus for either the VBM or SBM analyses.

DISCUSSION

Structural analysis of neuroanatomy offers a unique approach to unraveling the mystery of tinnitus. Different morphological techniques have unique strengths and limitations and results can vary depending on specific algorithms used to register or segment the brain and quantify changes in tissue type. Our study applied a range of these techniques to the same dataset—bringing novel insights into just how variable the findings from structural analysis of the brain can be.

While the large cohort of participants allowed us to control confounding effects of hearing loss and age, these two variables were correlated and so their independent effects cannot be isolated with any degree of precision. Controlling hearing loss and tinnitus severity, we found moderate changes in brain anatomy associated with tinnitus. Important and somewhat disappointing, many of these significant changes were different and some even contradicted findings from previous studies (see Adjamian et al., 2014).

Tinnitus-related Changes—Comparison With Previous Findings

The results of both our VBM and SBM analyses reveal differences between tinnitus and non-tinnitus participants in both cortical and subcortical auditory structures, but only when the analysis was focused on these regions (masked voxel/vertexwise analyses or ROI analysis). Furthermore, as shown in **Table 4**, there was a limited overlap between the location and direction of our effects and those of previously published VBM and SBM studies.

At the subcortical level, our VBM analysis showed an increase in gray matter concentration in the SOC and a reduction in white matter probability in the MGN (**Figure 7A**) for tinnitus participants. None of these effects have been reported before, although there are conflicting reports of changes in gray matter concentration in the medial geniculate body (MGB), with Mühlau et al. (2006) reporting an increase, and Mahoney et al. (2011) a decrease in tinnitus compared to controls. At the cortical level, we found small decreases in gray matter probability and/or thickness in right HG using both the VBM and SBM analyses (**Figures 8C,D**), as well as slightly larger decreases in cortical thickness in left AC (outside HG, **Figure 8A**). This is fairly consistent with previous SBM studies which reported a decrease in cortical thickness in right HG and STG bilaterally (Aldhafeeri et al., 2012) and a decrease in cortical volume in HG (Schneider et al., 2009). It should be borne in mind however that not all SBM studies have found this effect (see Leaver et al., 2012) and that VBM studies have tended to find increases rather than decreases in gray matter concentration in HG or STG in participants with tinnitus (Husain et al., 2011; Mahoney et al., 2011; Boyen et al., 2013).

The only two significant clusters of change related to tinnitus that we found in our whole-brain analysis were located outside sensory auditory structures: we found a decrease in cortical thickness for tinnitus participants in the left SFG and a decrease in cortical volume with tinnitus severity in the right precuneus

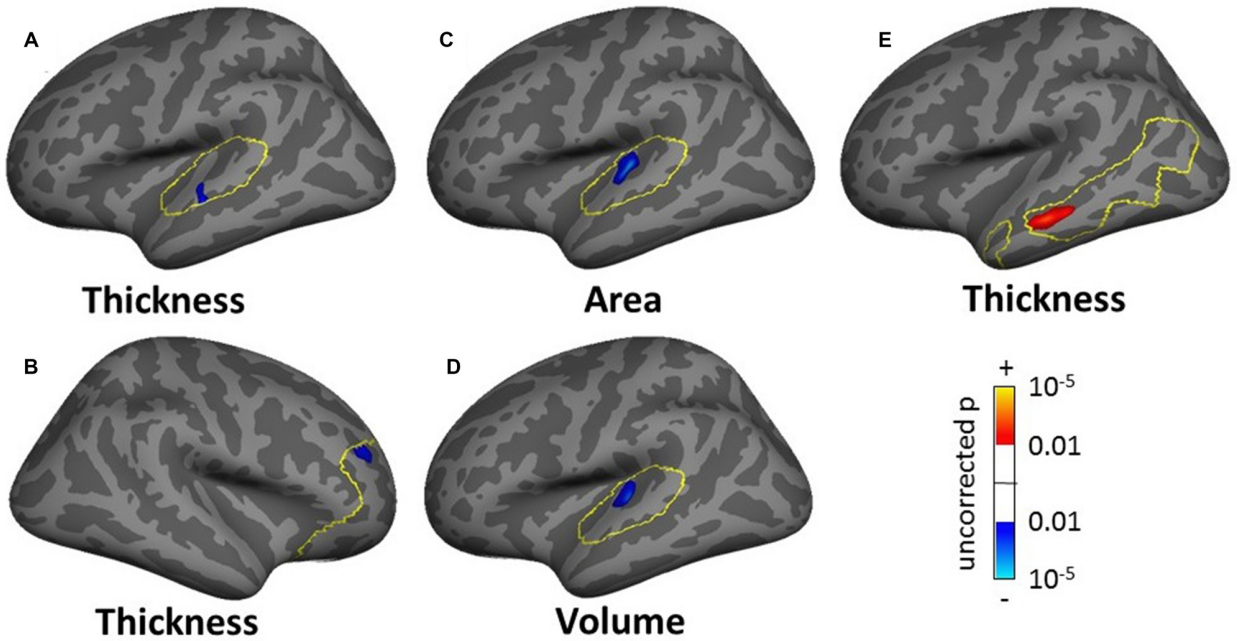

FIGURE 11 | Clusters showing a significant effect ($p < 0.05$ FWE-corrected) of tinnitus in left AC (A) and right rostromedial frontal cortex (B) of tinnitus severity in left HG (C,D) and left MTG (E) in the masked SBM analyses. The yellow outlines depict the masks used to restrict the vertexwise analysis (AC for **A,C,D**; PFC for **B**; DMN for **E**). Blue areas correspond to a negative effect (decrease in thickness for the normal-hearing tinnitus group vs. matched controls in **(A,B)** decrease in area and volume with increasing tinnitus severity in **(C,D)** and the red areas to a positive effect (increasing thickness for increasing tinnitus severity in **(E)**.

(**Figure 6**). Whereas the effect in precuneus has not been reported before, the location of the effect in the SFG is very similar to that of the gray matter (thickness) reduction reported by Leaver et al. (2012) in dorsomedial PFC using both VBM and SBM, although theirs was on the right side. Aldhafeeri et al. (2012) also reported a general decrease in cortical thickness in the right PFC using SBM. In contrast, Husain et al. (2011) reported an increase in gray matter concentration in tinnitus participants using VBM.

When restricting the analysis to ROIs, we found a few additional effects of tinnitus in non-auditory structures (e.g., increase in cortical thickness with tinnitus severity in MTG in DMN mask). One area of particular interest is the vmPFC: in separate VBM or SBM studies, Mühlau et al. (2006), Leaver et al. (2011) and Leaver et al. (2012) found a decrease in gray matter concentration in tinnitus participants in the subcallosal region, which is included in the vmPFC. On the other hand, at least three other studies have failed to replicate these effects (Landgrebe et al., 2009; Husain et al., 2011; Melcher et al., 2013) even though some of them were specifically designed to do so. Here we find an effect that clearly contradicts previous studies, since our SBM analysis shows a general increase in cortical area in vmPFC for the tinnitus group (ROI analysis) as well as a more focused increase in cortical thickness with tinnitus severity in the right rostro medial frontal gyrus (in the vmPFC-masked vertexwise analysis, **Figure 8F**).

Finally, we note that many significant effects of tinnitus reported in earlier studies (in cingulate cortex, hippocampus, insula, supramarginal gyrus, occipito-parietal cortex; Landgrebe et al., 2009; Leaver et al., 2011; Mahoney et al., 2011; Aldhafeeri et al., 2012; Boyen et al., 2013; Husain and Schmidt, 2014) were not replicated here. Therefore, even though we did find effects that replicated previous studies, the overall picture is one of non-replicability and contradiction.

In this study, we used both VBM and SBM analysis techniques to assess gray/white matter concentration, and thickness, area, and volume of the gray matter, respectively. One might expect that any regions found in the gray matter VBM analysis would also appear in the SBM analysis (at least for the cortical volume measurement), since we applied these to the exact same data. However, we observed a proportionally larger and a higher number of significant clusters in SBM than VBM, with differing locations. There are several differences in the exact details of the VBM and SBM procedures that could explain these discrepancies. First, the nature of the measurement is different: VBM as implemented in SPM measures the probability that a certain voxel belongs to white or gray matter and this is modulated by the amplitude of the deformation necessary to register individual brains onto a common template. On the other hand, SBM as implemented in Freesurfer measures actual geometric properties of the cortical sheet (thickness, area and volume) at each vertex of each subject (modulated by the deformation fields for area and volume). Second, the registration procedure is different: SPM uses non-linear registration in volumetric space, whereas FreeSurfer uses spherical registration in surface space. Whereas in Freesurfer, deformation fields are necessarily limited to the cortical sheet, in SPM, they can occur in

TABLE 4 | Summary of main results of previous tinnitus VBM and SBM studies compared to present results (table adapted from Adjamian et al., 2014).

	Group differences		Modulations	
Brain structure	**Decreases**	**Increases**	**HL**	**TIN**
Auditory gray matter				
Superior olivary complex	K			
Inferior colliculus	B	–	–	-
Medial geniculate body	FK	A	–	-
Heschl's gyrus (A1)	***CG***K***K***	I	***CG***K	I
Superior temporal gyrus (A2)	***GK***	DF	DI	H***H***
Non-auditory gray matter				
vmPFC/subcallosal gyrus	AE***G***H***H***	***(K)***	IJK	***K***
dmPFC	***G***H***H (K)***	D	DIJ	**-**
Nucleus accumbens	–	–	K	-
Anterior cingulate	***G***	D	D	-
Posterior cingulate	***G***	–	J	-
Hippocampus	BI	–	I	-
Insula	–	–	–	H***H***
Supramarginal gyrus	H	–	I	H***H***
Occipito-parietal cortex	–	I	I	-
Orbito-frontal cortex	F	–	–	-
Superior frontal gyrus	***(K)***	–	–	-
Middle frontal gyrus	–-	***(K)***	–	-
Middle temporal gyrus	–	I***K***	–	-
Precuneus	–	–	–	***K***
Fusiform gyrus	–	–	***K***	-

Present results are denoted by the letter K and are from grouping 1 only. Regular font letters denote VBM analyses and bold/italics letters SBM analyses. A = Mühlau et al. (2006); B = Landgrebe et al. (2009); C = Schneider et al. (2009); D = Husain et al. (2011); E = Leaver et al. (2011); F = Mahoney et al. (2011); G = Aldhafeeri et al. (2012); H = Leaver et al. (2012); I = Boyen et al. (2013); J = (Melcher et al. (2013); HL, Hearing loss; TIN, Tinnitus severity; vmPFC, ventromedial pre-frontal cortex; dmPFC, dorsomedial prefrontal Cortex.

any direction in volume space. As a result, errors due to imperfect registration will be different in the two techniques. Finally, even though we used almost identical statistical models, there were at least two important differences: we did not correct for total/average brain thickness/area/volume in the SBM analysis and we used different types of FWE correction. The latter could explain why significant cluster sizes are proportionally larger in the SBM analysis: in the VBM analysis, the cluster size corresponds to the voxelwise-corrected *p*-value whereas in the SBM analysis, it corresponded to the uncorrected *p*-value (and is therefore larger). It is also likely that the cluster-based FWE correction used in Freesurfer is more sensitive to large clusters of relatively low significance, which could explain why more clusters were found overall in the SBM analysis.

Reasons for Variability Between Studies

Tinnitus is a heterogeneous disorder, typically based on only a single criterion: the perception of a phantom sound. As yet there is no universally agreed separation of tinnitus patients into subgroups based on clinical and etiological factors. Tinnitus etiology, severity, hearing loss, comorbid medical conditions, age of onset, duration and laterality, among others, are factors which can effect brain morphology (Adjamian et al., 2014). Indeed, age and hearing loss seriously confound the interpretation of many results in this field. It is likely that the differences between participants across different studies, exaggerated by the lack of meaningful definitions of tinnitus subgroups, explain the reasons for diversity in findings.

A recent European-funded Cooperation in Science and Technology program (COST Action) for a Tinnitus research Network (TINNET[3]) aims to identify subtypes of tinnitus, and their neural correlates and thus develop an innovative hypothesis-driven treatment approaches. Until such time, future studies should attempt to collect as much information from participants as possible and attempt to recruit participants that are clinically and characteristically homogeneous as far as possible. Studies should ideally administer tinnitus questionnaires, depression questionnaires, measure audiograms at least up to 12 kHz, ascertain the duration, lateralization and cause of their tinnitus and basic demographic information. Participants should be matched on these characteristics as far as possible. Many of these variables were measured in the studies from which the data for the current analysis were obtained.

CONCLUSION

Given the results of the present study, and in the context of previous discrepant findings, we conclude that it is not yet possible with any confidence to associate tinnitus with anatomical changes in specific parts of the brain. This is likely due to the heterogeneity of tinnitus characteristics, and the lack meaningful subtyping. Exploratory analyses might propose a subtyping classification which could then generate hypotheses for future testing. However, the

[3]http://tinnet.tinnitusresearch.net/

more stringent the eligibility criteria for inclusion, the more challenging it will be to recruit sufficient number of participants in each subgroup for valid statistical inference.

AUTHOR CONTRIBUTIONS

PA and DRML conceived the study, wrote the discussion. PA and JD collected the data. TWA and JB analyzed the data and wrote the article. ARP and DAH supervised and provided guidance.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Adjamian, P., Hall, D. A., Palmer, A. R., Allan, T. W., and Langers, D. R. M. (2014). Neuroanatomical abnormalities in chronic tinnitus in the human brain. *Neurosci. Biobehav. Rev.* 45C, 119–133. doi: 10.1016/j.neubiorev.2014.05.013

Adjamian, P., Sereda, M., and Hall, D. A. (2009). The mechanisms of tinnitus: perspectives from human functional neuroimaging. *Hear. Res.* 253, 15–31. doi: 10.1016/j.heares.2009.04.001

Adjamian, P., Sereda, M., Zobay, O., Hall, D. A., and Palmer, A. R. (2012). Neuromagnetic indicators of tinnitus and tinnitus masking in patients with and without hearing loss. *J. Assoc. Res. Otolaryngol.* 13, 715–731. doi: 10.1007/s10162-012-0340-5

Aldhafeeri, F. M., Mackenzie, I., Kay, T., Alghamdi, J., and Sluming, V. (2012). Neuroanatomical correlates of tinnitus revealed by cortical thickness analysis and diffusion tensor imaging. *Neuroradiology* 54, 883–892. doi: 10.1007/s00234-012-1044-6

Ashburner, J. (2007). A fast diffeomorphic image registration algorithm. *Neuroimage* 38, 95–113. doi: 10.1016/j.neuroimage.2007.07.007

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Bookstein, F. L. (2001). "Voxel-based morphometry" should not be used with imperfectly registered images. *Neuroimage* 14, 1454–1462. doi: 10.1006/nimg.2001.0770

Boyen, K., Langers, D. R. M., de Kleine, E., and van Dijk, P. (2013). Gray matter in the brain: differences associated with tinnitus and hearing loss. *Hear. Res.* 295, 67–78. doi: 10.1016/j.heares.2012.02.010

British Society of Audiology. (2011). Recommended procedure; pure-tone air-conduction and bone-conduction threshold audiometry with and without masking. Available online at: http://www.thebsa.org.uk/wp-content/uploads/2014/04/BSA_RP_PTA_FINAL_24Sept11_MinorAmend06Feb12.pdf.

Crippa, A., Lanting, C. P., van Dijk, P., and Roerdink, J. B. (2010). A diffusion tensor imaging study on the auditory system and tinnitus. *Open Neuroimag. J.* 4, 16–25. doi: 10.2174/1874440001004010016

Dale, A. M., Fischl, B., and Sereno, M. I. (1999). Cortical surface-based analysis. I. Segmentation and surface reconstruction. *Neuroimage* 9, 179–194. doi: 10.1006/nimg.1998.0395

Davies, J., Gander, P. E., Andrews, M., and Hall, D. A. (2014). Auditory network connectivity in tinnitus patients: a resting-state fMRI study. *Int. J. Audiol.* 53, 192–198. doi: 10.3109/14992027.2013.846482

Draganski, B., Gaser, C., Busch, V., Schuierer, G., Bogdahn, U., and May, A. (2004). Neuroplasticity: changes in grey matter induced by training. *Nature* 427, 311–312. doi: 10.1038/427311a

Fackrell, K., Hall, D. A., Barry, J. G., and Hoare, D. J. (2014). "Tools for tinnitus measurement: development and validity of questionnaires to assess handicap and treatment effects," in *Tinnitus: Causes, Treatment and Short and Long-Term Health Effects*, eds F. Signorelli and F. Turjman (New York, NY: Nova Science Publishers), 13–60.

Fischl, B., and Dale, A. M. (2000). Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proc. Natl. Acad. Sci. U S A* 97, 11050–11055. doi: 10.1073/pnas.200033797

Fischl, B., Sereno, M. I., and Dale, A. M. (1999a). Cortical surface-based analysis. II: inflation, flattening and a surface-based coordinate system. *Neuroimage* 9, 195–207. doi: 10.1006/nimg.1998.0396

Fischl, B., Sereno, M. I., Tootell, R. B., and Dale, A. M. (1999b). High-resolution intersubject averaging and a coordinate system for the cortical surface. *Hum. Brain Mapp.* 8, 272–284. doi: 10.1002/(SICI)1097-0193(1999)8:4<272::AID-HBM10>3.0.CO;2-4

Good, C. D., Johnsrude, I. S., Ashburner, J., Henson, R. N., Friston, K. J., and Frackowiak, R. S. (2001). A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage* 14, 21–36. doi: 10.1006/nimg.2001.0786

Greicius, M. D., Krasnow, B., Reiss, A. L., and Menon, V. (2003). Functional connectivity in the resting brain: a network analysis of the default mode hypothesis. *Proc. Natl. Acad. Sci. U S A* 100, 253–258. doi: 10.1073/pnas.0135058100

Hagler, D. J. Jr., Saygin, A. P., and Sereno, M. I. (2006). Smoothing and cluster thresholding for cortical surface-based group analysis of fMRI data. *Neuroimage* 33, 1093–1103. doi: 10.1016/j.neuroimage.2006.07.036

Hayasaka, S., and Nichols, T. E. (2003). Validating cluster size inference: random field and permutation methods. *Neuroimage* 20, 2343–2356. doi: 10.1016/j.neuroimage.2003.08.003

Henry, J. A., Dennis, K. C., and Schechter, M. A. (2005). General review of tinnitus: prevalence, mechanisms, effects and management. *J. Speech Lang. Hear. Res.* 48, 1204–1235. doi: 10.1044/1092-4388(2005/084)

House, J. W., and Brackmann, D. E. (1981). Tinnitus: surgical treatment. *Ciba Found. Symp.* 85, 204–216. doi: 10.1002/9780470720677.ch12

Husain, F. T., Medina, R. E., Davis, C. W., Szymko-Bennett, Y., Simonyan, K., Pajor, N. M., et al. (2011). Neuroanatomical changes due to hearing loss and chronic tinnitus: a combined VBM and DTI study. *Brain Res.* 1369, 74–88. doi: 10.1016/j.brainres.2010.10.095

Husain, F. T., and Schmidt, S. A. (2014). Using resting state functional connectivity to unravel networks of tinnitus. *Hear. Res.* 307, 153–162. doi: 10.1016/j.heares.2013.07.010

Kuk, F. K., Tyler, R. S., Russell, D., and Jordan, H. (1990). The psychometric properties of a tinnitus handicap questionnaire. *Ear Hear.* 11, 434–445. doi: 10.1097/00003446-199012000-00005

Landgrebe, M., Langguth, B., Rosengarth, K., Braun, S., Koch, A., Kleinjung, T., et al. (2009). Structural brain changes in tinnitus: grey matter decrease in auditory and non-auditory brain areas. *Neuroimage* 46, 213–218. doi: 10.1016/j.neuroimage.2009.01.069

Lanting, C. P., De Kleine, E., Bartels, H., and Van Dijk, P. (2008). Functional imaging of unilateral tinnitus using fMRI. *Acta Otolaryngol.* 128, 415–421. doi: 10.1080/00016480701793743

Lanting, C. P., de Kleine, E., and van Dijk, P. (2009). Neural activity underlying tinnitus generation: results from PET and fMRI. *Hear. Res.* 255, 1–13. doi: 10.1016/j.heares.2009.06.009

Leaver, A. M., Renier, L., Chevillet, M. A., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2011). Dysregulation of limbic and auditory networks in tinnitus. *Neuron* 69, 33–43. doi: 10.1016/j.neuron.2010.12.002

Leaver, A. M., Seydell-Greenwald, A., Turesky, T. K., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2012). Cortico-limbic morphology separates tinnitus from tinnitus distress. *Front. Syst. Neurosci.* 6:21. doi: 10.3389/fnsys.2012.00021

Lee, Y. J., Bae, S. J., Lee, S. H., Lee, J. J., Lee, K. Y., Kim, M. N., et al. (2007). Evaluation of white matter structures in patients with tinnitus using diffusion tensor imaging. *J. Clin. Neurosci.* 14, 515–519. doi: 10.1016/j.jocn.2006.10.002

Maguire, E. A., Woollett, K., and Spiers, H. J. (2006). London taxi drivers and bus drivers: a structural MRI and neuropsychological analysis. *Hippocampus* 16, 1091–1101. doi: 10.1002/hipo.20233

Mahoney, C. J., Rohrer, J. D., Goll, J. C., Fox, N. C., Rossor, M. N., and Warren, J. D. (2011). Structural neuroanatomy of tinnitus and hyperacusis in semantic dementia. *J. Neurol. Neurosurg. Psychiatry* 82, 1274–1278. doi: 10.1136/jnnp.2010.235473

McCombe, A., Baguley, D., Coles, R., McKenna, L., McKinney, C., Windle-Taylor, P., et al. (2001). Guidelines for the grading of tinnitus severity: the results of a working group commissioned by the British association of otolaryngologists, head and neck surgeons, 1999. *Clin. Otolaryngol. Allied Sci.* 26, 388–393. doi: 10.1046/j.1365-2273.2001.00490.x

Melcher, J. R., Knudson, I. M., and Levine, R. A. (2013). Subcallosal brain structure: correlation with hearing threshold at supra-clinical frequencies (>8 kHz), but not with tinnitus. *Hear. Res.* 295, 79–86. doi: 10.1016/j.heares.2012.03.013

Melcher, J. R., Sigalovsky, I. S., and Guinan, J. J. Jr., Levine, R. A. (2000). Lateralized tinnitus studied with functional magnetic resonance imaging: abnormal inferior colliculus activation. *J. Neurophysiol.* 83, 1058–1072.

Mirz, F., Gjedde, A., Ishizu, K., and Pedersen, C. B. (2000a). Cortical networks subserving the perception of tinnitus—a PET study. *Acta Otolaryngol. Suppl.* 543, 241–243. doi: 10.1080/000164800454503

Mirz, F., Gjedde, A., Sødkilde-Jrgensen, H., and Pedersen, C. B. (2000b). Functional brain imaging of tinnitus-like perception induced by aversive auditory stimuli. *Neuroreport* 11, 633–637. doi: 10.1097/00001756-200002280-00039

Mirz, F., Gjedde, A., Stodkilde-Jorgensen, H., and Pedersen, C. B. (1999). "Neuroanatomical correlates of tinnitus," in *Proceedings of the Sixth International Tinnitus Seminar*, ed J. Hazell (London: Tinnitus and Hyperacusis Centre), 323–327.

Mühlau, M., Rauschecker, J. P., Oestreicher, E., Gaser, C., Röttinger, M., Wohlschläger, A. M., et al. (2006). Structural brain changes in tinnitus. *Cereb. Cortex* 16, 1283–1288. doi: 10.1093/cercor/bhj070

Newman, C. W., Jacobson, G. P., and Spitzer, J. B. (1996). Development of the Tinnitus Handicap inventory. *Arch Otolaryngol. Head Neck Surg.* 122, 143–148. doi: 10.1001/archotol.1996.01890140029007

Pfefferbaum, A., Mathalon, D. H., Sullivan, E. V., Rawles, J. M., Zipursky, R. B., and Lim, K. O. (1994). A quantitative magnetic resonance imaging study of changes in brain morphology from infancy to late adulthood. *Arch. Neurol.* 51, 874–887. doi: 10.1001/archneur.1994.00540210046012

Rauschecker, J. P., Leaver, A. M., and Mühlau, M. (2010). Tuning out the noise: limbic-auditory interactions in tinnitus. *Neuron* 66, 819–826. doi: 10.1016/j.neuron.2010.04.032

Reyes, S. A., Salvi, R. J., Burkard, R. F., Coad, M. L., Wack, D. S., Galantowicz, P. J., et al. (2002). Brain imaging of the effects of lidocaine on tinnitus. *Hear. Res.* 171, 43–50. doi: 10.1016/s0378-5955(02)00346-5

Schecklmann, M., Lehner, A., Poeppl, T. B., Kreuzer, P. M., Hajak, G., Landgrebe, M., et al. (2012). Cluster analysis for identifying subtypes of tinnitus: a positron emission tomography and voxel-based morphometry study. *Brain Res.* 1485, 3–9. doi: 10.1016/j.brainres.2012.05.013

Schecklmann, M., Lehner, A., Poeppl, T. B., Kreuzer, P. M., Rupprecht, R., Rackl, J., et al. (2013). Auditory cortex is implicated in tinnitus distress: a voxel-based morphometry study. *Brain Struct. Funct.* 218, 1061–1070. doi: 10.1007/s00429-013-0520-z

Schneider, P., Andermann, M., Wengenroth, M., Goebel, R., Flor, H., Rupp, A., et al. (2009). Reduced volume of Heschl's gyrus in tinnitus. *Neuroimage* 45, 927–939. doi: 10.1016/j.neuroimage.2008.12.045

Seki, S., and Eggermont, J. J. (2003). Changes in spontaneous firing rate and neural synchrony in cat primary auditory cortex after localized tone-induced hearing loss. *Hear. Res.* 180, 28–38. doi: 10.1016/s0378-5955(03)00074-1

Sereda, M., Adjamian, P., Edmondson-Jones, M., Palmer, A. R., and Hall, D. A. (2013). Auditory evoked magnetic fields in individuals with tinnitus. *Hear. Res.* 302, 50–59. doi: 10.1016/j.heares.2013.04.006

Seydell-Greenwald, A., Raven, E. P., Leaver, A. M., Turesky, T. K., and Rauschecker, J. P. (2014). Diffusion imaging of auditory and auditory-limbic connectivity in tinnitus: preliminary evidence and methodological challenges. *Neural Plast.* 2014:145943. doi: 10.1155/2014/145943

Winkler, A. M., Kochunov, P., Blangero, J., Almasy, L., Zilles, K., Fox, P. T., et al. (2010). Cortical thickness or grey matter volume? The importance of selecting the phenotype for imaging genetics studies. *Neuroimage* 53, 1135–1146. doi: 10.1016/j.neuroimage.2009.12.028

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Disrupted Brain Functional Network Architecture in Chronic Tinnitus Patients

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Purpose: Resting-state functional magnetic resonance imaging (fMRI) studies have demonstrated the disruptions of multiple brain networks in tinnitus patients. Nonetheless, several studies found no differences in network processing between tinnitus patients and healthy controls (HCs). Its neural bases are poorly understood. To identify aberrant brain network architecture involved in chronic tinnitus, we compared the resting-state fMRI (rs-fMRI) patterns of tinnitus patients and HCs.

Materials and Methods: Chronic tinnitus patients (n = 24) with normal hearing thresholds and age-, sex-, education- and hearing threshold-matched HCs (n = 22) participated in the current study and underwent the rs-fMRI scanning. We used degree centrality (DC) to investigate functional connectivity (FC) strength of the whole-brain network and Granger causality to analyze effective connectivity in order to explore directional aspects involved in tinnitus.

Results: Compared to HCs, we found significantly increased network centrality in bilateral superior frontal gyrus (SFG). Unidirectionally, the left SFG revealed increased effective connectivity to the left middle orbitofrontal cortex (OFC), left posterior lobe of cerebellum (PLC), left postcentral gyrus, and right middle occipital gyrus (MOG) while the right SFG exhibited enhanced effective connectivity to the right supplementary motor area (SMA). In addition, the effective connectivity from the bilateral SFG to the OFC and SMA showed positive correlations with tinnitus distress.

Conclusions: Rs-fMRI provides a new and novel method for identifying aberrant brain network architecture. Chronic tinnitus patients have disrupted FC strength and causal connectivity mostly in non-auditory regions, especially the prefrontal cortex (PFC). The current findings will provide a new perspective for understanding the neuropathophysiological mechanisms in chronic tinnitus.

Keywords: chronic tinnitus, degree centrality, effective connectivity, resting-state fMRI

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INTRODUCTION

Tinnitus is the perception of a sound in the absence of an external sound source. Roughly 12% of adults experience tinnitus, but the prevalence skyrockets to 50% in combat personnel (McFadden, 1982; Meikle, 1997). Chronic tinnitus usually leads to problems ranging from mild discomfort such as sleep disturbance to strong anxiety and depression

(Leske, 1981; Lockwood et al., 2002). Although most tinnitus patients localize tinnitus to one or both ears, the severity of the phantom sound could not be eliminated after sectioning the auditory nerve (Berliner et al., 1992; Jackler and Whinney, 2001). Tinnitus patients show various pathophysiological changes, such as increased spontaneous activity, enhanced neural synchrony, tonotopic map reorganization, abnormal consciousness gating mechanisms and coupling of networks involving auditory and non-auditory structures (Lenarz et al., 1993; Lockwood et al., 1998; Kaltenbach et al., 2005; Adjamian et al., 2009; Henry et al., 2014). Nonetheless, the mechanisms that maintain the disorder remain poorly understood.

Previous neuroimaging studies using electroencephalography (EEG), magnetoencephalography (MEG), positron emission tomography (PET), and functional magnetic resonance imaging (fMRI), have investigated the neuropathophysiological mechanisms implicated in chronic tinnitus (Shulman and Strashun, 1998; Mirz et al., 1999; Adjamian et al., 2009; Schlee et al., 2009; Roberts et al., 2010; Vanneste and De Ridder, 2012). Resting-state fMRI (rs-fMRI) is a promising noninvasive technique that could reflect the brain functional architecture in low frequency fluctuations (0.01–0.1 Hz) of blood oxygenation level-dependent (BOLD; Biswal et al., 1995; Fox and Raichle, 2007). Using rs-fMRI, multiple brain networks relevant to neural mechanisms of tinnitus have been demonstrated, such as the auditory network (Burton et al., 2012; Kim et al., 2012; Maudoux et al., 2012a,b; Schmidt et al., 2013; Hinkley et al., 2015; Minami et al., 2015; Leaver et al., 2016), dorsal attention network (DAN; Burton et al., 2012; Schmidt et al., 2013), ventral attention network (VAN; Burton et al., 2012), default mode network (DMN; Schmidt et al., 2013; Chen et al., 2014, 2015d; Leaver et al., 2016), and visual network (Burton et al., 2012; Chen et al., 2014). However, these results have been variable due to applied analytic methods. Burton et al. (2012) implied that there is dissociation between activity in auditory cortex and visual, attention and control networks using seed-based functional connectivity (FC) analysis. Through independent component analysis (ICA), Kim et al. (2012) found enhanced FC between the attention network and auditory network, suggesting that this network might contribute to the perception or salience of tinnitus. Schmidt et al. (2013) identified specific alterations in the connectivity of the DMN, DAN, and auditory networks due to tinnitus. Similarly, Maudoux et al. (2012a,b) provided fMRI evidence for a distributed network of auditory and non-auditory cortical and sub-cortical regions associated with chronic tinnitus pathology using ICA approach. Using the algorithms of amplitude of low-frequency fluctuations (ALFF) and regional homogeneity (ReHo), Chen et al. (2014, 2015c,d) found abnormal spontaneous neural activity within multiple cerebral networks, such as the DMN and the attention network. Nonetheless, other studies have failed to detect any differences in network processing between tinnitus patients and controls (Wineland et al., 2012; Davies et al., 2014). Heterogeneity among tinnitus cohorts may contribute to network state variations. According to these recent theories and observations, we speculated that abnormal brain FC and network might underlie the pathophysiology in chronic tinnitus.

The prefrontal cortex (PFC) exerts early inhibitory modulation of input to primary auditory cortex in humans and has been found to be associated with auditory attention (Lewis et al., 2000; Voisin et al., 2006). The vital role for the PFC in subserving tinnitus mechanism has been postulated (Jastreboff, 1990) and previous neuroimaging studies have confirmed the involvement of PFC for tinnitus (Vanneste et al., 2010, 2012; Vanneste and De Ridder, 2012; De Ridder et al., 2014). Furthermore, latest rs-fMRI studies identified abnormalities in the PFC associated with tinnitus (Burton et al., 2012; Kim et al., 2012; Schmidt et al., 2013; Ueyama et al., 2013; Chen et al., 2014, 2015b,c,d; Leaver et al., 2016). Among them, Ueyama et al. (2013) observed disrupted FC strength in distinct brain regions, especially in the left superior frontal gyrus (SFG), which was negatively correlated with tinnitus loudness. Taken together, the PFC is considered as a key region involved in tinnitus. Investigations exploring the role of PFC in the brain functional network architecture of tinnitus will provide valuable insight into the neural mechanisms underlying the chronic tinnitus.

Seed-based FC and ICA approaches have proved extremely useful in exploring connectivity patterns for specific components of interest. However, few studies have investigated the tinnitus-related alterations of whole-brain FC pattern or large-scale brain network. Degree centrality (DC) is a voxel-wise data-driven method that can quantify the importance of each node in brain network. This graph theory based network analysis can assess the network centrality without *a priori* selection of nodes or networks of interest (Zuo et al., 2012). This algorithm has been used to observe the alterations of resting-state functional networks in diverse diseases, such as Alzheimer's disease (AD), autism, and hepatic encephalopathy (Buckner et al., 2009; Di Martino et al., 2013; Chen et al., 2015a). Since the neural mechanisms underlying tinnitus are poorly understood and multiple brain systems are involved, we applied DC to analyze FC within the whole-brain network. To examine the directional connectivity network involved in tinnitus, we further used the granger causality analysis (GCA), which is a statistical method originally used in the field of economics to assess directional influences between simultaneously recorded time series (Granger, 1969; Zhou et al., 2011). GCA has been widely used to reveal the causal effects among brain regions in various neurological or psychiatric disorders, such as AD, schizophrenia, depression and hepatic encephalopathy (Qi et al., 2013; Zhong et al., 2014; Guo et al., 2015a,b). Thus, to unravel the details of the brain functional network architecture in tinnitus, we sought to evaluate the brain regions that show aberrant FC across the entire brain networks in tinnitus patients using DC and then use GCA to analyze effective connectivity to understand the directional aspect of these alterations. We hypothesized that the intrinsic dysconnectivity pattern of the PFC might play a crucial role in the brain functional network architecture of tinnitus patients.

MATERIALS AND METHODS

Subjects

All the subjects provided written informed consent before their participation in the study protocol, which was approved by the Research Ethics Committee of the Nanjing Medical University (Reference No. 2016067).

According to both the inclusion and exclusion criteria of this study, a final sample of 47 subjects including 25 chronic tinnitus patients and 22 healthy controls (HCs) were recruited through community health screening and newspaper advertisements. The tinnitus patients and healthy subjects were group-matched in terms of age, sex, and education. One tinnitus patient was subsequently excluded because the limits for head motion were exceeded during MR scanning. Ten patients reported a predominantly left-sided tinnitus, six a predominantly right-sided tinnitus and eight patients described their tinnitus as bilateral or originating within the head. All subjects were right-handed and completed at least 8 years of education. The severity of tinnitus and related distress were assessed by the Iowa version of the Tinnitus Handicap Questionnaires (THQ; Kuk et al., 1990). Hearing thresholds were determined by puretone audiometry (PTA). All of the participants had normal hearing (defined as thresholds <25 dB HL) at the frequencies of 0.25 kHz, 0.5 kHz, 1 kHz, 2 kHz, 4 kHz, and 8 kHz. There were no significant differences in auditory thresholds between tinnitus and control groups. In addition, none of the participants had symptoms of depression and anxiety according to the Self-Rating Depression Scale (SDS) and Self-Rating Anxiety Scale (SAS; overall scores <50, respectively; Zung, 1971, 1986). According to previous study (Khalfa et al., 2002), we used the Hyperacusis Questionnaire to exclude the participants with hyperacusis in the current study. Participants were also excluded from the study if they suffered from pulsatile tinnitus or Meniere's diseases, or if they had a past history of severe smoking, stroke, alcoholism, brain injury, Parkinson's disease, AD, epilepsy, major depression, neurological or psychiatric disorders that could affect cognitive function, major medical illness (e.g., anemia, thyroid dysfunction and cancer), MRI contraindications (e.g., cochlear implants, pacemakers, cerebral aneurysm clips, prosthetic valves, a history of intraocular metal fragments, and claustrophobia), or severe visual loss. The characteristics of the chronic tinnitus patients and healthy subjects are summarized in **Table 1**.

TABLE 1 | Characteristics of the tinnitus patients and healthy controls (HCs).

	Tinnitus patients (n = 24)	HCs (n = 22)	p value
Age (year)	50.8 ± 12.4 (26–67)	44.7 ± 15.4 (26–70)	0.144
Gender (male: female)	9:15	9:13	0.813
Education levels (years)	12.3 ± 3.1 (8–18)	13.4 ± 3.8 (8–22)	0.313
Tinnitus duration (months)	46.5 ± 39.1 (6–120)	–	–
THQ score	49.5 ± 15.5	–	–
Hearing thresholds (left)	13.0 ± 2.7	13.6 ± 2.2	0.414
Hearing thresholds (right)	14.6 ± 3.6	13.9 ± 3.4	0.470

Data are represented as Mean ± SD (range of min-max).

MRI Acquisition

MRI data were acquired at our hospital using a 3.0 T MRI scanner (Ingenia, Philips Medical Systems, Netherlands). Head motion and scanner noise were reduced using foam padding and earplugs. The earplugs (Hearos Ultimate Softness Series, USA) were used to attenuate scanner noise by approximately 32 dB. Subjects were instructed to lie quietly with their eyes closed without falling asleep, not think of anything in particular, and avoid any head motion during the scan. Functional images were obtained axially using a gradient echo-planar imaging (EPI) sequence as follows: repetition time (TR) = 2000 ms; echo time (TE) = 30 ms; slices = 36; thickness = 4 mm; gap = 0 mm; field of view (FOV) = 240 mm × 240 mm; acquisition matrix = 64 × 64; and flip angle (FA) = 90°. The fMRI sequence took 8 min and 8 s. Structural images were acquired with a three-dimensional turbo fast echo (3D-TFE) T1WI sequence with high resolution as follows: TR/TE = 8.1/3.7 ms; slices = 170; thickness = 1 mm; gap = 0 mm; FA = 8°; acquisition matrix = 256 × 256; FOV = 256 mm × 256 mm. The structural sequence took 5 min and 29 s.

Functional Data Preprocessing

Functional data analyses were conducted using Data Processing Assistant for Resting-State fMRI (DPARSF) programs (Chao-Gan and Yu-Feng, 2010) based on statistical parametric mapping (SPM8[1]) and rs-fMRI data analyses toolkits (REST[2]). A total of 240 volumes were scanned, and the first 10 volumes were discarded to allow for signal equilibrium of the initial magnetic resonance signals and adaptation of the subjects to scanner. The remaining 230 consecutive volumes were used for data analysis. Afterwards, the following procedures were carried out as follows: slice-timing adjustment, realignment for head-motion correction, spatial normalization to the Montreal Neurological Institute (MNI) template (resampling voxel size = 3 × 3 × 3 mm^3) and smoothing with an isotropic Gaussian kernel (full width at half maximum (FWHM) = 6 mm), detrending and filtering (0.01–0.08 Hz). Any subjects with a head motion >2.0 mm translation or a 2.0° rotation in any direction were excluded.

Degree Centrality Analysis

We restricted our voxel-wise centrality analyses to a predefined gray matter (GM) mask that included tissue with GM probabilities greater than 20% as previously described (Zuo et al., 2012). Within the mask, individual network centrality maps were generated in a voxel-wise fashion. First, the preprocessed functional runs were subjected to voxel-based whole-brain correlation analysis. The time course of each voxel from each participant was correlated with the time course of every other voxel, which resulted in a correlation matrix. An undirected adjacency matrix was then obtained by thresholding each correlation at $r > 0.25$ (Buckner et al., 2009; Zuo et al., 2012; Yan et al., 2013). As previously reported, the negative correlations were not included in DC calculation, given their ambiguous interpretation and detrimental effects

[1]http://www.fil.ion.ucl.ac.uk/spm
[2]http://www.restfmri.net

on test-retest reliability (Buckner et al., 2008; Vincent et al., 2008; Murphy et al., 2009). A high threshold was chosen to eliminate counting voxels that had low temporal correlation attributable to signal noise. Different threshold selections did not qualitatively change the results for cortex (Buckner et al., 2009). Then, the DC was computed as the number of significant correlations (binarized) or as the sum of the weights of the significant connections (weighted) for each voxel (Buckner et al., 2009; Zuo et al., 2012). This measure of connectivity (degree, D) for each voxel (i) with all other voxels (j) is given by the following: $D_i = \sum d_{ij}$ where, $j = 1 \ldots N$, $i \neq j$. The map of the connectivity was then standardized by converting to z scores so that maps across participants could be averaged and compared. The z score transformation is given by:

$$Z_i = \frac{D_i - \overline{D}}{\sigma_D} \; i = 1 \ldots N \tag{1}$$

The $\overline{D}$ is the mean degree across all the voxels in the whole-brain map and σ_D is the standard deviation of the map. DC has been shown to represent the most local and directly quantifiable centrality measure and has been widely used to examine node characteristics of intrinsic network connectivity (Zuo et al., 2012). The DC maps were transferred to z-values for group comparisons. Within brain network, the DC value of a node indicates its connectivity strength to all the other nodes and reflects its importance in functional integration.

We first estimated spatial distribution of mean DC in the tinnitus group and healthy group, respectively. The individual z values were entered into the SPM8 software for a random effect one-sample t-test in a voxel-wise way to show the average DC maps within each group. The significant threshold was set at $p < 0.01$, with multiple comparisons correction using the AFNI AlphaSim program[3] determined by Monte Carlo simulation (AlphaSim program with following parameters: single voxel p value of 0.01, a minimum cluster size of 40 voxels, 5000 simulations, cluster connection radius r = 5 mm, FWHM = 6 mm). This correction was confined within the aforementioned GM mask.

To find the disrupted brain hub regions, two-tailed two-sample t-test were then conducted to investigate the differences in the DC maps between tinnitus patients and HCs. Between-group comparisons of the DC maps were performed in the SPM8 software using general linear model (GLM) analysis, with age, sex and education included as nuisance covariates. A correction for multiple comparisons was performed by a Monte Carlo simulation using the AlphaSim program, resulting in a corrected threshold of $p < 0.01$ and minimum cluster size of 40 voxels (parameters were single voxel p value of 0.01, 5000 simulations, cluster connection radius r = 5 mm, FWHM = 6 mm). For between-group analysis, a mask was created by combining the significant clusters in both groups, which were obtained from one-sample t-test results.

[3]https://afni.nimh.nih.gov/pub/dist/doc/manual/AlphaSim.pdf

Effective Connectivity Analysis

Using DC approach, we were able to show that the bilateral SFG is a region of special functional importance in tinnitus patients revealing increased FC. To further investigate the influence of directionality, we applied GCA to evaluate changes in effective connectivity. Based on the results of the DC analysis we selected the seed regions, which showed significant differences between tinnitus patients and HCs (left and right SFG: MNI coordinates (x, y, z) ±18, 42, 27). Effective connectivity was analyzed using REST-GCA in the REST toolbox (Zang et al., 2012). In this study, two separate time series of the left and right SFG were defined as the seed time series x, and the time series y denotes the time series of all voxels in the brain. The linear direct influence of x on y ($F_{x\to y}$), and the linear direct influence of y on x ($F_{y\to x}$) were calculated voxel by voxel across the brain. Thus, two Granger causality maps were generated based on the influence measures for each subjects. The residual-based F was normalized (F′) and standardized to Z score for each voxel ($Z_{x\to y}$ and $Z_{y\to x}$, subtracting the global mean F′values, divided by standard deviation).

For the group analysis on the effective connectivity, mean values of $Z_{x\to y}$ and $Z_{y\to x}$ maps were computed for each group. All eight Granger causality maps were acquired, with four for each direction and four for each group (the left SFG with $Z_{x\to y}$ and $Z_{y\to x}$ and the right SFG with $Z_{x\to y}$ and $Z_{y\to x}$ for both tinnitus patient and HCs). Then these Granger causality maps were entered into SPM8 software for group comparison. A random effect two-sample t-test in a voxel-wise manner was performed to determine the differences of effective connectivity of the SFG between tinnitus patient and HCs, with age, sex and education including as nuisance covariates. A correction for multiple comparisons was also conducted by a Monte Carlo simulation using the AlphaSim program, with a corrected threshold of $p < 0.01$ and minimum cluster size of 40 voxels.

Correlation Analysis

To investigate the relationship between clinical characteristic of tinnitus patients and DC and effective connectivity measures, these regions showing significant differences in DC or effective connectivity between groups were extracted. Mean z values within these clusters were correlated against each tinnitus characteristic using the Pearson's correlation analysis by SPSS software (version 18.0; SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant, corrected for age, sex and education. Bonferroni correction for multiple comparisons was applied in the correlation analysis.

RESULTS

Degree Centrality Analysis

In both HC (**Figure 1A**) and tinnitus patients (**Figure 1B**), the spatial distribution of the weighted DC was highly localized in the occipital lobe, inferior parietal lobe (IPL), PFC, cingulate cortex, insula and thalamus. After two-sample t-test analysis, significantly increased DC within the bilateral SFG was found

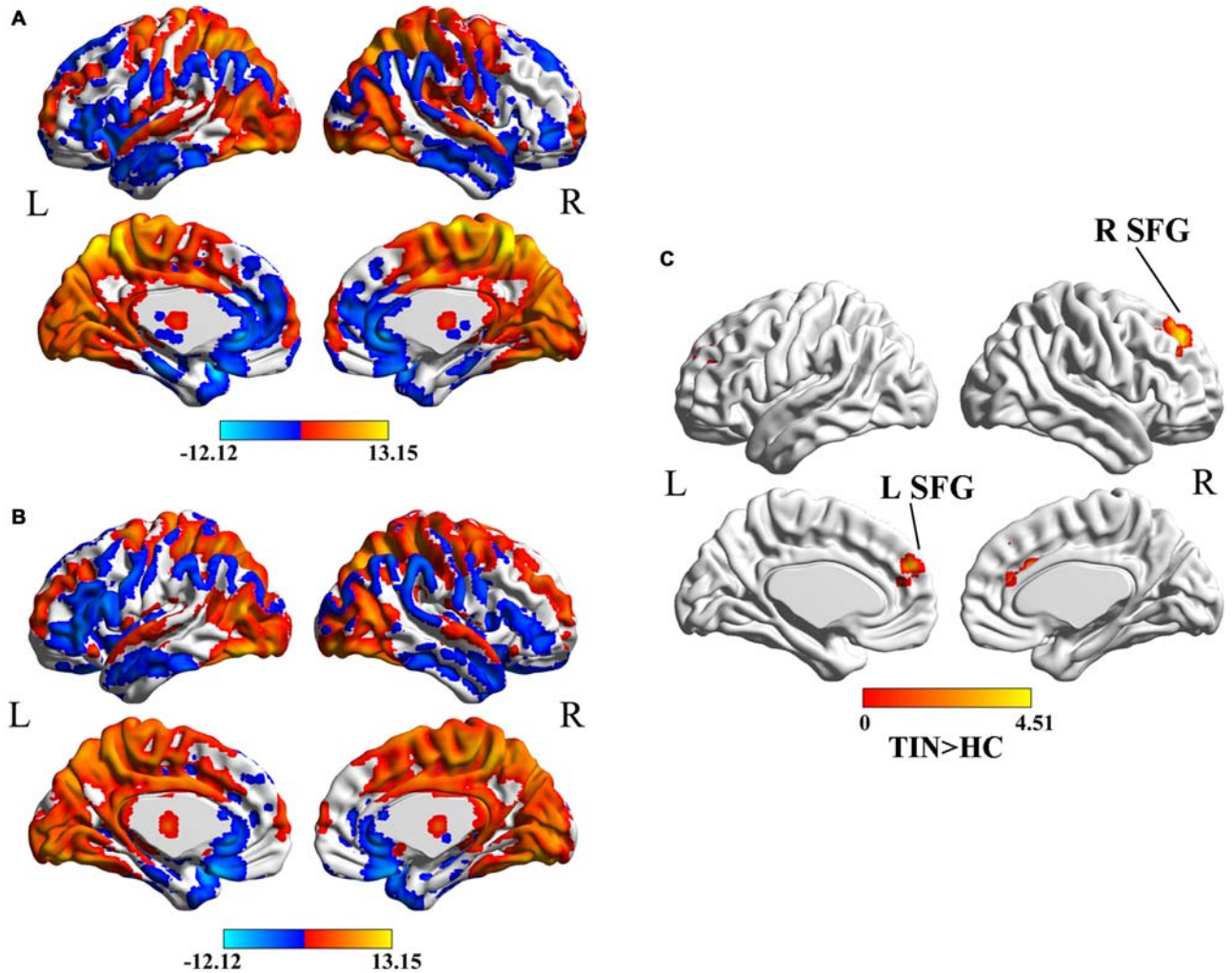

FIGURE 1 | (A) Spatial distribution of the degree centrality (DC) in the healthy controls (HCs). **(B)** Spatial distribution of the DC in the chronic tinnitus patients. **(C)** Significantly increased DC within the bilateral superior frontal gyrus (SFG) between tinnitus patients and HCs. TIN, tinnitus; HCs, healthy controls.

in tinnitus patients compared to HCs ($p < 0.01$, AlphaSim corrected; **Figure 1C**).

Effective Connectivity Analysis

Compared to HCs, patients with chronic tinnitus demonstrated significantly increased effective connectivity from the left SFG to the left middle orbitofrontal cortex (OFC), left posterior lobe of cerebellum (PLC), left postcentral gyrus (PoCG), and right middle occipital gyrus (MOG). Moreover, the right SFG exhibited enhanced effective connectivity to the right supplementary motor area (SMA; $p < 0.01$, AlphaSim corrected; **Figure 2** and **Table 2**). However, we did not find any abnormal feedback effect to the bilateral SFG in the tinnitus patients.

Correlation Results

We found no significant correlations between the DC of the bilateral SFG and the tinnitus characteristics. However, the THQ scores positively correlated with the increased effective connectivity from the left SFG to left OFC ($r = 0.504$, $p = 0.020$), and from the right SFG to right SMA ($r = 0.526$, $p = 0.014$; **Figure 3**). The other regions with enhanced effective connectivity revealed no significant correlations with tinnitus duration or THQ scores.

DISCUSSION

This is the first study to use both DC and GCA approaches to explore intrinsic functional network architecture related to tinnitus. Using DC analysis, we found significantly increased network centrality within the bilateral SFG regions in chronic tinnitus patients. Using GCA algorithm, we employed the bilateral SFG as seeds to examine their causal effect with the whole brain. Unidirectionally, bilateral SFG showed increased effective connectivity to several non-auditory regions including the prefrontal, motor, visual cortex and cerebellum. Of note,

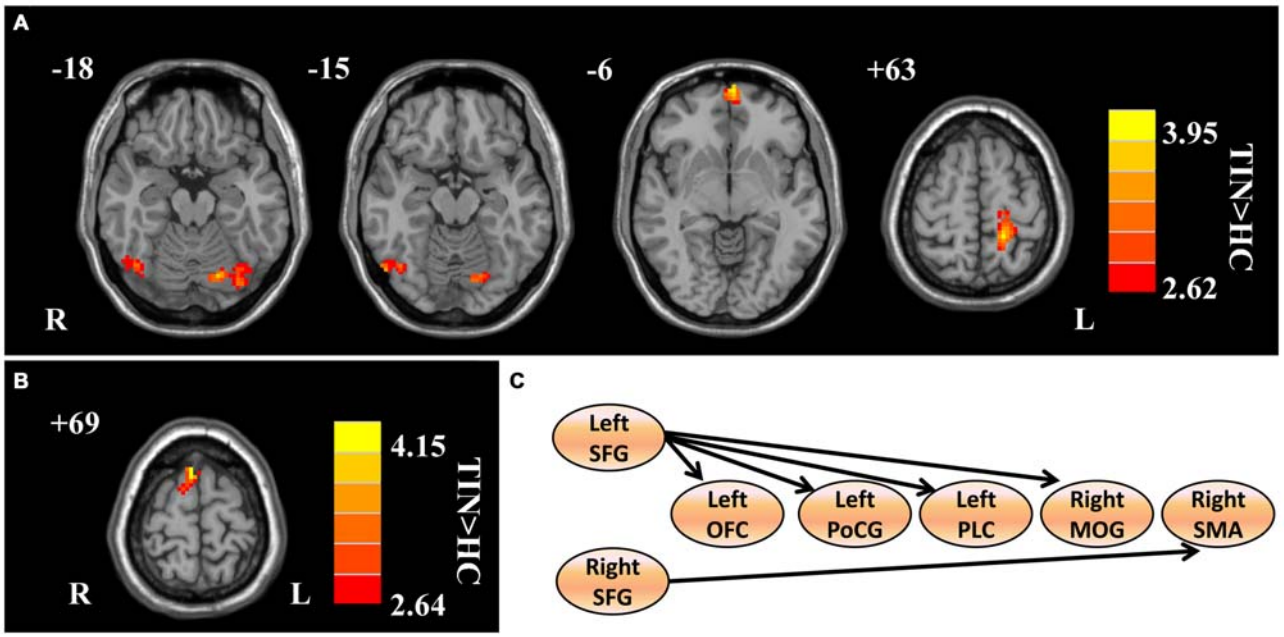

FIGURE 2 | Aberrant effective connectivity from the bilateral SFG in tinnitus patients. (A) Increased effective connectivity from the left SFG to the left orbitofrontal cortex (OFC), left PoCG, left posterior lobe of cerebellum (PLC), and right middle occipital gyrus (MOG; $p < 0.01$, AlphaSim corrected). **(B)** Increased effective connectivity from the right SFG to the right supplementary motor area (SMA; $p < 0.01$, AlphaSim corrected). **(C)** Schematic overview of changes in effective connectivity from the bilateral SFG. SFG, superior frontal gyrus; OFC, orbitofrontal cortex; PoCG, precentral gyrus; PLC, posterior lobe of cerebellum; MOG, middle occipital gyrus; SMA, supplementary motor area; TIN, tinnitus; HCs, healthy controls.

the abnormal connectivity to the OFC and the SMA revealed significant correlations with the tinnitus distress.

Increased Network Centrality in the SFG

Certain regions show strong connections with other regions within large-scale cortical networks that constitute an emerging feature of brain architecture (Sporns et al., 2007; Buckner et al., 2009; Zuo et al., 2012). Our findings show that the SFG is the main cortical hub in the brain network architecture affected by tinnitus, which is in line with the hypothesis that the dysconnectivity pattern of the PFC involved in tinnitus perception. The PFC has been regarded as a critical region by Jastreboff (1990), who suggested that the PFC integrates sensory and emotional aspects of tinnitus. Several neurophysiological models based on neuroimaging have been raised involved in tinnitus subsequently (De Ridder et al., 2014). Rauschecker et al. (2010) developed a model to demonstrate structural and functional differences in ventromedial prefrontal cortex (vmPFC) that were associated with tinnitus subjective loudness, indicating that PFC may contribute to certain perceptual features of tinnitus (Leaver et al., 2011). The current integrative model attempts to unify the different brain areas and networks associated with tinnitus in one model and propose the hypothetical neural core of conscious phantom sound perception (De Ridder et al., 2011, 2014; Vanneste and De Ridder, 2012), which is more extensive than the initially proposed auditory system (Jastreboff and Hazell, 2008).

Our current study suggested that the PFC, specifically the SFG, might be a major integrative hub of the model that should be involved to perceive tinnitus. However, we did not observe any abnormal neural activity in auditory system. The most parsimonious explanation for this is the absence of any hearing loss out to 8 kHz and the absence of hyperacusis in our tinnitus patients. We speculated that aberrant neural activity of the PFC may exist prior to the disruption of the auditory system in tinnitus patients with normal hearing. In addition, since our tinnitus patients showed no obvious symptoms of depression,

TABLE 2 | Regions showing significant differences in effective connectivity between tinnitus patients and HCs.

Brain regions	BA	Peak MNI coordinates *x, y, z* (mm)	Peak *T* value	Voxels
Increased effective connectivity from left SFG				
Left OFC	11	−3, 66, −6	4.1302	158
Left PoCG	3	−18, −42, 63	4.0470	132
Left PLC	–	−18, −75, −18	3.7193	100
Right MOG	19	54, −69, −15	3.6185	46
Increased effective connectivity from right SFG				
Right SMA	6	6, 15, 69	4.4562	44

A corrected threshold of $p < 0.01$ was determined by Monte Carlo simulation. BA, Brodmann's area; MNI, Montreal Neurological Institute; SFG, superior frontal gyrus; OFC, orbitofrontal cortex; PoCG, precentral gyrus; PLC, posterior lobe of cerebellum; MOG, middle occipital gyrus; SMA, supplementary motor area.

anxiety or cognitive decline, we did not detect any abnormalities in the limbic system. Based on previous fMRI studies, the SFG is a significant part of auditory connection cortex that can receive and integrate all kinds of information from different parts of the brain from inside and outside the body. Besides, it can also timely organize efferent impulses to ensure the coordination of the central nervous system (CNS) as a whole (Mathiak et al., 2007; Melloni et al., 2007). A possible interpretation for our results was that the increased DC in bilateral SFG might be due to feedback inhibition of the over activity in auditory network.

Previous neuroimaging studies have pointed out that the abnormalities of the SFG could act as a direct mechanism of tinnitus chronification (Giraud et al., 1999; Mirz et al., 1999; Haller et al., 2010). Wunderlich et al. (2010) detected the SFG activation after acoustic stimulation in a pitch discrimination task, suggesting the perception of auditory inputs in a more emotional context due to tinnitus. Chen et al. (2014) found increased ALFF values in the right SFG in chronic tinnitus patients, which was linked with tinnitus duration and distress. The SFG was also proved to be influenced by chronic tinnitus in recent rs-fMRI studies (Maudoux et al., 2012a; Ueyama et al., 2013; Chen et al., 2015c; Zhang et al., 2015). In line with these valuable findings, our results have implications for understanding the specific role of SFG abnormalities in chronic tinnitus, suggesting the PFC may provide valuable insight into the neural mechanisms underlying tinnitus.

Increased Effective Connectivity from the SFG

Using a GCA method, the present study exhibited that the information flow is unidirectionally affected (seed to whole brain) within non-auditory areas due to tinnitus. We found increased influence from the SFG to the frontal cortex (OFC and SMA), cerebellum (PLC), visual cortex (MOG) and somatosensory cortex (PoCG). Previous neuroimaging studies have identified abnormalities in the frontal cortex which could account for tinnitus. Firstly, it has been revealed that the OFC is critical for emotional processing of sounds (Damasio et al., 1996; Blood et al., 1999). The alterations of the OFC have been found in the functional coupling of long-range cortical networks between tinnitus patients and HCs by using EEG or MEG (Schlee et al., 2009; Vanneste et al., 2012; Song et al., 2014). Moreover, recent rs-fMRI studies have also demonstrated the abnormal FC of the OFC in tinnitus patients (Maudoux et al., 2012a; Chen et al., 2015d; Zhang et al., 2015). The OFC is also regarded as part of the reward system, which might integrate the aversive information of the perceived tinnitus (Rolls, 2004; Kringelbach, 2005). The heightened effective connectivity from the SFG to the OFC might be interpreted as a dysfunctional inhibitory response directing attention away from phantom sound perception. Our finding of positive correlation between the effective connection from the SFG to the OFC and tinnitus distress also emphasized the pivotal role of the OFC in tinnitus. Furthermore, we also found positive correlation between the THQ score and increased influence from the SFG to the SMA, which is regarded as a part of the primate cerebral cortex that contributes to the control of movement (Roland et al., 1980). By using quantitative EEG (qEEG), Vanneste et al. (2011) observed aberrant neuronal activity in the SMA in unilateral or bilateral tinnitus (Vanneste and De Ridder, 2012). It was hypothesized that synchronized theta activity in the SMA might be accountable for part of the conscious perception of the phantom sound (Vanneste and De Ridder, 2012). Nevertheless, further research is needed to clarify the role of the SMA in tinnitus.

Although the cerebellum is primarily involved in motor actions and control, some cerebellar regions such as the paraflocculus and vermis receive inputs from auditory centers (Petacchi et al., 2005). Auditory sensory processing in the cerebellum has been reported. Osaki et al. (2005) revealed that the right cerebellum was involved in tinnitus and showed a decreased regional blood flow during residual inhibition. Using rs-fMRI, chronic tinnitus patients showed increased FC in the cerebellar hemisphere that was linked with tinnitus distress (Maudoux et al., 2012a; Ueyama et al., 2013), confirming the involvement of cerebellum in auditory system. Nevertheless, the association of the cerebellum with tinnitus has not been substantially elucidated. Moreover, increased effective connectivity to MOG and PoCG raised the question of whether tinnitus might be linked to phantom visual or somatosensory perceptions. Such changes seem reasonable given the multisensory interactions known to exist between auditory, visual and somatosensory regions. Possible neural correlates of visual or somatosensory modulation of tinnitus were assessed (Murray et al., 2005). One interpretation of these results is that as patients attend to their phantom auditory sensation they contemporaneously activate visual or somatosensory areas. This interpretation is consistent with previous rs-fMRI studies showing abnormal neural activity in visual network (Burton et al., 2012; Maudoux et al., 2012a; Chen et al., 2014, 2015b,c,d) or somatosensory network (Maudoux et al., 2012a; Ueyama et al., 2013) in tinnitus. Therefore, tinnitus can be regarded as the consequence of multiple brain subnetworks involved in the different aspects of tinnitus, both acoustic and affective.

Tinnitus Lateralization

Interestingly, results of effective connectivity analysis were lateralized to the left hemisphere region in tinnitus patients. Asymmetry for the tinnitus patients has been reported both structurally and functionally (Mühlau et al., 2006; Smits et al., 2007; Schecklmann et al., 2013; Chen et al., 2014). Previous PET studies have demonstrated an overactivation of the left auditory cortex independent of tinnitus laterality and anatomical hemispheric differences (Arnold et al., 1996; Langguth et al., 2006; Schecklmann et al., 2013). This lateralization may be explained as an increase in activation on the side of the perceived tinnitus, or a decrease in activation on the side contralateral to the side of perceived tinnitus. The interpretation would indicate an increased spontaneous neural activity of the affected brain area in tinnitus patients (Smits et al., 2007). However, several studies also confirmed the right-

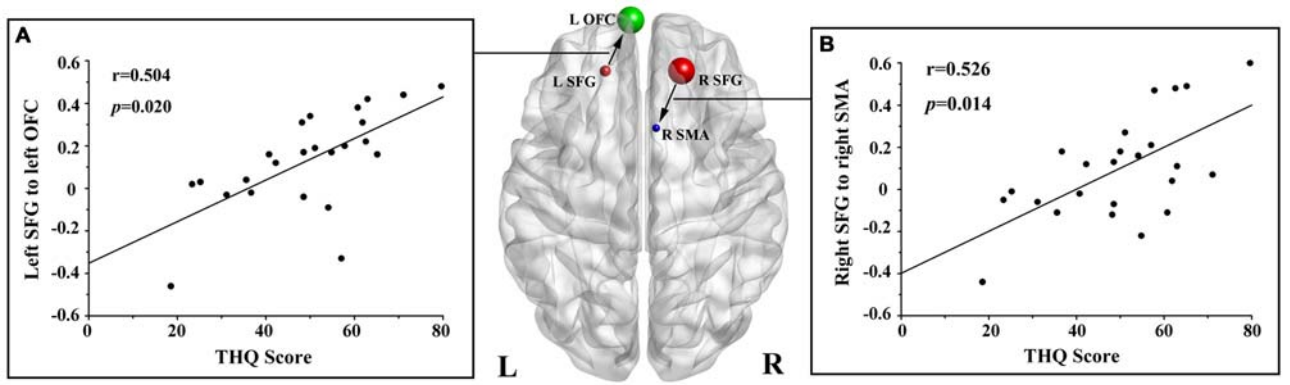

FIGURE 3 | Correlations between the increased effective connectivity of the bilateral SFG and the tinnitus handicap questionnaires (THQ) scores. **(A)** The THQ scores positively correlated with the increased effective connectivity from the left SFG to left OFC (r = 0.504, p = 0.020). **(B)** The THQ scores positively correlated with the increased effective connectivity from the right SFG to right SMA (r = 0.526, p = 0.014). L, left; R, right; THQ, tinnitus handicap questionnaires; SFG, superior frontal gyrus; OFC, orbitofrontal cortex; SMA, supplementary motor area.

lateralization in tinnitus (Chen et al., 2014; Geven et al., 2014). The inconsistencies between studies may be due to the different neuroimaging methods used to investigate tinnitus or heterogeneity of the tinnitus patients. Therefore, further studies are required to determine if the observed left hemispheric dominance is related specifically to tinnitus or some other factors.

Limitations

Study limitations must be acknowledged. First, we admit that it is difficult to make direct causal inferences regarding the relationships between the brain functional network architecture and tinnitus characteristics in tinnitus patients, considering the cross-sectional nature of our experimental design and limited sample size. Further longitudinal studies involving a greater number of subjects are required. The very nature of BOLD-fMRI makes it difficult to measure resting-state network activity given that amplitude is not measured only correlations of BOLD signals (Logothetis et al., 2001). The relationship between the measured BOLD-fMRI signal and the underlying neural activity is still largely unknown. Further studies combining fMRI with EEG may help understand the exact relationship. Second, common criticisms regarding GCA algorithm for rs-fMRI data have been raised. For example, the fallacious effects might occur because of the systematic differences across brain regions in hemodynamic delays. GCA has been confounded by the presence of vascular anatomy (Webb et al., 2013). Additionally, changes in directionality could be caused by the differences in hemodynamic coupling in different regions (Smith et al., 2011). Furthermore, we attempted to exclude subjects with hyperacusis from our study because subjects with hyperacusis exhibited robust activation in selected brain regions, such as the primary auditory cortex, thalamus, and auditory midbrain (Gu et al., 2010). However, it would be useful to include subjects with hyperacusis in future studies so as to examine if intrinsic functional network architecture is disrupted in a similar manner to that was observed in our tinnitus patients without hyperacusis. Finally, although we attempt to reduce the MR scanner noise with earplugs, the subjects cannot be completely prevented from hearing some noise that probably alters the resting-state brain networks to varying degree (Logothetis et al., 2009). This confounding factor should be taken into consideration for all rs-fMRI studies related to the auditory systems.

CONCLUSION

In this study, we show that disrupted functional network architecture within non-auditory regions revealed by DC and GCA are present in chronic tinnitus patients with normal hearing, without hyperacusis. Chronic tinnitus patients exhibited enhanced FC strength in bilateral SFG regions, which showed unidirectionally increased causal connectivity to frontal, motor, visual, somatosensory cortex and cerebellum. Furthermore, increased effective connectivity from bilateral SFG to OFC and SMA were positively correlated with tinnitus distress. Disturbance in brain functional network architecture will contribute to a better understanding of the neuropathophysiological mechanisms in tinnitus perception.

AUTHOR CONTRIBUTIONS

Y-CC and YF designed the experiment, collected the data, performed the analysis and wrote the manuscript. J-JX and C-NM collected the data. WX, JR, and XY contributed to the discussion and manuscript revision.

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REFERENCES

Adjamian, P., Sereda, M., and Hall, D. A. (2009). The mechanisms of tinnitus: perspectives from human functional neuroimaging. *Hear. Res.* 253, 15–31. doi: 10.1016/j.heares.2009.04.001

Arnold, W., Bartenstein, P., Oestreicher, E., Römer, W., and Schwaiger, M. (1996). Focal metabolic activation in the predominant left auditory cortex in patients suffering from tinnitus: a PET study with [18F] deoxyglucose. *ORL J. Otorhinolaryngol. Relat. Spec.* 58, 195–199. doi: 10.1159/000276835

Berliner, K. I., Shelton, C., Hitselberger, W. E., and Luxford, W. M. (1992). Acoustic tumors: effect of surgical removal on tinnitus. *Otol. Neurotol.* 13, 13–17. doi: 10.1097/00129492-199201000-00005

Biswal, B., Zerrin Yetkin, F., Haughton, V. M., and Hyde, J. S. (1995). Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn. Reson. Med.* 34, 537–541. doi: 10.1002/mrm.1910340409

Blood, A. J., Zatorre, R. J., Bermudez, P., and Evans, A. C. (1999). Emotional responses to pleasant and unpleasant music correlate with activity in paralimbic brain regions. *Nat. Neurosci.* 2, 382–387.

Buckner, R. L., Andrews-Hanna, J. R., and Schacter, D. L. (2008). The brain's default network. *Ann. N Y Acad. Sci.* 1124, 1–38. doi: 10.1196/annals.1440.011

Buckner, R. L., Sepulcre, J., Talukdar, T., Krienen, F. M., Liu, H., Hedden, T., et al. (2009). Cortical hubs revealed by intrinsic functional connectivity: mapping, assessment of stability and relation to Alzheimer's disease. *J. Neurosci.* 29, 1860–1873. doi: 10.1523/jneurosci.5062-08.2009

Burton, H., Wineland, A., Bhattacharya, M., Nicklaus, J., Garcia, K. S., and Piccirillo, J. F. (2012). Altered networks in bothersome tinnitus: a functional connectivity study. *BMC Neurosci.* 13:3. doi: 10.1186/1471-2202-13-3

Chao-Gan, Y., and Yu-Feng, Z. (2010). DPARSF: a MATLAB toolbox for "pipeline" data analysis of resting-state fMRI. *Front. Syst. Neurosci.* 4:13. doi: 10.3389/fnsys.2010.00013

Chen, H.-J., Jiang, L.-F., Sun, T., Liu, J., Chen, Q.-F., and Shi, H.-B. (2015a). Resting-state functional connectivity abnormalities correlate with psychometric hepatic encephalopathy score in cirrhosis. *Eur. J. Radiol.* 84, 2287–2295. doi: 10.1016/j.ejrad.2015.08.005

Chen, Y.-C., Xia, W., Feng, Y., Li, X., Zhang, J., Feng, X., et al. (2015b). Altered interhemispheric functional coordination in chronic tinnitus patients. *Biomed. Res. Int.* 2015:345647. doi: 10.1155/2015/345647

Chen, Y.-C., Xia, W., Luo, B., Muthaiah, V. P., Xiong, Z., Zhang, J., et al. (2015c). Frequency-specific alternations in the amplitude of low-frequency fluctuations in chronic tinnitus. *Front. Neural Circuits* 9:67. doi: 10.3389/fncir.2015.00067

Chen, Y.-C., Zhang, J., Li, X.-W., Xia, W., Feng, X., Qian, C., et al. (2015d). Altered intra-and interregional synchronization in resting-state cerebral networks associated with chronic tinnitus. *Neural Plast.* 2015:475382. doi: 10.1155/2015/475382

Chen, Y.-C., Zhang, J., Li, X.-W., Xia, W., Feng, X., Gao, B., et al. (2014). Aberrant spontaneous brain activity in chronic tinnitus patients revealed by resting-state functional MRI. *Neuroimage Clin.* 6, 222–228. doi: 10.1016/j.nicl.2014.09.011

Damasio, A. R., Everitt, B., and Bishop, D. (1996). The somatic marker hypothesis and the possible functions of the prefrontal cortex [and discussion]. *Philos. Trans. R. Soc. B Biol. Sci.* 351, 1413–1420. doi: 10.1098/rstb.1996.0125

Davies, J., Gander, P., Andrews, M., and Hall, D. (2014). Auditory network connectivity in tinnitus patients: a resting-state fMRI study. *Int. J. Audiol.* 53, 192–198. doi: 10.3109/14992027.2013.846482

De Ridder, D., Elgoyhen, A. B., Romo, R., and Langguth, B. (2011). Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc. Natl. Acad. Sci. U S A* 108, 8075–8080. doi: 10.1073/pnas.1018466108

De Ridder, D., Vanneste, S., Weisz, N., Londero, A., Schlee, W., Elgoyhen, A. B., et al. (2014). An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci. Biobehav. Rev.* 44, 16–32. doi: 10.1016/j.neubiorev.2013.03.021

Di Martino, A., Zuo, X.-N., Kelly, C., Grzadzinski, R., Mennes, M., Schvarcz, A., et al. (2013). Shared and distinct intrinsic functional network centrality in autism and attention-deficit/hyperactivity disorder. *Biol. Psychiatry* 74, 623–632. doi: 10.1016/j.biopsych.2013.02.011

Fox, M. D., and Raichle, M. E. (2007). Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat. Rev. Neurosci.* 8, 700–711. doi: 10.1038/nrn2201

Geven, L. I., de Kleine, E., Willemsen, A. T., and van Dijk, P. (2014). Asymmetry in primary auditory cortex activity in tinnitus patients and controls. *Neuroscience* 256, 117–125. doi: 10.1016/j.neuroscience.2013.10.015

Giraud, A. L., Chéry-Croze, S., Fischer, G., Fischer, C., Vighetto, A., Grégoire, M.-C., et al. (1999). A selective imaging of tinnitus. *Neuroreport* 10, 1–5. doi: 10.1097/00001756-199901180-00001

Granger, C. W. (1969). Investigating causal relations by econometric models and cross-spectral methods. *Econometrica* 37, 424–438. doi: 10.2307/1912791

Gu, J. W., Halpin, C. F., Nam, E.-C., Levine, R. A., and Melcher, J. R. (2010). Tinnitus, diminished sound-level tolerance and elevated auditory activity in humans with clinically normal hearing sensitivity. *J. Neurophysiol.* 104, 3361–3370. doi: 10.1152/jn.00226.2010

Guo, W., Liu, F., Liu, J., Yu, L., Zhang, J., Zhang, Z., et al. (2015a). Abnormal causal connectivity by structural deficits in first-episode, drug-naive schizophrenia at rest. *Schizophr. Bull.* 41, 57–65. doi: 10.1093/schbul/sbu126

Guo, W., Liu, F., Zhang, Z., Liu, J., Yu, M., Zhang, J., et al. (2015b). Unidirectionally affected causal connectivity of cortico-limbic-cerebellar circuit by structural deficits in drug-naive major depressive disorder. *J. Affect. Disord.* 172, 410–416. doi: 10.1016/j.jad.2014.10.019

Haller, S., Birbaumer, N., and Veit, R. (2010). Real-time fMRI feedback training may improve chronic tinnitus. *Eur. Radiol.* 20, 696–703. doi: 10.1007/s00330-009-1595-z

Henry, J. A., Roberts, L. E., Caspary, D. M., Theodoroff, S. M., and Salvi, R. J. (2014). Underlying mechanisms of tinnitus: review and clinical implications. *J. Am. Acad. Audiol.* 25, 5–22. doi: 10.3766/jaaa.25.1.2

Hinkley, L. B., Mizuiri, D., Hong, O., Nagarajan, S. S., and Cheung, S. W. (2015). Increased striatal functional connectivity with auditory cortex in tinnitus. *Front. Hum. Neurosci.* 9:568. doi: 10.3389/fnhum.2015.00568

Jackler, R. K., and Whinney, D. (2001). A century of eighth nerve surgery. *Otol. Neurotol.* 22, 401–416. doi: 10.1097/00129492-200109000-00029

Jastreboff, P. J. (1990). Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci. Res.* 8, 221–254. doi: 10.1016/0168-0102(90)90031-9

Jastreboff, P. J., and Hazell, J. W. (2008). *Tinnitus Retraining Therapy: Implementing the Neurophysiological Model.* Cambridge, UK: Cambridge University Press.

Kaltenbach, J. A., Zhang, J., and Finlayson, P. (2005). Tinnitus as a plastic phenomenon and its possible neural underpinnings in the dorsal cochlear nucleus. *Hear. Res.* 206, 200–226. doi: 10.1016/j.heares.2005.02.013

Khalfa, S., Dubal, S., Veuillet, E., Perez-Diaz, F., Jouvent, R., and Collet, L. (2002). Psychometric normalization of a hyperacusis questionnaire. *ORL J. Otorhinolaryngol. Relat. Spec.* 64, 436–442. doi: 10.1159/000067570

Kim, J.-Y., Kim, Y.-H., Lee, S., Seo, J.-H., Song, H.-J., Cho, J. H., et al. (2012). Alteration of functional connectivity in tinnitus brain revealed by resting-state fMRI? a pilot study. *Int. J. Audiol.* 51, 413–417. doi: 10.3109/14992027.2011.652677

Kringelbach, M. L. (2005). The human orbitofrontal cortex: linking reward to hedonic experience. *Nat. Rev. Neurosci.* 6, 691–702. doi: 10.1038/nrn1747

Kuk, F. K., Tyler, R. S., Russell, D., and Jordan, H. (1990). The psychometric properties of a tinnitus handicap questionnaire. *Ear Hear.* 11, 434–445. doi: 10.1097/00003446-199012000-00005

Langguth, B., Eichhammer, P., Kreutzer, A., Maenner, P., Marienhagen, J., Kleinjung, T., et al. (2006). The impact of auditory cortex activity on characterizing and treating patients with chronic tinnitus–first results from a PET study. *Acta Otolaryngol. Suppl.* 126, 84–88. doi: 10.1080/03655230600895317

Leaver, A. M., Renier, L., Chevillet, M. A., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2011). Dysregulation of limbic and auditory networks in tinnitus. *Neuron* 69, 33–43. doi: 10.1016/j.neuron.2010.12.002

Leaver, A. M., Turesky, T. K., Seydell-Greenwald, A., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2016). Intrinsic network activity in tinnitus investigated using functional MRI. *Hum. Brain Mapp.* doi: 10.1002/hbm.23204 [Epub ahead of print].

Lenarz, T., Schreiner, C., Snyder, R. L., and Ernst, A. (1993). Neural mechanisms of tinnitus. *Eur. Arch. Otorhinolaryngol.* 249, 441–446. doi: 10.1007/bf00168851

Leske, M. C. (1981). Prevalence estimates of communicative disorders in the US Language, hearing and vestibular disorders. *ASHA* 23, 229–237.

Lewis, J. W., Beauchamp, M. S., and DeYoe, E. A. (2000). A comparison of visual and auditory motion processing in human cerebral cortex. *Cereb. Cortex* 10, 873–888. doi: 10.1093/cercor/10.9.873

Lockwood, A. H., Salvi, R. J., and Burkard, R. F. (2002). Tinnitus. *N. Engl. J. Med.* 347, 904–910. doi: 10.1056/NEJMra013395

Lockwood, A. H., Salvi, R., Coad, M., Towsley, M., Wack, D., and Murphy, B. (1998). The functional neuroanatomy of tinnitus Evidence for limbic system links and neural plasticity. *Neurology* 50, 114–120. doi: 10.1212/wnl.50.1.114

Logothetis, N. K., Murayama, Y., Augath, M., Steffen, T., Werner, J., and Oeltermann, A. (2009). How not to study spontaneous activity. *Neuroimage* 45, 1080–1089. doi: 10.1016/j.neuroimage.2009.01.010

Logothetis, N. K., Pauls, J., Augath, M., Trinath, T., and Oeltermann, A. (2001). Neurophysiological investigation of the basis of the fMRI signal. *Nature* 412, 150–157. doi: 10.1038/35084005

Mathiak, K., Menning, H., Hertrich, I., Mathiak, K. A., Zvyagintsev, M., and Ackermann, H. (2007). Who is telling what from where? A functional magnetic resonance imaging study. *Neuroreport* 18, 405–409. doi: 10.1097/wnr.0b013e328013cec4

Maudoux, A., Lefebvre, P., Cabay, J.-E., Demertzi, A., Vanhaudenhuyse, A., Laureys, S., et al. (2012a). Auditory resting-state network connectivity in tinnitus: a functional MRI study. *PLoS One* 7:e36222. doi: 10.1371/journal.pone.0036222

Maudoux, A., Lefebvre, P., Cabay, J.-E., Demertzi, A., Vanhaudenhuyse, A., Laureys, S., et al. (2012b). Connectivity graph analysis of the auditory resting state network in tinnitus. *Brain Res.* 1485, 10–21. doi: 10.1016/j.brainres.2012.05.006

McFadden, D. (1982). *Tinnitus: Facts, Theories and Treatments.* Washington, DC: National Academies.

Meikle, M. B. (1997). Electronic access to tinnitus data: the oregon tinnitus data archive. *Otolaryngol. Head Neck Surg.* 117, 698–700. doi: 10.1016/s0194-5998(97)70055-x

Melloni, L., Molina, C., Pena, M., Torres, D., Singer, W., and Rodriguez, E. (2007). Synchronization of neural activity across cortical areas correlates with conscious perception. *J. Neurosci.* 27, 2858–2865. doi: 10.1523/jneurosci.4623-06.2007

Minami, S. B., Oishi, N., Watabe, T., Uno, K., Kaga, K., and Ogawa, K. (2015). Auditory resting-state functional connectivity in tinnitus and modulation with transcranial direct current stimulation. *Acta Otolaryngol.* 135, 1286–1292. doi: 10.3109/00016489.2015.1068952

Mirz, F., Pedersen, B., Ishizu, K., Johannsen, P., Ovesen, T., Stødkilde-Jørgensen, H., et al. (1999). Positron emission tomography of cortical centers of tinnitus. *Hear. Res.* 134, 133–144. doi: 10.1016/s0378-5955(99)00075-1

Mühlau, M., Rauschecker, J., Oestreicher, E., Gaser, C., Röttinger, M., Wohlschläger, A., et al. (2006). Structural brain changes in tinnitus. *Cereb. Cortex* 16, 1283–1288. doi: 10.1093/cercor/bhj070

Murphy, K., Birn, R. M., Handwerker, D. A., Jones, T. B., and Bandettini, P. A. (2009). The impact of global signal regression on resting state correlations: are anti-correlated networks introduced? *Neuroimage* 44, 893–905. doi: 10.1016/j.neuroimage.2008.09.036

Murray, M. M., Molholm, S., Michel, C. M., Heslenfeld, D. J., Ritter, W., Javitt, D. C., et al. (2005). Grabbing your ear: rapid auditory-somatosensory multisensory interactions in low-level sensory cortices are not constrained by stimulus alignment. *Cereb. Cortex* 15, 963–974. doi: 10.1093/cercor/bhh197

Osaki, Y., Nishimura, H., Takasawa, M., Imaizumi, M., Kawashima, T., Iwaki, T., et al. (2005). Neural mechanism of residual inhibition of tinnitus in cochlear implant users. *Neuroreport* 16, 1625–1628. doi: 10.1097/01.wnr.0000183899.85277.08

Petacchi, A., Laird, A. R., Fox, P. T., and Bower, J. M. (2005). Cerebellum and auditory function: an ALE meta-analysis of functional neuroimaging studies. *Hum. Brain Mapp.* 25, 118–128. doi: 10.1002/hbm.20137

Qi, R., Zhang, L. J., Zhong, J., Zhang, Z., Ni, L., Jiao, Q., et al. (2013). Altered effective connectivity network of the basal ganglia in low-grade hepatic encephalopathy: a resting-state fMRI study with Granger causality analysis. *PLoS One* 8:e53677. doi: 10.1371/journal.pone.0053677

Rauschecker, J. P., Leaver, A. M., and Mühlau, M. (2010). Tuning out the noise: limbic-auditory interactions in tinnitus. *Neuron* 66, 819–826. doi: 10.1016/j.neuron.2010.04.032

Roberts, L. E., Eggermont, J. J., Caspary, D. M., Shore, S. E., Melcher, J. R., and Kaltenbach, J. A. (2010). Ringing ears: the neuroscience of tinnitus. *J. Neurosci.* 30, 14972–14979. doi: 10.1523/JNEUROSCI.4028-10.2010

Roland, P. E., Larsen, B., Lassen, N., and Skinhøj, E. (1980). Supplementary motor area and other cortical areas in organization of voluntary movements in man. *J. Neurophysiol.* 43, 118–136.

Rolls, E. T. (2004). The functions of the orbitofrontal cortex. *Brain Cogn.* 55, 11–29. doi: 10.1016/S0278-2626(03)00277-X

Schecklmann, M., Landgrebe, M., Poeppl, T. B., Kreuzer, P., Männer, P., Marienhagen, J., et al. (2013). Neural correlates of tinnitus duration and distress: a positron emission tomography study. *Hum. Brain Mapp.* 34, 233–240. doi: 10.1002/hbm.21426

Schlee, W., Mueller, N., Hartmann, T., Keil, J., Lorenz, I., and Weisz, N. (2009). Mapping cortical hubs in tinnitus. *BMC Biol.* 7:80. doi: 10.1186/1741-7007-7-80

Schmidt, S. A., Akrofi, K., Carpenter-Thompson, J. R., and Husain, F. T. (2013). Default mode, dorsal attention and auditory resting state networks exhibit differential functional connectivity in tinnitus and hearing loss. *PLoS One* 8:e76488. doi: 10.1371/journal.pone.0076488

Shulman, A., and Strashun, A. (1998). Descending auditory system/cerebellum/tinnitus. *Int. Tinnitus J.* 5, 92–106.

Smith, S. M., Miller, K. L., Salimi-Khorshidi, G., Webster, M., Beckmann, C. F., Nichols, T. E., et al. (2011). Network modelling methods for FMRI. *Neuroimage* 54, 875–891. doi: 10.1016/j.neuroimage.2010.08.063

Smits, M., Kovacs, S., De Ridder, D., Peeters, R. R., Van Hecke, P., and Sunaert, S. (2007). Lateralization of functional magnetic resonance imaging (fMRI) activation in the auditory pathway of patients with lateralized tinnitus. *Neuroradiology* 49, 669–679. doi: 10.1007/s00234-007-0231-3

Song, J.-J., De Ridder, D., Weisz, N., Schlee, W., Van de Heyning, P., and Vanneste, S. (2014). Hyperacusis-associated pathological resting-state brain oscillations in the tinnitus brain: a hyperresponsiveness network with paradoxically inactive auditory cortex. *Brain Struct. Funct.* 219, 1113–1128. doi: 10.1007/s00429-013-0555-1

Sporns, O., Honey, C. J., and Kötter, R. (2007). Identification and classification of hubs in brain networks. *PLoS One* 2:e1049. doi: 10.1371/journal.pone.0001049

Ueyama, T., Donishi, T., Ukai, S., Ikeda, Y., Hotomi, M., Yamanaka, N., et al. (2013). Brain regions responsible for tinnitus distress and loudness: a resting-state fMRI study. *PLoS One* 8:e67778. doi: 10.1371/journal.pone.0067778

Vanneste, S., and De Ridder, D. (2012). The auditory and non-auditory brain areas involved in tinnitus. An emergent property of multiple parallel overlapping subnetworks. *Front. Syst. Neurosci.* 6:31. doi: 10.3389/fnsys.2012.00031

Vanneste, S., Joos, K., and De Ridder, D. (2012). Prefrontal cortex based sex differences in tinnitus perception: same tinnitus intensity, same tinnitus distress, different mood. *PLoS One* 7:e31182. doi: 10.1371/journal.pone.0031182

Vanneste, S., Plazier, M., Van der Loo, E., Van de Heyning, P., Congedo, M., and De Ridder, D. (2010). The neural correlates of tinnitus-related distress. *Neuroimage* 52, 470–480. doi: 10.1016/j.neuroimage.2010.04.029

Vanneste, S., Plazier, M., van der Loo, E., Van de Heyning, P., and De Ridder, D. (2011). The difference between uni-and bilateral auditory phantom percept. *Clin. Neurophysiol.* 122, 578–587. doi: 10.1016/j.clinph.2010.07.022

Vincent, J. L., Kahn, I., Snyder, A. Z., Raichle, M. E., and Buckner, R. L. (2008). Evidence for a frontoparietal control system revealed by intrinsic functional connectivity. *J. Neurophysiol.* 100, 3328–3342. doi: 10.1152/jn.90355.2008

Voisin, J., Bidet-Caulet, A., Bertrand, O., and Fonlupt, P. (2006). Listening in silence activates auditory areas: a functional magnetic resonance imaging study. *J. Neurosci.* 26, 273–278. doi: 10.1523/jneurosci.2967-05.2006

Webb, J. T., Ferguson, M. A., Nielsen, J. A., and Anderson, J. S. (2013). BOLD granger causality reflects vascular anatomy. *PLoS One* 8:e84279. doi: 10.1371/journal.pone.0084279

Wineland, A. M., Burton, H., and Piccirillo, J. (2012). Functional connectivity networks in nonbothersome tinnitus. *Otolaryngol. Head Neck Surg.* 147, 900–906. doi: 10.1177/0194599812451414

Wunderlich, A. P., Schönfeldt-Lecuona, C., Wolf, R. C., Dorn, K., Bachor, E., and Freund, W. (2010). Cortical activation during a pitch discrimination task in tinnitus patients and controls-an fMRI study. *Audiol. Neurootol.* 15, 137–148. doi: 10.1159/000241094

Yan, C.-G., Craddock, R. C., Zuo, X.-N., Zang, Y.-F., and Milham, M. P. (2013). Standardizing the intrinsic brain: towards robust measurement of inter-individual variation in 1000 functional connectomes. *Neuroimage* 80, 246–262. doi: 10.1016/j.neuroimage.2013.04.081

Zhang, J., Chen, Y.-C., Feng, X., Yang, M., Liu, B., Qian, C., et al. (2015). Impairments of thalamic resting-state functional connectivity in patients with chronic tinnitus. *Eur. J. Radiol.* 84, 1277–1284. doi: 10.1016/j.ejrad.2015.04.006

Zang, Z.-X., Yan, C.-G., Dong, Z.-Y., Huang, J., and Zang, Y.-F. (2012). Granger causality analysis implementation on MATLAB: a graphic user interface toolkit for fMRI data processing. *J. Neurosci. Methods* 203, 418–426. doi: 10.1016/j.jneumeth.2011.10.006

Zhong, Y., Huang, L., Cai, S., Zhang, Y., von Deneen, K. M., Ren, A., et al. (2014). Altered effective connectivity patterns of the default mode network in Alzheimer's disease: an fMRI study. *Neurosci. Lett.* 578, 171–175. doi: 10.1016/j.neulet.2014.06.043

Zhou, Z., Wang, X., Klahr, N. J., Liu, W., Arias, D., Liu, H., et al. (2011). A conditional Granger causality model approach for group analysis in functional magnetic resonance imaging. *Magn. Reson. Imaging* 29, 418–433. doi: 10.1016/j.mri.2010.10.008

Zung, W. W. (1971). A rating instrument for anxiety disorders. *Psychosomatics* 12, 371–379. doi: 10.1016/s0033-3182(71)71479-0

Zung, W. (1986). "Zung self-rating depression scale and depression status inventory," in *Assessment of Depression*, eds N. Sartorius and T. A. Ban (Berlin: Springer), 221–231.

Zuo, X.-N., Ehmke, R., Mennes, M., Imperati, D., Castellanos, F. X., Sporns, O., et al. (2012). Network centrality in the human functional connectome. *Cereb. Cortex* 22, 1862–1875. doi: 10.1093/cercor/bhr269

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Resting-State Brain Abnormalities in Chronic Subjective Tinnitus: A Meta-Analysis

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Purpose: The neural mechanisms that give rise to the phantom sound of tinnitus have not been fully elucidated. Neuroimaging studies have revealed abnormalities in resting-state activity that could represent the neural signature of tinnitus, but there is considerable heterogeneity in the data. To address this issue, we conducted a meta-analysis of published neuroimaging studies aimed at identifying a common core of resting-state brain abnormalities in tinnitus patients.

Methods: A systematic search was conducted for whole-brain resting-state neuroimaging studies with SPECT, PET and functional MRI that compared chronic tinnitus patients with healthy controls. The authors searched PubMed, Science Direct, Web of Knowledge and Embase databases for neuroimaging studies on tinnitus published up to September 2016. From each study, coordinates were extracted from clusters with significant differences between tinnitus subjects and controls. Meta-analysis was performed using the activation likelihood estimation (ALE) method.

Results: Data were included from nine resting-state neuroimaging studies that reported a total of 51 distinct foci. The meta-analysis identified consistent regions of increased resting-state brain activity in tinnitus patients relative to controls that included, bilaterally, the insula, middle temporal gyrus (MTG), inferior frontal gyrus (IFG), parahippocampal gyrus, cerebellum posterior lobe and right superior frontal gyrus. Moreover, decreased brain activity was only observed in the left cuneus and right thalamus.

Conclusions: The current meta-analysis is, to our knowledge, the first to demonstrate a characteristic pattern of resting-state brain abnormalities that may serve as neuroimaging markers and contribute to the understanding of neuropathophysiological mechanisms for chronic tinnitus.

Keywords: tinnitus, neuroimaging, meta-analysis, resting-state fMRI, brain networks

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INTRODUCTION

Tinnitus is the conscious perception of sound in the absence of an internal or external acoustic signal (Jastreboff, 1990). In the United States, an estimated 50 million adults have experienced tinnitus occasionally, and 16 million adults are estimated to experience frequent tinnitus (Shargorodsky et al., 2010). The central nervous system is believed to play a major role in its

development and maintenance of tinnitus (Rauschecker et al., 2010; Leaver et al., 2011; De Ridder et al., 2014b; Chen et al., 2015a). Previous electrophysiological and neuroimaging studies (Lockwood et al., 1998; Kaltenbach et al., 2005) suggest that tinnitus may arise from aberrant firing patterns or high levels of spontaneous neural activity in the central auditory pathway rather than the cochlea. However, evidences suggest that other brain regions outside the classical auditory pathway may involve attentional mechanisms that contribute to the persistent awareness of the phantom sound as well as the development of anxiety and distress leading to disabling features of chronic tinnitus (Mirz et al., 2000; Schmidt et al., 2013; Henry et al., 2014). Despite extensive research, the brain abnormalities and neuropathophysiological mechanisms underlying chronic tinnitus remain poorly understood. One of the major questions is what regions of the human brain are involved in tinnitus, a question that has been explored in numerous imaging studies (Jastreboff et al., 1994; Lockwood et al., 1998; Mirz et al., 2000; Rauschecker et al., 2010; De Ridder et al., 2014b).

A number of existing neurophysiological mechanisms and models have been proposed to account for the pathophysiology of tinnitus patients, such as central gain (Schaette and McAlpine, 2011), neural synchrony (Seki and Eggermont, 2003), frontostriatal gating (Rauschecker et al., 2015), thalamocortical dysrhythmia (Llinás et al., 1999), noise-canceling deficit (Rauschecker et al., 2010; Leaver et al., 2011), global workspace (De Ridder et al., 2014b), and precision/predictive coding models (Sedley et al., 2016). However, there is a lack of consensus as to which neural mechanism(s) and what regions of the central nervous system are common to the diverse population of tinnitus patients participating in the imaging studies. Human neuroimaging studies have revealed augmented activity in tinnitus patients in several brain regions within and/or beyond classical auditory pathways. According to the model of De Ridder et al. (2014b) tinnitus is underpinned by the integration of multiple nonspecific subnetworks of the brain involving general components of cognition, emotion and memory. Communication between these different subnetworks occurs at specific hubs, brain areas that are involved in multiple subnetworks simultaneously. These different subnetworks, which interact at different oscillatory frequencies, communicate with one another at partially overlapping hubs. Nonetheless, the role of the potential hubs involved in multiple subnetworks of tinnitus still remains unclear.

Since about 85% of chronic tinnitus patients perceived the phantom sound constantly (Schecklmann et al., 2014), the resting-state fMRI measurements seem well suited to identify the neural structures, subnetworks and hubs involved in tinnitus. A growing number of studies have used resting-state fMRI to investigate tinnitus (Husain and Schmidt, 2014) and multiple brain networks implicated in tinnitus have been identified, such as the auditory network (Burton et al., 2012; Kim et al., 2012; Maudoux et al., 2012a,b; Schmidt et al., 2013; Hinkley et al., 2015; Minami et al., 2015; Leaver et al., 2016b), default mode network (DMN; Schmidt et al., 2013; Chen et al., 2014, 2015d; Leaver et al., 2016b), dorsal attention network (DAN; Burton et al., 2012; Schmidt et al., 2013), ventral attention network (VAN; Burton et al., 2012), and visual network (Burton et al., 2012; Chen et al., 2014, 2015d). As such, tinnitus can be seen as the interaction of multiple brain subnetworks, each contributing to different aspects of tinnitus such as its acoustic features, emotional affect and awareness or attention. However, these studies reported relatively inconsistent results. For instance, most researches demonstrated the increased resting-state brain activity between tinnitus patients and healthy controls (Maudoux et al., 2012a; Chen et al., 2014, 2016; Laureano et al., 2014; Yang et al., 2014; Ueyama et al., 2015), while others failed to identify any regions of increased brain activity (Geven et al., 2014; Leaver et al., 2016b). Moreover, several studies have failed to detect any differences in network processing between tinnitus patients and controls (Wineland et al., 2012; Davies et al., 2014). These reported discrepancies can potentially be attributed to the limited sample size, variable clinical demographics and use of different methods. Furthermore, in many studies tinnitus groups were not compared to an appropriate control group (Leaver et al., 2011; Schecklmann et al., 2013; Lanting et al., 2014).

Tinnitus is a very heterogeneous condition with respect to the characteristics of the perceived sound, degree of associated awareness and distress, duration and comorbidities (Landgrebe et al., 2012). Given the high heterogeneity, it is not surprising that inconsistencies across studies are encountered. Nevertheless, there may be some commonalities across these diverse studies. One approach to identifying a common core is to perform a meta-analysis of the existing resting-state studies of patients with chronic tinnitus with the goal of identifying brain network hubs in tinnitus patients common to neuroimaging studies of tinnitus. Activation likelihood estimation (ALE) is the most common coordinate-based meta-analytic method used to analyze the neuroimaging literature; this approach seeks to identify brain locations with a consistent pattern of response across experiments. This approach is based on the collection of peak coordinates from each study included in the meta-analysis rather than the input of raw images (Turkeltaub et al., 2002; Wager et al., 2004; Laird et al., 2005a; Eickhoff et al., 2009). ALE technique has been successfully applied to neuroimaging studies of various neurological or psychiatric disorders, such as epilepsy (Li et al., 2012), Parkinson's disease (Shao et al., 2015), schizophrenia (Ellison-Wright and Bullmore, 2010), and narcolepsy (Weng et al., 2015). Song et al. (2012) performed a meta-analysis exclusively of PET studies of tinnitus using the ALE method to retrieve the most consistent activation areas across different task dimensions. However, this meta-analysis did not include the large body of fMRI studies that might prove useful in detecting resting-state brain abnormalities in tinnitus patients. Therefore the goal of the current study was to conduct a quantitative meta-analysis of several different types of resting state neuroimaging data in the tinnitus literature that met our inclusion criterion. To accomplish this, we used the ALE algorithm to determine the resting-state brain abnormalities in tinnitus patients compared to healthy controls. Our working hypothesis was that the ALE analysis would identify a

common core of brain regions linked to tinnitus generation despite the heterogeneity of the patients and experimental methods.

MATERIALS AND METHODS

Search Strategies and Study Selection

Our analysis was performed according to the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) criteria (Stroup et al., 2000). A comprehensive literature search up to September, 2016 was conducted in PubMed, Science Direct, Web of Knowledge, and Embase using the following search terms: (1) "neuroimaging" <OR > "PET" <OR > "fMRI"; (2) "resting state"; and (3) "tinnitus". Our search was restricted to humans. In addition, we manually reviewed the references cited in articles that were retrieved.

Studies were selected according to the following inclusion criteria: (1) published as an article (and not a letter or an abstract); (2) comparisons of tinnitus patients with healthy control groups on a whole-brain level; and (3) clearly reported Montreal Neurological Institute (MNI) or Talairach coordinates of the activation areas (x, y, z). Studies reporting only findings for specific ROIs were not included in the present meta-analysis. In accordance with many previous ALE meta-analyses (Laird et al., 2005b; Petacchi et al., 2005; Li et al., 2010; Kühn and Gallinat, 2013), we included coordinates resulting from fMRI as well as from PET data. We included data from PET and fMRI studies and other data analysis techniques despite the fact that they have different physiological bases and theoretical assumptions since both methods have been used to identify differences between the intrinsic brain function in patients compared with controls. The rationale was to provide an all-encompassing overview of attempts to identify resting-state abnormalities in tinnitus patients. Our search criteria yielded a total of 67 peer-reviewed published articles. Of the 67 studies, 26 studies were excluded as these studies were not about comparisons between tinnitus patients and healthy controls; nine articles were excluded since these were electroencephalography (EEG) or magnetoencephalography (MEG) studies; another four pulsatile tinnitus studies and four animal studies were excluded. Fifteen studies were further excluded since these did not report detailed peak coordinates over the whole brain. Finally, nine resting-state neuroimaging studies, six fMRI, two SPECT and one PET, were included in the ALE meta-analysis (**Figure 1** and **Table 1**).

Two independent reviewers (CYC and FW) evaluated the methodology and the risk of bias of the eligible studies. First, they assessed the titles of the search results and retrieved the relevant articles. Second, the articles that remained eligible were assessed based on their abstract to determine whether any of the inclusion criteria were not met. The full text of all remaining articles was then assessed with a data extraction template, which was constructed for the purpose of organizing and extracting information from the included articles and excluding articles without peak values. The reviewers analyzed all articles in terms of patient selection and their comparable controls, blinding, diagnostic criteria and regression methods. Any disagreements were assessed by the third reviewer (FB). The demographic data were extracted from each article, including the first author's name, year of publication, total patient number, sex distribution, mean patient age and range, statistic thresholds, hearing and psychological status.

Data Extraction

The x, y, and z peak activation coordinates of all eligible contrasts constituted the meta-analysis input. The data originally reported in Talairach spaces were converted to MNI coordinates (Lancaster et al., 2007). The data from MNI coordinates were texted and implemented in GingerALE 2.3.3[1], Research Imaging Institute of the University of Texas Health Science Center, San Antonio, TX, USA). Coordinates in each study were independently extracted by two authors (CYC and FW).

ALE Meta-Analysis

Ginger ALE software was used to analyze the resting-state brain activity between tinnitus patients and healthy controls. The reported loci of maximal anatomical differences were modeled as the peaks of three-dimensional Gaussian probability density functions defined by the full-width at half-maximum (FWHM), which was set according to a quantitative uncertainty model (Laird et al., 2005a; Eickhoff et al., 2009). ALE values were calculated on a voxel-by-voxel basis by measuring the union model activation maps modeled above. This revised analysis tested for convergence by studies (random effects) instead of foci (fixed effects). Following the method described by Turkeltaub et al. (2002, 2012), 1000 permutations were used to determine which tests were statistically significant and threshold determined for the resultant ALE map. A whole-brain histogram was computed in which the null hypothesis of uniformly distributed foci was rejected for voxels with an ALE value greater than the critical threshold, defined as the $100(1-\alpha)$th percentile of the permutation distribution, where α refers to the desired level of significance. The analyses were performed at a cluster forming threshold (reported with each p value and ALE thresholds in the results, ALE values greater than this threshold are statistically significant) computed using a $p < 0.05$, corrected for multiple comparisons using false-discovery rate (FDR; Genovese et al., 2002; Laird et al., 2005a). The volume of the minimum cluster threshold was set at 200 mm^3. The coordinates of the weighted center were generated for each cluster. The resulting significant anatomical areas were labeled based on probabilistic cytoarchitectonic maps of the human brain using the SPM Anatomy Toolbox v2.1 (Eickhoff et al., 2005). Results were visualized with Mango software[2], using the Colin brain template in the MNI space[3].

RESULTS

Using our inclusion/exclusion criteria, we identified nine eligible neuroimaging studies utilizing different methods, including

[1]http://brainmap.org/ale/
[2]http://ric.uthscsa.edu/mango/mango.html
[3]http://www.brainmap.org/ale

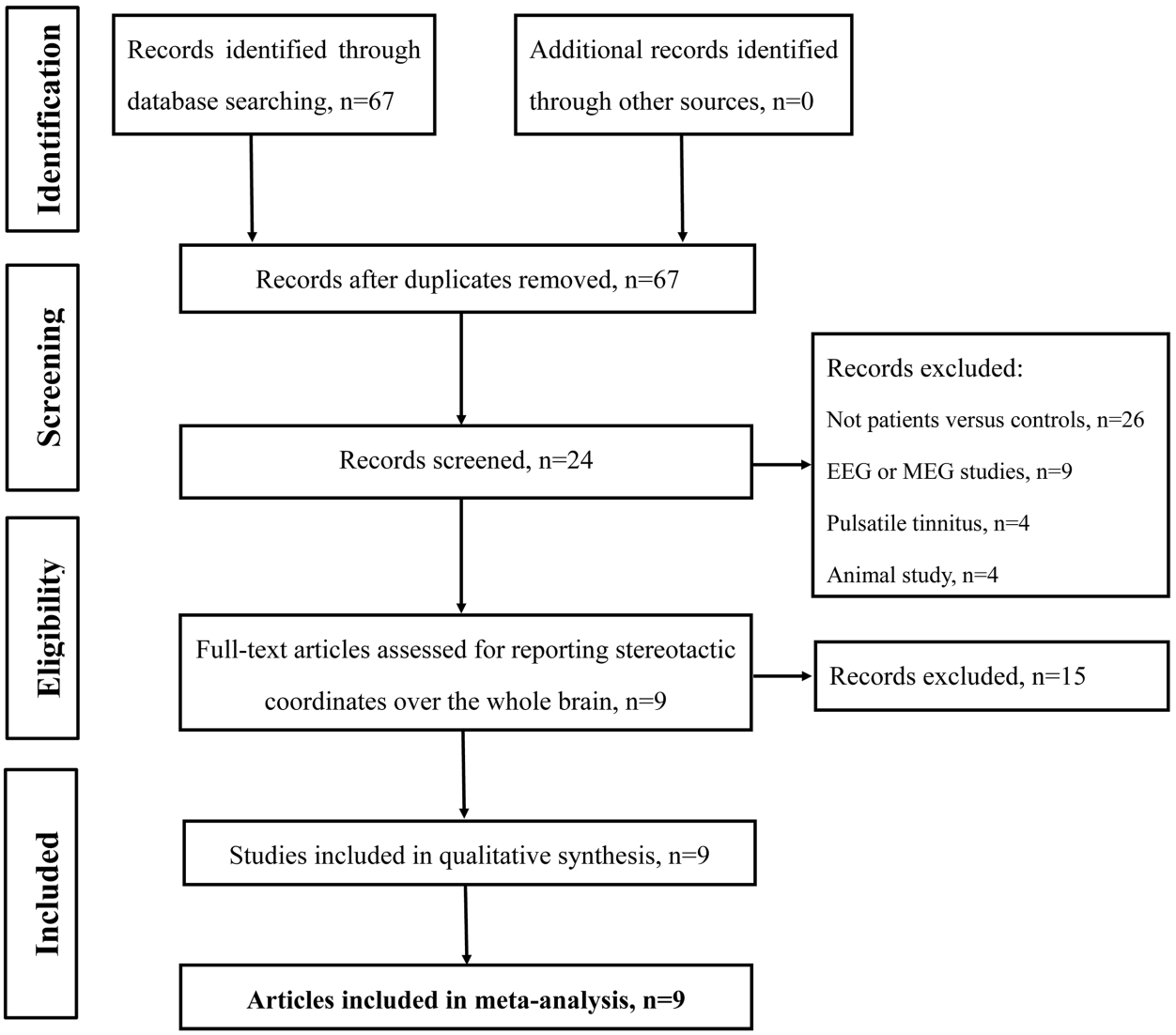

FIGURE 1 | Flow diagram of the literature search. Flow diagram shows the results of the systematic search for the selected studies in this meta-analysis.

SPECT (Laureano et al., 2014; Ueyama et al., 2015), PET (Geven et al., 2014), and fMRI (Maudoux et al., 2012a; Chen et al., 2014, 2015d, 2016; Leaver et al., 2016b). **Figure 1** is a flow diagram showing the steps in the identification, exclusion and inclusion of the studies. The clinical and demographic data of the participants from all recruited studies are presented in **Table 1**. The subjects of patients and controls from each study are generally comparable by age, sex, and education. The hearing and psychological status are also shown in **Table 2**.

As illustrated in **Figure 2**, a total of 13 peak foci were reported in this meta-analysis. Compared with healthy controls, tinnitus patients showed increased resting-state neural activity bilaterally in the middle temporal gyrus (MTG), inferior frontal gyrus (IFG), parahippocampal gyrus, insula, cerebellar posterior lobe and right superior frontal gyrus (SFG). Moreover, decreased brain activity was also observed in the left cuneus and right thalamus. **Table 3** displays the coordinates of cluster maxima.

DISCUSSION

The current study is the first whole-brain meta-analysis exploring the resting-state brain abnormalities in chronic tinnitus patients compared to healthy controls. By analyzing nine neuroimaging studies, the meta-analysis identified consistent regions of aberrant neural activity mainly in the non-auditory brain regions, including the MTG, frontal cortex, parahippocampus, insula, cerebellum, cuneus, and thalamus, in different aspects of tinnitus. Surprisingly, the auditory cortex was not detected in this study. This may be due to the fact that most patients in these studies had little or mild hearing loss.

TABLE 1 | List of all studies included in the meta-analysis: subjects' demographic and clinical characteristics.

Study	Journal	Modality/ Method of analysis	Male: Female		Mean age ± SD		Reported contrasts	Foci NO.	Scanner	Processing software	Smoothing kernel (mm)	Statistical threshold	MNI or Tal
			Patients	Control	Patients	Control							
Maudoux et al. (2012a)	Plos One	fMRI/ICA	7:6	9:6	52 ± 11	51 ± 13	TIN > HC HC > TIN	11 6	Siemens 3.0T	Brain Voyager	8	$P < 0.05$, FDR corrected	Tal
Geven et al. (2014)	Neuroscience	PET	10:10	9:10	51.0 ± 10.0	50.8 ± 9.5	HC > TIN	2	Siemens	SPM5	8	$p < 0.001$, uncorrected	MNI
Laureano et al. (2014)	Plos One	SPECT	6:14	6:11	42.95 ± 9.03	41.41 ± 9.98	TIN > HC	1	GE	SPM8	8	$P < 0.05$, FWE corrected	MNI
Chen et al. (2014)	NeuroImage: Clinical	fMRI/ALFF	17:14	17:15	41.9 ± 10.8	46.5 ± 12.6	TIN > HC HC > TIN	3 4	Siemens 3.0T	SPM8	4	$P < 0.05$, AlphaSim corrected	MNI
Yang et al. (2014)*	Journal of Otology	fMRI/ReHo	14:4	15:5	43	42	TIN > HC HC > TIN	1 1	Philips 3.0T	SPM5	NA	$P < 0.05$, FWE corrected	MNI
Ueyama et al. (2015)	Plos One	SPECT	10:7	NA	NA	NA	TIN > HC HC > TIN	6 2	FUJI FILM	SPM8	8	$P < 0.05$, AlphaSim corrected	MNI
Chen et al. (2015d)	Neural Plasticity	fMRI/ReHo	16:13	15:15	40.9 ± 10.5	46.2 ± 11.9	TIN > HC HC > TIN	4 1	Siemens 3.0T	SPM8	4	$P < 0.01$, AlphaSim corrected	MNI
Leaver et al. (2016b)	Human Brain Mapping	fMRI/ICA	10:11	9:10	47.33 ± 13.47	48.89 ± 12.49	HC > TIN	5	Siemens 3.0T	Brain Voyager	6	$P < 0.0005$, uncorrected	Tal
Chen et al. (2016)	Frontiers in Aging Neuroscience	fMRI/DC	9:15	9:13	50.8 ± 12.4	44.7 ± 15.4	TIN > HC	2	Philips 3.0T	SPM8	6	$P < 0.01$, AlphaSim corrected	MNI

*Note: TIN, tinnitus; HC, healthy control; fMRI, functional magnetic resonance imaging; PET, positron emission tomography; SPECT, single photon emission computed tomography; ALFF, amplitude of low-frequency fluctuations; ReHo, regional homogeneity; ICA, independent component analysis; DC, degree centrality; SPM, statistical parametric mapping; MNI, Montreal Neurological Institute; FDR, false discovery rate; FWE, family-wise error; NA, not available. *Only reported mean age in this study.*

TABLE 2 | The hearing and psychological status of the subjects included in all the studies.

Study	Hearing status		Psychological status
	Patients	Controls	
Maudoux et al. (2012a)	2 severe HL 7 mild/moderate HL 4 no HL	No HL	No major neurological neurosurgical or psychiatric history
Geven et al. (2014)	NA	NA	No major medical, neurological or psychiatric diagnoses
Laureano et al. (2014)	No HL	No HL	No neurologic or psychiatric disorders
Chen et al. (2014)	No HL	No HL	No depression or anxiety or other neurologic or psychiatric disorders
Yang et al. (2014)	1 profound HL 1 severe HL 4 moderate HL 9 mild HL 3 no HL	No HL	NA
Ueyama et al. (2015)	11 mild to severe HL 6 no HL	No HL	No neurologic or psychiatric disorders
Chen et al. (2015d)	No HL	No HL	No depression or anxiety or other neurologic or psychiatric disorders
Leaver et al. (2016b)	15 mild to severe HL 6 no HL	10 mild to severe HL 9 no HL	No significant symptoms of anxiety or depression
Chen et al. (2016)	No HL	No HL	No depression or anxiety or other neurologic or psychiatric disorders

Note: HL, hearing loss; NA, not available.

These non-auditory areas are parts of separable subnetworks representing multiple clinical cognitive and emotional aspects of tinnitus; resting-state disruptions in these areas may provide new insights on the neuropathological mechanisms of this disorder.

Middle Temporal Gyrus

The MTG has been suggested to be involved in cognitive processes including language, semantic memory and multimodal sensory integration (Cabeza and Nyberg, 2000). A quantitative EEG study has demonstrated higher α2-band activity in the

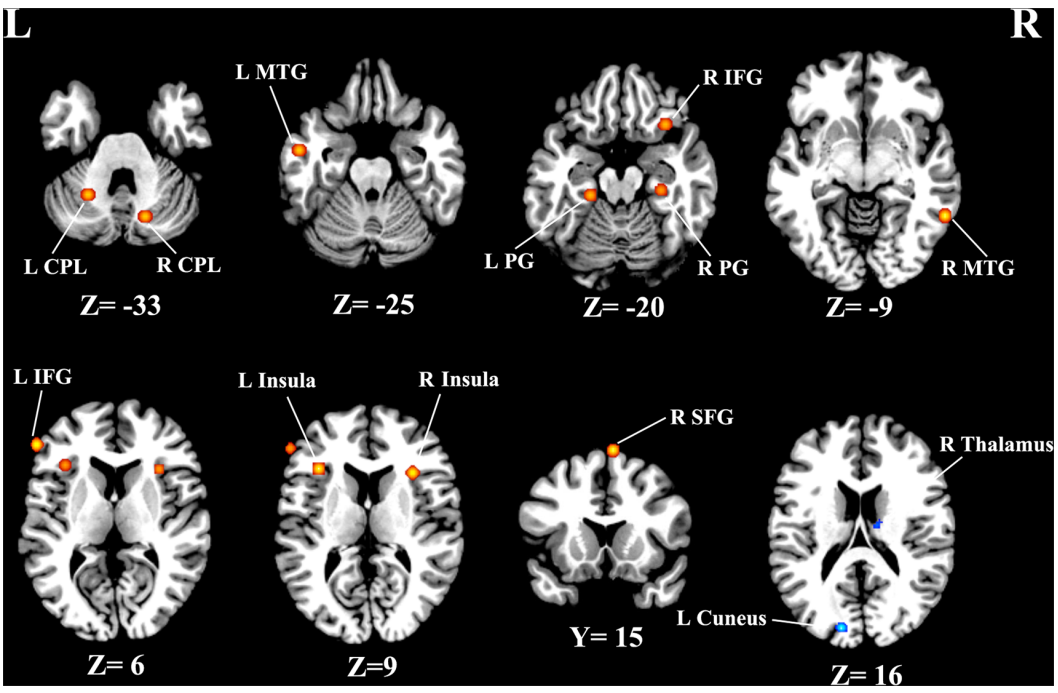

FIGURE 2 | Resting-state brain activity alterations in chronic tinnitus patients compared with healthy controls. Results are from the activation likelihood estimation (ALE) software for meta-analyses. All activations are significant at $p < 0.05$ corrected for multiple comparisons using the false-discovery rate (FDR) correction.

TABLE 3 | Regions of altered brain activity in tinnitus patients relative to healthy controls.

Brain regions	BA	MNI coordinates *x, y, z* (mm)	ALE extrema value	Cluster size (mm^3)
Tinnitus > Controls				
L Insula	13	−36, 24, 9	0.0088	880
R Cerebellar Posterior Lobe	–	17, −72, −33	0.0075	768
L Cerebellar Posterior Lobe	–	−25, −56, −33	0.0075	768
L Middle Temporal Gyrus	21	−53, −6, −25	0.0075	768
R Middle Temporal Gyrus	37	60, −51, −9	0.0086	768
R Inferior Frontal Gyrus	47	34, 22, −20	0.0072	736
R Superior Frontal Gyrus	6	6, 15, 69	0.0086	728
L Parahippocampal Gyrus	35	−22, −30, −20	0.0073	720
R Parahippocampal Gyrus	35	30, −26, −18	0.0073	720
L Inferior Frontal Gyrus	46	−57, 39, 6	0.0085	712
R Insula	13	34, 20, 10	0.0082	704
Tinnitus < Controls				
L Cuneus	17	−14, −88, 16	0.013	488
R Thalamus	–	14, −10, 14	0.0101	368

ALE, activation likelihood estimation; BA, Brodmann area; MNI, Montreal Neurological Institute; L, left; R, right. Each ALE map was thresholded using a FDR-corrected $p < 0.05$.

MTG in tinnitus patients with higher distress (Vanneste et al., 2010a). Voxel-based morphometry (VBM) analyses revealed that the gray matter increases in the MTG in tinnitus patients with hearing impairment (Boyen et al., 2013). Using resting-state fMRI, significantly increased spontaneous neural activity was observed in the right MTG in tinnitus patients (Chen et al., 2014). Moreover, Zhang et al. (2015) showed decreased functional connectivity between right MTG and left thalamus, which was negatively correlated with tinnitus severity. In addition, the MTG has been regarded as a key region of the DMN (Raichle et al., 2001). The DMN, consisting of nodes in the MTG, posterior cingulate/precuneus, angular gyrus and medial frontal gyrus, is most active at rest and shows reduced activity when a subject enters a task-based state involving attention or goal-directed behavior (Raichle et al., 2001; Mantini et al., 2007). As a condition involving the perception of a phantom auditory sensation, tinnitus might lead to dysfunction of the DMN. Previous fMRI studies found abnormal functional connectivity within the DMN associated with tinnitus distress (Burton et al., 2012; Maudoux et al., 2012b; Schmidt et al., 2013). Nevertheless, the source or type of aberrant neural activity within DMN regions in tinnitus remains unclear. Our results indicate that increased neuronal activity in the MTG may be responsible for disrupting the DMN in tinnitus patients.

Frontal Cortex

The frontal cortex, including the SFG and IFG, exhibited increased neural activity in tinnitus patients in the current study. Rauschecker et al. (2010) developed a model to demonstrate structural and functional differences in ventromedial prefrontal cortex that were associated with tinnitus subjective loudness, indicating that frontal cortex may contribute to certain perceptual features of tinnitus. Resting-state fMRI studies have pointed out that the abnormalities of the frontal cortex could act as a direct mechanism of tinnitus chronification (Burton et al., 2012; Schmidt et al., 2013; Chen et al., 2014, 2015c,d, 2016), which are confirmed by the current meta-analysis. Based on the previous fMRI studies, the SFG has been regarded as a major integrative hub of the tinnitus network architecture (Chen et al., 2016), which can receive and integrate all kinds of information from different parts of the brain from inside and outside the body. Besides, it can also timely organize efferent impulses to ensure the coordination of the central nervous system as a whole (Mathiak et al., 2007; Melloni et al., 2007). A possible explanation for our result was that the increased activity of the SFG might be due to feedback inhibition of an over active auditory network (Downar et al., 2000). Furthermore, the IFG serves as the core region of response inhibition and IFG activity might mirror the attempt to control the bottom-up attention allocation to the tinnitus percept in a top-down manner (Aron et al., 2014). In one hypothetical model, the fronto-insular cortex is part of a salience network that drives switching by a central executive control network important to maintaining and adjusting attention (Sridharan et al., 2008). In another model, the IFG and the insula act as executive control components in the attention system that regulates dorsal and VANs, which lack direct interconnections (Shulman et al., 2009). Taken together, we suggest that tinnitus distress, salience, or attentional focus is associated with increased resting-state activity in these brain subnetworks.

Parahippocampus

The parahippocampal area has been hypothesized to play a central role in memory recollection and transferring information from the hippocampus to the association areas, which might explain its involvement in the generation of simple auditory phantom percepts such as tinnitus (De Ridder et al., 2014a; Vanneste and De Ridder, 2016). EEG study suggested that tinnitus patients differed from healthy controls by increased

delta and theta activity in the parahippocampus (Moazami-Goudarzi et al., 2010). Prior resting-state fMRI studies also provided further support linking tinnitus physiopathology with parahippocampal region involved in mnemonic network (Maudoux et al., 2012a; Chen et al., 2015b; Leaver et al., 2016b).

Insula

The increased response in the insula, mainly the anterior part, may be an indication of successful adaption to the tinnitus perception (van der Loo et al., 2011). On the basis of resting-state quantitative EEG, the insula has been implicated in tinnitus and specific tinnitus characteristics (van der Loo et al., 2011; Vanneste et al., 2011a,b). Greater synchrony of alpha activity was observed bilaterally in the anterior insula of patients with more severe tinnitus-related distress (Vanneste et al., 2010a). In addition, chronic tinnitus patients showed enhanced spontaneous neuronal activity and functional connectivity in bilateral anterior insula were revealed by resting-state fMRI (Burton et al., 2012; Chen et al., 2015d).

Furthermore, the insula is one of key nodes in the common brain circuit of both tinnitus and chronic pain (Rauschecker et al., 2015). Although there also exist important differences between the two disorders, the striking similarities and associations indicate that tinnitus and chronic pain share common neuropathological mechanisms. Now advances in neuroimaging have demonstrated that similar structures and functional systems are involved and probably play a central role in both disorders (Rauschecker et al., 2015). Significant loss of gray matter and compromised circuit function are detected in several specific regions, such as the ventromedial prefrontal cortex, nucleus accumbens and insula (De Ridder et al., 2011; Rauschecker et al., 2015). These areas act as a central gatekeeping system for perceptual sensations, which determines the affective value of sensory stimuli and modulates information flow in the brain (Rauschecker et al., 2015). Tinnitus and chronic pain occur when this system is compromised.

Although the EEG has been used to determine resting-state long-range functional coupling in tinnitus, it has many differences from the resting-state fMRI (Vanneste et al., 2010a,b). EEG does not offer the spatial resolution of fMRI, but offer the advantage of being quiet and not influencing the resting-state network of tinnitus. Thus these two techniques may measure different aspects of spontaneous neuronal activity (Tagliazucchi et al., 2012). Britz et al. (2010) extracted four resting-state networks from the EEG data that were the equivalent of stereotypical fMRI networks dedicated to auditory, attetional, visual and self-referential processing, but DMN was not detected by EEG in Britz's study. Other studies have correlated the DMN with beta-2 (Laufs et al., 2003) or with delta (Mantini et al., 2007) spectral bands of EEG. Therefore, a direct comparison of EEG and resting-state fMRI is complicated by the fact that similar EEG power bands may be correlated with varying fMRI-generated spatial maps and a single resting-state network may be linked with different EEG spectral patterns (Laufs et al., 2008; Musso et al., 2010).

Cerebellum

Furthermore, although the cerebellum is primarily involved in motor actions and control, several cerebellar regions such as the paraflocculus and vermis receive inputs from auditory centers (Petacchi et al., 2005). Brozoski et al. (2007) observed increased activity in the parafloccular lobe of the cerebellum in animals with tinnitus confirmed and suggested that the cerebellum acts as a gating control mechanism comparing the afferent input from the cochlea with descending signals from the auditory cortex (Bauer et al., 2013; Chen et al., 2015b). Consistent with this view, hyperactivity in the auditory cortex and increased functional connectivity between the auditory cortex and cerebellum were observed in rats with salicylate-induced tinnitus (Chen et al., 2015a). If this cerebellar-tinnitus gating hypothesis is correct, then inactivating the cerebellum could possibly suppress the sound perception of tinnitus. Based on these findings, we suggest that cerebellum may play a pivotal role of gating control in tinnitus. Furthermore, using resting-state fMRI, chronic tinnitus patients exhibited enhanced functional connectivity in the cerebellar hemisphere that was associated with tinnitus distress (Maudoux et al., 2012a; Ueyama et al., 2013), indicating the involvement of cerebellum in auditory system.

Cuneus

Our meta-analysis found decreased brain activity in the cuneus of tinnitus patients. We speculate that the connections between auditory and visual regions make it possible to alter the brain activity in the visual areas (Kaltenbach et al., 2004; Cate et al., 2009). This is consistent with prior fMRI studies showing negative correlations of functional connectivity between auditory and visual resting-state subnetworks in tinnitus patients (Burton et al., 2012; Maudoux et al., 2012b). One possibility is that the phantom sounds might act to decrease spontaneous activity in visual areas because of the salience of the tinnitus perception (Chen et al., 2014, 2015d). Thus, tinnitus may be regarded as the consequence of multisensory interactions between auditory and visual regions.

Thalamus

The thalamus, which regulates the flow of sensory information to and from the auditory cortex, has been thought to play a key role in tinnitus (Richardson et al., 2012). Llinás et al. (1999) hypothesized that tinnitus results from thalamocortical dysrhythmias triggered by peripheral damage. Prior MRI studies have identified structural and functional abnormalities involved in thalamocortical network of tinnitus (Mühlau et al., 2006; Rauschecker et al., 2010; Benson et al., 2014; Chen et al., 2014; Lanting et al., 2014; Zhang et al., 2015). Consistent with our results, Zhang et al. (2015) showed decreased functional connectivity between the thalamus and auditory cortical areas in tinnitus, which may be a reflection of disrupted thalamic gating mechanism (Rauschecker et al., 2010). The thalamus is regarded as the center of ascending noise canceling system, and thus if it is dysfunctional, noise canceling is no longer possible and the subject may

perceive tinnitus (Rauschecker et al., 2010). However, the mechanisms responsible for these functional changes are unknown, but could involve aberrant inhibition (Richardson et al., 2012).

Limitations

Several inevitable limitations should be noted in our study. First, the heterogeneity of the included studies could affect the current results. In particular, hearing loss is always a complicating factor in structural and functional studies of tinnitus. Melcher et al. (2013) compared tinnitus patients with controls who were matched for hearing thresholds in the standard clinical frequencies and found that gray matter changes were not related to tinnitus but instead negatively correlated with hearing thresholds at frequencies above 8 kHz. Therefore, a more complete characterization of hearing loss is desirable. Moreover, tinnitus can become a significant psychological problem and comorbid symptoms such as depression and anxiety are potential confounds on the observed brain activity in tinnitus patients (Leaver et al., 2016a). It is necessary to characterize the psychological health problems as covariates in the analyses. The problems of differential tinnitus variables on structural and functional changes in neural activity can be solved using correlational analyses of large samples (Schecklmann et al., 2013) and comparisons of different tinnitus subtypes (Vanneste and De Ridder, 2012). Second, voxel-wise meta-analytic approach like ALE provides excellent control of false positive results, but it is more difficult to avoid false negatives (Radua et al., 2012). Voxel-wise meta-analysis is based on the pooling of peak stereotactic coordinates rather than on raw statistical brain maps from the original studies, which could give rise to less accurate results (Radua et al., 2012). Furthermore, fMRI produces aversive scanner noise which has been shown to interfere with auditory processing in the human brain at the physiological and psychological level (Perrachione and Ghosh, 2013). The concept of resting state is somewhat problematic in tinnitus studies because the auditory pathway is likely to be activated by scanner noise which is nearly impossible to completely eliminate even with ear plugs or active noise reduction (Logothetis et al., 2009). Therefore, this limitation should be taken into consideration when interpreting the resting-state fMRI data in auditory system-related researches. Finally, the number of included neuroimaging studies is relatively small. Further explorations are needed to perform the subgroup analyses or meta-regression analyses for the control of confounding factors that might influence resting-state brain function in tinnitus, such as the imaging methodology and data analysis method. Most importantly, we should establish standard protocols for resting-state fMRI researches to minimize heterogeneity on the experimental side.

CONCLUSION

Using the ALE-based meta-analysis, our study demonstrated abnormal resting-state neural activity mainly in the non-auditory brain areas, including the MTG, frontal cortex, parahippocampus, insula, cerebellum, cuneus and thalamus. In accordance to the hypothesis, these results show that there exist several important brain hubs in specific tinnitus network encompassing DMN, attentional, mnemonic, salience, visual and thalamocortical subnetworks. Aberrant resting-state brain patterns in tinnitus-related networks may help enhance our understanding of the neuropathological mechanisms underlying chronic subjective tinnitus. Nonetheless, a theoretical pathophysiological framework capable of explaining all these aspects in one model is still highly required. There is thus hope that a single cure can be found that would target a common mechanism.

AUTHOR CONTRIBUTIONS

Y-CC and FW designed the experiment, reviewed the literatures, performed the analysis and wrote the manuscript. JW, FB and WX reviewed the literatures. J-PG and XY contributed to the discussion and manuscript revision.

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REFERENCES

Aron, A. R., Robbins, T. W., and Poldrack, R. A. (2014). Inhibition and the right inferior frontal cortex: one decade on. *Trends Cogn. Sci.* 18, 177–185. doi: 10.1016/j.tics.2013.12.003

Bauer, C. A., Kurt, W., Sybert, L. T., and Brozoski, T. J. (2013). The cerebellum as a novel tinnitus generator. *Hear. Res.* 295, 130–139. doi: 10.1016/j.heares.2012.03.009

Benson, R. R., Gattu, R., and Cacace, A. T. (2014). Left hemisphere fractional anisotropy increase in noise-induced tinnitus: a diffusion tensor imaging (DTI) study of white matter tracts in the brain. *Hear. Res.* 309, 8–16. doi: 10.1016/j.heares.2013.10.005

Boyen, K., Langers, D. R., de Kleine, E., and van Dijk, P. (2013). Gray matter in the brain: differences associated with tinnitus and hearing loss. *Hear. Res.* 295, 67–78. doi: 10.1016/j.heares.2012.02.010

Britz, J., Van De Ville, D., and Michel, C. M. (2010). BOLD correlates of EEG topography reveal rapid resting-state network dynamics. *Neuroimage* 52, 1162–1170. doi: 10.1016/j.neuroimage.2010.02.052

Brozoski, T. J., Ciobanu, L., and Bauer, C. A. (2007). Central neural activity in rats with tinnitus evaluated with manganese-enhanced magnetic resonance imaging (MEMRI). *Hear. Res.* 228, 168–179. doi: 10.1016/j.heares.2007.02.003

Burton, H., Wineland, A., Bhattacharya, M., Nicklaus, J., Garcia, K. S., and Piccirillo, J. F. (2012). Altered networks in bothersome tinnitus: a functional connectivity study. *BMC Neurosci.* 13:3. doi: 10.1186/1471-2202-13-3

Cabeza, R., and Nyberg, L. (2000). Imaging cognition II: an empirical review of 275 PET and fMRI studies. *J. Cogn. Neurosci.* 12, 1–47. doi: 10.1162/08989290051137585

Cate, A. D., Herron, T. J., Yund, E. W., Stecker, G. C., Rinne, T., Kang, X., et al. (2009). Auditory attention activates peripheral visual cortex. *PLoS One* 4:e4645. doi: 10.1371/journal.pone.0004645

Chen, Y.-C., Feng, Y., Xu, J.-J., Mao, C.-N., Xia, W., Ren, J., et al. (2016). Disrupted brain functional network architecture in chronic tinnitus patients. *Front. Aging Neurosci.* 8:174. doi: 10.3389/fnagi.2016.00174

Chen, Y.-C., Li, X., Liu, L., Wang, J., Lu, C.-Q., Yang, M., et al. (2015a). Tinnitus and hyperacusis involve hyperactivity and enhanced connectivity in auditory-limbic-arousal-cerebellar network. *Elife* 4:e06576. doi: 10.7554/eLife.06576

Chen, Y.-C., Xia, W., Feng, Y., Li, X., Zhang, J., Feng, X., et al. (2015b). Altered interhemispheric functional coordination in chronic tinnitus patients. *Biomed Res. Int.* 2015:345647. doi: 10.1155/2015/345647

Chen, Y.-C., Xia, W., Luo, B., Muthaiah, V. P., Xiong, Z., Zhang, J., et al. (2015c). Frequency-specific alternations in the amplitude of low-frequency fluctuations in chronic tinnitus. *Front. Neural Circuits* 9:67. doi: 10.3389/fncir.2015.00067

Chen, Y.-C., Zhang, J., Li, X.-W., Xia, W., Feng, X., Qian, C., et al. (2015d). Altered intra-and interregional synchronization in resting-state cerebral networks associated with chronic tinnitus. *Neural Plast.* 2015:475382. doi: 10.1155/2015/475382

Chen, Y.-C., Zhang, J., Li, X.-W., Xia, W., Feng, X., Gao, B., et al. (2014). Aberrant spontaneous brain activity in chronic tinnitus patients revealed by resting-state functional MRI. *Neuroimage Clin.* 6, 222–228. doi: 10.1016/j.nicl.2014.09.011

Davies, J., Gander, P., Andrews, M., and Hall, D. (2014). Auditory network connectivity in tinnitus patients: a resting-state fMRI study. *Int. J. Audiol.* 53, 192–198. doi: 10.3109/14992027.2013.846482

Downar, J., Crawley, A. P., Mikulis, D. J., and Davis, K. D. (2000). A multimodal cortical network for the detection of changes in the sensory environment. *Nat. Neurosci.* 3, 277–283. doi: 10.1038/72991

Eickhoff, S. B., Laird, A. R., Grefkes, C., Wang, L. E., Zilles, K., and Fox, P. T. (2009). Coordinate–based activation likelihood estimation meta–analysis of neuroimaging data: a random–effects approach based on empirical estimates of spatial uncertainty. *Hum. Brain Mapp.* 30, 2907–2926. doi: 10.1002/hbm.20718

Eickhoff, S. B., Stephan, K. E., Mohlberg, H., Grefkes, C., Fink, G. R., Amunts, K., et al. (2005). A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage* 25, 1325–1335. doi: 10.1016/j.neuroimage.2004.12.034

Ellison-Wright, I., and Bullmore, E. (2010). Anatomy of bipolar disorder and schizophrenia: a meta-analysis. *Schizophr. Res.* 117, 1–12. doi: 10.1016/j.schres.2009.12.022

Genovese, C. R., Lazar, N. A., and Nichols, T. (2002). Thresholding of statistical maps in functional neuroimaging using the false discovery rate. *Neuroimage* 15, 870–878. doi: 10.1006/nimg.2001.1037

Geven, L., de Kleine, E., Willemsen, A., and van Dijk, P. (2014). Asymmetry in primary auditory cortex activity in tinnitus patients and controls. *Neuroscience* 256, 117–125. doi: 10.1016/j.neuroscience.2013.10.015

Henry, J. A., Roberts, L. E., Caspary, D. M., Theodoroff, S. M., and Salvi, R. J. (2014). Underlying mechanisms of tinnitus: review and clinical implications. *J. Am. Acad. Audiol.* 25, 5–22; quiz 126. doi: 10.3766/jaaa.25.1.2

Hinkley, L. B., Mizuiri, D., Hong, O., Nagarajan, S. S., and Cheung, S. W. (2015). Increased striatal functional connectivity with auditory cortex in tinnitus. *Front. Hum. Neurosci.* 9:568. doi: 10.3389/fnhum.2015.00568

Husain, F. T., and Schmidt, S. A. (2014). Using resting state functional connectivity to unravel networks of tinnitus. *Hear. Res.* 307, 153–162. doi: 10.1016/j.heares.2013.07.010

Jastreboff, P. J. (1990). Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci. Res.* 8, 221–254. doi: 10.1016/0168-0102(90)90031-9

Jastreboff, P. J., Hazell, J. W., and Graham, R. L. (1994). Neurophysiological model of tinnitus: dependence of the minimal masking level on treatment outcome. *Hear. Res.* 80, 216–232. doi: 10.1016/0378-5955(94)90113-9

Kaltenbach, J. A., Zacharek, M. A., Zhang, J., and Frederick, S. (2004). Activity in the dorsal cochlear nucleus of hamsters previously tested for tinnitus following intense tone exposure. *Neurosci. Lett.* 355, 121–125. doi: 10.1016/j.neulet.2003.10.038

Kaltenbach, J. A., Zhang, J., and Finlayson, P. (2005). Tinnitus as a plastic phenomenon and its possible neural underpinnings in the dorsal cochlear nucleus. *Hear. Res.* 206, 200–226. doi: 10.1016/j.heares.2005.02.013

Kim, J.-Y., Kim, Y.-H., Lee, S., Seo, J.-H., Song, H.-J., Cho, J. H., et al. (2012). Alteration of functional connectivity in tinnitus brain revealed by resting-state fMRI?: a pilot study. *Int. J. Audiol.* 51, 413–417. doi: 10.3109/14992027.2011.652677

Kühn, S., and Gallinat, J. (2013). Resting-state brain activity in schizophrenia and major depression: a quantitative meta-analysis. *Schizophr. Bull.* 39, 358–365. doi: 10.1093/schbul/sbr151

Laird, A. R., Fox, P. M., Price, C. J., Glahn, D. C., Uecker, A. M., Lancaster, J. L., et al. (2005a). ALE meta-analysis: controlling the false discovery rate and performing statistical contrasts. *Hum. Brain Mapp.* 25, 155–164. doi: 10.1002/hbm.20136

Laird, A. R., McMillan, K. M., Lancaster, J. L., Kochunov, P., Turkeltaub, P. E., Pardo, J. V., et al. (2005b). A comparison of label-based review and ALE meta-analysis in the Stroop task. *Hum. Brain Mapp.* 25, 6–21. doi: 10.1002/hbm.20129

Lancaster, J. L., Tordesillas-Gutiérrez, D., Martinez, M., Salinas, F., Evans, A., Zilles, K., et al. (2007). Bias between MNI and Talairach coordinates analyzed using the ICBM-152 brain template. *Hum. Brain Mapp.* 28, 1194–1205. doi: 10.1002/hbm.20345

Landgrebe, M., Azevedo, A., Baguley, D., Bauer, C., Cacace, A., Coelho, C., et al. (2012). Methodological aspects of clinical trials in tinnitus: a proposal for an international standard. *J. Psychosom. Res.* 73, 112–121. doi: 10.1016/j.jpsychores.2012.05.002

Lanting, C. P., de Kleine, E., Langers, D. R., and van Dijk, P. (2014). Unilateral tinnitus: changes in connectivity and response lateralization measured with FMRI. *PLoS One* 9:e110704. doi: 10.1371/journal.pone.0110704

Laufs, H., Daunizeau, J., Carmichael, D. W., and Kleinschmidt, A. (2008). Recent advances in recording electrophysiological data simultaneously with magnetic resonance imaging. *Neuroimage* 40, 515–528. doi: 10.1016/j.neuroimage.2007.11.039

Laufs, H., Krakow, K., Sterzer, P., Eger, E., Beyerle, A., Salek-Haddadi, A., et al. (2003). Electroencephalographic signatures of attentional and cognitive default modes in spontaneous brain activity fluctuations at rest. *Proc. Natl. Acad. Sci. U S A* 100, 11053–11058. doi: 10.1073/pnas.1831638100

Laureano, M. R., Onishi, E. T., Bressan, R. A., Castiglioni, M. L. V., Batista, I. R., Reis, M. A., et al. (2014). Memory networks in tinnitus: a functional brain image study. *PLoS One* 9:e87839. doi: 10.1371/journal.pone.0087839

Leaver, A. M., Renier, L., Chevillet, M. A., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2011). Dysregulation of limbic and auditory networks in tinnitus. *Neuron* 69, 33–43. doi: 10.1016/j.neuron.2010.12.002

Leaver, A. M., Seydell-Greenwald, A., and Rauschecker, J. P. (2016a). Auditory-limbic interactions in chronic tinnitus: challenges for neuroimaging research. *Hear. Res.* 334, 49–57. doi: 10.1016/j.heares.2015.08.005

Leaver, A. M., Turesky, T. K., Seydell-Greenwald, A., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2016b). Intrinsic network activity in tinnitus investigated using functional MRI. *Hum. Brain Mapp.* 37, 2717–2735. doi: 10.1002/hbm.23204

Li, H., Chan, R. C., McAlonan, G. M., and Gong, Q. Y. (2010). Facial emotion processing in schizophrenia: a meta-analysis of functional neuroimaging data. *Schizophr. Bull.* 36, 1029–1039. doi: 10.1093/schbul/sbn190

Li, J., Zhang, Z., and Shang, H. (2012). A meta-analysis of voxel-based morphometry studies on unilateral refractory temporal lobe epilepsy. *Epilepsy Res.* 98, 97–103. doi: 10.1016/j.eplepsyres.2011.10.002

Llinás, R. R., Ribary, U., Jeanmonod, D., Kronberg, E., and Mitra, P. P. (1999). Thalamocortical dysrhythmia: a neurological and neuropsychiatric syndrome characterized by magnetoencephalography. *Proc. Natl. Acad. Sci. U S A* 96, 15222–15227. doi: 10.1073/pnas.96.26.15222

Lockwood, A. H., Salvi, R. J., Coad, M. L., Towsley, M. L., Wack, D. S., and Murphy, B. W. (1998). The functional neuroanatomy of tinnitus: evidence for limbic system links and neural plasticity. *Neurology* 50, 114–120. doi: 10.1212/WNL.50.1.114

Logothetis, N. K., Murayama, Y., Augath, M., Steffen, T., Werner, J., and Oeltermann, A. (2009). How not to study spontaneous activity. *Neuroimage* 45, 1080–1089. doi: 10.1016/j.neuroimage.2009.01.010

van der Loo, E., Congedo, M., Vanneste, S., Van De Heyning, P., and De Ridder, D. (2011). Insular lateralization in tinnitus distress. *Auton. Neurosci.* 165, 191–194. doi: 10.1016/j.autneu.2011.06.007

Mantini, D., Perrucci, M. G., Del Gratta, C., Romani, G. L., and Corbetta, M. (2007). Electrophysiological signatures of resting state networks in the human brain. *Proc. Natl. Acad. Sci. U S A* 104, 13170–13175. doi: 10.1073/pnas.0700668104

Mathiak, K., Menning, H., Hertrich, I., Mathiak, K. A., Zvyagintsev, M., and Ackermann, H. (2007). Who is telling what from where? A functional magnetic resonance imaging study. *Neuroreport* 18, 405–409. doi: 10.1097/WNR.0b013e328013cec4

Maudoux, A., Lefebvre, P., Cabay, J.-E., Demertzi, A., Vanhaudenhuyse, A., Laureys, S., et al. (2012a). Auditory resting-state network connectivity in tinnitus: a functional MRI study. *PLoS One* 7:e36222. doi: 10.1371/journal.pone.0036222

Maudoux, A., Lefebvre, P., Cabay, J. E., Demertzi, A., Vanhaudenhuyse, A., Laureys, S., et al. (2012b). Connectivity graph analysis of the auditory resting state network in tinnitus. *Brain Res.* 1485, 10–21. doi: 10.1016/j.brainres.2012.05.006

Melcher, J. R., Knudson, I. M., and Levine, R. A. (2013). Subcallosal brain structure: correlation with hearing threshold at supra-clinical frequencies (>8 kHz), but not with tinnitus. *Hear. Res.* 295, 79–86. doi: 10.1016/j.heares.2012.03.013

Melloni, L., Molina, C., Pena, M., Torres, D., Singer, W., and Rodriguez, E. (2007). Synchronization of neural activity across cortical areas correlates with conscious perception. *J. Neurosci.* 27, 2858–2865. doi: 10.1523/JNEUROSCI.4623-06.2007

Minami, S. B., Oishi, N., Watabe, T., Uno, K., Kaga, K., and Ogawa, K. (2015). Auditory resting-state functional connectivity in tinnitus and modulation with transcranial direct current stimulation. *Acta Otolaryngol.* 135, 1286–1292. doi: 10.3109/00016489.2015.1068952

Mirz, F., Gjedde, A., Ishizu, K., and Pedersen, C. B. (2000). Cortical networks subserving the perception of tinnitus–a PET study. *Acta Otolaryngol. Suppl.* 543, 241–243. doi: 10.1080/000164800454503

Moazami-Goudarzi, M., Michels, L., Weisz, N., and Jeanmonod, D. (2010). Temporo-insular enhancement of EEG low and high frequencies in patients with chronic tinnitus. QEEG study of chronic tinnitus patients. *BMC Neurosci.* 11:40. doi: 10.1186/1471-2202-11-40

Mühlau, M., Rauschecker, J. P., Oestreicher, E., Gaser, C., Röttinger, M., Wohlschlager, A. M., et al. (2006). Structural brain changes in tinnitus. *Cereb. Cortex* 16, 1283–1288. doi: 10.1093/cercor/bhj070

Musso, F., Brinkmeyer, J., Mobascher, A., Warbrick, T., and Winterer, G. (2010). Spontaneous brain activity and EEG microstates. A novel EEG/fMRI analysis approach to explore resting-state networks. *Neuroimage* 52, 1149–1161. doi: 10.1016/j.neuroimage.2010.01.093

Perrachione, T. K., and Ghosh, S. S. (2013). Optimized design and analysis of sparse-sampling FMRI experiments. *Front. Neurosci.* 7:55. doi: 10.3389/fnins.2013.00055

Petacchi, A., Laird, A. R., Fox, P. T., and Bower, J. M. (2005). Cerebellum and auditory function: an ALE meta-analysis of functional neuroimaging studies. *Hum. Brain Mapp.* 25, 118–128. doi: 10.1002/hbm.20137

Radua, J., Mataix-Cols, D., Phillips, M., El-Hage, W., Kronhaus, D., Cardoner, N., et al. (2012). A new meta-analytic method for neuroimaging studies that combines reported peak coordinates and statistical parametric maps. *Eur. Psychiatry* 27, 605–611. doi: 10.1016/j.eurpsy.2011.04.001

Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., and Shulman, G. L. (2001). A default mode of brain function. *Proc. Natl. Acad. Sci. U S A* 98, 676–682. doi: 10.1073/pnas.98.2.676

Rauschecker, J. P., Leaver, A. M., and Mühlau, M. (2010). Tuning out the noise: limbic-auditory interactions in tinnitus. *Neuron* 66, 819–826. doi: 10.1016/j.neuron.2010.04.032

Rauschecker, J. P., May, E. S., Maudoux, A., and Ploner, M. (2015). Frontostriatal gating of tinnitus and chronic pain. *Trends Cogn. Sci.* 19, 567–578. doi: 10.1016/j.tics.2015.08.002

Richardson, B. D., Brozoski, T. J., Ling, L. L., and Caspary, D. M. (2012). Targeting inhibitory neurotransmission in tinnitus. *Brain Res.* 1485, 77–87. doi: 10.1016/j.brainres.2012.02.014

De Ridder, D., Elgoyhen, A. B., Romo, R., and Langguth, B. (2011). Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc. Natl. Acad. Sci. U S A* 108, 8075–8080. doi: 10.1073/pnas.1018466108

De Ridder, D., Vanneste, S., and Freeman, W. (2014a). The Bayesian brain: phantom percepts resolve sensory uncertainty. *Neurosci. Biobehav. Rev.* 44, 4–15. doi: 10.1016/j.neubiorev.2012.04.001

De Ridder, D., Vanneste, S., Weisz, N., Londero, A., Schlee, W., Elgoyhen, A. B., et al. (2014b). An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci. Biobehav. Rev.* 44, 16–32. doi: 10.1016/j.neubiorev.2013.03.021

Schaette, R., and McAlpine, D. (2011). Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J. Neurosci.* 31, 13452–13457. doi: 10.1523/JNEUROSCI.2156-11.2011

Schecklmann, M., Landgrebe, M., Langguth, B., and TRI Database Study Group. (2014). Phenotypic characteristics of hyperacusis in tinnitus. *PLoS One* 9:e86944. doi: 10.1371/journal.pone.0086944

Schecklmann, M., Landgrebe, M., Poeppl, T. B., Kreuzer, P., Männer, P., Marienhagen, J., et al. (2013). Neural correlates of tinnitus duration and distress: a positron emission tomography study. *Hum. Brain Mapp.* 34, 233–240. doi: 10.1002/hbm.21426

Schmidt, S. A., Akrofi, K., Carpenter-Thompson, J. R., and Husain, F. T. (2013). Default mode, dorsal attention and auditory resting state networks exhibit differential functional connectivity in tinnitus and hearing loss. *PLoS One* 8:e76488. doi: 10.1371/journal.pone.0076488

Sedley, W., Friston, K. J., Gander, P. E., Kumar, S., and Griffiths, T. D. (2016). An integrative tinnitus model based on sensory precision. *Trends Neurosci.* 39, 799–812. doi: 10.1016/j.tins.2016.10.004

Seki, S., and Eggermont, J. J. (2003). Changes in spontaneous firing rate and neural synchrony in cat primary auditory cortex after localized tone-induced hearing loss. *Hear. Res.* 180, 28–38. doi: 10.1016/s0378-5955(03)00074-1

Shao, N., Yang, J., and Shang, H. (2015). Voxelwise meta-analysis of gray matter anomalies in Parkinson variant of multiple system atrophy and Parkinson's disease using anatomic likelihood estimation. *Neurosci. Lett.* 587, 79–86. doi: 10.1016/j.neulet.2014.12.007

Shargorodsky, J., Curhan, G. C., and Farwell, W. R. (2010). Prevalence and characteristics of tinnitus among US adults. *Am. J. Med.* 123, 711–718. doi: 10.1016/j.amjmed.2010.02.015

Shulman, G. L., Astafiev, S. V., Franke, D., Pope, D. L., Snyder, A. Z., McAvoy, M. P., et al. (2009). Interaction of stimulus-driven reorienting and expectation in ventral and dorsal frontoparietal and basal ganglia-cortical networks. *J. Neurosci.* 29, 4392–4407. doi: 10.1523/JNEUROSCI.5609-08.2009

Song, J. J., De Ridder, D., Van de Heyning, P., and Vanneste, S. (2012). Mapping tinnitus-related brain activation: an activation-likelihood estimation metaanalysis of PET studies. *J. Nucl. Med.* 53, 1550–1557. doi: 10.2967/jnumed.112.102939

Sridharan, D., Levitin, D. J., and Menon, V. (2008). A critical role for the right fronto-insular cortex in switching between central-executive and default-mode networks. *Proc. Natl. Acad. Sci. U S A* 105, 12569–12574. doi: 10.1073/pnas.0800005105

Stroup, D. F., Berlin, J. A., Morton, S. C., Olkin, I., Williamson, G. D., Rennie, D., et al. (2000). Meta-analysis of observational studies in epidemiology: a proposal for reporting. *JAMA* 283, 2008–2012. doi: 10.1001/jama.283.15.2008

Tagliazucchi, E., von Wegner, F., Morzelewski, A., Brodbeck, V., and Laufs, H. (2012). Dynamic BOLD functional connectivity in humans and its electrophysiological correlates. *Front. Hum. Neurosci.* 6:339. doi: 10.3389/fnhum.2012.00339

Turkeltaub, P. E., Eden, G. F., Jones, K. M., and Zeffiro, T. A. (2002). Meta-analysis of the functional neuroanatomy of single-word reading: method and validation. *Neuroimage* 16, 765–780. doi: 10.1006/nimg.2002.1131

Turkeltaub, P. E., Eickhoff, S. B., Laird, A. R., Fox, M., Wiener, M., and Fox, P. (2012). Minimizing within-experiment and within-group effects in Activation Likelihood Estimation meta-analyses. *Hum. Brain Mapp.* 33, 1–13. doi: 10.1002/hbm.21186

Ueyama, T., Donishi, T., Ukai, S., Ikeda, Y., Hotomi, M., Yamanaka, N., et al. (2013). Brain regions responsible for tinnitus distress and loudness: a resting-state fMRI study. *PLoS One* 8:e67778. doi: 10.1371/journal.pone.0067778

Ueyama, T., Donishi, T., Ukai, S., Yamamoto, Y., Ishida, T., Tamagawa, S., et al. (2015). Alterations of regional cerebral blood flow in tinnitus patients as assessed using single-photon emission computed tomography. *PLoS One* 10:e0137291. doi: 10.1371/journal.pone.0137291

Vanneste, S., and De Ridder, D. (2012). The auditory and non-auditory brain areas involved in tinnitus. An emergent property of multiple parallel overlapping subnetworks. *Front. Syst. Neurosci.* 6:31. doi: 10.3389/fnsys.2012.00031

Vanneste, S., and De Ridder, D. (2016). Deafferentation-based pathophysiological differences in phantom sound: tinnitus with and without hearing loss. *Neuroimage* 129, 80–94. doi: 10.1016/j.neuroimage.2015.12.002

Vanneste, S., Plazier, M., der Loo, E., de Heyning, P. V., Congedo, M., and De Ridder, D. (2010a). The neural correlates of tinnitus-related distress. *Neuroimage* 52, 470–480. doi: 10.1016/j.neuroimage.2010.04.029

Vanneste, S., Plazier, M., van der Loo, E., Van de Heyning, P., and De Ridder, D. (2010b). The differences in brain activity between narrow band noise and pure tone tinnitus. *PLoS One* 5:e13618. doi: 10.1371/journal.pone.0013618

Vanneste, S., Plazier, M., van der Loo, E., Van de Heyning, P., and De Ridder, D. (2011a). The difference between uni- and bilateral auditory phantom percept. *Clin. Neurophysiol.* 122, 578–587. doi: 10.1016/j.clinph.2010.07.022

Vanneste, S., van de Heyning, P., and De Ridder, D. (2011b). The neural network of phantom sound changes over time: a comparison between recent-onset and chronic tinnitus patients. *Eur. J. Neurosci.* 34, 718–731. doi: 10.1111/j.1460-9568.2011.07793.x

Wager, T. D., Jonides, J., and Reading, S. (2004). Neuroimaging studies of shifting attention: a meta-analysis. *Neuroimage* 22, 1679–1693. doi: 10.1016/j.neuroimage.2004.03.052

Weng, H.-H., Chen, C.-F., Tsai, Y.-H., Wu, C.-Y., Lee, M., Lin, Y.-C., et al. (2015). Gray matter atrophy in narcolepsy: an activation likelihood estimation meta-analysis. *Neurosci. Biobehav. Rev.* 59, 53–63. doi: 10.1016/j.neubiorev.2015.03.009

Wineland, A. M., Burton, H., and Piccirillo, J. (2012). Functional connectivity networks in nonbothersome tinnitus. *Otolaryngol. Head Neck Surg.* 147, 900–906. doi: 10.1177/0194599812451414

Yang, H., Zheng, Y., Ou, Y., and Huang, X. (2014). Regional homogeneity on resting state fMRI in patients with tinnitus. *J. Otol.* 9, 173–178. doi: 10.1016/j.joto.2014.10.001

Zhang, J., Chen, Y. C., Feng, X., Yang, M., Liu, B., Qian, C., et al. (2015). Impairments of thalamic resting-state functional connectivity in patients with chronic tinnitus. *Eur. J. Radiol.* 84, 1277–1284. doi: 10.1016/j.ejrad.2015.04.006

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Does Chronic Tinnitus Alter the Emotional Response Function of the Amygdala?: A Sound-Evoked fMRI Study

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Tinnitus is often associated with strong negative thoughts and emotions which can contribute to a distressing and chronic long-term condition. The amygdala, the "feeling and reacting" part of the brain, may play a key role in this process. Although implicated in several theoretical models of tinnitus, quantification of activity in the human amygdala has only been made possible more recently through neuroimaging methods such as functional magnetic resonance imaging (fMRI) but benefits from modified scanning parameters using a double-echo acquisition for improved BOLD sensitivity. This study thus examined the role of the amygdala in emotional sound processing in people with tinnitus using a novel double-echo imaging sequence for optimal detectability of subcortical activity. Our hypotheses were: (1) emotionally evocative sound clips rated as pleasant or unpleasant would elicit stronger amygdalar activation than sound clips rated as neutral, (2) people with tinnitus have greater amygdalar activation in response to emotionally evocative sounds (relative to neutral sounds) compared to controls.

Methods: Twelve participants all with chronic, constant tinnitus took part. We also recruited 11 age and hearing-matched controls. Participants listened to a range of emotionally evocative sound clips; rated as pleasant, unpleasant or neutral. A region-of-interest analysis was chosen to test our a priori hypotheses.

Results: Both groups displayed a robust and similar overall response to sounds vs. silence in the following ascending auditory pathways; inferior colliculus, medial geniculate body and the primary auditory cortex. In support of our first hypothesis, the amygdala's response to pleasant and unpleasant sound clips was significantly greater than neutral sounds. Opposing our second hypothesis, we found that the amygdala's overall response to pleasant and unpleasant sounds (compared to neutral sounds) was actually lower in the tinnitus group as compared to the controls.

Conclusions: The "muted" amygdala activation observed in the tinnitus group could reflect an internal modification of emotional response perhaps as a result of successful habituation to emotionally negative sound. This interpretation would predict a heightened amygdala emotional response in individuals with a more clinically bothersome tinnitus.

Keywords: amygdala, double echo, functional magnetic resonance imaging (fMRI), tinnitus, valence, emotion, distress, fMRI

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INTRODUCTION

Tinnitus describes the phantom perception of sound, in the absence of an external sound source. Most individuals who experience tinnitus will habituate such that it does not impact on quality of life or emotional state (Davis and El Rafaie, 2000). However, for some the experience of tinnitus can be distressing, affecting sleep, concentration, and mood (Folmer et al., 1999; Cronlein et al., 2016; Tegg-Quinn et al., 2016). Interestingly, the amount of distress attributed to tinnitus is not predicted by its psychoacoustic composition e.g., perceived loudness (Jakes et al., 1985; Leaver et al., 2012). Instead, the emotional evaluation of stimuli appears strongly linked to the limbic system.

The limbic system is a convenient way of describing a number of functionally and anatomically connected brain structures that regulate autonomic and endocrine function, particularly in response to emotional stimuli (Le Doux, 2000). There is no universal agreement on the total list of structures which comprise the limbic system but it includes the cingulate gyrus, parahippocampal gyrus, hippocampal formation, amygdala, septal area, and hypothalamus (Rajmohan and Mohandas, 2007). The limbic system forms the "feeling and reacting brain" but many of the brain areas within the limbic system are also implicated in memory, particularly emotional memory (Rajmohan and Mohandas, 2007).

The amygdala may be particularly significant in its role of mediating emotional responses to sensory stimuli. To decipher specifically how information is relayed between the auditory cortex and amygdala, Kumar et al. (2012) used a range of aversive sound clips in a group of 16 young adults (aged 22–35) with normal hearing. Their analysis focused on the interactions between auditory cortex and amygdala (effective connectivity) using a technique known as dynamic causal modeling. According to the models tested, they concluded the following: unpleasant sound stimuli are first processed and decoded in the auditory cortex before any emotional response can be assigned by the amygdala. Forward connections from the auditory cortex to the amygdala are modulated by acoustic features. The amygdala then modulates the auditory cortex in accordance with the (un)pleasantness of sounds.

Irwin et al. (2011) compared responses to sound clips of real world soundscapes rated as pleasant, neutral or unpleasant to examine the role of auditory and limbic brain networks in normal hearing adults (aged 21–55 years). Irwin et al. (2011) found that highly pleasant or highly unpleasant soundscapes relative to neutral soundscapes evoked greater activation of a number of auditory brain regions including the auditory cortex and the posterior insula, and non-auditory brain regions such as the amygdala. A large response was found to emotionally evocative sounds, irrespective of whether they were pleasant or unpleasant. Irwin described this as a "U-shaped function." A direct between-hemisphere comparison of amygdala response activation revealed no differences.

The amygdala has been proposed in models of tinnitus to account for the emotional distress that can occur for some individuals in response to "phantom" sounds. Jastreboff's neurophysiological model of tinnitus (1990) includes the amygdala as a key component. Central to this model is the concept that sounds which evoke strong emotional reactions activate limbic and autonomic systems. Sounds can be real physical sounds or phantom sounds such as tinnitus. Typically, repeated exposure to the same sound results in habituation, where the person becomes less aware of that sound. However, when there is an emotional reaction to the sound, any subsequent exposure to the same sound stimulus maintains a conscious awareness of the sound, without habituation. For De Ridder et al. (2011, 2014), a key factor in chronic tinnitus concerns the role of emotional memories. Such memory mechanisms play a role in persistent tinnitus because they result in an extended state of hypervigilance which promotes a sustained state of awareness about the tinnitus. The amygdala is highlighted as a structure of major functional importance because it is not only part of the "distress network" but it also overlaps with brain areas involved in central control of the autonomic system, consistent with Jastreboff's neurophysiological model.

Despite its projected role in tinnitus, the involvement of the amygdala has not been directly measured until more recently (e.g., Shulman et al., 1995; Roy et al., 2009; Crippa et al., 2010; Irwin et al., 2011; Kumar et al., 2012). And even so has been considered as a single functional structure, rather than its known anatomical substructures. Generally these study findings are consistent with the view that the amygdala works with auditory brain regions in the perception of emotionally evocative sounds (both real sounds and phantom sounds). Emotional information (auditory and visual) can be described by two main factors (Bradley and Lang, 2000; Hall et al., 2013). The first factor relates to ratings of pleasantness (valence) and the second factor relates to ratings of vibrancy (arousal). While brain imaging research tends to focus on the coding of pleasantness, for example by measuring the differential response to stimuli that have previously been rated as unpleasant, neutral, or pleasant, Irwin et al. (2011) reported that vibrancy had little effect on the overall brain response.

A majority of the studies examining the response to the emotional dimension of stimulus processing have enrolled young healthy participants (Bradley and Lang, 2000; Costa et al., 2010; Irwin et al., 2011; Kumar et al., 2012). But there are a small number of relevant fMRI studies that have recruited people with tinnitus. Golm et al. (2013) used a non-auditory sentence reading task to stimulate cognitive emotional processing in individuals with varying degrees of tinnitus distress. The task comprised three sentence types, neutral (e.g., regularly I look at my watch), negative (e.g., I often feel sorry for myself), and tinnitus-related (e.g., I will never get rid of the noise). Compared to healthy age- and hearing-matched controls, tinnitus patients showed stronger activations when reading tinnitus-related sentences relative to neutral sentences in many parts of the limbic system: anterior cingulate cortex, mid-cingulate cortex, posterior cingulate cortex, retrosplenial cortex, and insula as well as frontal areas. The tinnitus group were also divided according to levels of perceived tinnitus distress. Individuals with a global score of 31 or higher on the Tinnitus Questionnaire (Hallam, 1996) were assigned into the high distress group. Direct group comparisons (high distress vs. low distress) revealed stronger activity in the left

middle frontal gyrus in the high tinnitus distress group, a brain region which Jastreboff (1990) had previously implicated in the integration of sensory and emotional characteristics of tinnitus. Although this study showed some limbic activity, specific amygdala involvement was not found.

Carpenter-Thompson et al. (2014) used emotionally evocative sounds chosen from the International Affective Digital Sounds database (Bradley and Lang, 2007) to directly assess the effects of tinnitus on emotional processing. The authors were motivated by the hypothesis that alterations of the limbic system due to the presence of chronic aversive internal sounds (i.e., tinnitus) are also manifested when processing external affective sounds. They expected to observe an elevated response in the amygdala, parahippocampus, and insula regions of the tinnitus group in response to emotionally evocative sounds, relative to the control groups. They also hypothesized that the tinnitus group would show a heightened response in auditory regions relative to the other two groups. To examine these questions their stimulus set comprised 30 pleasant, 30 unpleasant, and 30 neutral sound clips. In an effort to control for hearing loss, three participant groups were included: "hearing loss with tinnitus" ($n = 13$), "hearing loss without tinnitus," ($n = 12$) and "normal hearing without tinnitus" ($n = 12$). All groups were gender matched and age matched (mean and SD). Contrary to the author's hypothesis, the tinnitus group did not show an elevated amygdala response in either the pleasant > neutral or unpleasant > neutral contrasts. Direct between-group comparisons failed to show any statistically significant differences in the amygdala response, but the authors highlighted a trend across groups such that "normal hearing without tinnitus" > "hearing loss without tinnitus" > "hearing loss with tinnitus" for the two statistical contrasts described above. And these reached statistical significance only at an uncorrected threshold level of $p < 0.001$ in the normal hearing group for emotionally evocative sounds. The authors suggested two reasons for this: (1) individuals with tinnitus might re-route their emotional signaling pathway to avoid the amygdala and its connections to the auditory cortex, (2) because their participants had mildly bothersome tinnitus, and so may have habituated to the tinnitus. However, we propose a third explanation; it is well known that detecting signal from the amygdala is challenging with fMRI due to its location leading to MR signal loss (Chen et al., 2003; Irwin et al., 2012). It is therefore conceivable that the sensitivity to detecting responses relevant for emotional coding may have been restricted by their choice of fMRI parameters, which were not optimally suited for detection Blood Oxygen Level Dependent (BOLD) contrast in the peri-amygdalar area of the brain.

The present study advances our understanding of how chronic tinnitus impacts upon emotional processing by seeking to conduct an independent confirmation of the previous findings reviewed here. For completeness of reporting, we describe the pattern of sound-related activity in the ascending auditory system, but the focus of this article is on two specific directional hypotheses:

1) Sound clips rated as pleasant and/or unpleasant elicit greater amygdalar activity than neutral sound clips.
2) Compared to age and hearing-matched controls, people with tinnitus have greater amygdalar activity in response to pleasant and unpleasant sounds, relative to neutral sounds.

Our study design sought to overcome several limitations of some of the previous studies. First, we took great care to match groups for age and hearing loss accounting for these known confounding effects (Adjamian et al., 2009). Second, we applied a novel double-echo Echo Planar Imaging (EPI) sequence to improve BOLD sensitivity. This sequence acquires two EPI images per pulse and a sum of these two datasets was created for image analysis (Marciani et al., 2006).

An exploratory research question explored whether differential responses could be detected within the three subdivisions of the amygdala. This was of interest because while previous studies (Golm et al., 2013; Carpenter-Thompson et al., 2014) consider the amygdala as a single homogenous body, this structure can be anatomically delineated into three major subdivisions (Amunts et al., 2005); the laterobasal nuclei (LB), the superficial subnuclei (SF), and the centromedial subnuclei (CM).

MATERIALS AND METHODS

Participants

Twelve participants (mean age 65.8 years) with chronic subjective tinnitus and 11 age- and hearing-matched controls (mean age 68.5 years) were recruited through Nottingham Audiology Services or the Ear, Nose and Throat (ENT) department, Queen's Medical Centre, Nottingham. All participants were aged 49–75 years without a history of neurological disorder. See **Table 1**. for participant demographics and tinnitus characteristics. This study was approved by the National Research Ethics Committee (REC: 09/H0407/8). All participants gave written informed consent prior to taking part. Participants reported in this current study are also described in a previous article (Davies et al., 2014).

Clinical Profile

All participants underwent extended frequency audiometry (125–14 kHz) prior to scanning. No participants presented with unilateral or asymmetrical hearing loss (as indicated by a between-ear air conduction threshold difference of 15 dB at two or more consecutive frequencies). The average hearing status of both groups could be described as a bilateral, mild to moderately severe sloping sensorineural hearing loss, typical of presbyacusis (see **Figure 1**). All participants were not considered to have hyperacusis, as indicated by a score of <29 on the hyperacusis questionnaire (Khalfa et al., 2002).

Questionnaire scores are given in **Table 1**. All participants completed the Hyperacusis Questionnaire (HQ: Khalfa et al., 2002), the Beck Anxiety Inventory (BAI: Beck et al., 1988) and the Beck Depression Inventory—Fast Screen (BDI-FS: Beck et al., 2000). Groups were comparable in terms of clinical profile of co-morbidities. No participant had a HQ score greater than the 28 point cut-off for hyperacusis (Khalfa et al., 2002). All participants had low anxiety, as evidenced by scores <21 on the BAI scale. And all but one participant in each group

TABLE 1 | Group demographics, questionnaire scores and tinnitus characteristics.

	No tinnitus group					Tinnitus group					Tinnitus characteristics			
	Sex	Age	HQ	BAI	BDI-FS	Sex	Age	HQ	BAI	BDI-FS	Laterality	Duration (yrs)	THQ	TCHQ % annoy
	F	68	6	2	0	M	72	24	10	4	L	15	25.1	28
	F	71	9	8	3	M	64	14	2	2	L&R	2	35.5	10
	M	58	13	2	0	M	72	14	2	2	L&R	2	60.4	70
	M	68	19	0	3	F	67	8	6	0	L&R	4	61.3	50
	M	75	8	3	0	F	73	11	4	2	IN HEAD	70	21.1	5
	M	68	9	0	0	F	57	18	11	0	IN HEAD	2	63.3	50
	M	60	2	0	0	M	71	22	3	0	IN HEAD	6	68.4	30
	F	75	18	13	4	M	71	11	5	0	L&R	10	32.2	20
	M	66	8	0	0	M	64	11	0	0	L&R	20	30	20
	M	74	2	0	1	F	72	17	3	0	R	13	50.6	35
	M	70	11	14	0	M	49	10	4	2	L&R	2	57.5	50
	∼	∼	∼	∼	∼	F	58	15	1	1	L&R	40	18.7	25
MEAN	8M/3F	68.5	9.6	3.8	1	7M/5F	65.8	14.6	4.3	1.1	∼	15.5	43.7	32.8
SD	∼	∼	5.54	5.34	1.55	∼	∼	4.9	3.36	1.31	∼	20.4	18.32	

M, male; F, female; L, left; R, right; HEAD, central tinnitus; HQ, hyperacusis questionnaire; BAI, beck anxiety inventory; BDI-FS, beck depression inventory—fast screen; THQ, tinnitus handicap questionnaire; TCHQ, tinnitus case history questionnaire.

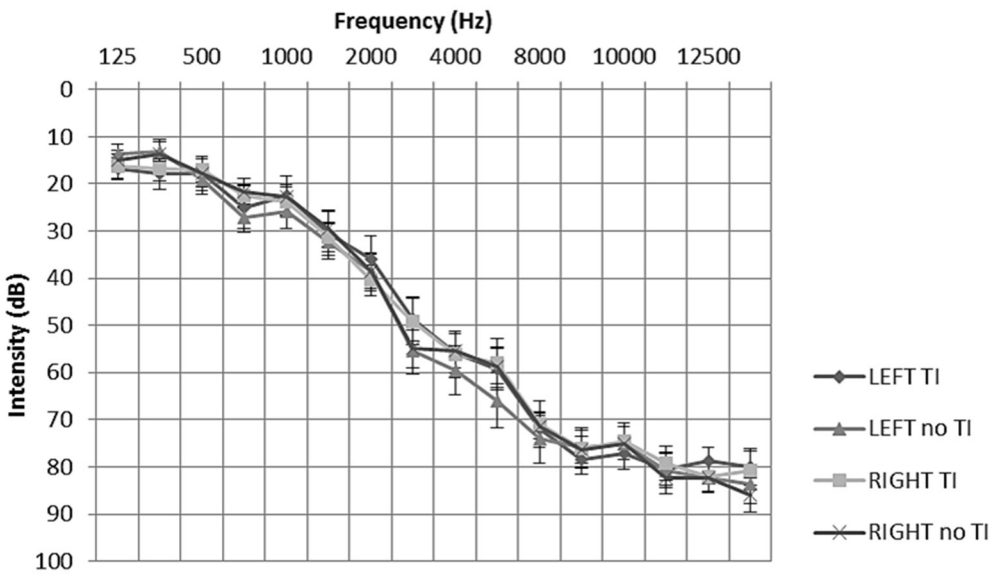

FIGURE 1 | Mean average audiometric thresholds for tinnitus and no tinnitus groups. *Post hoc t*-tests of average hearing thresholds found no significant differences between or within participant groups ($P > 0.05$). Error bars represent one standard error of the mean. TI, tinnitus.

had a minimal depression, as evidenced by scores ≤3 on the BDI-FS scale.

Tinnitus participants also completed the Tinnitus Handicap Questionnaire (THQ: Kuk et al., 1990) and the Tinnitus Case History Questionnaire (TCHQ: Langguth et al., 2007). The tinnitus group had an average global THQ score of 43.7 out of 100 and subjectively rated their tinnitus annoyance at 32.8 out of 100.

Sound Stimuli and Task

The present study used 84 sound clips derived from a previously published fMRI study (Irwin et al., 2011). This subset of sound clips were chosen to vary among natural and mechanical real-world sound sources and were previously rated as being pleasant e.g., bird song, unpleasant e.g., car crash or neutral e.g., footsteps. The strategy used to rate the sound clips (see Hall et al., 2013) was

as follows; five raters (aged between 21 and 40) rated a total of 219 sound clips using a 9-point visual analog scale with anchor points at either end e.g., 1 = unpleasant/unhappy, 5 = neutral and 9 = pleasant/happy. Scores for each sound clip were comparable across the five raters. In the present study, these sound clips were further reduced down to 84 sound clips (28 pleasant, 28 neutral, and 28 unpleasant) by omitting sounds that were rated either between the anchor end-points (pleasant and unpleasant) or not in the center (neutral) of the 9-point scale. Although our study participants did not rate the sound clips, many of their subjective comments regarding their pleasantness aligned with the formal ratings. Furthermore, we did not expect people with tinnitus to rate sounds differently to that of individuals without tinnitus, for example see Carpenter-Thompson et al. (2014). The intensity of all sound clips was matched at 71 dB A by taking a root-mean-square level average over the 7.8 s clip duration. In an effort to preserve the ecological validity of the listener experience, frequency content was not altered to compensate for participant hearing levels.

Figure 2 shows an illustrative example of the sound clip sequence design which participants had to listen to whilst in the MRI scanner. Each sound clip had a 50 ms onset and offset ramp. Sound clips were presented to participants in a pseudo-randomized order, such that the three categories of sound (neutral, pleasant, and unpleasant) and silence period occurred within a single block that was repeated 16 times. Within each block two segments of sound clips of the same category (or silence) were played in succession (brief inter-stimulus gap of 450 ms) and no repeated sounds were allowed in the two segments. Each participant therefore listened to each sound category (and silent periods) a total of 32 times. The sequence of sounds was constrained to avoid two sequential blocks of the same sound category. Three different unique orderings of sounds were created (i.e., not similar within or across blocks) and randomized across subjects.

fMRI Acquisition

Data were obtained using a Philips Achieva 3.0 Tesla MR scanner (Philips Medical Systems, The Netherlands) and an 8-channel SENSE receiver head coil. For improved BOLD sensitivity in the peri-amygdala area, whole-brain EPI data were acquired by collecting two MR time-series at echo times of 20 and 45 ms after each radio-frequency (RF) pulse (see Marciani et al., 2006). Other EPI parameters were: $TR = 8250$ ms, acquisition time = 2,420 ms, 36 slices, 0 mm slice gap, $FOV = 240 \times 240$ mm along the AC-PC line, voxel size $= 3 \times 3 \times 3$ mm, acquisition matrix $= 80 \times 77$, 120 volumes, SENSE factor = 2.3, descending slice order). Slice acquisition angle was tilted in line with the axis of the supratemporal plane to capture as much of the brain as possible, with the same negative sloping pitch in the sagittal plane for each subject.

A sparse sampling sequence was adopted, which gave long periods of no scanner noise (5830 ms) in between acquisitions (Hall et al., 1999). Sound clips were presented as shown in **Figure 3**. Our previous methodological work supports our belief that the small overlap of the sound and the scan acquisition does not affect the BOLD response to the extent that it would affect the statistical contrasts of interest. A 5 min MPRAGE anatomical image was also acquired for each participant (160 slices, $FOV = 256$, voxel size $1 \times 1 \times 1$ mm).

The participants were instructed to keep still with their eyes closed and listen to the sounds. Scanning duration was approximately 16 min. Sound stimuli were delivered through customized circum-aural ear defenders which could provide up to 40 dB attenuation. The ear defenders also employed active noise cancelation, helping to reduce scanner noise by up to an additional 35 dB (Hall et al., 2009). The noise reduction procedures were considered critical for the perception of the sounds, but also to make the scanner environment more suitable for people with tinnitus, whose tinnitus sound could otherwise be masked by the scanner noise or even exacerbated.

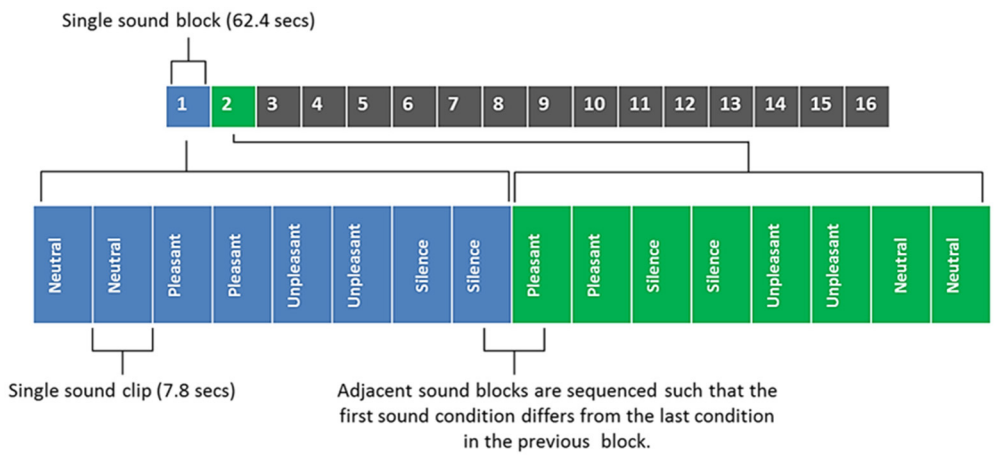

FIGURE 2 | Sound clip sequence, with blocks 1 and 2 being broken down into their component parts for illustrative purposes. Each sound block contains 4 different sound conditions/8 sound clips (each sound clip is repeated in quick succession; with a brief inter-stimulus gap of 450 ms). Total duration of all 16 sounds blocks was 16 min 38 s.

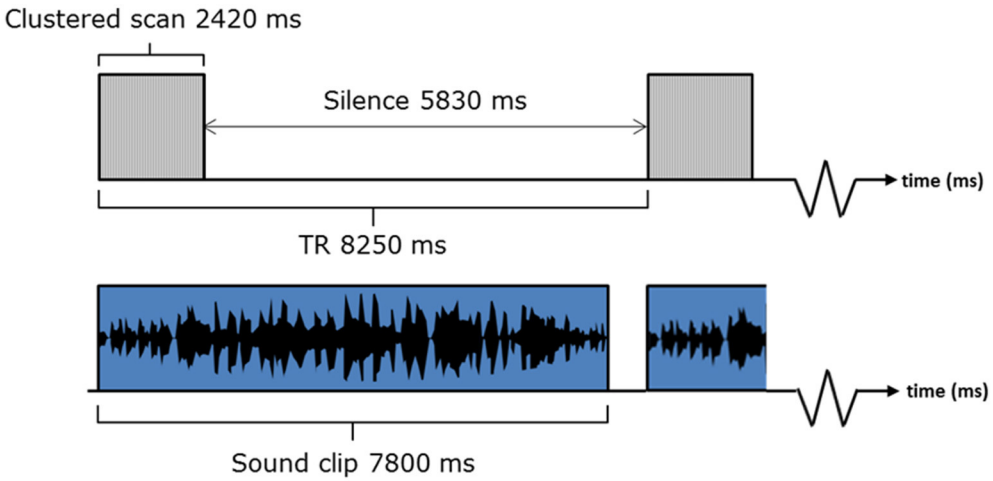

FIGURE 3 | fMRI listening task paradigm.

Preprocessing and Analysis

First, the two time-series (i.e., at 20 and 45 ms echo times) were combined using a weighted sum; with equal 50/50 weighting using a custom MATLAB script. The single functional time-series was then processed using statistical parametric mapping software SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/). Images were realigned, co-registered with the participant's high resolution anatomical scan, normalized to the Montreal Neurological Institute (MNI152) template and spatially smoothed (4 mm full-width at half maximum). A 4 mm smoothing kernel was chosen because of the desire to limit signal spread in small brain regions (Morawetz et al., 2008). The precision of the normalization process was checked visually using the inferior colliculus as a marker since this is easily visible as a discrete structure on both anatomical and functional images.

We adopted a random effects general linear model approach. A first-level fixed-effects analysis was performed on each individual's data, specifying the following t contrasts (sound>silence, pleasant>neutral, unpleasant>neutral, neutral>silence and salient>neutral). Here, "sound" is defined as the sum of all sound conditions. "Salient" is defined as the sum of both pleasant and unpleasant sound conditions such that the contrast "salient>neutral" defines the U-shaped function identified by Irwin et al. (2011). A second-level random effects analysis was then specified to interrogate the hypotheses, with the variance between individuals within a group and between groups accounted in the model.

Regions of Interest

The ascending auditory system comprises the inferior colliculus, medical geniculate nucleus, and auditory cortex in both hemispheres. Localization of activity in the inferior colliculus and medial geniculate body was guided by known co-ordinates (±SEM) for the center of each region. A single inferior colliculus has a volume of about 135 mm^3 (Kang et al., 2008) which, at our given acquisition resolution, corresponds to 5 voxels, with an MNI co-ordinate center of +/−4, −4, −10 mm. A single medical geniculate nucleus has a volume of about 129 mm^3 (Kitajima et al., 2015) which corresponds to 5 voxels with a center at ±15, −27, −7 mm.

To test our *a priori* hypotheses relating to sound-evoked activation, analysis targeted pre-defined regions relevant to the hypotheses concerning amygdala in both hemispheres. The average size of the "classic" amygdala is estimated to be 1.24 cm^3 ($SD = 0.14$), while the average size of the amygdala with wider borders was 1.63 cm^3 ($SD = 0.2$) (Brabec et al., 2010). At our given acquisition resolution, this corresponded to 23–53 voxels. To localize the amygdala, we used a probabilistic map of the amygdala and its subdivisions based on anatomical data and transformed into MNI space and implemented in the SPM anatomy toolbox v1.8 (Amunts et al., 2005; Eickhoff et al., 2005). This map defined a wide amygdala border as it was thresholded to include those voxels with a <10% probability. While this method reduces the impact of normalization errors on the quantification of overall amygdala activity, it does reduce the precision of segmenting the three amygdalar subdivisions.

RESULTS

Sound-Related Activation in the Ascending Auditory System

The first analysis sought to demonstrate sound-related brain activity using a second-level random effects one-sample *t*-test for the contrast "sound>silence" in SPM8 ($n = 23$). Results were whole-brain corrected for family wise error (FWE) and thresholded at $p < 0.05$. We observed robust sound-evoked activation within the inferior colliculus, medial geniculate body and primary auditory cortex, across both hemispheres (see **Figure 4**). There were no significant differences between groups ($p > 0.05$ FWE corrected).

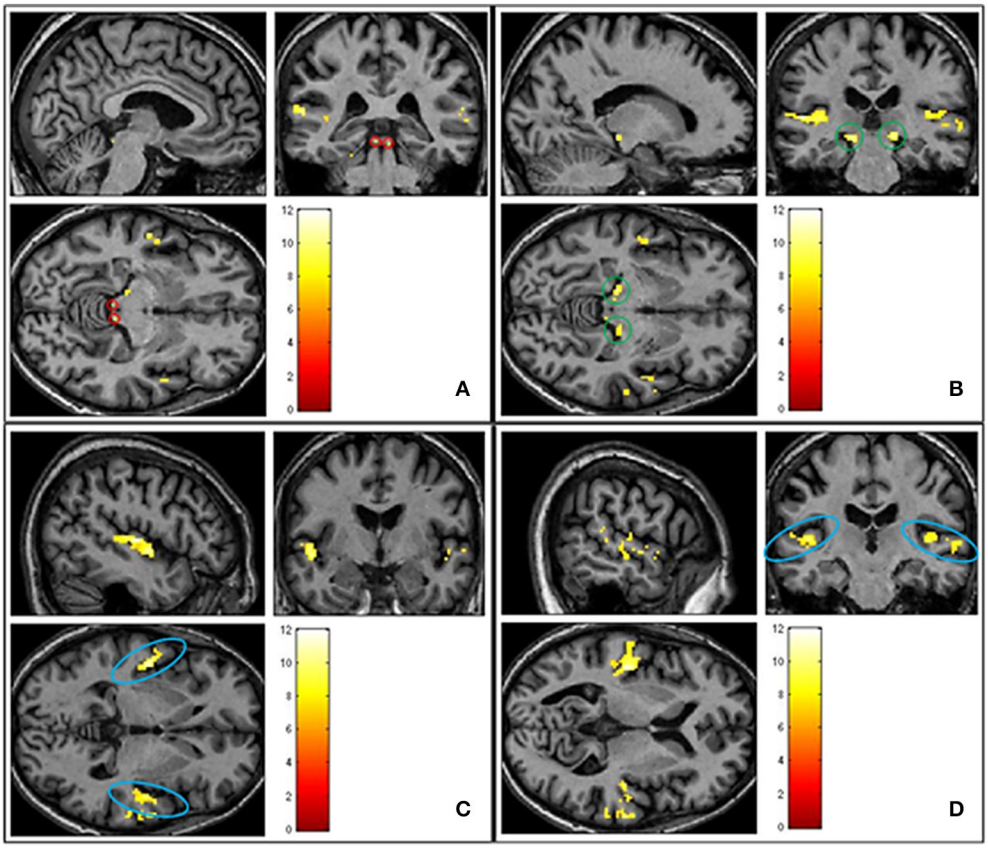

FIGURE 4 | Shows group averaged ($n = 23$) activation of ascending auditory structures in response to all sound conditions (pleasant, neutral and unpleasant) > silence ($p < 0.05$ FWE corrected). (A) Inferior colliculus circled in red. **(B)** Medial geniculate body circled in green. **(C)** Primary auditory cortex circled in blue. **(D)** Primary auditory cortex circled in blue.

Differential Sound-Evoked Activity in Amygdala

The next analysis addressed the first hypothesis by examining the data using a second-level random effects one-sample *t*-test for the contrast "salient>neutral" in SPM8 ($n = 23$). Because we were testing a specific hypothesis in a pre-defined region of the brain we report uncorrected *p*-values. Within the thresholded borders of the probabilistic map, bilateral amygdala activity was observed to be greater in response to pleasant sounds and unpleasant sounds compared with neutral sounds ($p < 0.05$, uncorrected), potentially consistent with a U-shaped function. The uncorrected result is shown in **Figure 5**. However, this did not survive statistical thresholding after implementing FWE small volume correction.

Effects of Tinnitus on the Response to Pleasant and Unpleasant Sounds

The second hypothesis predicted that people with tinnitus have greater amygdala activity in response to emotionally evocative sounds (relative to neutral sounds) compared to age and hearing-matched controls. Overall amygdala activity was quantified by selecting the peak maxima for the contrast "salient>neutral," separately in the left and right hemisphere for each individual. Peak maxima fell within the borders of the probabilistic map, as above. For each peak maxima, we extracted the general linear model beta values for each sound condition. These beta values were then submitted to a mixed model ANalysis Of VAriance (ANOVA) in SPSS, with two within-group factors; valence (pleasant, neutral, and unpleasant) and hemisphere (left and right) and one between-group factor (no tinnitus and tinnitus). Results are displayed in **Figure 6**. Again results in these selected voxels confirmed there was a strong effect of valence [$F_{(2, 42)} = 102.99$, $p < 0.0001$], with the amygdala showing the greatest response to pleasant sounds and unpleasant sounds compared with neutral sounds. However, the response was statistically equivalent in both people with tinnitus and no tinnitus controls [$F_{(1, 21)} = 3.58$, $p = 0.072$] and across hemispheres [$F_{(1, 42)} = 0.27$, $p = 0.608$]. There was no interaction between hemisphere and group [$F_{(1, 42)} = 2.06$, $p = 0.166$].

A significant interaction was observed between valence and group [$F_{(2, 42)} = 3.63$, $p = 0.035$]. *Post-hoc* testing showed that the no tinnitus group had a significantly greater amygdala response to pleasant>neutral sounds ($p = 0.024$) and unpleasant>neutral sounds ($p = 0.043$) compared to the tinnitus group. Direct comparison of the amygdala's response to the neutral>silence

condition were not significantly different between groups ($p = 0.77$) suggesting that both groups had a similar "baseline response" to neutral sounds. These main effects and U-shaped pattern were confirmed even when a more conservative ANOVA was re-run to include only those participants with peaks confirmed within the amygdala according to the probabilistic atlas.

Amygdala Subnuclei Activation

The voxel-wise statistics and probability value associated with each peak maxima for the contrast "salient>neutral" are presented in **Table 2** (tinnitus group) and **Table 3** (no tinnitus controls). The assignment of peak voxel to subdivisions of the amygdala provides preliminary exploratory information about the distribution of the greatest response to emotionally evocative sounds (relative to neutral sounds) within this brain region. Whilst all peak voxel amygdala co-ordinates fell within the borders of the probabilistic map, we note that some contiguous voxel clusters extend beyond those borders.

Bilateral peak activity occurred in 9 out of 12 (75%) tinnitus participants and 9 out of 11 (81.8%) of the no tinnitus controls. Unilateral peak activity occurred in six participants (3 from each group). Only one participant (subject 85 in the no tinnitus group) showed no amygdala activation in either the left or right hemisphere. Peak activity was most likely to be found in the LB subdivision of the amygdala, and least likely to be found in the CM subdivision of the amygdala. This pattern was true across both hemispheres and both for people with tinnitus and no tinnitus controls.

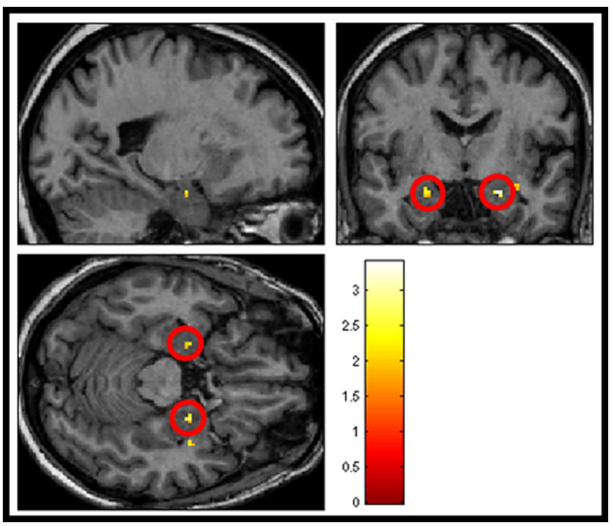

FIGURE 5 | Shows group averaged ($n = 23$) activation of amygdala structures (circled in red) in response to salience > neutral sound condition, masked with amygdala template ($p < 0.05$ uncorrected).

DISCUSSION

This study examined how the amygdala responds to emotionally evocative sounds in people with and without chronic tinnitus. Using an experimental protocol adapted from a previously published study (Irwin et al., 2011) we were able to measure activation of auditory brain areas and the amygdala in response to emotionally evocative sounds. The main findings of all analyses are now discussed.

Sound-Related Activation of Ascending Auditory Pathways

We found significant sound-related activity in several portions of the ascending auditory pathways including the inferior colliculus, medial geniculate body and the primary auditory cortex, across both brain hemispheres. As expected, this replicates the findings of several earlier sound-evoked studies (Hunter et al., 2010; Husain et al., 2011; Irwin et al., 2011; Carpenter-Thompson et al., 2014). Upon direct statistical comparison between groups, we found no differences in activation amongst auditory brain regions. This finding mimics that of Carpenter-Thompson et al. (2014) study which implemented a similar experimental design and also controlled for hearing loss.

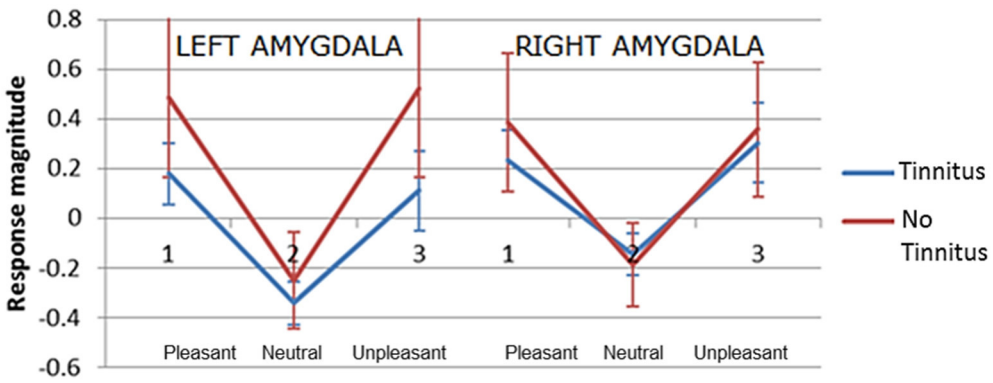

FIGURE 6 | Plots of mean response magnitude (beta values $p < 0.05$, uncorrected) for the left and right amygdala across three categories of pleasantness (1) pleasant (2) neutral (3) unpleasant. Error bars represent 95% confidence intervals.

TABLE 2 | Tinnitus group: Amygdala response to the "salient>neutral" contrast ($p < 0.05$ uncorrected).

Subject No.	Peak voxel co-ordinates and associated statistics							Localization probability		
	x	*y*	*z*	*T*-stat	*Z*-score	T2* intensity	Cluster size	LB	SF	CM
LEFT AMYGDALA										
1	–24	–14	–8	2.19	2.17	35.07	24	20	30	40
6	–26	–6	–30	2.75	2.7	26.95	7	50	0	0
17	–22	–10	–24	3.01	2.94	59.42	36	100	10	0
19	–20	–8	–12	2.41	2.38	29.54	10	80	100	20
24	–22	–6	–16	3.29	3.2	50.14	133	60	50	10
25	–26	2	–28	2.68	2.63	34.64	71	0	0	0
29	–26	–6	–24	1.93	1.91	20.62	2	100	0	0
30	–30	–2	–18	1.63 n.s	1.63 n.s	57.08	4	20	0	0
34	–34	–2	–22	2.4	2.36	40.93	4	0	0	0
45	–16	0	–18	2.94	2.87	55.26	9	10	0	0
54	–32	–6	–32	2.44	2.39	41.64	16	0	0	0
74	–28	–6	–20	3.88	3.72	57.78	134	100	0	0
Average						42.42				
SD						13.35				
RIGHT AMYGDALA										
1	26	–2	–18	2.4	2.37	55.21	17	10	10	0
6	32	–10	–10	2.55	2.51	55.45	3	60	20	0
17	18	–6	–20	2.44	2.4	65.28	17	0	10	0
19	28	–14	–8	2.83	2.77	43.75	10	40	20	20
24	32	–2	–36	3.41	3.31	37.96	42	10	0	0
25	30	2	–24	2.95	3.88	57.84	43	0	0	0
29	30	4	–32	1.49 n.s	1.49 n.s	0	2	10	0	0
30	28	–2	–18	1.84	1.83	69.45	3	30	10	0
34	30	–4	–22	3.05	2.97	49.25	79	70	0	0
45	24	–4	–12	2.47	2.43	60.07	10	20	60	0
54	20	–10	–12	2.23	2.19	59.98	10	10	80	0
74	20	–8	–12	2.27	2.23	45	15	0	80	0
Average						49.93				
SD						18.19				
(*T*-stat of 1.66/$p = 0.049$)										

Statistical outputs are reported for each individual's peak voxel amygdala co-ordinates (across hemispheres). Amygdala subnuclei: SF, superficial; CM, centromedial; LB, laterobasal. n.s, not significant ($p > 0.05$).

The Effects of Pleasantness on Amygdala Activity

In support of our first hypothesis we found that the amygdala's response to sound was significantly modulated by emotional valence. That is, compared with neutral sound clips, the amygdala's response to pleasant and unpleasant sound clips was significantly enhanced. This overall "U-shaped" response to pleasantness reflects the same amygdala response pattern found by Irwin et al. (2011) in young adults with normal hearing. Also in agreement with Irwin et al.'s (2011) data, we found no main effect of hemisphere, suggesting a lack of amygdala dominance. However, the distinctive amygdala U-shaped response pattern that has been independently replicated across both studies seems to suggest a genuine neurophysiological difference in amygdala function between sound conditions.

Contrary to our second hypothesis, we found no significant main effect of group, indicating that the amygdala's overall response to emotionally evocative sounds was similar between groups. Surprisingly however, a consistent trend for higher activation in response to salient sounds compared with neutral sounds was observed in the "no tinnitus group." Planned contrasts revealed the specific nature of this relationship. Compared to the "tinnitus group," the "no tinnitus" group had significantly greater amygdala response magnitude to both pleasant and unpleasant sounds (compared to neutral sounds). By comparing the neutral>silence conditions we determined that there was no difference in baseline activation to non-valent sound between our tinnitus and control groups, discounting the possibility of a ceiling effect caused by a raised baseline response from either group. Our findings are able to build

TABLE 3 | No tinnitus group: Amygdala response to the "salient>neutral" contrast ($p < 0.05$ uncorrected).

Subject No.	Peak voxel co-ordinates and associated statistics							Localization probability		
	x	*y*	*z*	*T*-stat	*Z*-score	T2* intensity	Cluster size	LB	SF	CM
LEFT AMYGDALA										
79	–22	–6	–16	2.6	2.54	41.7	23	60	50	10
80	–22	0	–22	3.18	3.09	137.02	60	20	10	0
81	–20	0	–22	4.07	3.88	31.85	53	10	10	0
82	–22	–8	–20	3.2	3.1	39.82	55	90	30	0
83	–24	–2	–22	3.08	2.99	53.07	50	30	10	0
84	–22	–6	–28	2.5	2.45	38.23	12	50	0	0
85	–26	2	–26	1.8	1.78	48.41	3	0	0	0
86	–20	–4	–10	1.57 n.s	1.57 n.s	30.39	1	0	70	10
88	–22	–6	–14	3	2.92	46.41	24	20	50	20
89	–20	–6	–6	2.89	2.82	22.56	5	10	80	20
90	–26	–6	–28	2.55	2.5	40.54	23	60	0	0
Average						48.14				
SD						30.70				
RIGHT AMYGDALA										
79	20	–6	–12	2.23	2.2	77.17	9	0	80	0
80	32	–2	–22	2.5	2.45	62.82	17	60	0	0
81	32	0	–20	2.41	2.36	54.44	8	0	0	0
82	36	–4	–32	2.7	2.64	39.77	15	10	0	0
83	28	–16	–8	2.63	2.57	45.23	6	30	20	20
84	24	–4	–30	3.21	3.11	35.56	27	0	0	0
85	28	2	–26	1.8	1.78	37.39	1	0	0	0
86	30	–8	–14	1.88	1.87	35.71	3	80	30	0
88	34	–6	–20	2.62	2.57	52.74	14	80	10	0
89	34	2	–26	2.38	2.34	28.96	14	0	0	0
90	30	–2	–20	2.43	2.39	62.67	10	50	0	0
Average						48.40				
SD						14.84				
(*T* stat of 1.66/$p = 0.049$)										

Statistical outputs are reported for each individual's peak voxel amygdala co-ordinates (across hemispheres). Amygdala subnuclei: SF, superficial; CM, centromedial; LB, laterobasal. n.s, not significant ($p > 0.05$).

on this uncertainty discussed by Carpenter-Thompson et al. (2014) who did not use a silence condition. In opposition to our original hypothesis, these findings seems to indicate a "muting" of the amygdala response amongst individuals with tinnitus. This finding agrees with Carpenter-Thompson et al. (2014) who observed a decreasing trend in amygdala response activation for NH>HL>TIN groups (note this trend was not statistically significant). Two potential interpretations of this finding include: (1) given the chronic nature of tinnitus, amygdala resources could be automatically be recruited to process the valent tinnitus sound, or (2) people with tinnitus may be actively controlling their emotional response to tinnitus. It may therefore be plausible that in an effort to reduce one's emotional reaction to tinnitus, affected individuals suppress amygdala activation through self-modulation in an effort to divert attention away from the experience of chronic tinnitus. In doing so, this leaves less available "resources" for assignment to other emotional stimuli. Supporting this notion, Domes et al. (2010) found that a group of healthy adults were able to modulate activation of their amygdala up or down by increasing or decreasing their emotional response to affective visual stimuli. Notably, our participants had mild to moderate tinnitus distress and may be better / more successful tinnitus habituators.

Amygdala Subnuclei Activation

Amygdala activation was found in the vast majority of participants ($n = 22/23$). This number is considerably higher than Irwin et al. (2011) study from which this experimental protocol was adapted, where only 3/16 participants demonstrated suprathreshold amygdala activity. This large difference in amygdala detectability between studies may reflect our application of a double echo imaging sequence, which is known to provide improved signal

contrast across the brain and improved BOLD sensitivity across a range of tissues (Posse et al., 1999; Marciani et al., 2006).

In our exploratory research question which explored whether differential responses could be detected within the three subdivisions of the amygdala, we found that peak activity was most likely to be found in the LB subdivision of the amygdala, and least likely to be found in the CM subdivision of the amygdala. This pattern was consistent across both hemispheres and for both study groups. Within the animal literature, it is well known that the LB nuclei acts as the "gateway" for sensory information to the amygdala, receiving input from both the auditory thalamus and from association areas of the auditory cortex (Bordi and LeDoux, 1992). Support for similar involvement of the LB nuclei when processing emotionally evocative auditory stimuli has been presented in more recent human neuroimaging studies (Ball et al., 2007; Kumar et al., 2012). Kumar et al. (2012) found both the LB and the SF nucleus to encode acoustic features necessary for attributing valence. An earlier study by Ball et al. (2007) also found activation of the LB nuclei but in response to both pleasant and unpleasant sounds. Here, the authors thought this finding may reflect a predominance of auditory inputs to LB subnuclei. In line with this literature, our observed decreasing trend of LB>SF>CM subnuclei activation seems to suggest that the LB nuclei played the most active role in processing the emotional auditory stimuli. However, Ball et al. (2007) discuss an important caveat regarding the choice of voxel resolution. Given that the centers of the different amygdala nuclei are at most 1 cm apart (Mai et al., 1997), subdivision-level investigation of the human amygdala is not optimized at 3 mm^3 resolution. This finding should therefore be interpreted cautiously.

CONCLUSION

To summarize, this study used a double-echo imaging sequence to optimally detect amygdala response patterns to emotionally evocative sounds in people with mild to moderately distressing tinnitus. Our main results show a strong modulatory effect of emotional valence on the amygdala's response. This pattern of activation was reduced in individuals with tinnitus, contrary to our expectations.

By using micro-anatomically defined probabilistic maps, we were able to estimate the origins of amygdala peak level activity. In line with previous research, this found the LB nucleus to be most active when processing emotional auditory stimuli. Based on these findings, the amygdala does appear to provide some useful information which could help in the identification and objectification of tinnitus. However, such activation patterns are, up to now, unlikely to be able to differentiate between the true presence or absence of tinnitus on a single subject level. Future studies targeting amygdala function should carefully consider fMRI parameters to ensure sufficient signal quality from the amygdala regions.

AUTHOR CONTRIBUTIONS

JD, PG, and DH conceived the study. JD and PG collected the data. JD analyzed the data and wrote the article. PG and DH supervised and provided guidance.

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REFERENCES

Adjamian, P., Magdalena, S., and Hall, D. (2009). The mechanisms for tinnitus: perspectives from human functional neuroimaging. *Hear. Res.* 253, 15–31. doi: 10.1016/j.heares.2009.04.001

Amunts, K., Kedo, O., Kindler, M., Pieperhoff, P., Mohlberg, H., Shah, N., et al. (2005). Cytoarchitectonic mapping of the human amygdala, hippocampal region and entorhinal cortex: intersubject variability and probability maps. *Anatomy Embryol.* 210, 343–352. doi: 10.1007/s00429-005-0025-5

Ball, T., Rahm, B., Eickhoff, S. B., Schulze-Bonhage, A., Speck, O., and Mutschler, I. (2007). Response properties of human amygdala subregions: evidence based on functional MRI combined with probabilistic anatomical maps. *PLoS ONE* 2:e307. doi: 10.1371/journal.pone.0000307

Beck, A. T., Epstein, N., Brown, G., and Steer, R. A., (1988). An inventory for measuring clinical anxiety: psychometric properties. *J. Consult. Clin. Psychol.* 56, 893–897. doi: 10.1037/0022-006X.56.6.893

Beck, A. T., Steer, R. A., and Brown, G. K. (2000). *BDI-Fast Screen for Medical Patients: Manual*. San Antonio, TX: Psychological Corporation.

Bordi, F., and LeDoux, J. (1992). Sensory tuning beyond the sensory system: an initial analysis of auditory response properties of neurons in the lateral amygdaloid nucleus and overlying areas of the striatum. *J. Neurosci.* 12, 2493–2503.

Brabec, J., Rulseh, A., Hoyt, B., Vizek, M., Horinek, D., Hort, J., et al. (2010). Volumetry of the human amygdala - an anatomical study. *Psychiatry Res.* 182, 67–72. doi: 10.1016/j.pscychresns.2009.11.005

Bradley, M. M., and Lang, P. J. (2000). Affective reactions to acoustic stimuli. *Psychophysiology* 37, 204–215. doi: 10.1111/1469-8986.3720204

Bradley, M. M., and Lang, P. J. (2007). *The International Affective Digitized Sounds (IADS-2): Affective Ratings of Sounds and Instruction Manual*. Gainesville, FL: University of Florida.

Carpenter-Thompson, J. R., Akrofi, K., Schmidt, S. A., Dolcos, F., and Husain, F. T. (2014). Alterations of the emotional processing system may underlie preserved rapid reaction time in tinnitus. *Brain Res.* 1567, 28–41. doi: 10.1016/j.brainres.2014.04.024

Chen, N. K., Dickey, C. C., Yoo, S.-S., Guttman, C. R. G., and Panych, L. P. (2003). Selection of voxel size and slice orientation for fMRI in the presence of susceptibility field gradients: application to imaging of the amygdala. *NeuroImage* 19, 817–825. doi: 10.1016/S1053-8119(03)00091-0

Costa, V. D., Lang, P. J., Sabatinelli, D., Versace, F., and Bradley, M. M. (2010). Emotional imagery: assessing pleasure and arousal in the brain's reward circuitry. *Hum. Brain Mapp.* 31, 1446–1457. doi: 10.1002/hbm.20948

Crippa, A., Lanting, C. P., van Dijk, P., and Roerdink, J. B. (2010). A diffusion tensor imaging study on the auditory system and tinnitus. *Open Neuroimage J.* 4, 16–25. doi: 10.2174/1874440001004010016

Cronlein, T., Langguth, B., Pregler, M., Kreuzer, P. M., Wetter, T. C., and Schecklmann, M. (2016). Insomnia in patients with chronic tinnitus: Cognitive and emotional distress as moderator variables. *J. Psychosom. Res.* 83, 65–68. doi: 10.1016/j.jpsychores.2016.03.001

Davies, J., Gander, P. E., Andrews, M., and Hall, D. A. (2014). Auditory network connectivity in tinnitus patients: a resting-state fMRI study. *Int. J. Audiol.* 53, 192–198. doi: 10.3109/14992027.2013.846482

Davis, A., and El Rafaie, A. (2000). "Epidemiology of tinnitus," in *Tinnitus Handbook*, ed R. S. Tyler (San Diego, CA: Singular Thomson Learning), 1–23.

De Ridder, D., Elgoyhen, A. B., Romo, R., and Langguth, B. (2011). Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8075–8080. doi: 10.1073/pnas.1018466108

De Ridder, D., Vanneste, S., Weisz, N., Londero, A., Schlee, W., Elgoyhen, A., et al. (2014). An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci. Biobehav. Rev.* 44, 16–32. doi: 10.1016/j.neubiorev.2013.03.021

Domes, G., Schulze, L., Bottger, M., Grossmann, A., Hauenstein, K., Wirtz, P. H., et al. (2010). The neural correlates of sex differences in emotional reactivity and emotion regulation. *Hum. Brain Mapp.* 31, 758–769. doi: 10.1002/hbm.20903

Eickhoff, S., Stephan, K. E., Mohlberg, H., Grefkes, C., Fink, G. R., Amunts, K., et al. (2005). A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *NeuroImage* 25, 1325–1335. doi: 10.1016/j.neuroimage.2004.12.034

Folmer, R., Griest, S., Meikle, M., and Martin, W. (1999). Tinnitus severity, loudness, and depression. *Otolaryngol. Head Neck Surg.* 121, 48–51.

Golm, D., Schmidt-Samoa, C., Dechent, P., and Kroner-Herwig, B. (2013). Neural correlates of tinnitus related distress: an fMRI-study. *Hear. Res.* 295, 87–99. doi: 10.1016/j.heares.2012.03.003

Hall, D. A., Chambers, J., Akeroyd, M. A., Foster, J. R., Coxon, R., and Palmer, A. R. (2009). Acoustic, psychophysical, and neuroimaging measurements of the effectiveness of active cancellation during auditory functional magnetic resonance imaging. *J. Acoust. Soc. Am.* 125, 347–359. doi: 10.1121/1.3021437

Hall, D. A., Haggard, M. P., Akeroyd, M. A., Palmer, A. R., Summerfield, A. Q., Elliott, M. R., et al. (1999). Sparse temporal sampling in auditory fMRI. *Hum. Brain Mapp.* 7, 213–223. doi: 10.1002/(SICI)1097-0193(1999)7:3<213::AID-HBM5>3.0.CO;2-N

Hall, D. A., Irwin, A., Edmondson-Jones, M., Phillips, S., and Poxon, J. E. W. (2013). An exploratory evaluation of perceptual, psychoacoustic and acoustical properties of urban soundscapes. Special Issue. *Appl. Acoust.* 74, 248–254. doi: 10.1016/j.apacoust.2011.03.006

Hallam, R. S. (1996). *Manual of the Tinnitus Questionnaire*. London: The Psychological Corporation.

Hunter, M. D., Eickhoff, S. B., Pheasant, R. J., Douglas, M. J., Watts, G. R., Farrow, T. F., et al. (2010).The state of tranquility: subjective perception is shaped by contextual modulation of auditory connectivity. *Neuroimage* 53, 611–618. doi: 10.1016/j.neuroimage.2010.06.053

Husain, F. T., Pajor, N. M., Smith, J. F., Kim, J. H., Rudy, S., Zalewski, C., et al. (2011). Discrimination task reveals differences in neural bases of tinnitus and hearing impairment. *PLoS ONE* 6:e26639. doi: 10.1371/journal.pone.0026639

Irwin, A., Gander, P. E., and Hall, D. A. (2012). "Listening to emotion: auditory processing and the amygdala," in *Insights into the Amygdala: Structure Function and Implications for Disorders,* ed D. Yilmazer-Hanke (New York, NY: Nova Science Publishers Inc.), 255–275.

Irwin, A., Hall, D. A., Peters, A., and Plack, C. J. (2011). Listening to urban soundscapes: physiological validity of perceptual dimensions. *Psychophysiology* 48, 258–268. doi: 10.1111/j.1469-8986.2010.01051.x

Jakes, S. C., Hallam, R. S., Chambers, C., and Hinchcliffe, R. (1985). A factor analytical study of tinnitus complaint behaviour. *Int. J. Audiol.* 24, 195–206. doi: 10.3109/00206098509070103

Jastreboff, P. J. (1990). Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci. Res.* 8, 221–254. doi: 10.1016/0168-0102(90)90031-9

Kang, D., Kwon, K., Gu, B., Choi, J., Jang, J., and Kwon, J. (2008). Structural abnormalities of the right inferior colliculus in schizophrenia. *Psychiatry Res.* 164, 160–165. doi: 10.1016/j.pscychresns.2007.12.023

Khalfa, S., Dubal, S., Veuillet, E., Perez-Diaz, F., Jouvent, R., and Collet, L. (2002). Psychometric normalization of a hyperacusis questionnaire. *ORL J. Otorhinolaryngol. Relat. Spec.* 64, 436–442. doi: 10.1159/000067570

Kitajima, M., Hirai, T., Yoneda, T., Iryo, Y., Azuma, M., Tateishi, M., et al. (2015). Visualization of the medial and lateral Geniculate nucleus on phase difference enhanced imaging. *Am. J. Neuroradiol.* 36, 1669–1674. doi: 10.3174/ajnr.A4356

Kuk, F. K., Tyler, R. S., Russell, D., and Jordan, H. (1990). The psychometric properties of a tinnitus handicap questionnaire. *Ear Hear.* 11, 434–445. doi: 10.1097/00003446-199012000-00005

Kumar, S., von Kriegstein, K., Friston, K., and Griffiths, T. D. (2012). Features versus feelings: dissociable representations of the acoustic features and valence of aversive sounds. *J. Neurosci.* 32, 14184–14192. doi: 10.1523/JNEUROSCI.1759-12.2012

Langguth, B., Goodey, R., Azevedo, A., Bjorne, A., Cacace, A., Crocetti, A., et al. (2007). Consensus for tinnitus patient assessment and treatment outcome measurement: tinnitus research initiative meeting, Regensburg, July 2006. *Prog. Brain Res.* 166, 525–536. doi: 10.1016/S0079-6123(07)66050-6

Leaver, A. M., Seydell-Greenwald, A., Turesky, T. K., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2012). Cortico-limbic morphology separates tinnitus from tinnitus distress. *Front. Syst. Neurosci.* 6:21. doi: 10.3389/fnsys.2012.00021

Le Doux, J. E. (2000). Emotion circuits in the brain. *Annu. Rev. Neurosci.* 23, 155–184. doi: 10.1146/annurev.neuro.23.1.155

Mai, J. K., Assheuer, J., and Paxinos, G. (1997). *Atlas of the Human Brain*. San Diego, CA: Academic Press.

Marciani, L., Pfeiffer, J. C., Hort, J., Head, K., Bush, D., Taylor, A. J., et al. (2006). Improved methods for fMRI studies of combined taste and aroma stimuli. *J. Neurosci. Methods* 158, 186–194. doi: 10.1016/j.jneumeth.2006.05.035

Morawetz, C., Holz, P., Lange, C., Baudewig, J., Weniger, G., Irle, E., et al. (2008). Improved functional mapping of the human amygdala using a standard functional magnetic resonance imaging sequence with simple modifications. *Magn. Reson. Imaging* 26, 45–53. doi: 10.1016/j.mri.2007.04.014

Posse, S., Wiese, S., Gembris, D., Mathiak, K., Kessler, C., Grosse-Ruyken, M., et al. (1999). Enhancement of BOLD-contrast sensitivity by single-shot multi-echo functional MR imaging. *Magn. Reson. Med.* 42, 87–97. doi: 10.1002/(SICI)1522-2594(199907)42:1<87::AID-MRM13>3.0.CO;2-O

Rajmohan, V., and Mohandas, E. (2007). The limbic system. *Indian J. Psychiatry* 49, 132–139. doi: 10.4103/0019-5545.33264

Roy, A. K., Shehzad, Z., Margulies, D. S., Kelly, A. M., Uddin, L. Q., Gotimer, K., et al. (2009). Functional connectivity of the human amygdala using resting state fMR. *NeuroImage* 45, 614–626. doi: 10.1016/j.neuroimage.2008.11.030

Shulman, A., Strashun, A. M., Afriyie, M., Aronson, F., Abel, W., and Goldstein, B. (1995). SPECT imaging of brain and tinnitus–neurotologic/neurologic implications. *Int. Tinnitus J.* 1, 13–29.

Tegg-Quinn, S., Bennett, R., Eikelboom, R., and Baguley, D. (2016). The impact of tinnitus upon cognition in adults: A systematic review. *Int. J. Audiol.* 55, 533–540. doi: 10.1080/14992027.2016.1185168

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Paired Associative Stimulation of the Temporal Cortex: Effects on the Auditory Steady-State Response

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Background: Paired associative stimulation (PAS) is the repeated combination of a sensory stimulus with transcranial magnetic stimulation (TMS) in close temporal association. Recently, a study demonstrated that PAS of an auditory stimulus together with TMS of the temporal cortex is capable of changing the amplitude of auditory evoked potentials (AEP).

Objective: This study examined the influence of tone duration and habituation in temporal cortex PAS as elicited by 40 and 20 Hz amplitude modulated auditory steady-state responses (aSSR).

Methods: Eighteen subjects participated in two experiments, including two PAS protocols each, which consisted of 200 auditory stimuli (4 kHz) paired with temporal cortex TMS with an interstimulus interval (ISI) of 45 ms between tone onset and TMS pulse, delivered at 0.1 Hz. Experiment 1 compared auditory stimuli with different lengths [PAS (23 ms) vs. PAS (400 ms)]. Experiment 2 investigated verum vs. sham PAS. aSSR for the paired tone (4 kHz) and a control tone (1 kHz) were measured pre- and post-interventional—using 40 Hz aSSR in experiment 1 and both 20 and 40 Hz aSSR in experiment 2.

Results: A statistically significant, sham-controlled decrease in amplitude was observed for the 20 Hz aSSR using the 4 kHz PAS carrier frequency in experiment 2.

Conclusion: Frequency-specific effects for the 20 Hz aSSR confirm the feasibility of auditory PAS and highlight the secondary auditory cortex as its target site, introducing new possible treatment protocols for patients suffering from tinnitus. The amplitude decrease can be explained by principles of spike timing-dependent plasticity and the superposition model of aSSR.

Keywords: paired associative stimulation, auditory steady-state response, temporal cortex, tinnitus, spike-timing dependent plasticity

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INTRODUCTION

Transcranial magnetic stimulation (TMS) is a non-invasive stimulation technique which uses a coil placed on the scalp to apply magnetic stimulation to possible target areas of the cortex (1). A series of TMS pulses is called repetitive TMS (rTMS), which can induce changes of excitability *via* processes similar to long term potentiation (LTP) and long term depression (LTD) (2). Paired associative

stimulation (PAS) is the pairing of external sensory stimuli with TMS pulses applied to the corresponding cortical region of the peripheral stimulus capable of inducing changes in neuroplasticity (3). Based on the concept of spike timing-dependent plasticity (STDP), the effects of PAS depend strongly on the order of the cortical processing of the peripheral stimulus and the TMS pulse. If cortical neurons are stimulated post-synaptically with TMS before they are excited pre-synaptically by the sensory stimulus, synaptic connectivity is reduced *via* LTD-like effects (3). If this order is reversed, LTP-like effects are expected (3). A recent pilot study revealed that the principles of PAS apply not only to the motor cortex (4) and the primary somatosensory cortex (5) but to the human secondary auditory cortex as well (6).

Tones of a specific carrier frequency can have sinusoidally modulated sound levels. These amplitude modulated tones (AM) are used to evoke auditory steady-state responses (aSSR) in the auditory cortex (7). They are recorded in the electroencephalogram as sinusoidal waves of the same frequency as the frequency of the amplitude modulation of the tone (8). So far there is no complete understanding of the mechanism underlying the aSSR. In theory, depending on the modulation frequency different parts of the auditory cortex can be activated and the generated neural responses are thought to correspond with those of transient auditory evoked potentials (AEP) (7). For example, 40-Hz AM aSSR have a modulated sound level with a period of 25 ms. Therefore, the 40-Hz AM aSSR most likely correlates with the Pa-component, a middle latency AEP with a latency of about 25 ms (9–12). There is a lot of evidence that the source of the 40 Hz aSSR is localized in the Heschl's gyrus, which is considered to be the primary auditory cortex (13–15), which is also be presumed to be the origin of the Pa-component (16). Equivalent to the 40 Hz AM tone, a 20-Hz aSSR may reflect the P1-component, a late AEP with a latency of 50 ms generated in the secondary auditory cortex (16).

A pilot study showed that PAS of the auditory cortex is capable to induce timing- and tone-specific inhibitory effects as indicated by amplitude decreases of long-latency AEP (6). PAS (45 ms) showed greater decreases than PAS (10 ms) [PAS protocol with an interstimulus interval (ISI) of 45 ms between tone onset and TMS pulse vs. a PAS protocol with an ISI of 10 ms] (6). Schecklmann et al. assumed that the auditory evoked signal reaches the secondary auditory cortex, which has been stimulated with TMS, after about 50 ms (6). Thus, the more pronounced amplitude reduction after PAS (45 ms) was interpreted as a consequence of the shorter interval between pre- and postsynaptic excitation as compared to PAS (10 ms) (6). The effects seemed also to be frequency specific, as the amplitude decrease was more pronounced for the 4 kHz tone which had been used for the PAS intervention in contrast to a 1 kHz control tone (6). No significant effects on the AEP were observed after 0.1 or 1 Hz rTMS without acoustic stimulation that were used as control conditions (6). In this pilot study, the paired tone had a duration of 400 ms which represents a relatively long duration as PAS of the somatosensory or motor system uses electric stimuli in the range of microseconds (3). Thus, the long duration might have contributed to the inhibitory effect. One further limitation of the pilot study was the lack of a control condition that consisted of auditory stimulation in combination with sham stimulation (6). Therefore, habituation effects induced by numerous repetitions of the presented tones could not be ruled out as a potential confounder, even if the timing-specific effects (same number of presented acoustic stimuli) argued against pure habituation effects as an explanation for the observed amplitude decreases (6). Furthermore, only effects on the secondary auditory cortex were evaluated by assessing late AEP (6).

The aims of the present work were to control for effects of the duration of the paired auditory stimulus and for unspecific effects such as habituation. For this purpose, we conducted two experiments contrasting long- and short PAS tones (experiment 1) and verum (using a defined stimulation intensity) and sham stimulation (experiment 2) for the PAS stimulation. Effects were measured *via* aSSR using 40 Hz amplitude (experiment 1) and both 40 and 20 Hz amplitude modulation (experiment 2). Therefore, effects on the primary (40 Hz AM aSSR) and on the secondary (20 Hz aSSR) auditory cortex can be evaluated.

We hypothesized that PAS of the temporal cortex can induce changes in neuroplasticity. According to the model of STDP the chosen ISI of 45 ms between tone onset and the TMS pulse will lead to an increase in amplitude of the 40 Hz AM aSSR representing the primary auditory cortex and to a decrease in amplitude of the 20 Hz AM aSSR representing the secondary auditory cortex (16).

MATERIALS AND METHODS

Subjects and Recruitment

Eighteen students from the University of Regensburg participated in the study. We recruited all subjects by word of mouth. All volunteers received a monetary compensation and had no relevant neurological or medical disorders. Seventeen subjects completed a multiple choice vocabulary test ("Mehrfach-Wortschatz-Intelligenztest", third edition, MWT-B) (17). Participant 18 was excluded from this test as she was not a German native speaker. Hearing function was assessed by pure tone audiometry testing seven frequencies between 125 Hz and 8 kHz (Midimate 622D, Madsen Electronics, GN Otometry, Denmark). All participants had a hearing threshold below 30 dB HL for all tested frequencies. All subjects gave written informed consent after being informed about contraindications, side effects (3), and study procedure. The study was approved by the Ethics Committee of the University of Regensburg and performed in accordance with the last revision of the Declaration of Helsinki.

General Study Procedure

All participants completed four different sessions of PAS, two within each experiments. We (Sarah Engel and Robert Daniel Heinrich Markewitz) conducted the experiments in a quiet room of the Department of Psychiatry and Psychotherapy of the University of Regensburg at the Bezirksklinikum Regensburg. One of us operated the TMS stimulator, while the other one overviewed the stimulus presenting computer program. Within experiment 1 and 2, we presented the different PAS-conditions in a randomized order 1 week apart with a 6-month interval between experiments 1 and 2. We measured aSSR before and after each PAS-condition (see **Figure 1**).

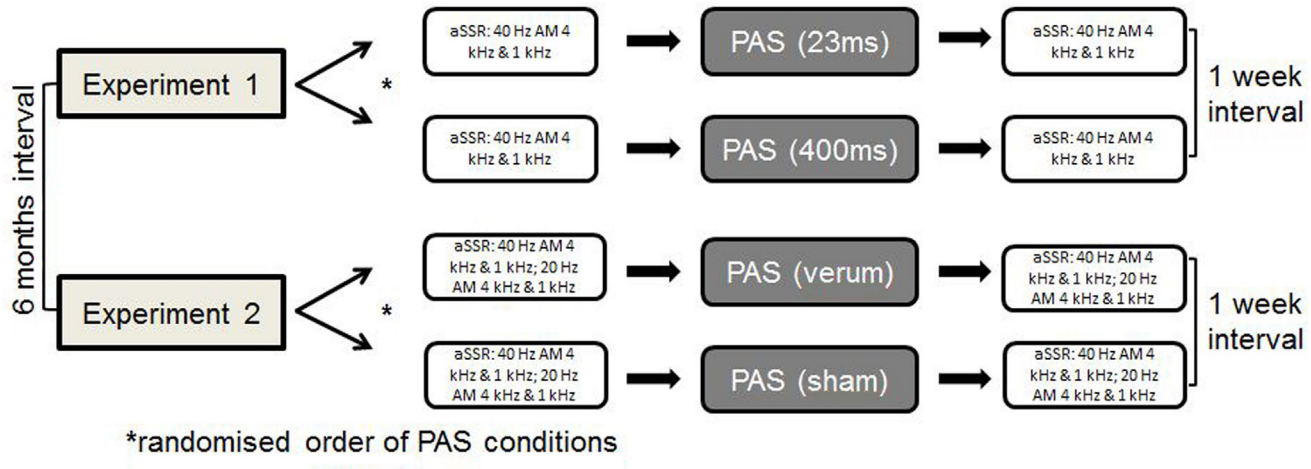

FIGURE 1 | General study procedure.

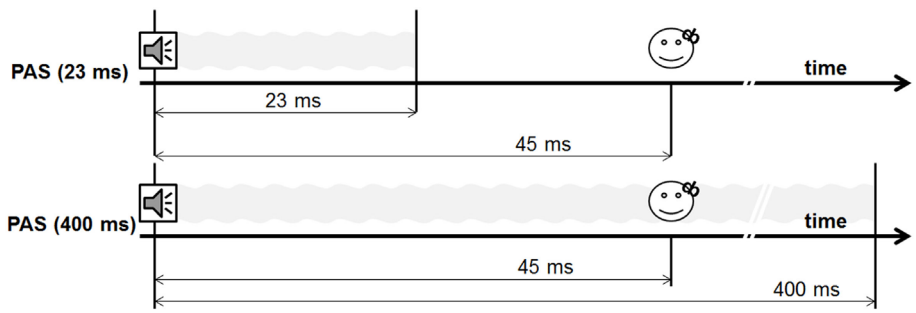

FIGURE 2 | Design experiment 1.

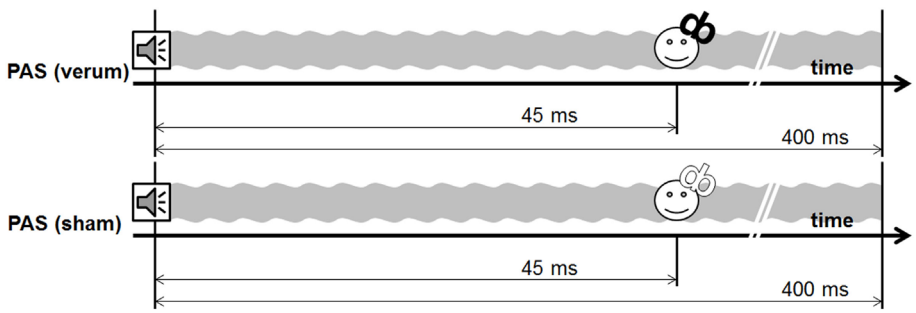

FIGURE 3 | Design experiment 2.

In the first session of each experiment, we determined the stimulation intensity [110% resting motor threshold (RMT)] for each subject following the protocol of Schecklmann and colleagues (6, 18).

For each experiment, we evaluated the sensation levels for the tones used during the experiments using Adobe audition 3.0 (Adobe Systems, DE, USA). We presented all tones binaurally through inserted earphones (E-A-RLINK, Foam Eartips for Insert Earphones, 3M, E-A-R, Etymotic Research, Inc.) at 60 dB sensation level.

PAS Protocols

All PAS protocols lasted around 33 min and consisted of 200 stimulus pairs of an auditory stimulus of 4 kHz and a TMS pulse with an ISI of 45 ms presented with a stimulation frequency of 0.1 Hz. We used an ISI of 45 ms as the pilot study showed the largest effects for this condition (6).

During experiment 1, we performed two different PAS protocols, using a 400-ms tone and a 23-ms tone [PAS (23 ms) vs. PAS (400 ms)] (see **Figures 2** and **3**). The shortest tone length enabling

a pure tone percept was 23 ms as evaluated by subjective judgment and fourier analysis as implemented in Adobe Audition.

Experiment 2 included a sham and a verum PAS protocol, using a 400-ms tone [PAS (sham) vs. PAS (verum)]. The PAS (verum) protocol was the same protocol as the PAS (400 ms) protocol used in experiment 1. In the sham condition, the water-cooled figure of eight coil was reverted in a way that the back of the coil was directed to the head of the subject. The magnetic field is decreased to one-sixth on this side of the coil as indicated by own measurements [compare technique in van Doren et al. (19)]. This sham condition guaranteed comparable sensations with respect to the click sound and the vibration of the coil.

We placed the coil over the left auditory cortex following the protocol of the pilot study (6) by using a standard procedure based on EEG coordinates (20). The TMS pulses were presented with a water-cooled figure of eight coil (MAGPRO, Medtronic, USA, outer diameter: 90 mm; water-cooled double coil). The computer software Presentation (Neurobehavioral Systems, Inc., USA) triggered the TMS pulse and presented the auditory stimuli. To ensure the exact timing of the ISI (45 ms), we measured the acoustic stimuli from the insert earplugs with a sound-level meter linked to one channel of the EEG amplifier and the TMS artifact which is induced by stimulation of the electrode cap.

aSSR Recording and Measurement

We recorded aSSR with an EEG cap (Braincap Fast'n Easy 64 Ch for TMS, Standard Layout, Easycap, Germany), reduced impedances below 10 kΩ. We sampled EEG data with a frequency of 500 Hz (BrainAmp MR plus, Germany). We used AM tones of 800 ms duration (rise- and fall time: 75 ms) with a carrier frequency of 4 kHz (paired tone) or 1 kHz (control tone), presented in a randomized order with a variable ISI (2,800–3,200 ms). For recording EEG, we used BrainVision (Brain Products GmbH, Germany).

Experiment 1

In experiment 1, we compared two PAS protocols, one using a 23-ms tone of 4 kHz [PAS (23 ms)] and the other one using a 400 ms tone of 4 kHz [PAS (400 ms)]. As read-out parameter we used 40 Hz AM aSSR, measured as described above, in order to evaluate the effects on the primary auditory cortex. We used two different carrier frequencies: a 4-kHz tone, correlating with the 4 kHz we used during the PAS intervention (paired tone), and a 1-kHz tone (control tone). The aSSR measurments before and after the PAS intervention lasted about 7.5 min each.

Experiment 2

In experiment 2, we compared a verum condition (actual stimulation of the auditory cortex) with a sham condition [PAS (verum) vs. PAS (sham)]. As in experiment 1, we also used a 400-ms tone with a carrier frequency of 4 kHz for the PAS intervention. In order to evaluate the effects on the primary and secondary cortex, we measured 20 and 40 Hz AM aSSR before and after the intervention with two different carrier frequencies, 4 (paired tone) and 1 kHz (control tone). Accordingly, four acoustic stimuli were presented (40 Hz AM aSSR of 4 kHz carrier frequency, 40 Hz AM aSSR of 1 kHz carrier frequency, 20 Hz AM aSSR of a 4 kHz carrier frequency, and 20 Hz AM aSSR of 1 kHz carrier frequency). Measurements of the aSSR lasted about 15 min each.

Data Analysis and Statistical Evaluation

We transferred all recorded EEG data to EEGLAB (21), created epochs of 4.5 s (from 2 s before till 2.5 s after tone onset), and processed the EEG data using a high (0.1 Hz) and a low (90 Hz) pass filter. After visual inspection, we excluded segments containing muscle artifacts, electrodes with signal loss, and segments with strong background noise. Further artifacts were rejected using independent component analysis.

After a subsequent visual inspection for any remaining artifacts, we interpolated the EEG data and re-referenced it to an average reference. The electrode FCz, which was used as a reference electrode during measurements, was reconstructed. EEG channels which were omitted before due to artifacts were then reconstructed using surrounding electrodes for interpolation purposes.

For the analysis of the 20-Hz aSSR, we filtered the data with 18–22 Hz, while a filter of 38–42 Hz was used for analysis of the 40-Hz aSSR.

After manually inspecting all segments of each participant for artifacts, we identified 59 as the minimum number of segments, i.e., the measurement with the smallest number of remaining segments counted 59 segments. Therefore, we used the first 59 trials of each participant and of each condition for further calculations.

Then we transferred the EEG data to FieldTrip (21). We calculated and rectified the mean voltage of all trials. Thereafter, we performed a baseline correction for the interval of 300 ms before the tone onset. We inspected the averaged and rectified trials for plausibility using topographies and trajectories. We decided to use time-locked data (averaging of the single segments) and evoked activity as the principle of STDP is related to an exact and constant timing of two stimuli.

For further statistical analysis and based on plausibility checks, we chose a time of interest of 500–800 ms to avoid interference with long-latency AEP. Our region of interest was in the fronto-parietal area (F1, Fz, F2, FC1, FCz, FC2, C1, Cz, C2). We extracted the data from these electrodes and imported it into SPSS 18.0.0 (SPSS, USA).

We computed 2 × 2 analyses of variance with two within-subjects factors "time" (pre vs. post) and "PAS-condition" (experiment 1: short vs. long tone; experiment 2: sham vs. verum condition), for both tones (1 kHz control tone and 4 kHz paired tone) and both types of AM tones in experiment 2 (20 Hz AM and 40 Hz AM). We used a two tailed paired Student's *t*-test for *post hoc* analysis for statistically significant interaction effects. We performed corrections for multiple comparisons using Bonferroni correction.

RESULTS

All participants had a mean age of 21.28 years [±2.37 standard deviation (SD)] with an age range from 19 to 28 years. All participants were right handed, 10 were female. The mean hearing level (dB HL) was 13.318 ± 2.572 SD with a range (dB HL) of 8.890–17.78. There was no significant difference between the

RMT for experiment 1 and 2 ($T = -0.414$; $df = 1;17$; $p = 0.684$). Experiment 1 and 2 were completed by all 18 participants without any reports of side effects.

Explanation of Plausibility

For all subjects, we could identify the typical topography of both the 20- and the 40-Hz aSSRs as a positive maximum in the fronto-central region for the sensitive interval of 500–800 ms including the electrodes: F1, Fz, F2, FC1, FCz, FC2, C1, Cz, C2.

Effects of PAS Adjusted for Multiple Comparisons (Bonferroni)

Experiment 1 showed no significant effects for the 1 kHz tone (all F-values < 5.375; all p-values > 0.066) and no significant effect for the 4 kHz tone (all F-values < 2.232; all p- values > 0.306).

Looking at the results of experiment 2, we found a significant result for the 20 Hz AM 4 kHz paired tone for the main effect PAS condition ($F = 8.816$; $df = 1;17$; $p = 0.018$) as well as for the interaction "time by PAS condition" ($F = 6.11$; $df = 1;17$; $p = 0.048$) (see **Figure 4**), but not for the time main effect ($F = 0.167$; $df = 1;17$; $p = 1.376$). The *post hoc* paired Student's t-test showed a significant decrease in the amplitude from pre to post stimulation for the verum condition ($t = 3.505$; $df = 1;17$; $p = 0.012$) but no significant decrease in amplitude of the verum condition in contrast to the sham condition after the stimulation ($t = 2.120$; $df = 1;17$; $p = 0.196$). For the sham condition there were no differences between the pre- and post-measurement ($t = 0.253$; $df = 1;17$; $p = 1.606$). Before stimulation verum and sham differed not significant ($t = -1.374$; $df = 1;17$; $p = 0.374$). Therefore, a significant decrease of the 20 Hz AM 4 kHz tone—which was paired in the PAS—could be observed for the verum condition with no changes for the sham condition. There were no significant effects for the 40 Hz AM 4 kHz tone (all F-values < 1.521, all p-values > 0.468). All effects for both 40 and 20 Hz AM tones with the carrier frequency of 1 kHz were not significant (all F-values < 2.188; all p-values > 0.314).

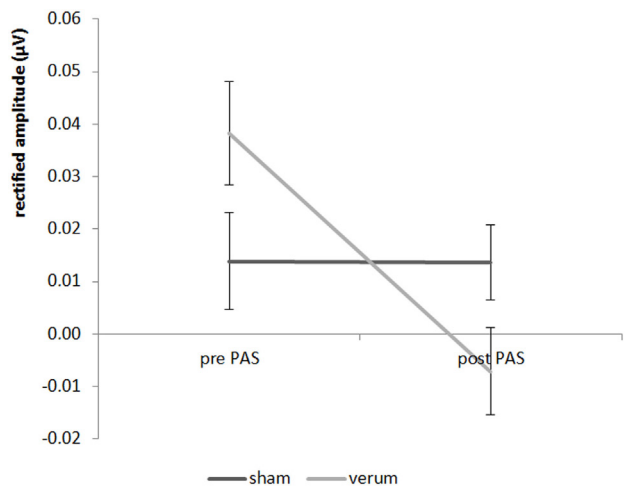

FIGURE 4 | Results for the 20 Hz AM 4 kHz paired tone in experiment 2 (± standard deviation).

DISCUSSION

The main finding of our experiments was a significant interaction effect showing a sham-controlled PAS induced decrease of the 20 Hz aSSR amplitude. This effect was frequency specific as it occurred only for the 4 kHz tone (carrier frequency which was used for pairing in the PAS) but not for the 1 kHz control tone. We could not find any statistically significant results for the 40 Hz aSSR, neither for the 1 kHz nor for the 4 kHz carrier frequency, including experiment 1 (short tone vs. long tone) and 2 (verum vs. sham condition). The significant frequency-specific interaction effect may support the notion that PAS with combined auditory and TMS induces an inhibitory mechanism by inducing STDP. A pure habituation effect can be excluded as auditory stimulation combined with sham TMS (experiment 2) did not induce a significant amplitude reduction of the aSSR. The observed frequency specificity is in line with the results of the pilot study (6), where inhibitory effects were also observed primarily for the frequency of the tone that was paired with TMS in the PAS protocol. The frequency specificity is a further argument for the assumption that combined auditory stimulation and TMS is critical for the observed inhibitory effects.

The reduction of the 20 Hz aSSR after PAS fits well with superposition theory which explains the generation of aSSR (9–12). Based on the theory of STDP (2, 3) the PAS protocol with an ISI of 45 ms should lead to LTD-like effects for external stimuli which arrive in the stimulated cortical area later than 45 ms after auditory stimulation. Under the assumption that the 20 Hz aSSR is generated by superposition of the P1/P50 (which has a latency of 50 ms), effects of a PAS (45 ms) protocol should lead to amplitude decrease which was the case in the present study.

There were no significant effects for the 40 Hz aSSR neither in experiment 1 nor in experiment 2. As shown in the plausibility check, we were able to evoke the 40 Hz aSSR, but the PAS protocols did not induce any changes of the amplitude of the 40 Hz aSSR. 40 Hz aSSR are presumably generated by the primary auditory cortex (12), whereas the 20 Hz aSSR are most likely generated in the secondary cortex (16). Therefore, the significant decrease in amplitude of the 20 Hz aSSR as compared to no significant change in amplitude for the 40 Hz aSSR might be explained by the different anatomical origins of the 20- and the 40-Hz aSSR as described above. While the primary auditory cortex occupies most of the Heschl's gyrus deep in the Sylvian fissure (22), the secondary auditory cortex (22), lies next to the primary auditory cortex on the external surface of the cortex. Due to its superficial location the secondary auditory cortex can be better reached with TMS than the primary auditory cortex. The individual stimulation intensity for the PAS intervention was determined as 110% of the RMT and, therefore, depended on the anatomy of the motor cortex, which lies as part of the precentral gyrus on the outer surface of the cortex as well (22). As such, we assumed that a 10% increase of the RMT will also be able to reach the auditory cortex. However, whether the stimulation intensity is high enough to have a direct effect on the primary auditory cortex is questionable. Since effects on the secondary auditory cortex could be observed, but none on the primary auditory cortex, we can assume that the intensity level of 110% of the RMT, we used

during the intervention, may not be high enough to induce direct changes in neuroplasticity in the primary auditory cortex. For further experiments, we should take into account that the intensity of the electromagnetic field is inversely proportional to the distance from the TMS-coil (3).

Notably, we cannot exclude that TMS effects propagate from the secondary to the primary auditory cortex (23). However, such transsynaptically propagated effects on the primary auditory cortex would be too late to induce any STDP like effects in the investigated PAS protocols. Because we did not see any statistically significant results for the 40 Hz aSSRs neither in experiment 1 nor in experiment 2, we cannot draw any conclusions about the influence of different tone lengths of the paired tone. Further experiments using 20 Hz aSSR as read-out parameter will be needed to investigate the relevance of the tone length of the paired tone. Moreover, further experiments investigating the impact of different PAS intervals would be useful to confirm STDP as the underlying mechanism for the observed results.

Further experiments will also be necessary for additional evaluation of tonotopical effects of PAS on aSSR (e.g., using different frequencies as different stimulation conditions for the tones paired with the TMS-pulse during the intervention). Only then can an assessment of the potential of PAS as a tool both for research purposes and treatment of medical conditions be undertaken. For instance, PAS could be applied therapeutically to attenuate tinnitus symptoms. Pathogenesis of tinnitus, a phantom perception of sound (24), is thought to originate from abnormal neural activity (24, 25) and a decrease of functional inhibiting pathways (26). Our study contributes to the findings of the previous study (6) that depending on timing PAS is capable of inhibiting neural activities in the auditory cortex. Therefore, PAS of the auditory cortex could be used to reduce abnormal neural activity and to compensate the missing inhibiting pathways found in patients with tinnitus. If PAS proves to have a strictly tonotopical effect on aSSR it might even offer an individualized therapy option for people with tonal tinnitus, who could be treated with an inhibitory PAS intervention using their individual tinnitus pitch as a paired tone, other than rTMS which has shown moderate effects lasting from weeks to several months (27, 28). The potential therapeutic value of PAS in this context remains speculative.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the last revision of the Declaration of Helsinki with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Ethics Committee of the University of Regensburg.

AUTHOR CONTRIBUTIONS

All authors conceived and designed the research, as well as interpreted the results of the experiments. SE and RM performed the experiments. SE drafted the manuscript and prepared figures. MS and BL edited and revised the manuscript. All authors approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/article/10.3389/fpsyt.2017.00227/full#supplementary-material.

REFERENCES

1. Hallett M. Transcranial magnetic stimulation: a primer. *Neuron* (2007) 55(2):187–99. doi:10.1016/j.neuron.2007.06.026
2. Pascual-Leone A, Valls-Sole J, Wassermann EM, Hallett M. Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. *Brain* (1994) 117(Pt 4):847–58. doi:10.1093/brain/117.4.847
3. Siebner HR, Ziemann U. *Das TMS-Buch: Handbuch der transkraniellen Magnetstimulation*. Heidelberg: Springer Medizin (2007).
4. Stefan K, Kunesch E, Cohen LG, Benecke R, Classen J. Induction of plasticity in the human motor cortex by paired associative stimulation. *Brain* (2000) 123(Pt 3):572–84. doi:10.1093/brain/123.3.572
5. Wolters A, Sandbrink F, Schlottmann A, Kunesch E, Stefan K, Cohen LG, et al. A temporally asymmetric Hebbian rule governing plasticity in the human motor cortex. *J Neurophysiol* (2003) 89(5):2339–45. doi:10.1152/jn.00900.2002
6. Schecklmann M, Volberg G, Frank G, Hadersdorfer J, Steffens T, Weisz N, et al. Paired associative stimulation of the auditory system: a proof-of-principle study. *PLoS One* (2011) 6(11):e27088. doi:10.1371/journal.pone.0027088
7. Santarelli R, Maurizi M, Conti G, Ottaviani F, Paludetti G, Pettorossi VE. Generation of human auditory steady-state responses (SSRs). II: addition of responses to individual stimuli. *Hear Res* (1995) 83(1–2):9–18. doi:10.1016/0378-5955(94)00185-S
8. Müller N, Schlee W, Hartmann T, Lorenz I, Weisz N. Top-down modulation of the auditory steady-state response in a task-switch paradigm. *Front Hum Neurosci* (2009) 3:1. doi:10.3389/neuro.09.001.2009
9. Plourde G, Stapells DR, Picton TW. The human auditory steady-state evoked potentials. *Acta Otolaryngol Suppl* (1991) 491:153–9; discussion 160. doi:10.3109/00016489109136793
10. Gutschalk A, Mase R, Roth R, Ille N, Rupp A, Hahnel S, et al. Deconvolution of 40 Hz steady-state fields reveals two overlapping source activities of the human auditory cortex. *Clin Neurophysiol* (1999) 110(5):856–68. doi:10.1016/S1388-2457(99)00019-X
11. Suzuki T, Kobayashi K, Umegaki Y. Effect of natural sleep on auditory steady state responses in adult subjects with normal hearing. *Audiology* (1994) 33(5):274–9. doi:10.3109/00206099409071887
12. Presacco A, Bohorquez J, Yavuz E, Ozdamar O. Auditory steady-state responses to 40-Hz click trains: relationship to middle latency, gamma band and beta band responses studied with deconvolution. *Clin Neurophysiol* (2010) 121(9):1540–50. doi:10.1016/j.clinph.2010.03.020
13. Brugge JF, Nourski KV, Oya H, Reale RA, Kawasaki H, Steinschneider M, et al. Coding of repetitive transients by auditory cortex on Heschl's gyrus. *J Neurophysiol* (2009) 102(4):2358–74. doi:10.1152/jn.91346.2008
14. Godey B, Schwartz D, de Graaf JB, Chauvel P, Liegeois-Chauvel C. Neuromagnetic source localization of auditory evoked fields and intracerebral evoked potentials: a comparison of data in the same patients. *Clin Neurophysiol* (2001) 112(10):1850–9. doi:10.1016/S1388-2457(01)00636-8
15. Bidet-Caulet A, Fischer C, Besle J, Aguera P, Giard M, Bertrand O. Effects of selective attention on the electrophysiological representation of concurrent sounds in the human auditory cortex. *J Neurosci* (2007) 27(35):9252–61. doi:10.1523/JNEUROSCI.1402-07.2007

16. Eckert J, Lang N, Maurer K. *Praxis der evozierten Potentiale: SEP, AEP, MEP, VEP; mit 60 Tabellen*. 2nd ed. Darmstadt: Steinkopff (2005).
17. Lehrl S. *Manual zum MWT-B*. 5th ed. Balingen: Spitta-Verl (2005).
18. Pridmore S, Fernandes Filho JA, Nahas Z, Liberatos C, George MS. Motor threshold in transcranial magnetic stimulation: a comparison of a neurophysiological method and a visualization of movement method. *J ECT* (1998) 14(1):25–7. doi:10.1097/00124509-199803000-00004
19. van Doren J, Langguth B, Schecklmann M. Electroencephalographic effects of transcranial random noise stimulation in the auditory cortex. *Brain Stimul* (2014) 7:807–12. doi:10.1016/j.brs.2014.08.007
20. Langguth B, Zowe M, Landgrebe M, Sand P, Kleinjung T, Binder H, et al. Transcranial magnetic stimulation for the treatment of tinnitus: a new coil positioning method and first results. *Brain Topogr* (2006) 18(4):241–7. doi:10.1007/s10548-006-0002-1
21. Delorme A, Makeig S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J Neurosci Methods* (2004) 134(1):9–21. doi:10.1016/j.jneumeth.2003.10.009
22. Benninghoff A, Drenckhahn D. *Taschenbuch Anatomie*. 1st ed. München: Elsevier, Urban & Fischer (2008).
23. Gueguin M, Le Bouquin-Jeannes R, Faucon G, Chauvel P, Liegeois-Chauvel C. Evidence of functional connectivity between auditory cortical areas revealed by amplitude modulation sound processing. *Cereb Cortex* (2006) 17(2):304–13. doi:10.1093/cercor/bhj148
24. Møller AR, Langguth B, de Ridder D, Kleinjung T. *Textbook of Tinnitus*. New York, NY: Springer New York (2011).
25. Norena AJ, Eggermont JJ. Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. *Hear Res* (2003) 183(1–2):137–53. doi:10.1016/S0378-5955(03)00225-9
26. Roberts LE, Moffat G, Baumann M, Ward LM, Bosnyak DJ. Residual inhibition functions overlap tinnitus spectra and the region of auditory threshold shift. *J Assoc Res Otolaryngol* (2008) 9(4):417–35. doi:10.1007/s10162-008-0136-9
27. Langguth B, de Ridder D. Chapter 36 – Tinnitus: therapeutic use of superficial brain stimulation. In: Lozano AM, Hallett M, editors. *Handbook of Clinical Neurology: Brain Stimulation*. Elsevier (2013). p. 441–67. Available from: http://www.sciencedirect.com/science/article/pii/B978044453497200036X
28. Langguth B, Schecklmann M, Lehner A, Landgrebe M, Poeppl TB, Kreuzer PM, et al. Neuroimaging and neuromodulation: complementary approaches for identifying the neuronal correlates of tinnitus. *Front Syst Neurosci* (2012) 6:15. doi:10.3389/fnsys.2012.00015

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Targeting Heterogeneous Findings in Neuronal Oscillations in Tinnitus: Analyzing MEG Novices and Mental Health Comorbidities

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Tinnitus is a prevalent phenomenon and bothersome for people affected by it. Its occurrence and maintenance have a clear neuroscientific tie and one aspect are differences in the neuronal oscillatory pattern, especially in auditory cortical areas. As studies in this field come to different results, the aim of this study was to analyze a large number of participants to achieve more stable results. Furthermore, we expanded our analysis to two variables of potential influence, namely being a novice to neuroscientific measurements and the exclusion of psychological comorbidities. Oscillatory brain activity of 88 subjects (46 with a chronic tinnitus percept, 42 without) measured in resting state by MEG was investigated. In the analysis based on the whole group, in sensor space increased activity in the delta frequency band was found in tinnitus patients. Analyzing the subgroup of novices, a significant difference in the theta band emerged additionally to the delta band difference (sensor space). Localizing the origin of the activity, we found a difference in theta and gamma band for the auditory regions for the whole group and the same significant difference in the subgroup of novices. However, no differences in oscillatory activity were observed between tinnitus and control groups once subjects with mental health comorbidity were excluded. Against the background of previous studies, the study at hand underlines the fragility of the results in the field of neuronal cortical oscillations in tinnitus. It supports the body of research arguing for low frequency oscillations and gamma band activity as markers associated with tinnitus.

Keywords: tinnitus, neuronal oscillations, spectral analysis, magnetencephalography, auditory cortex

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INTRODUCTION

Tinnitus is a phantom auditory percept, which exists unrelated to any external source. It is – at least temporarily – familiar to up to 30% of the population and affects up to 14% as a chronic condition (Axelsson and Ringdahl, 1989; Sindhusake et al., 2003). Next to its perception as being annoying, between 1 and 3% of all people report a meaningful decrease in quality of life through the tinnitus, which can be expressed for example in sleeping disorders or depressed mood (Dobie, 2003). Hearing loss is strongly associated with tinnitus and triggering underlying maladaptive subcortical and cortical processes such as hypersynchronicity, hyperactivity, and burst firing (Shore et al., 2016). Markers of ongoing cortical activity are neuronal oscillations which represent the "rhythmic fluctuations in the excitability of neurons or populations of neurons" (Cohen, 2014).

Several studies in the past focused on neuronal oscillations in tinnitus (see Zobay et al., 2015) and tried to establish theories based on their results. Weisz et al. (2005) found an increase in delta and a decrease in alpha frequency band over temporal regions, nicely fitting into the established framework of alpha as a mechanism of an inhibitory balancing rhythm (Jensen and Mazaheri, 2010). A lack of alpha power – especially in the temporal regions where the auditory cortex is located – might thus represent a lack of inhibition in these areas. This might correspond to the above mentioned hyperactivity and increased spontaneous firing rate of the auditory cortex. Additional support for the idea of alpha oscillations as a key mechanism for tinnitus comes from research on comparable phenomena such as auditory hallucinations: Müller et al. (2014) for instance presented popular melodies to their subjects. In short gaps in the music, which were filled with pink noise, subjects who reported that they had the impression the melody would continue, showed decreased levels of alpha activity compared to those who did not report that the melody continued for them. Leske et al. (2014) demonstrated that the strength of the illusionary percept of the Zwicker tone negatively correlated with alpha frequency band power. Therefore, both studies show some form of reduced alpha activity in auditory areas if an auditory illusion is present. Recent neurofeedback studies further support this framework (Hartmann et al., 2014): if tinnitus patients were able to alternate, e.g., increase the power of alpha oscillations in their temporal areas of their brain, their tinnitus percept was altered as well, as it got more quiet. Despite its appealing and intuitive character of the findings in the alpha power band, results are not conclusive, as there are studies which do not report any anomalies in the alpha spectrum of tinnitus patients (Adjamian et al., 2012).

Two other prominent models explaining the role of neuronal oscillations in tinnitus make assumptions about theta/delta and gamma oscillations. One model thereby focuses on thalamocortical dysrhythmia (TCD, Llinas et al., 1999; Adjamian et al., 2012; De Ridder et al., 2015). A disrupted communication between the thalamus and cortical areas, that results in symptomatology, is the central idea of this model. This pathological communication is thought to be expressed in an increase in the theta and gamma activity (Llinas et al., 1999; Adjamian et al., 2012; De Ridder et al., 2015). The other model focuses on predictive coding, a current trend in neuroscience. This approach assumes that the brain is constantly producing predictions of the input and if those predictions are violated a prediction error is encoded. Sedley et al. (2016) state that spontaneous activity in the sub-cortical auditory pathway, the so-called tinnitus precursor, is existent but usually neglected in favor of the concept "silence." Tinnitus emerges, if the prediction and the precursor converge. This means, the concept or prediction of silence is abandoned. This occurs, if the precursor gains precision and lastly replaces the prediction "silence." Another option is, that the precursor codes a more intense tinnitus than the prediction would forecast. So in both cases, no-tinnitus and (less intense) tinnitus, the model would anticipate a prediction error, as there is a discrepancy between the prediction (silence or less intense tinnitus) and the precursor. This prediction error reflects a suppression of the tinnitus. Following this hypothesis, in case of tinnitus the prediction error would be reduced as prediction and precursor match. Their model also covers the specific prediction of neuronal oscillations: low frequencies as theta or delta and gamma oscillations, representing the incident of prediction errors, are expected. There is also some support for these suppositions in the research body of tinnitus, yet the results are still quite heterogeneous (Zobay et al., 2015). Taken together, results of previous studies offered heterogeneous results, which can be divided in evidence favoring the model of an importance of alpha oscillations, as well as models based on TCD or predictive coding, which underline the importance of low frequency oscillations.

Possible reasons for this heterogeneity in the different studies are hard to trace. They could represent systematic changes between the compared groups, yet influences through many other factors are likely, which may smear or veil the true effects. An overview of additional technical factors and aspects regarding data handling and data analysis of oscillatory brain activity, which could affect the results, are covered in a paper by Gross et al. (2013). Besides these technical and analyses-specific aspects pointed out in that paper, also biological factors [e.g., arousal level, hearing loss (Adjamian et al., 2012), the menstrual cycle (Brötzner et al., 2014)] as well as psychological variables (instruction, state of mind, mental health status) may affect neuronal oscillations. Additionally, sample sizes are varying between studies and are often considered to be too small (cf. Adjamian, 2014). In general the question was raised, if healthy subjects and patients have the same experience with the performed measurements. Experienced participants (e.g., persons who participated in multiple experiments) might show less arousal, less vigilance, than unexperienced participants leading to differential oscillatory resting state activity such as reduced alpha. A need to quantify those possible differences was acclaimed (Diaz et al., 2013). This question is of special importance for the research on neural oscillation in tinnitus, as tinnitus patients are often novices to the MEG and the healthy controls often function as control subjects on a regular basis for different studies and therefore they are no novices to the MEG measurement any more.

Of course, those confounding variables are always present in neuroscientific research. It is typically assumed that those variables are equally distributed between the groups and therefore their veiling effect is canceled out. However, in resting state measurements they may be more influential, as the measured signal is more subtle than for example evoked brain activity. Past research has shown, that there is powerful and meaningful output arising from this mode of measurement (resting state) in basic as well as clinical research (e.g., see Başar and Güntekin, 2008). Electrophysiological resting state measurements in tinnitus might be even more interesting to look at as these measurements are with high ecological validity as they are typically recorded in rather quiet surroundings without external activity/stimulation (e.g., no external source, permanently present).

Another important factor, which might add to the heterogeneity of the results in the research on tinnitus, are psychological comorbidities. Those are high in tinnitus patients (Zirke et al., 2010) and some comorbidities are known to have oscillatory correlates (e.g., for Depression: Jiang et al., 2016, for bipolar disorder: Degabriele and Lagopoulos, 2009). In tinnitus research is a focus on identifying underlying neural circuits of tinnitus distress and depression. For the former correlates in high alpha and beta band have been found, especially in right frontal areas, and for the latter in some studies alpha band correlations, here more left frontal lateralized; both constructs have a presumed neuronal overlap in parahippocampal areas (Vanneste et al., 2010; Joos et al., 2012; Meyer et al., 2017). Thus, another aim of the study at hand was to evaluate the impact of comorbid psychological disorders or rather the effect left, if psychological comorbidities in the sample are ruled out. In clinical research, conducting a DSM (Diagnostic and statistical Manual of Mental Disorders) based semi-structured interview covering psychological disorders (SKID, German version, Wittchen et al., 1997) is considered to be the gold standard for detecting and assessing mental health. Therefore, we screened a subgroup of tinnitus patients as well as healthy controls for psychological comorbidities with this instrument in order to detect any mental health disorders.

Taken the current body of research, we tried to shed more light onto the neuronal oscillations in tinnitus based on (a) a sufficiently large sample size (b) by approximation of potential confounding variables of being a novice in the measuring procedure and (c) by ruling out any mental health comorbidity.

MATERIALS AND METHODS

Subjects

Fifty-nine participants having chronic tinnitus were recruited and measured. Due to artifacts (e.g., eye blinks, muscle artifacts), 13 dropped out, leaving 46 participants (mean age ± standard deviation: 41.59 ± 9.92 years; 46% female) in the tinnitus group for data analysis. Fifty-seven healthy controls were recruited, in 15 cases the data did not meet our requirements for analysis (i.e., 90 artifact-free trials) so we conducted our analysis with 42 healthy control participants without tinnitus (39.52 ± 12.05 years; 62% female). There was no significant group difference regarding age and gender ($p > 0.05$). The study protocol was approved by the ethics committee of the Department of Psychology of the University of Münster and was conducted according to the Declaration of Helsinki. Each participant signed informed consent prior to the investigation. Recruitment was conducted through advertisements in local newspapers, flyers in public places, doctor's offices (ENT) and our homepage.

Subgroup of Novices

Twenty-nine subjects with a chronic tinnitus perception (mean age ± standard deviation: 39.00 ± 10.69 years; 48% female) and 26 healthy control participants without any tinnitus perception (42.65 ± 11.95 years; 62% female) were analyzed as a subgroup. All subjects participated for the first time in a MEG measurement. There was no significant group difference regarding age and gender ($p > 0.05$) between these subgroups.

Subgroup of Psychological Comorbidity Free Subjects

Out of the whole sample 40 subjects (20 per group) were screened for psychological disorders with the SKID. Two participants of the tinnitus group fulfilled the criteria of a mental health disorder (Major depressive Episode, Alcohol Abuse) and were therefore excluded from analysis. The other drop-outs ($n = 10$) were eliminated as their artifact free number of epochs was not sufficient. So 14 subjects with tinnitus (44.36 ± 13.45 years; 50% female) and 14 subjects without tinnitus (42.86 ± 10.26 years; 43% female) having no psychological disorder were analyzed. There was no significant group difference regarding age and gender ($p > 0.05$). For a graphical overview of the different groups analyzed in this paper see **Figure 1**.

Procedures

Five minutes resting state MEG recordings were collected by means of a 275 channel whole-head MEG system (OMEGA 2005 WC, CTF Systems, Inc., Port Coquitlam, BC, Canada) with first order axial gradiometers placed in a magnetically shielded and acoustically quiet room. The participants sat upright in a comfortable position; head position was supported through cotton pad stabilization in the dewar and constantly monitored. During data acquisition participants were instructed to look at a black screen with their eyes open [52 cm × 40 cm (WxH)] at a distance of 90 cm and 'to relax doing nothing.' A continuous data stream was acquired and digitally sampled at a rate of 600 Hz. The 5 min recordings of resting state were always performed prior to further MEG acquisitions used for other studies (Stein et al., 2014; Wunderlich et al., 2015; Domschke et al., 2016; Zwanzger et al., 2016).

Preprocessing and Data Analysis

Sensor Space

The recorded data were analyzed using the FieldTrip toolbox (Oostenveld et al., 2011) under Matlab (The MathWorks, Natick, MA, United States, Version R2013b). An independent component analysis by means of "runica algorithm" (Makeig et al., 1997) was performed in order to identify the strongest components corresponding to ocular, cardiac, and muscle artifacts. Based on their statistical performance, the individual independent components for each subject were rejected from the data in an automated procedure (Dammers et al., 2008). The continuous data stream was then segmented into 150 epochs of 2 s each. A 50 Hz notch filter (plus its harmonics) was applied to eliminate disturbances by the electric power supply. In order to analyze relevant brain waves, an offline 4^{th} order Butterworth low-pass filter (cut-off frequency of 45 Hz) was applied to the data that were DC offset corrected based on the whole epoch length. Additionally, trials containing channels with a signal range larger than 2.5 pT were regarded as artifact-contaminated and excluded. Ninety of the remaining trials were

randomly chosen for further analysis. Participants with a residual trial number below 90 were excluded. To account for intra-individual head positions during the MEG measurements, the data was projected onto a common sensor space (Knösche et al., 2002). Axial gradiometer recorded data were further transformed to planar gradiometers by taking the norm of the first spatial derivatives in polar and azimuthal directions for each sensor (Knösche and Bastiaansen, 2002). This was done for a better interpretation of the location of the activation picked up by the coils.

In the following, a spectral analysis was performed by means of a fast Fourier transformation (FFT) between DC and 30 Hz after applying a Hanning window to each epoch. Then, for each subject the power spectral density averaged across epochs was normalized by the individual overall spectral power (power spectral density averaged across all channels and frequencies).

Source Space

As most models have a prediction of neuronal activity in the auditory cortex (Zobay et al., 2015), we restricted the source space analysis to this area. To analyze the neuronal activity in the estimation of the auditory areas, the Brain Electrical Source Analysis software (BESA Research 6.0, Megis Software) was used. The recorded data was imported into BESA. In order to circumvent referring to data being processed by FieldTrip routines for the sensor space analysis, the data preprocessing has been repeated for the source space analysis using almost the same steps in BESA. Thereby we followed the FieldTrip approach described above as closely as possible. To eliminate artifact activity as eye blinks, movements or cardiac activity from the brain activity, an ICA was calculated as implemented in BESA (Lee et al., 1999). Independent component analysis using an extended infomax algorithm for mixed subgaussian and supergaussian sources was used (Lee and Lewicki, 2002) and identified artifact contaminated components were subtracted from the raw data. Data was again cut into epochs of 2 s and 1 Hz high pass and 180 Hz low pass filters were applied to the data. Trials with an amplitude range over 2.5 pT at any MEG sensor were regarded as contaminated and rejected.

Based on a source montage template for auditory brain regions (three temporal sources in the left and three symmetric sources in the right hemisphere; see also **Supplementary Figure S1**), we reconstructed the source strength waveforms (cf. Scherg et al., 2002) using BESA. This auditory source montage is based on literature on auditory activity (an overview of the exact procedure is provided in Scherg et al., 2002). Afterward a FFT frequency analysis was calculated for each source comprising the source montage. The obtained normalized power spectrum was exported to SPSS (IBM SPSS Statistics for Macintosh, Version 24.0) for further statistical analysis.

Statistical Analysis

Sensor Space

To check for group differences (tinnitus vs. no tinnitus) in the *a priori* defined frequency bands {delta [1–4 Hz], theta [5–7 Hz], alpha [8–12 Hz] (low alpha [8–10 Hz] and high alpha [10–12 Hz]), beta [13–30 Hz], gamma [31–45 Hz]}, non-parametric statistical tests were calculated. Cluster-based statistics were calculated to determine significance probabilities based on a permutation distribution (Monte Carlo). For each frequency band 1000 permutations were drawn. The Monte Carlo p-values were set to $p < 0.025$ on sensor and $p < 0.05$ on cluster level and the used statistic for the permuting were an independent t- tests.

These analyses were done for the whole group comparison (1), the subgroups of novices (2) and psychological comorbidity-free subjects (3) (all: tinnitus vs. no tinnitus).

Source Space

The spectral components of the temporal source montage extracted from the BESA analysis were averaged over the six individual sources in the auditory area for each frequency band and normalized (over all auditory sources and frequencies). Using a one-way ANOVA in SPSS we compared the frequency bands. As we tested for seven frequency bands, all p-values were Bonferroni-corrected for seven comparisons.

RESULTS

Sensor Space

All Participants

Visual inspection of the spectral power resembles the power distribution reported by Weisz et al. (2005), indicating an overall reduced alpha power in the tinnitus group and a slight increase at delta frequencies (cf. **Figure 2**) compared to the control group. A significant difference in the delta frequency band ($p = 0.048$) was located at a right fronto-temporal area (cf. **Figure 2**). In spite of its visual appearance, groups did not differ in the alpha band (8–12 Hz, $p = 0.18$) nor in the alpha sub-bands (low alpha; 8–10 Hz, $p = 0.178$, high alpha; 10–12 Hz, $p = 0.247$).

Groups did also not differ in the other frequency band (theta, beta, gamma; all $p > 0.05$). To explain the discrepancy between the visualized data (the plot, see **Figure 2**) and the statistics, we

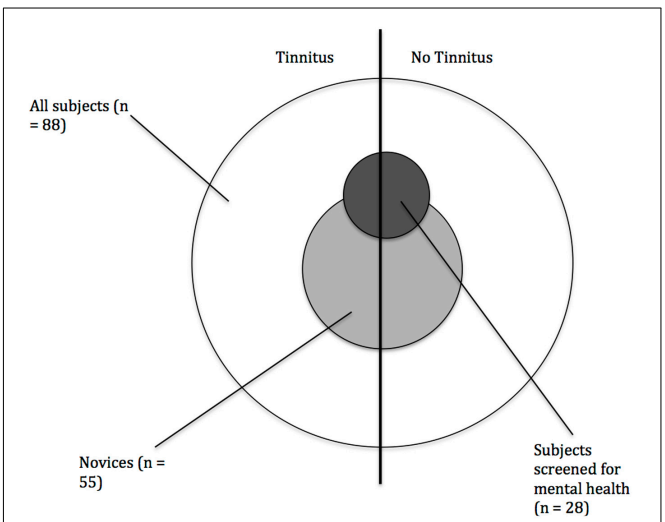

FIGURE 1 | Scheme of the organization of the subjects.

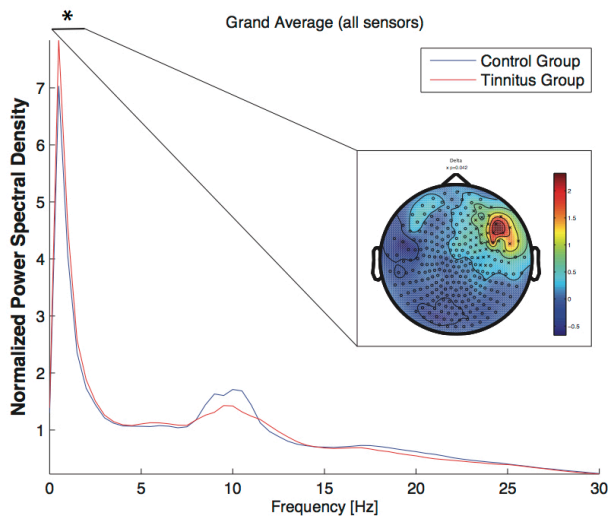

FIGURE 2 | Power spectral density across all sensors for the tinnitus group (blue line) and the control group (red line). The power spectral density was averaged across all epochs and was normalized by the overall spectral power (channels × frequency bins). The significant difference (*) in the delta band is depicted as topographic plot (tinnitus minus controls). The circles ('o') mark a sensor, the cross ('x') stands for a sensor, where a significant difference was found between the groups. The spectrum was cut off at 30 Hz to highlight the relevant frequency bands.

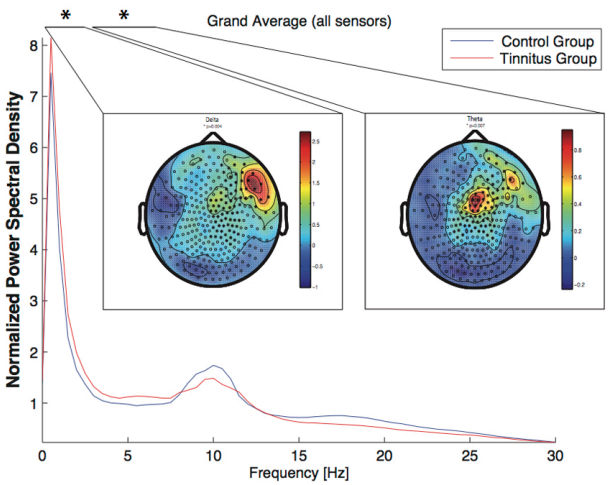

FIGURE 3 | Power spectral density across all sensors for the *Novices* group (tinnitus subjects: blue line; control group: red line). The power spectral density was averaged across all epochs and was normalized by the overall spectral power (channels × frequency bins). The significant difference (*) in the delta and the theta band is depicted as topographic plot (tinnitus minus controls). The circles ('o') mark a sensor, the star ('*') stands for a sensor, where a significant difference was found between the groups. The spectrum was cut off at 30 Hz to highlight the relevant frequency bands.

did a *post hoc* analysis of the alpha power, which displayed a considerable variance of the alpha power values in the patient group and therefore impeding the statistics to become significant.

Novices Only

Again, per visual inspection, the alpha frequency power in the novices (tinnitus vs. no tinnitus) appears to be different compared to those of the group of all subjects, yet this could not be affirmed through our statistical analysis. In the analysis of the MEG-Novices (tinnitus vs. no tinnitus) we found a significant difference in the delta (1–4 Hz, $p < 0.01$) and theta (4–8 Hz, $p < 0.01$, see **Figure 3**) frequency band. Again, all other frequency bands did not differ significantly between tinnitus subjects and healthy controls ($p > 0.05$).

Psychological Comorbidity Free Subjects Only

No significant differences between the group of mentally healthy tinnitus subjects and subjects without tinnitus occurred in the oscillatory resting state activity using the permutation testing on sensor level ($p > 0.05$, see **Figure 4**).

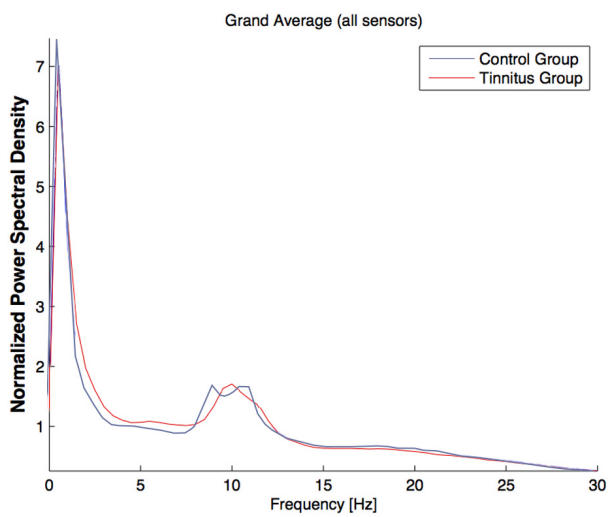

FIGURE 4 | Power spectral density across all sensors for the *Psychologically Healthy* group (tinnitus subjects: blue line; control group: red line). The power spectral density was averaged across all epochs and was normalized by the overall spectral power (channels × frequency bins). The spectrum was cut off at 30 Hz to highlight the relevant frequency bands.

Source Space

All Participants

When comparing the complete tinnitus group against the healthy controls using the BESA source reconstruction of oscillatory activity in the auditory areas, we find a significant difference in the theta and gamma band [one-way ANOVA with group as factor; theta: $F(1,86) = 8.403$, $p = 0.005$; gamma: $F(1,86) = 9.408$, $p = 0.003$, see **Figure 5**]. No significant difference emerged in the other frequency bands [$F(1,86) < 1.603$, $p > 0.209$].

Novices Only

A significant difference resulted in the theta and gamma band in the temporally located sources [theta: $F(1,53) = 18.026$, $p < 0.001$; gamma: $F(1,53) = 10.760$, $p = 0.002$; see **Figure 6**]. The other frequency bands did not differ significantly ($p > 0.05$).

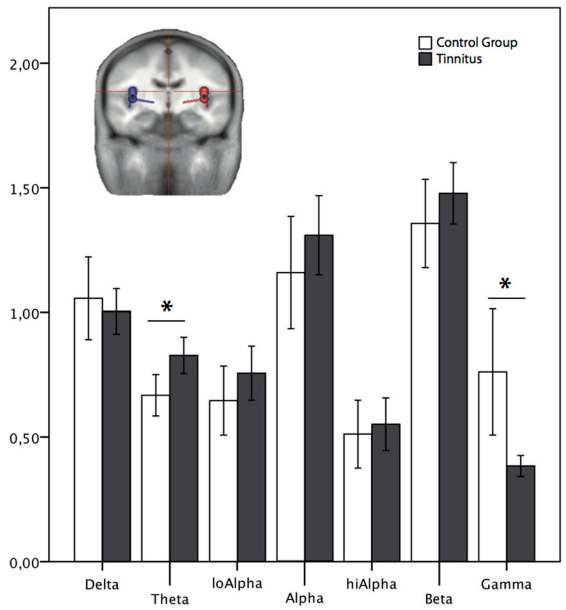

FIGURE 5 | Normalized power spectrum of the source waveforms, reconstructed in auditory areas. *Marks a significant difference between the groups. The dipoles (blue: left, red: right side) in the head scheme show exemplarily four of six locations of the source montage. The same locations and orientations were used for both groups. LoAlpha = low alpha frequency range (8–10 Hz); hiAlpha = High alpha frequency range (10–12 Hz). Error bars symbolize ±2 standard errors.

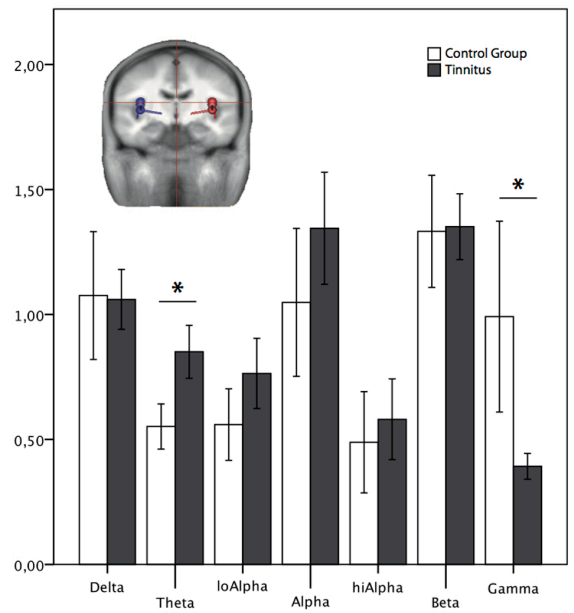

FIGURE 6 | Normalized power spectrum of the source waveforms, reconstructed in auditory areas for the group of *Novices* only. * Marks a significant difference between the groups. The dipoles (blue: left, red: right side) in the head scheme show exemplarily four of six locations of the source montage. The same locations and orientations were used for both groups. LoAlpha = low alpha frequency range (8–10 Hz); hiAlpha = high alpha frequency range (10–12 Hz). Error bars symbolize ±2 standard errors.

Psychological Comorbidity Free Subjects Only

There was no significant difference for any of the frequency bands between the control and the experimental group for the group, in which psychological disorders were measured and excluded [$F(1,26) < 2.587$; $p > 0.05$, see **Figure 7**].

DISCUSSION

To the best of our knowledge, this is the first study investigating the neural oscillations of a sample comprising 88 subjects with and without tinnitus. Due to this large sample size, results of the current study can be assumed to be valuable with regard to questions concerning the role of neural oscillations in tinnitus.

Encompassing the whole group of participants, we found an increase in the delta spectrum in sensor space in the right frontal–temporal area of the coils. This means we found differences in the slow wave power of delta between the tinnitus and the control group in the directly picked up signal, the sensor space. The depicted activity here could correspond to auditory areas, but other sources are also imaginable. When localizing the origin of the signal within the brain, the analysis in source space, we found a difference when focusing on the auditory cortical areas for theta, another slow wave frequency. Sensor and source space findings agree in the coinciding occurrence of low oscillatory frequencies. Besides this, we found a difference in high frequency activity, namely a significant reduction in gamma band in source space, which is not detectable in our sensor space analysis.

Following the goal to rule out one confounding factor by restricting the analysis to subjects from both groups who were in MEG for the first time, the novices, we saw similar results: again, we found the difference in delta activity in sensor space, but additionally also in the theta frequency band. Furthermore, in source space we found a significant difference in the theta and gamma band in areas associated with auditory processing.

When analyzing the subgroup of subjects, who had been screened for psychological disorders and had been adjudged to be without mental health comorbidity, we did not find any difference between the groups of tinnitus subjects and healthy controls neither in sensor nor in source space.

According to Zobay et al. (2015), the TCD model would rather predict differences in the theta than the delta band. Yet, delta and theta frequencies are thought to be originating from the same source and are often treated equally (Pierzycki et al., 2016). The increase in slow wave activity (delta/theta) is in line with the TCD as well as the predictive coding model and previous findings (Weisz et al., 2005; Adjamian, 2014; De Ridder et al., 2015; Sedley et al., 2016). And, according to these models, we would also have expected changes in the gamma activity. With respect to the model of De Ridder et al. (2015) one would expect an increase in gamma power, whereas the integrative model of Sedley et al. (2016) would be predict a gamma decrease in the tinnitus population. In our sample we found a reduction in gamma band activity in the tinnitus group. So, these findings would be in line with the theoretical assumptions of Sedley et al. (2016)

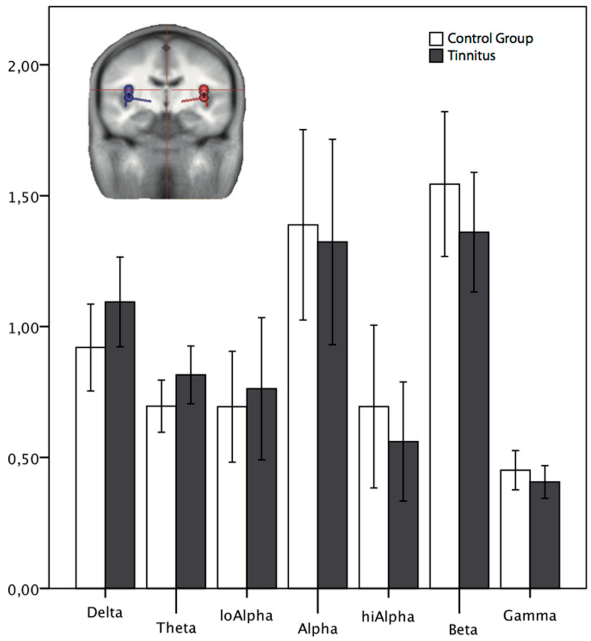

FIGURE 7 | Normalized power spectrum of the source waveforms, reconstructed in auditory areas for the group of *psychologically healthy*. The dipoles (blue: left, red: right side) in the head scheme show exemplarily four of six locations of the source montage. The same locations and orientations were used for both groups. LoAlpha, low alpha frequency range (8–10 Hz); hiAlpha, high alpha frequency range (10–12 Hz). Error bars symbolize ±2 standard errors.

that the mismatch in tinnitus patients is reduced. As described above, here prediction ('tinnitus') and the precursor would be consistent and therefore no mismatch would be elicited. Evidence for this assumptions is derived from intracranial measurements in a single-case study (Sedley et al., 2015) as well as a group study (Sedley et al., 2012), where residual inhibition was used to manipulate the tinnitus. Additionally, a decrease in gamma activity was reported by Müller et al. (2013) after they used rTMS stimulation and was associated with an increase in tinnitus loudness. But gamma activity in general might be difficult to measure: research from other fields of domains, e.g., in the visual field (Muthukumaraswamy et al., 2010), has shown, how unpredictable and therefore problematic it is to trace gamma oscillations. Furthermore, gamma networks seem to be less reliable, too (Jin et al., 2011), which adds up to the intricacy of analyzing gamma activity. This might also explain, why we found the gamma results in source space only. In source space we have a better signal to noise ratio and therefore were more prone to pick up subtle signals as presumably the gamma band.

A priori we defined our source of interest in the auditory areas and therefore limited out analysis to the estimation of this region. An analysis with less beforehand defined restrictions or even network analysis, could possibly add insight to the findings of this paper. Especially, a replication of the findings of the gamma activity would be needed to validate our results. As shown in this paper, calculating both analysis (sensor and source space) is fruitful, as they show overlapping, yet not redundant results, which can trigger more comprehension.

To shed light on possible confounders which influence the neuronal activity we examined the factor of "being a novice." Next to a different result in sensor space (finding a difference in theta and delta band), we saw in source space the same results as for the whole group. A comparison between novices and non-novices within the two groups (looking at tinnitus novices vs. tinnitus non-novices and the same for the control group) also would have been interesting; unfortunately these analyses were not possible as the two groups differed significantly in their age. In our sample, the novices were older than the non-novices. Students are our main resource of subjects and the novices were specifically recruited in the public and covered a broader age range. Concluding, our findings hint that this factor ("being a novice") does not seem to have a meaningful impact on the results.

If we follow the above mentioned idea, that the more you control for confounders, the more precise are the results, this effect should also have been visible in the second subgroup, the group of mentally healthy participants. Conversely, this did not appear. As effects are expected to be subtle (Adjamian, 2014), the most probable reason for that might be the sample size of this subgroup (14 per group). This might have been too small to see a significant effect. Another possibility is, that, if you carefully control for mental health comorbidities, some oscillatory effects between the groups (tinnitus and non-tinnitus) disappear. Psychological disorders do reflect in the neuronal firing pattern and might account for some variance between the groups if comparing a tinnitus group against a group of non-tinnitus sufferers. The connection between tinnitus and psychological disorders is complex and its direction (e.g., causality, mediation, vulnerability) unclear. It is likely that comorbid psychological disorders might interfere with tinnitus perception, as postulated, e.g., for somatoform disorders (Signal-Filter-Modell according to Rief and Barsky) (Voderholzer and Hohagen, 2017). Here, the assumption is, that attentional processes, anxieties and depressive mood influence the cortical perception of the bodily symptoms. Next to this enhancement of bodily symptoms (the tinnitus correlate would most likely be tinnitus loudness), Zirke et al. (2010) state, that psychological comorbid disorders might hinder the habituation to the tinnitus itself. Furthermore, it is likely that the mental health status might also influence the overall distress perceived through the tinnitus. Handing out a questionnaire covering, e.g., the depressive symptomatic, is a good tool to estimate symptoms, yet these do not cover the full spectrum of mental health and are more a clue, not a tool to diagnose a person. Therefore the approach of this paper, performing the structured interviews covering the majority of psychological disorders, like the SKID or the CIDI (see Zirke et al., 2013), could be a step into the future to surely be aware of all mental health disorders in the participant group. The question, which oscillatory pattern between the two groups remains, if we control for as many confounders as possible, is yet to be answered.

Next to the two factors analyzed in this paper, many others may contribute to the heterogeneity within the measurement of neural oscillations. One step evolving from this issue is the

development and handing out of questionnaires covering, e.g., cognitive states. Diaz et al. (2013) define the resting state "as a multi-faceted cognitive construct" and developed a questionnaire to quantify these cognitions. Seven dimensions were identified which were present during resting state measurements: discontinuity of mind, theory of mind, self, planning, sleepiness, comfort, and somatic awareness. Quantifying patient-control differences in the cognitive states might be a helpful tool for future measurements, to control more possible confounders and conduct measurements, which narrow down the true underlying difference of symptoms of tinnitus. To address this issue, other fields started projects of gathering multi-center data and meta data sharing in order to increase replicability and validity of the data (see Kafkafi et al., 2016).

One overall attempt to deal with the heterogeneity in tinnitus findings, is to define subtypes of tinnitus. TINNET (a European project on Tinnitus http://tinnet.tinnitusresearch.net) is aiming at quantifying and analyzing all aspects and facets of tinnitus and hopes to break down the heterogeneity. A workgroup of this project suggested guidelines for M/EEG measurements in tinnitus[1]. Those should make sure a better comparability of results between different labs and studies. The guideline advocates, next to other factors, the measurement of tinnitus distress, hearing loss, hyperacusis, and a resting state questionnaire. Taking them into consideration as confounders would have been helpful to rule out possible explanations. We think having quantified those factors would have helped to come to a more comprehensive and target-oriented interpretation of our results.

Another option to control for the heterogeneous characteristics in tinnitus research is to use a between-subjects-design with using tinnitus subjects only. Control group and tinnitus group may differ substantially in factors as, e.g., hearing loss or hyperacusis. With comparing subgroups within the group of tinnitus could therefore yield in more homogenous and more comparable groups. In this study we chose a comparison of tinnitus-experiencing subjects to subjects without tinnitus because we wanted to keep the comparability with other studies in the field (Weisz et al., 2005; Adjamian et al., 2012) and controlled that key aspects, as age and gender, are distributed equally in both groups.

Taken together, this study ruled out criticism of a too small sample size in the neuronal oscillations research in tinnitus. We were able to show that an analysis based on a sufficient sample size supports the significance of slow oscillatory frequencies and gamma band activity in the neuroscientific conceptualization of tinnitus. Yet, our data does not support a single model only. Targeting the heterogeneity of tinnitus on different levels, e.g., as focusing subtyping of tinnitus or, as done here, controlling more confounding variables, seems to be a necessary step to understand this phenomenon. To control the naiveté in studies is an approach to narrow down a manifold set of confounders (here: vigilance, arousal, stimulation due unfamiliarity to the situation). However, in our analysis of novices only, results did not hint toward a reduced variance in the sample and therefore to clearer results. Next to controlling factors forcefully (with excluding anyone who had experience in experiments beforehand or with a psychological comorbidity), quantifying the experience during the experiment is another option. This could, most likely, be done with questionnaires to assess those variable and take them into statistical analysis or at least into consideration when interpreting the findings. Controlling mental health comorbidities is, derived from the literature, clearly a worthy, yet very resource consuming approach. The lack of a statistical difference between the second subgroup in our study (*psychologically healthy*), could either show that whether or not those variables are controlled in a tinnitus sample, differences do disappear or that our sample size, thinned out through a relatively high drop-out rate and the time and a certain professional training demanding screening procedure, was too small to produce meaningful differences.

To sum up, we agree with previously published papers that neuronal oscillations in tinnitus are presently no distinctly defined biomarker (Pierzycki et al., 2016) and future research is needed to test the theoretically postulated models for tinnitus (e.g., TCD, predictive coding). Our study could contribute with highlighting the need of a thorough assessment of mental health in tinnitus studies and the rather minor importance of the factor 'being a novice' to the measurement. Overall, we could demonstrate a support for low neuronal frequencies as well as high frequencies to be connected with the percept of tinnitus.

AUTHOR CONTRIBUTIONS

PL, RW, AE, AL, and MJ recruited the participants. PL and AW developed and performed the data analysis. PL, AW, AE, RW, AL, and CP wrote the manuscript. All authors approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyg.2018.00235/full#supplementary-material

FIGURE S1 | Location of the AEP montage by BESA covering the auditory areas.

[1]http://tinnet.tinnitusresearch.net/images/pdf/WG3/Standardisation_Report_V5.pdf

REFERENCES

Adjamian, P. (2014). The application of electro- and magneto-encephalography in tinnitus research – methods and interpretations. *Front. Neurol.* 5:228. doi: 10.3389/fneur.2014.00228

Adjamian, P., Sereda, M., Zobay, O., Hall, D. A., and Palmer, A. R. (2012). Neuromagnetic indicators of tinnitus and tinnitus masking in patients with and without hearing loss. *J. Assoc. Res. Otolaryngol.* 13, 715–731. doi: 10.1007/s10162-012-0340-5

Axelsson, A., and Ringdahl, A. (1989). Tinnitus—a study of its prevalence and characteristics. *Br. J. Audiol.* 23, 53–62. doi: 10.3109/03005368909077819

Başar, E., and Güntekin, B. (2008). A review of brain oscillations in cognitive disorders and the role of neurotransmitters. *Brain Res.* 1235, 172–193. doi: 10.1016/j.brainres.2008.06.103

Brötzner, C. P., Klimesch, W., Doppelmayr, M., Zauner, A., and Kerschbaum, H. H. (2014). Resting state alpha frequency is associated with menstrual cycle phase, estradiol and use of oral contraceptives. *Brain Res.* 1577, 36–44. doi: 10.1016/j.brainres.2014.06.034

Cohen, M. X. (2014). *Analyzing Neural Time Series Data*. Boston, MA: Institute of Technology.

Dammers, J., Schiek, M., Boers, F., Silex, C., Zvyagintsev, M., Pietrzyk, U., et al. (2008). Integration of amplitude and phase statistics for complete artifact removal in independent components of neuromagnetic recordings. *IEEE Trans. Biomed. Eng.* 55, 2353–2362. doi: 10.1109/TBME.2008.926677

De Ridder, D., Vanneste, S., Langguth, B., and Llinas, R. (2015). Thalamocortical dysrhythmia: a theoretical update in tinnitus. *Front. Neurol.* 6:124. doi: 10.3389/fneur.2015.00124

Degabriele, R., and Lagopoulos, J. (2009). A review of EEG and ERP studies in bipolar disorder: review article. *Acta Neuropsychiatr.* 21, 58–66. doi: 10.1111/j.1601-5215.2009.00359.x

Diaz, B. A., Van Der Sluis, S., Moens, S., Benjamins, J. S., Migliorati, F., Stoffers, D., et al. (2013). The Amsterdam Resting-State questionnaire reveals multiple phenotypes of resting-state cognition. *Front. Hum. Neurosci.* 7:446. doi: 10.3389/fnhum.2013.00446

Dobie, R. A. (2003). Depression and tinnitus. *Otolaryngol. Clin. North Am.* 36, 383–388. doi: 10.1016/S0030-6665(02)00168-8

Domschke, K., Zwanzger, P., Rehbein, M. A., Steinberg, C., Knoke, K., Dobel, C., et al. (2016). Magnetoencephalographic correlates of emotional processing in major depression before and after pharmacological treatment. *Int. J. Neuropsychopharmacol.* 19:pyv093. doi: 10.1093/ijnp/pyv093

Gross, J., Baillet, S., Barnes, G. R., Henson, R. N., Hillebrand, A., Jensen, O., et al. (2013). Good practice for conducting and reporting MEG research. *Neuroimage* 65, 349–363. doi: 10.1016/j.neuroimage.2012.10.001

Hartmann, T., Lorenz, I., Müller, N., Langguth, B., and Weisz, N. (2014). The effects of neurofeedback on oscillatory processes related to tinnitus. *Brain Topogr.* 27, 149–157. doi: 10.1007/s10548-013-0295-9

Jensen, O., and Mazaheri, A. (2010). Shaping functional architecture by oscillatory alpha activity: gating by inhibition. *Front. Hum. Neurosci.* 4:186. doi: 10.3389/fnhum.2010.00186

Jiang, H., Popov, T., Jylänki, P., Bi, K., Yao, Z., Lu, Q., et al. (2016). Predictability of Depression severity based on posterior alpha oscillations. *Clin. Neurophysiol.* 127, 2108–2114. doi: 10.1016/j.clinph.2015.12.018

Jin, S.-H., Seol, J., Kim, J. S., and Chung, C. K. (2011). How reliable are the functional connectivity networks of meg in resting States? *J. Neurophysiol.* 106, 2888–2895. doi: 10.1152/jn.00335.2011

Joos, K., vanneste, S., and de Ridder, D. (2012). Disentangling depression and distress networks in the tinnitus brain. *PLoS One* 7:e40544. doi: 10.1371/journal.pone.0040544

Kafkafi, N., Agassi, J., Chesler, E. J., Crabbe, J. C., Crusio, W. E., Eilam, D., et al. (2016). Reproducibility and replicability of rodent phenotyping in preclinical studies. *Neurosci. Biobehav. Rev.* (in press). doi: 10.1016/j.neubiorev.2018.01.003

Knösche, T. R., and Bastiaansen, M. C. (2002). On the time resolution of event-related desynchronization: a simulation study. *Clin. Neurophysiol.* 113, 754–763. doi: 10.1016/S1388-2457(02)00055-X

Knösche, T. R., Lattner, S., Maess, B., Schauer, M., and Friederici, A. D. (2002). Early parallel processing of auditory word and voice information. *Neuroimage* 17, 1493–1503. doi: 10.1006/nimg.2002.1262

Lee, T. W., Girolami, M., and Sejnowski, T. J. (1999). Independent component analysis using an extended infomax algorithm for mixed subgaussian and supergaussian sources. *Neural. Comput.* 11, 417–441. doi: 10.1162/089976699300016719

Lee, T. W., and Lewicki, M. S. (2002). Unsupervised image classification, segmentation, and enhancement using ica mixture models. *IEEE Trans. Image Process.* 11, 270–279. doi: 10.1109/83.988960

Leske, S., Tse, A., Oosterhof, N. N., Hartmann, T., Müller, N., Keil, J., et al. (2014). NeuroImage the strength of alpha and beta oscillations parametrically scale with the strength of an illusory auditory percept. *Neuroimage* 88, 69–78. doi: 10.1016/j.neuroimage.2013.11.014

Llinas, R. R., Ribary, U., Jeanmonod, D., Kronberg, E., and Mitra, P. P. (1999). Thalamocortical dysrhythmia: a neurological and neuropsychiatric syndrome characterized by magnetoencephalography. *Proc. Natl. Acad. Sci. U.S.A.* 96, 15222–15227. doi: 10.1073/pnas.96.26.15222

Makeig, S., Jung, T. P., Bell, A. J., Ghahremani, D., and Sejnowski, T. J. (1997). Blind separation of auditory event-related brain responses into independent components. *Proc. Natl. Acad. Sci. U.S.A.* 94, 10979–10984. doi: 10.1073/pnas.94.20.10979

Meyer, M., Neff, P., Grest, A., Hemsley, C., Weidt, S., and Kleinjung, T. (2017). EEG oscillatory power dissociates between distress- and depression-related psychopathology in subjective tinnitus. *Brain Res.* 1663, 194–204. doi: 10.1016/j.brainres.2017.03.007

Müller, N., Leske, S., Hartmann, T., Szebényi, S., and Weisz, N. (2014). Listen to yourself: the medial prefrontal cortex modulates auditory alpha power during speech preparation. *Cereb. Cortex* 25, 4029–4037. doi: 10.1093/cercor/bhu117

Müller, N., Lorenz, I., Langguth, B., and Weisz, N. (2013). rTMS induced tinnitus relief is related to an increase in auditory cortical alpha activity. *PLoS One* 8:e55557. doi: 10.1371/journal.pone.0055557

Muthukumaraswamy, S. D., Singh, K. D., Swettenham, J. B., and Jones, D. K. (2010). Visual gamma oscillations and evoked responses: variability, repeatability and structural MRI correlates. *Neuroimage* 49, 3349–3357. doi: 10.1016/j.neuroimage.2009.11.045

Oostenveld, R., Fries, P., Maris, E., and Schoffelen, J. M. (2011). FieldTrip: open source software for advanced analysis of MEG, EEG, and invasive electrophysiological data. *Comput. Intell. Neurosci.* 2011:156869. doi: 10.1155/2011/156869

Pierzycki, R. H., McNamara, A. J., Hoare, D. J., and Hall, D. A. (2016). Whole scalp resting state eeg of oscillatory brain activity shows no parametric relationship with psychoacoustic and psychosocial assessment of tinnitus: a repeated measures study. *Hear. Res.* 331, 101–108. doi: 10.1016/j.heares.2015.11.003

Scherg, M., Ille, N., Bornfleth, H., and Berg, P. (2002). Advanced tools for digital EEG review: virtual source montages, whole-head mapping, correlation, and phase analysis. *J. Clin. Neurophysiol.* 19, 91–112. doi: 10.1097/00004691-200203000-00001

Sedley, W., Friston, K. J., Gander, P. E., Kumar, S., Griffiths, T. D., Shargorodsky, J., et al. (2016). An integrative tinnitus model based on sensory precision. *Trends Neurosci.* 39, 799–812. doi: 10.1016/j.tins.2016.10.004

Sedley, W., Gander, P. E., Kumar, S., Oya, H., Kovach, C. K., Nourski, K. V., et al. (2015). Intracranial mapping of a cortical tinnitus system using residual inhibition. *Curr. Biol.* 25, 1208–1214. doi: 10.1016/j.cub.2015.02.075

Sedley, W., Teki, S., Kumar, S., Barnes, G. R., Bamiou, D. E., and Griffiths, T. D. (2012). Single-Subject oscillatory gamma responses in tinnitus. *Brain* 135, 3089–3100. doi: 10.1093/brain/aws220

Shore, S. E., Roberts, L. E., and Langguth, B. (2016). Maladaptive plasticity in tinnitus–triggers, mechanisms and treatment. *Nat. Rev. Neurol.* 12, 150–160. doi: 10.1038/nrneurol.2016.12

Sindhusake, D., Mitchell, P., Newall, P., Golding, M., Rochtchina, E., and Rubin, G. (2003). Prevalence and characteristics of tinnitus in older adults: the blue mountains hearing study: prevalencia y características del acúfeno en adultos mayores: el estudio de audición blue mountains. *Int. J. Audiol.* 42, 289–294. doi: 10.3109/14992020309078348

Stein, A., Engell, A., Junghoefer, M., Wunderlich, R., Lau, P., Wollbrink, A., et al. (2014). Inhibition-Induced plasticity in tinnitus patients after repetitive exposure to tailor-made notched music. *Clin. Neurophysiol.* 126, 1007–1015. doi: 10.1016/j.clinph.2014.08.017

Vanneste, S., Plazier, M., Van der Loo, E., Van de Heyning, P., Congedo, M., and De Ridder, D. (2010). The neural correlates of tinnitus-related distress. *Neuroimage* 52, 470–480. doi: 10.1016/j.neuroimage.2010.04.029

Voderholzer, U., and Hohagen, F. (2017). *Therapie Psychischer Erkrankungen*. Zurich: Urban & Fische.

Weisz, N., Moratti, S., Meinzer, M., Dohrmann, K., and Elbert, T. (2005). Tinnitus perception and distress is related to abnormal spontaneous brain activity as measured by magnetoencephalography. *PLoS Med*. 2:e153. doi: 10.1371/journal.pmed.0020153

Wittchen, H. U., Zaudig, M., and Fydrich, T. (1997). *Strukturiertes Klinisches Interview Für DSM-IV, Hogrefe. Göttingen, Germany*. Available at: http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Strukturiertes+Klinisches+Interview+f?r+DSM-IV#6

Wunderlich, R., Lau, P., Stein, A., Engell, A., Wollbrink, A., Rudack, C., et al. (2015). Impact of spectral notch width on neurophysiological plasticity and clinical effectiveness of the tailor-made notched music training. *PLoS One* 10:e0138595. doi: 10.1371/journal.pone.0138595

Zirke, N., Goebel, G., and Mazurek, B. (2010). Tinnitus und psychische komorbiditäten. *HNO* 58, 726–732. doi: 10.1007/s00106-009-2050-9

Zirke, N. C., Seydel, D., Arsoy, B. F., Klapp, H., Haupt, A. J., Szczepek, H., et al. (2013). Analysis of mental disorders in tinnitus patients performed with composite international diagnostic interview. *Qual. Life Res.* 22, 2095–2104. doi: 10.1007/s11136-012-0338-9

Zobay, O., Palmer, A. R., Hall, D. A., Sereda, M., and Adjamian, P. (2015). Source space estimation of oscillatory power and brain connectivity in tinnitus. *PLoS One* 10:e0120123. doi: 10.1371/journal.pone.0120123

Zwanzger, P., Klahn, A. L., Arolt, V., Ruland, T., Zavorotnyy, M., Sälzer, J., et al. (2016). Impact of electroconvulsive therapy on magnetoencephalographic correlates of dysfunctional emotional processing in major depression. *Eur. Neuropsychopharmacol.* 26, 684–692. doi: 10.1016/j.euroneuro.2016.02.005.

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frontiers in Human Neuroscience

ORIGINAL RESEARCH
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A Quantitative Electroencephalography Study on Cochlear Implant-Induced Cortical Changes in Single-Sided Deafness with Tinnitus

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The mechanism of tinnitus suppression after cochlear implantation (CI) in single-sided deafness (SSD) is not fully understood. In this regard, by comparing pre- and post-CI quantitative electroencephalography (qEEG), we explored cortical changes relevant to tinnitus improvement. In SSD patients who underwent CI, qEEG data were collected: (1) before CI, (2) 6 months post-operatively with CI-on, and (3) 30 min after CI-off and source-localized cortical activity/functional connectivity analyses were performed. Compared to the pre-operative baseline, the CI-on condition demonstrated significantly decreased activity in the right auditory- and orbitofrontal cortices (OFC) for the delta frequency band as well as decreased connectivity between the auditory cortex/posterior cingulate cortex for the delta/beta2 bands. Meanwhile, compared to the CI-off condition, the CI-on condition displayed decreased activity in the right auditory cortices/OFC for the delta band, and in bilateral auditory cortices, left inferior frontal cortex/OFC for the gamma band. However, qEEG analyses showed no significant differences between the CI-off and baseline conditions. CI induced overall decreased cortical activity and functional connectivity. However, judging from no differences between the CI-off and baseline conditions, CI-induced cortical activity and functional connectivity changes are not by cortical plastic changes, but by dynamic peripheral reafferentation.

Keywords: single side deafness, tinnitus, cochlear implantation, electroencephalography, dynamic peripheral reafferentation

INTRODUCTION

Tinnitus, the conscious perception of sound in the absence of a corresponding external acoustic stimulus (Baguley et al., 2013), afflicts 10–15% of the adult population and interferes severely with the quality of life of 5–26% of the affected population (Heller, 2003; Krog et al., 2010). The development of tinnitus is frequently deemed to be a neuroplastic response to sensory

deprivation (Eggermont and Roberts, 2004; Song et al., 2012). This assumption is supported by a transient perception of tinnitus after experimentally induced partial (Schaette et al., 2012) and complete (Del Bo et al., 2008) temporary auditory deprivation in normal subjects, and was further reinforced by lack of tinnitus in congenitally deaf animal models (Eggermont and Kral, 2016). Furthermore, analogous to phantom limb pain, the tinnitus spectrum corresponds to auditory deprived frequencies (Norena et al., 2002).

In patients with severe peripheral auditory deafferentation, reafferentation of the ascending auditory nervous system with cochlear implants (CI) may abate tinnitus. Indeed, CI improved tinnitus significantly in 66–100%of CI users with bilateral profound hearing loss (Ruckenstein et al., 2001). Also, improvement of tinnitus by CI was reported in patients with single-sided deafness (SSD) and ipsilesional debilitating tinnitus (Punte et al., 2011). In a recent meta-analysis, CI showed a statistically significant improvement in the severity of tinnitus (Blasco and Redleaf, 2014). In this regard, CI is a promising treatment option for patients with SSD and combined severe tinnitus.

However, the mechanism of tinnitus suppression after CI in patients with SSD is not fully understood. In previous literature, several mechanisms of CI-mediated tinnitus suppression have been suggested. Some researchers have claimed that acoustic masking provided by CI is the primary mechanism of tinnitus suppression, by distracting attention from tinnitus (Andersson et al., 2009; Kleinjung et al., 2009), while others have suggested that plastic changes in the central auditory system by prolonged CI stimulation (Giraud et al., 2001) and electrical stimulation resulting in contralateral residual inhibition (Souliere et al., 1992) are possible mechanisms of tinnitus suppression. These assumptions are, however, based on inferential reasoning rather than data-driven analysis.

From this perspective, a study to explore post-CI changes in patients with SSD with regard to ongoing cortical activity may be of help in further understanding the mechanism of tinnitus alterations in SSD subjects after CI. By comparing pre- and post-CI source-localized quantitative electroencephalography (qEEG) findings, we attempted to find CI-driven cortical activity changes that may have abated subjective tinnitus in patients with SSD. Additionally, by analyzing changes in functional connectivity, we sought to reveal changes in functional connections of remote brain areas that may be responsible for the improvement of tinnitus after CI.

MATERIALS AND METHODS

Participants

Four patients (three men and one woman) with unilateral acquired SSD (pure tone threshold >90 dB at 0.5, 1, 2, and 4 kHz) and ipsilateral tinnitus underwent pre-operative EEG and subsequent CI with a Med-EL device (Med-EL, Innsbruck, Austria). All patients presented with left-sided SSD and the median duration of deafness was 4.5 years (range, 9 months to 5 years). All four patients' etiology of SSD was idiopathic sudden sensorineural hearing loss. The detailed demographic characteristics of the patients are summarized in **Table 1**.

The criteria for CI in patients with SSD and tinnitus were: (1) a duration of SSD $<$ 10 years, (2) tinnitus development after SSD onset, and (3) tinnitus loudness on a numeric rating scale (NRS) $\geq$ 6 of 10 for at least 6 months that was intractable to conventional therapies including medication, tinnitus retraining therapy, and non-invasive neuromodulation such as transcranial magnetic stimulation or transcranial direct current stimulation. The exclusion criteria were: (1) severe depression with a Beck Depression Index (Beck and Steer, 1984) score $>$ 16, and (2) a presumed etiology of tinnitus other than SSD. With regard to tinnitus loudness and tinnitus-related distress, all patients were evaluated using a NRS loudness score and a tinnitus questionnaire (TQ) (Goebel and Hiller, 1994) score.

This study and all related documents were approved by the ethics committee of Antwerp University Hospital. All patients gave written informed consent before enrollment. The study procedures were carried out in accordance with the relevant guidelines and regulations.

EEG Recording

Pre-operative and 6-month post-operative EEGs were performed in all four patients. Pre-operative EEGs were recorded during resting-state for 5 min, while post-operative EEGs were recorded for 5 min under the following two conditions: (1) CI switch-on with a music stimulus (classical music from a radio channel) to the CI ear presented directly to the external audio processor via an audio cable at the most comfortable loudness level for each patient (CI-on); and (2) CI switch-off with no sound stimulus (CI-off).

Electroencephalograms were measured using the WinEEG software version 2.84.44 (Mitsar, St. Petersburg, Russia) in a room shielded against sound and stray electric fields with patients sitting upright with their eyes closed to reduce resting-state skin conductance levels in overall frequency bands (Barry et al., 2007, 2009). The EEG was sampled with 19 electrodes in the standard 10–20 International placements referenced to linked ears. While recording, impedance was maintained below 5 kΩ at all electrodes. Data were recorded with a sampling rate of 1,024 Hz using a 0.15 Hz high-pass filter and a 200 Hz low-pass filter. After initial recording, the data were processed offline by resampling to 128 Hz and band-pass filtering at 2–44 Hz by employing a fast Fourier transform filter with application of a Hanning window, and then imported into the Eureka! Software (Sherlin and Congedo, 2005) for precise artifact rejection before source-localization. All artifacts in the recorded EEG stream were removed meticulously by manual inspection.

The vigilance of all participants was checked by monitoring EEG streams to prevent unwanted changes, caused by drowsiness, such as alpha rhythm slowing or the appearance of spindles (Moazami-Goudarzi et al., 2010); no enrolled participant exhibited drowsiness-related EEG changes.

TABLE 1 | Demographic characteristics of the included subjects.

Subject number	Age (years)/sex	Contralateral hearing threshold (average of 0.5, 1, 2, and 4 kHz) (dB HL)	Duration of single-sided deafness	Etiology	Psychoacoustic characteristics of tinnitus	Frequency matching (Hz)	Perceived tinnitus loudness (dB SL)	Side of the cochlear implant	Name of the implanted device
1	53/female	20	4 years	Sudden sensorineural hearing loss	Pure tone	6000	20	Left	MED-EL Sonata ti 100 FLEX Soft electrode
2	64/male	23	5 years	Sudden sensorineural hearing loss	Pure tone	3000	10	Left	MEDEL Sonata ti 100 FLEX Soft electrode
3	47/male	15	9 months	Sudden sensorineural hearing loss	Pure tone	8000	30	Left	MED-EL Sonata ti 100 FLEX 24 electrode
4	49/male	15	5 years	Sudden sensorineural hearing loss	Narrow band noise	6000	40	Left	MED-EL Pulsar ci 100 Standard electrode

dB HL, dB hearing level; dB SL, dB sensation level.

Artifact Removal by Band-Limited Independent Component Analysis

Localization of the cortical resting-state or auditory evoked potentials in CI users via qEEG is confounded by stimulus artifacts produced by the implanted device itself. In a previous article, we described a successful method of CI artifact removal from specific bands in the EEG streams of patients with CIs using band-limited independent component analysis [BL-ICA, for further information, please refer to Kim et al. (2015)]. BL-ICA successfully removes artifacts by applying a narrow band-pass filter, which limits the number of sources and enhances the signal to noise ratio, thus allowing CI artifacts to be clearly detected and separated from other brain sources. By applying BL-ICA, all post-operative EEG data measured while listening to music were cleaned.

Source Localization Analysis

Low-resolution brain electromagnetic tomography (LORETA)-KEY software[1], dedicated to functional localization of current densities based on certain electrophysiological and neuroanatomical constraints, (Pascual-Marqui, 2002) was utilized to localize the cortical sources that generated the scalp-recorded electrical activity in each of the following eight frequency bands: delta (2–3.5 Hz), theta (4–7.5 Hz), alpha 1 (8–10 Hz), alpha 2 (10–12 Hz), beta 1 (13–18 Hz), beta 2 (18.5–21 Hz), beta 3 (21.5–30 Hz), and gamma (30.5–44 Hz) (Song et al., 2013a,b, 2014, 2015a,b; Vanneste et al., 2013; Kim et al., 2015, 2016). This software implements the lead field of Fuchs et al. (2002) that was derived from standard electrode positions realigned to a standard Montreal Neurological Institute (MNI)-152 head in combination with a boundary element method derived from the same standard anatomy (Jurcak et al., 2007). The LORETA-KEY anatomical template divides the neocortical MNI-152 volume, including the hippocampus and anterior cingulate cortex, into 6,239 voxels with dimensions of 5 mm × 5 mm × 5 mm, based on the Daemon Atlas (Lancaster et al., 2000). Anatomical labeling of significant clusters was performed automatically by a toolbox implemented in LORETA-KEY. The locations of significant clusters were initially investigated using the Anatomy toolbox (Eickhoff et al., 2005), and were reconfirmed using the Talairach and Tournoux atlas (Talairach and Tornoux, 1988). Renders were generated using the BrainNet Viewer[2] (Xia et al., 2013).

Functional Connectivity

Using the pre- and post-operative qEEG data, the extent of phase synchronization and coherence between the time series corresponding to different regions of interest (ROIs) were calculated to analyze functional connectivity. To calculate functional connectivity, we employed the built-in connectivity toolbox of the LORETA-KEY. This toolbox defines measures of linear- and non-linear dependence (i.e., coherence and phase synchronization) between multivariate time series. In the

[1]http://www.uzh.ch/keyinst/NewLORETA/Software/Software.htm

[2]http://www.nitrc.org/projects/bnv/

current study, we have calculated lagged linear coherence that excludes non-lagged parts of coherence which comprises effects of volume conduction, and effects of non-recorded sources that simultaneously drive recorded sources (Milz et al., 2014). For lagged linear coherence connectivity analysis, a total of 28 ROIs defined by Brodmann areas (BA) were selected as possible nodes based on previous literature on tinnitus: bilateral primary and secondary auditory cortices (A1s and A2s) (Rolls, 2004; Kringelbach, 2005), bilateral parahippocampus (PHC) (Landgrebe et al., 2009), bilateral dorsal/pregenual/subgenual anterior cingulate cortices (dACC/pgACC/sgACC) (Vanneste et al., 2010; De Ridder et al., 2011), bilateral posterior cingulate cortices (PCC) (Vanneste et al., 2010; Schecklmann et al., 2011), bilateral insula, bilateral precuneus, and bilateral orbitofrontal cortices (OFC) (Vanneste et al., 2010; De Ridder et al., 2011).

Statistical Analysis

To identify cortical activity differences between pre-operative resting-state and post-operative sound stimuli-induced cortical activity, between pre-operative resting-state and post-operative device-off state activity, and between post-operative device-on with sound stimuli and device-off state activity ("CI-on – CI-off"), voxel-by-voxel analysis using LORETA-KEY was performed for the eight frequency band between-condition comparisons of the current density distribution. Also, regression analyses were performed to compare between "CI-on – CI-off" and percent improvement in tinnitus loudness and between "CI-on – CI-off" and percent improvement in TQ score. For source-localized group comparison analyses, statistical non-parametric mapping (SnPM) of LORETA-KEY images was performed for each contrast using LORETA-KEY's built-in voxelwise randomization tests (5000 permutations) and employing a log-*F*-ratio statistic for independent groups with a threshold of $P < 0.01$. A correction for multiple comparisons in SnPM using random permutations (5000 permutations in the current study) has been proven to give results similar to those obtained from a comparable Statistical Parametric Mapping approach using a general linear model with multiple comparison corrections derived from random field theory (Holmes et al., 1996; Nichols and Holmes, 2002). Additionally, power spectral density (PSD) was calculated by EEGLAB toolbox (Delorme and Makeig, 2004). Topography was described based on PSD in 19 channels. The red, green, and blue colors in the topography represent maximum, mean, and minimum power, respectively, in specific bands such as delta and gamma.

For lagged linear connectivity differences, we compared differences between the pre-operative baseline, post-operative CI-off, and post-operative CI-on with music stimuli conditions, employing the *t*-statistics for groups with a threshold of $P < 0.05$, and also corrected for multiple comparisons by performing LORETA-KEY-built-in voxelwise randomization tests (5000 permutations).

All other descriptive statistical analyses were performed using the SPSS software version 20.0 (SPSS Inc., Chicago, IL, USA). For all analyses, descriptive statistical significance was set at $P < 0.05$.

RESULTS

Comparison of Changes in Visual Analog Scale Tinnitus Loudness and Tinnitus Questionnaire Scores in all Patients

Table 2 summarizes the pre- and post-operative comparisons of NRS tinnitus loudness and TQ scores. All four patients showed improved NRS loudness and TQ scores under the post-operative CI-on state compared with the pre-operative baseline. Also, compared to pre-operative NRS loudness (median, 8.5; range, 7–9) and TQ scores (median, 61; range, 52–78), the post-operative CI-on state showed improved NRS loudness (median, 4; range, 3–6) and TQ scores (median, 42.5; range, 29–56) with a trend-level significance ($P = 0.068$; $Z = 1.826$ for both comparisons, Wilcoxon signed rank test). When we compared the post-operative CI-on and off states, the CI-on state showed tinnitus alleviation with regard to NRS loudness and TQ score compared with CI-off NRS loudness (median, 8.5; range, 6–9) and TQ score (median, 61; range, 52–76) with a trend-level significance ($P = 0.068$; $Z = 1.826$ for both comparisons, Wilcoxon signed rank test). However, the comparison between the pre-operative baseline and the post-operative CI-off state showed no differences with regard to NRS loudness and TQ score ($P = 0.317$; $Z = 1.00$ and $P = 0.564$; $Z = 0.577$, respectively, Wilcoxon signed rank test) (**Table 2**).

Meanwhile, regression analyses comparing between "CI-on – CI-off" and percent improvement in tinnitus loudness and between "CI-on – CI-off" and percent improvement in TQ score did not reveal any significant correlations between cortical activity changes and percent improvement in tinnitus loudness or TQ score.

Group Comparison with Regard to Source-Localized Activity and Functional Connectivity

Post-operative CI-On versus Pre-operative Baseline

Compared with the pre-operative baseline, the post-operative CI-on condition resulted in significantly decreased activity in the right A1 (BAs 41 and 42) and A2 (BAs 21 and 22) and in the right OFC (BAs 10 and 11) for the delta frequency band ($P < 0.01$) (**Figure 1**). For the other seven frequency bands, no significant differences with regard to source-localized activity were found between the two conditions.

On lagged linear connectivity comparison, the patients showed decreased functional connectivity between the PCC and A1 for the delta frequency band and between the PCC and A2 for the beta 2 band for the post-operative CI-on state compared with pre-operative baseline (**Figure 2**).

Post-Operative CI-On versus CI-Off

Compared to the cortical activity of the CI-off condition, the subjects demonstrated significantly decreased activity in the right A1 (BAs 41 and 42) and A2 (BAs 21 and 22) and right OFC (BAs 10 and 11) for the delta frequency band, and in the right A1 and A2, left A2, left temporopolar cortex (TPC, BA 38), left inferior

TABLE 2 | Pre- and post-operative comparison of numeric rating scale tinnitus loudness and tinnitus questionnaire scores in all patients (the order of the subjects are the same as Table 1).

Subject number	Pre-operative NRS loudness	Pre-operative TQ score	Post-operative NRS loudness (CI-on with music stimuli)	Post-operative TQ score (CI-on with music stimuli)	Post-operative NRS loudness (CI-off)	Post-operative TQ score (CI-off)
1	8	78	3	41	8	76
2	7	60	5	44	6	58
3	9	52	3	29	9	52
4	9	62	6	56	9	64

NRS, numeric rating scale; TQ, tinnitus questionnaire.

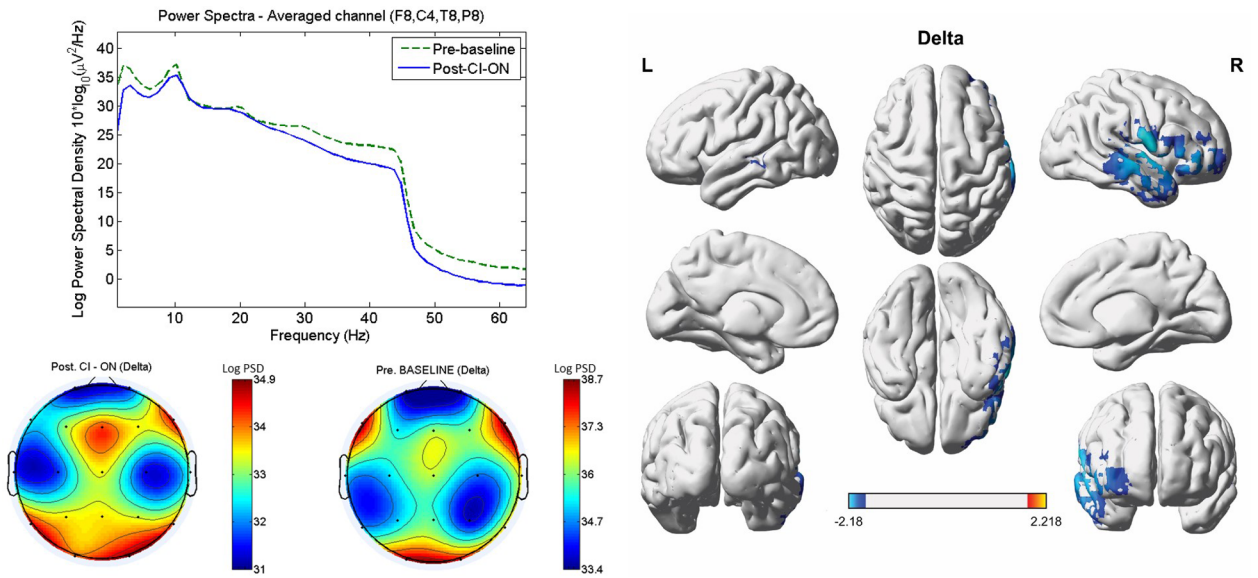

FIGURE 1 | Comparisons between the post-operative cochlear implant (CI)-on condition and the pre-operative baseline with regard to power spectra, sensor topography, and source-localized activity. As compared with the pre-operative baseline, the post-operative CI-on condition resulted in significantly decreased activity in the right primary (BAs 41 and 42) and secondary (BAs 21 and 22) auditory cortices, and in the right orbitofrontal cortex (BAs 10 and 11) for the delta frequency band ($P < 0.01$). L, left; R, right. Color bar (right bottom) presents *t*-value. PSD, power spectral density.

frontal cortex (IFC, BA 47), and left OFC for the gamma band of the CI-on condition ($P < 0.01$) (**Figure 3**).

On lagged linear connectivity comparison, no significant differences were found between the two conditions for all eight frequency bands.

Post-operative CI-Off versus Pre-operative Baseline

Neither source-localized cortical activity comparisons nor lagged linear functional connectivity analysis showed statistically significant differences between the CI-off and pre-operative baseline conditions for all eight frequency bands.

DISCUSSION

In the current study, we investigated post-CI changes in patients with SSD with regard to source-localized cortical activity and functional connectivity. In short, the CI-on condition resulted in decreased cortical activity as compared with both the CI-off and pre-operative baseline conditions, but the CI-off and pre-operative baseline conditions showed no significant differences.

Alleviation of Tinnitus and Tinnitus-Related Distress by Peripheral Reafferentation-Induced Cortical Deactivation

All four subjects in the current study showed improvements in NRS tinnitus loudness and TQ score. Although these improvements were only marginally significant both for NRS tinnitus loudness and TQ score ($P = 0.068$ for both parameters), considering the small number of included subjects, the improvements can be regarded to indicate a meaningful alleviation of tinnitus and tinnitus-related loudness.

On source-localized cortical activity analysis, as compared with the pre-operative baseline, the post-operative CI-on condition demonstrated significantly decreased activity in the right A1 and A2, and in the right OFC for the delta frequency

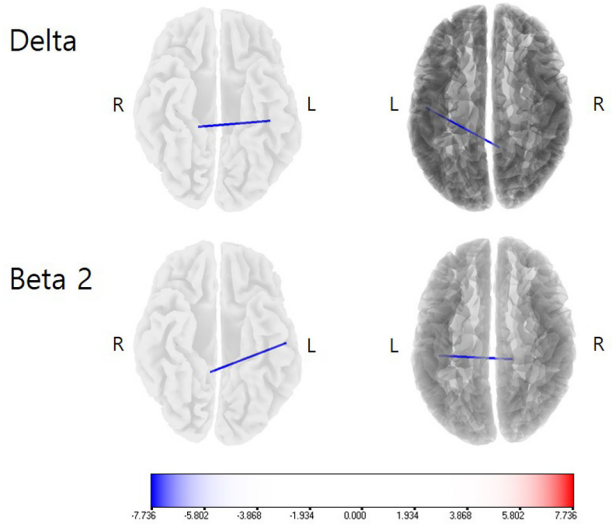

FIGURE 2 | Lagged linear connectivity comparisons between the post-operative cochlear implant (CI)-on condition and the pre-operative baseline. The patients showed decreased functional connectivity between the PCC and A1 for the delta frequency band and between the PCC and A2 for the beta 2 band for the post-operative CI-on state, as compared with the pre-operative baseline. Color bar presents *t*-value.

band. Previous studies both in animals (Engineer et al., 2011) and in human subjects (van der Loo et al., 2009) have demonstrated that the auditory cortex (AC) plays an important role in tinnitus perception. A recent meta-analysis on positron emission tomography (PET) studies in tinnitus patients has also reported increased regional cerebral blood flow in the A1 and A2 (Song et al., 2012). Moreover, perceived tinnitus loudness is correlated with increased contralateral source-localized activity in the AC (van der Loo et al., 2009). In this regard, the significantly decreased activity in the A1 and A2 in patients with SSD after CI, compared with the pre-operative baseline, may be associated with the improvement of tinnitus loudness in these subjects.

Moreover, significantly decreased functional connectivity between the A1 and PCC for the delta frequency band and between the A2 and PCC for the beta 2 band under the CI-on condition, as compared to those under the pre-operative baseline, may also be related to the improvement of tinnitus loudness in these SSD subjects. PCC has been posited to be an important component of the brain's default mode network (DMN) (Raichle et al., 2001; Raichle and Snyder, 2007), which is a set of cortical areas activated when a subject is occupied with internally focused tasks (Schlee et al., 2012). In persistent vegetative state patients, auditory stimulation-induced cortical activation is restricted to the A1, without functional connectivity to the areas comprising the DMN, including the PCC (Laureys et al., 2000; Boly et al., 2004). In other words, functional connectivity between the A1 and the PCC is crucial for conscious auditory perception. In a recent study that evaluated the correlation between pre-CI cortical activity and the extent of tinnitus improvement, increased activity of the PCC for the delta band and increased functional connectivity between the A1 and the PCC for the delta band were negatively correlated with the percent improvement of tinnitus loudness (Song et al., 2013b). This is in line with the current results showing the significantly decreased functional connectivity between the A1 and PCC for the delta frequency band under the CI-on condition as compared to those under the pre-operative baseline. That is, functional decoupling between the A1 and PCC for the delta band by CI may be associated with the improvement of tinnitus under the CI-on condition as compared with the pre-operative baseline condition. Also, the delta and beta 2 frequency bands has been found to be important in the integrity of the

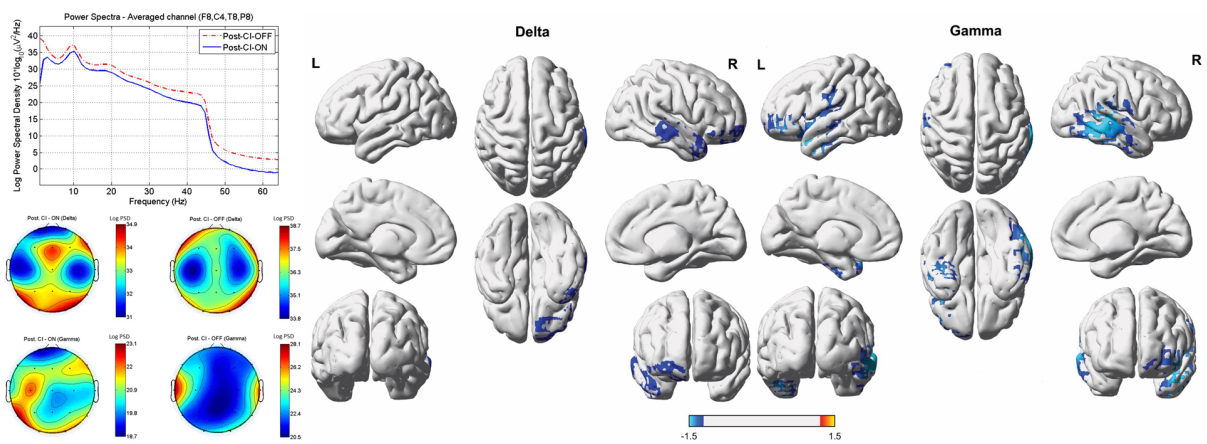

FIGURE 3 | Comparisons between the post-operative cochlear implant (CI)-on and off conditions with regard to power spectra, sensor topography, and source-localized activity. Compared to the cortical activity of the CI-off condition, the subjects demonstrated significantly decreased activity in the right primary (BAs 41 and 42) and secondary (BAs 21 and 22) auditory cortices (A1 and A2), and right orbitofrontal cortex (OFC, BAs 10 and 11) for the delta frequency band and in the right A1 and A2, left A2, left temporopolar cortex (BA 38), left inferior frontal cortex (BA 47), and left OFC for the gamma band for the CI-on with music stimulus condition. L, left; R, right. Color bar (right bottom) presents *t*-value. PSD, power spectral density.

DMN in previous EEG studies (Neuner et al., 2014; Thatcher et al., 2014). Considering this, functional decoupling of the A1/A2 from a component of DMN for the delta and beta 2 frequency bands may have hindered conscious perception of the abnormal activity in the auditory cortices and thus associated with the improvement of tinnitus loudness. Thus, decreased functional connectivity between the A1/A2 and the PCC may be associated with the improvement of tinnitus loudness in these subjects.

Meanwhile, significantly decreased activity in the right OFC for the delta frequency band after CI compared with the pre-operative baseline may be associated with the improvement of the TQ score (i.e., tinnitus-related distress). The OFC has been suggested to be important for emotional processing of sounds (Blood et al., 1999; Vanneste and De Ridder, 2012) and also plays an important role in the top-down modulation of peripheral physiological responses to emotional experiences (Critchley et al., 2004). Additionally, the aforementioned correlation study between pre-CI cortical activity and the amount of post-CI tinnitus improvement revealed that increased pre-CI connectivity between the AC and the OFC is a predictor of poor response to the improvement of tinnitus-related distress (Song et al., 2013b). In particular, a previous qEEG study has demonstrated that the OFC were more activated in highly distressed tinnitus patients than in less distressed patients for the delta band (Song et al., 2015b). In this regard, decreased activity in the OFC for the delta band after CI may be associated with the improvement of the TQ score in our case series.

One possible bias that might be crucial in the interpretation of the comparison between the CI-on condition and pre-operative baseline is the baseline activity of the subjects. In other words, the differences detected in the analysis above might also be partly affected by the changes in the baseline activity in the subjects that might have not be detected in the analysis comparing the CI-off condition and pre-operative baseline. To further clarify this issue, future studies comparing these conditions repeatedly in a larger number of subjects or comparing the CI-off condition and pre-operative baseline serially at different time points after turning the device off should be performed.

"Dynamic" Cortical Activity Modulation by Peripheral Reafferentation

Although CIs starkly improved tinnitus and distress in our current patients with SSD, when they were turned off, the NRS tinnitus loudness and the TQ score returned to levels close to those measured pre-operatively. Moreover, the CI-on condition resulted in significantly decreased activity in the right A1 and A2 and the right OFC for the delta band and in the right A1 and A2, left A2, left TPC, left IFC, and left OFC for the gamma band, compared with the CI-off condition. In addition to the role of A1/A2 in tinnitus perception and of the OFC in tinnitus-related distress described above, the left TPC and IFC were significantly deactivated when the CI was on. The TPC contributes to the processing of auditory concepts (Bonner and Price, 2013) and increased pre-operative activity of the TPC was found to be a negative predictor of tinnitus loudness improvement in SSD patients after CI (Song et al., 2013b). The aggravation of tinnitus loudness after turning off the CI device may be partly due to reactivation of the left TPC. Meanwhile, the left IFC is involved in non-spatial auditory cognition and congruity (Michelon et al., 2003) or cognitive reappraisal (Wager et al., 2008). In a previous meta-analysis of PET studies in tinnitus, the IFC, or the ventrolateral prefrontal cortex, has been found to be commonly activated in tinnitus patients (Song et al., 2012). Therefore, cognitive processing of tinnitus may have been disinhibited in the current subjects after turning off the CI device, and this disinhibition may have manifested as the aggravation of tinnitus loudness.

When the pre-operative baseline and post-operative CI-off conditions were compared, neither subjectively perceived tinnitus loudness/distress nor source-localized activity showed statistically significant differences. CI-induced peripheral reafferentation was effective in alleviating tinnitus only when the device was actively functioning, at least until 6 months post-operatively. In other words, CI-related improvements in tinnitus may be associated with peripheral auditory reafferentation-induced dynamic suppression of tinnitus-related maladaptive cortical activity.

Limitations of the Current Study and Proposed Future Studies

To our knowledge, this is the first study comparing pre- and post-operative cortical activity and functional connectivity in SSD patients who underwent CI. Although we found several significant findings, there are several limitations that should be further investigated in future studies. First, only four patients were included in this study. Although we found several cortical areas that showed significantly decreased activity and functional connectivity for the post-operative CI-on condition, as compared to the pre-operative baseline or post-operative CI-off conditions, we may have failed to discover other crucial areas that also contribute to the improvement of tinnitus, due to the limited statistical power. Additionally, the lack of differences between CI-off and baseline conditions or no significant correlations between cortical activity changes and percent improvement in tinnitus loudness or TQ score might have been due to small subject number-related insufficient statistical power inherent in the current study. Second, the current study revealed dynamic tinnitus suppression by peripheral sensory reafferentation, and these results should be reevaluated in a future study with both a larger number of subjects and a longer follow-up period. In the current study, the subjects' post-operative EEGs were measured 6 months post-operatively, which may not have been long enough to observe possible central changes induced by continuous peripheral stimulus. Further studies in SSD subjects with CI, with follow-up periods of at least 12–24 months, should be performed to explore possible plastic changes. Third, all four subjects in the current study were coincidentally left SSD subjects. This may have affected the results because previous researchers reported that cortical activity differences from normal hearing peers are reported to be larger when the hearing loss occurred in

the left ear compared with the right ear (Ponton et al., 2001; Hanss et al., 2009), and left and right unilateral sensorineural hearing loss subjects show different cortical activation patterns to sound stimuli (Schmithorst et al., 2005). Further studies comparing left- and right-SSD subjects with tinnitus after CI should be performed to further explore possible differences. Fourth, the lack of control group, composed of SSD subjects without tinnitus who underwent CI, limits the value of comparison between the CI-on and CI-off conditions. Future studies enrolling SSD subjects without tinnitus who underwent CI as a control group should be performed to further compare CI-on and CI-off conditions. Fifth, BL-ICA-based cleaning of the CI-on condition might have resulted in power decrease in the cleaned bands, and thus the direct comparison between the CI-on and CI-off conditions has inherent limitations. Future studies using auditory stimulation of the non-deaf side may give information on what extent the BL-ICA itself has an adverse effect on the interpretation of the results, and thus give us more precise results. Also, future studies using a similar study paradigm to the current study while measuring cortical activity changes by PET may give us additional precise information, as PET is not affected by device-related artifacts.

CONCLUSION

Taken together, our data demonstrated that the CI-on condition resulted in decreased cortical activity compared with both the CI-off and pre-operative baseline conditions, particularly in areas such as the A1/A2 and the OFC. Also, decreased functional connectivity between the A1/A2 and the PCC were observed in the CI-on condition compared with pre-operative baseline. However, the CI-off and pre-operative baseline conditions showed no significant differences with regard to source-localized activity and functional connectivity. In this regard, CI may alleviate tinnitus in patients with SSD not by sound stimuli-induced cortical plastic changes, but by suppressing abnormally active tinnitus-related cortical regions by dynamic peripheral reafferentation.

AUTHOR CONTRIBUTIONS

J-JS led the analysis and interpretation of the results, and drafted the first manuscript. K-JP, HC, and J-WC conceived the investigation, revised the manuscript for important intellectual content. KK, WS, S-YL, GM, PVdH, DDR, and SV contributed to all aspects of the investigation, including methodological design, data collection and analysis, interpretation of the results, and revision of the manuscript for important intellectual content. All authors approved the final version of the manuscript and agree to be accountable for all aspects of the work.

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REFERENCES

Andersson, G., Freijd, A., Baguley, D. M., and Idrizbegovic, E. (2009). Tinnitus distress, anxiety, depression, and hearing problems among cochlear implant patients with tinnitus. *J. Am. Acad. Audiol.* 20, 315–319. doi: 10.3766/jaaa.20.5.5

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/s0140-6736(13)60142-7

Barry, R. J., Clarke, A. R., Johnstone, S. J., and Brown, C. R. (2009). EEG differences in children between eyes-closed and eyes-open resting conditions. *Clin. Neurophysiol.* 120, 1806–1811. doi: 10.1016/j.clinph.2009.08.006

Barry, R. J., Clarke, A. R., Johnstone, S. J., Magee, C. A., and Rushby, J. A. (2007). EEG differences between eyes-closed and eyes-open resting conditions. *Clin. Neurophysiol.* 118, 2765–2773. doi: 10.1016/j.clinph.2007.07.028

Beck, A. T., and Steer, R. A. (1984). Internal consistencies of the original and revised Beck Depression Inventory. *J. Clin. Psychol.* 40, 1365–1367. doi: 10.1002/1097-4679(198411)40:6<1365::AID-JCLP2270400615>3.0.CO;2-D

Blasco, M. A., and Redleaf, M. I. (2014). Cochlear implantation in unilateral sudden deafness improves tinnitus and speech comprehension: meta-analysis and systematic review. *Otol. Neurotol.* 35, 1426–1432. doi: 10.1097/MAO.0000000000000431

Blood, A. J., Zatorre, R. J., Bermudez, P., and Evans, A. C. (1999). Emotional responses to pleasant and unpleasant music correlate with activity in paralimbic brain regions. *Nat. Neurosci.* 2, 382–387. doi: 10.1038/7299

Boly, M., Faymonville, M. E., Peigneux, P., Lambermont, B., Damas, P., Del Fiore, G., et al. (2004). Auditory processing in severely brain injured patients: differences between the minimally conscious state and the persistent vegetative state. *Arch. Neurol.* 61, 233–238. doi: 10.1001/archneur.61.2.233

Bonner, M. F., and Price, A. R. (2013). Where is the anterior temporal lobe and what does it do? *J. Neurosci.* 33, 4213–4215. doi: 10.1523/JNEUROSCI.0041-13.2013

Critchley, H. D., Wiens, S., Rotshtein, P., Ohman, A., and Dolan, R. J. (2004). Neural systems supporting interoceptive awareness. *Nat. Neurosci.* 7, 189–195. doi: 10.1038/nn1176

De Ridder, D., Vanneste, S., and Congedo, M. (2011). The distressed brain: a group blind source separation analysis on tinnitus. *PLoS ONE* 6:e24273. doi: 10.1371/journal.pone.0024273

Del Bo, L., Forti, S., Ambrosetti, U., Costanzo, S., Mauro, D., Ugazio, G., et al. (2008). Tinnitus aurium in persons with normal hearing: 55 years later. *Otolaryngol. Head Neck Surg.* 139, 391–394. doi: 10.1016/j.otohns.2008.06.019

Delorme, A., and Makeig, S. (2004). EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. *J. Neurosci. Methods* 134, 9–21. doi: 10.1016/j.jneumeth.2003.10.009

Eggermont, J. J., and Kral, A. (2016). Somatic memory and gain increase as preconditions for tinnitus: insights from congenital deafness. *Hear. Res.* 333, 37–48. doi: 10.1016/j.heares.2015.12.018

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Eickhoff, S. B., Stephan, K. E., Mohlberg, H., Grefkes, C., Fink, G. R., Amunts, K., et al. (2005). A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage* 25, 1325–1335. doi: 10.1016/j.neuroimage.2004.12.034

Engineer, N. D., Riley, J. R., Seale, J. D., Vrana, W. A., Shetake, J. A., Sudanagunta, S. P., et al. (2011). Reversing pathological neural activity using targeted plasticity. *Nature* 470, 101–104. doi: 10.1038/nature09656

Fuchs, M., Kastner, J., Wagner, M., Hawes, S., and Ebersole, J. S. (2002). A standardized boundary element method volume conductor model. *Clin. Neurophysiol.* 113, 702–712. doi: 10.1016/S1388-2457(02)00030-5

Giraud, A. L., Price, C. J., Graham, J. M., and Frackowiak, R. S. (2001). Functional plasticity of language-related brain areas after cochlear implantation. *Brain* 124(Pt 7), 1307–1316. doi: 10.1093/brain/124.7.1307

Goebel, G., and Hiller, W. (1994). [The tinnitus questionnaire. A standard instrument for grading the degree of tinnitus. Results of a multicenter study with the tinnitus questionnaire]. *HNO* 42, 166–172.

Hanss, J., Veuillet, E., Adjout, K., Besle, J., Collet, L., and Thai-Van, H. (2009). The effect of long-term unilateral deafness on the activation pattern in the auditory cortices of French-native speakers: influence of deafness side. *BMC Neurosci.* 10:23. doi: 10.1186/1471-2202-10-23

Heller, A. J. (2003). Classification and epidemiology of tinnitus. *Otolaryngol. Clin. North Am.* 36, 239–248. doi: 10.1016/S0030-6665(02)00160-3

Holmes, A. P., Blair, R. C., Watson, J. D., and Ford, I. (1996). Nonparametric analysis of statistic images from functional mapping experiments. *J. Cereb. Blood Flow Metab.* 16, 7–22. doi: 10.1097/00004647-199601000-00002

Jurcak, V., Tsuzuki, D., and Dan, I. (2007). 10/20, 10/10, and 10/5 systems revisited: their validity as relative head-surface-based positioning systems. *Neuroimage* 34, 1600–1611. doi: 10.1016/j.neuroimage.2006.09.024

Kim, K., Punte, A. K., Mertens, G., Van de Heyning, P., Park, K. J., Choi, H., et al. (2015). A novel method for device-related electroencephalography artifact suppression to explore cochlear implant-related cortical changes in single-sided deafness. *J. Neurosci. Methods* 255, 22–28. doi: 10.1016/j.jneumeth.2015.07.020

Kim, S. H., Jang, J. H., Lee, S. Y., Han, J. J., Koo, J. W., Vanneste, S., et al. (2016). Neural substrates predicting short-term improvement of tinnitus loudness and distress after modified tinnitus retraining therapy. *Sci. Rep.* 6:29140. doi: 10.1038/srep29140

Kleinjung, T., Steffens, T., Strutz, J., and Langguth, B. (2009). Curing tinnitus with a Cochlear Implant in a patient with unilateral sudden deafness: a case report. *Cases J.* 2:7462. doi: 10.1186/1757-1626-2-7462

Kringelbach, M. L. (2005). The human orbitofrontal cortex: linking reward to hedonic experience. *Nat. Rev. Neurosci.* 6, 691–702. doi: 10.1038/nrn1747

Krog, N. H., Engdahl, B., and Tambs, K. (2010). The association between tinnitus and mental health in a general population sample: results from the HUNT Study. *J. Psychosom. Res.* 69, 289–298. doi: 10.1016/j.jpsychores.2010.03.008

Lancaster, J. L., Woldorff, M. G., Parsons, L. M., Liotti, M., Freitas, C. S., Rainey, L., et al. (2000). Automated Talairach atlas labels for functional brain mapping. *Hum. Brain Mapp.* 10, 120–131. doi: 10.1002/1097-0193(200007)10:3<120::AID-HBM30>3.0.CO;2-8

Landgrebe, M., Langguth, B., Rosengarth, K., Braun, S., Koch, A., Kleinjung, T., et al. (2009). Structural brain changes in tinnitus: grey matter decrease in auditory and non-auditory brain areas. *Neuroimage* 46, 213–218. doi: 10.1016/j.neuroimage.2009.01.069

Laureys, S., Faymonville, M. E., Degueldre, C., Fiore, G. D., Damas, P., Lambermont, B., et al. (2000). Auditory processing in the vegetative state. *Brain* 123(Pt 8), 1589–1601. doi: 10.1093/brain/123.8.1589

Michelon, P., Snyder, A. Z., Buckner, R. L., McAvoy, M., and Zacks, J. M. (2003). Neural correlates of incongruous visual information. An event-related fMRI study. *Neuroimage* 19, 1612–1626. doi: 10.1016/S1053-8119(03)00111-3

Milz, P., Faber, P. L., Lehmann, D., Kochi, K., and Pascual-Marqui, R. D. (2014). sLORETA intracortical lagged coherence during breath counting in meditation-naive participants. *Front. Hum. Neurosci.* 8:303. doi: 10.3389/fnhum.2014.00303

Moazami-Goudarzi, M., Michels, L., Weisz, N., and Jeanmonod, D. (2010). Temporo-insular enhancement of EEG low and high frequencies in patients with chronic tinnitus. QEEG study of chronic tinnitus patients. *BMC Neurosci.* 11:40. doi: 10.1186/1471-2202-11-40

Neuner, I., Arrubla, J., Werner, C. J., Hitz, K., Boers, F., Kawohl, W., et al. (2014). The default mode network and EEG regional spectral power: a simultaneous fMRI-EEG study. *PLoS ONE* 9:e88214. doi: 10.1371/journal.pone.0088214

Nichols, T. E., and Holmes, A. P. (2002). Nonparametric permutation tests for functional neuroimaging: a primer with examples. *Hum. Brain Mapp.* 15, 1–25. doi: 10.1002/hbm.1058

Norena, A., Micheyl, C., Chery-Croze, S., and Collet, L. (2002). Psychoacoustic characterization of the tinnitus spectrum: implications for the underlying mechanisms of tinnitus. *Audiol. Neurootol.* 7, 358–369. doi: 10.1159/000066156

Pascual-Marqui, R. D. (2002). Standardized low-resolution brain electromagnetic tomography (sLORETA): technical details. *Methods Find. Exp. Clin. Pharmacol.* 24(Suppl. D), 5–12.

Ponton, C. W., Vasama, J. P., Tremblay, K., Khosla, D., Kwong, B., and Don, M. (2001). Plasticity in the adult human central auditory system: evidence from late-onset profound unilateral deafness. *Hear. Res.* 154, 32–44. doi: 10.1016/S0378-5955(01)00214-3

Punte, A. K., Vermeire, K., Hofkens, A., De Bodt, M., De Ridder, D., and Van de Heyning, P. (2011). Cochlear implantation as a durable tinnitus treatment in single-sided deafness. *Cochlear Implants Int.* 12(Suppl. 1), S26–S29. doi: 10.1179/146701011X13001035752336

Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., and Shulman, G. L. (2001). A default mode of brain function. *Proc. Natl. Acad. Sci. U.S.A.* 98, 676–682. doi: 10.1073/pnas.98.2.676

Raichle, M. E., and Snyder, A. Z. (2007). A default mode of brain function: a brief history of an evolving idea. *Neuroimage* 37, 1083–1090; discussion 1097–1089. doi: 10.1016/j.neuroimage.2007.02.041

Rolls, E. T. (2004). The functions of the orbitofrontal cortex. *Brain Cogn.* 55, 11–29. doi: 10.1016/S0278-2626(03)00277-X

Ruckenstein, M. J., Hedgepeth, C., Rafter, K. O., Montes, M. L., and Bigelow, D. C. (2001). Tinnitus suppression in patients with cochlear implants. *Otol. Neurotol.* 22, 200–204. doi: 10.1097/00129492-200103000-00014

Schaette, R., Turtle, C., and Munro, K. J. (2012). Reversible induction of phantom auditory sensations through simulated unilateral hearing loss. *PLoS ONE* 7:e35238. doi: 10.1371/journal.pone.0035238

Schecklmann, M., Landgrebe, M., Poeppl, T. B., Kreuzer, P., Manner, P., Marienhagen, J., et al. (2011). Neural correlates of tinnitus duration and Distress: a positron emission tomography study. *Hum. Brain Mapp.* 34, 233–240. doi: 10.1002/hbm.21426

Schlee, W., Leirer, V., Kolassa, I. T., Weisz, N., and Elbert, T. (2012). Age-related changes in neural functional connectivity and its behavioral relevance. *BMC Neurosci.* 13:16. doi: 10.1186/1471-2202-13-16

Schmithorst, V. J., Holland, S. K., Ret, J., Duggins, A., Arjmand, E., and Greinwald, J. (2005). Cortical reorganization in children with unilateral sensorineural hearing loss. *Neuroreport* 16, 463–467. doi: 10.1097/00001756-200504040-00009

Sherlin, L., and Congedo, M. (2005). Obsessive-compulsive dimension localized using low-resolution brain electromagnetic tomography (LORETA). *Neurosci. Lett.* 387, 72–74. doi: 10.1016/j.neulet.2005.06.069

Song, J. J., De Ridder, D., Schlee, W., Van de Heyning, P., and Vanneste, S. (2013a). "Distressed aging": the differences in brain activity between early- and late-onset tinnitus. *Neurobiol. Aging* 34, 1853–1863. doi: 10.1016/j.neurobiolaging.2013.01.014

Song, J. J., De Ridder, D., Van de Heyning, P., and Vanneste, S. (2012). Mapping tinnitus-related brain activation: an activation-likelihood estimation metaanalysis of PET studies. *J. Nucl. Med.* 53, 1550–1557. doi: 10.2967/jnumed.112.102939

Song, J. J., De Ridder, D., Weisz, N., Schlee, W., Van de Heyning, P., and Vanneste, S. (2014). Hyperacusis-associated pathological resting-state brain oscillations in the tinnitus brain: a hyperresponsiveness network with

paradoxically inactive auditory cortex. *Brain Struct. Funct.* 219, 1113–1128. doi: 10.1007/s00429-013-0555-1

Song, J. J., Punte, A. K., De Ridder, D., Vanneste, S., and Van de Heyning, P. (2013b). Neural substrates predicting improvement of tinnitus after cochlear implantation in patients with single-sided deafness. *Hear. Res.* 299, 1–9. doi: 10.1016/j.heares.2013.02.001

Song, J. J., Vanneste, S., and De Ridder, D. (2015a). Dysfunctional noise cancelling of the rostral anterior cingulate cortex in tinnitus patients. *PLoS ONE* 10:e0123538. doi: 10.1371/journal.pone.0123538

Song, J. J., Vanneste, S., Schlee, W., Van de Heyning, P., and De Ridder, D. (2015b). Onset-related differences in neural substrates of tinnitus-related distress: the anterior cingulate cortex in late-onset tinnitus, and the frontal cortex in early-onset tinnitus. *Brain Struct. Funct.* 220, 571–584. doi: 10.1007/s00429-013-0648-x

Souliere, C. R. Jr., Kileny, P. R., Zwolan, T. A., and Kemink, J. L. (1992). Tinnitus suppression following cochlear implantation. A multifactorial investigation. *Arch. Otolaryngol. Head Neck Surg.* 118, 1291–1297. doi: 10.1001/archotol.1992.01880120017004

Talairach, J., and Tornoux, P. (1988). *Co-Planar Stereotaxic Atlas of the Human Brain: 3-Dimensional Proportional System: An Approach to Cerebral Imaging.* Stuttgart: Georg Thieme.

Thatcher, R. W., North, D. M., and Biver, C. J. (2014). LORETA EEG phase reset of the default mode network. *Front. Hum. Neurosci.* 8:529. doi: 10.3389/fnhum.2014.00529

van der Loo, E., Gais, S., Congedo, M., Vanneste, S., Plazier, M., Menovsky, T., et al. (2009). Tinnitus intensity dependent gamma oscillations of the contralateral auditory cortex. *PLoS ONE* 4:e7396. doi: 10.1371/journal.pone.0007396

Vanneste, S., and De Ridder, D. (2012). The auditory and non-auditory brain areas involved in tinnitus. An emergent property of multiple parallel overlapping subnetworks. *Front. Syst. Neurosci.* 6:31. doi: 10.3389/fnsys.2012.00031

Vanneste, S., Plazier, M., der Loo, E., de Heyning, P. V., Congedo, M., and De Ridder, D. (2010). The neural correlates of tinnitus-related distress. *Neuroimage* 52, 470–480. doi: 10.1016/j.neuroimage.2010.04.029

Vanneste, S., Song, J. J., and De Ridder, D. (2013). Tinnitus and musical hallucinosis: the same but more. *Neuroimage* 82, 373–383. doi: 10.1016/j.neuroimage.2013.05.107

Wager, T. D., Davidson, M. L., Hughes, B. L., Lindquist, M. A., and Ochsner, K. N. (2008). Prefrontal-subcortical pathways mediating successful emotion regulation. *Neuron* 59, 1037–1050. doi: 10.1016/j.neuron.2008.09.006

Xia, M., Wang, J., and He, Y. (2013). BrainNet viewer: a network visualization tool for human brain connectomics. *PLoS ONE* 8:e68910. doi: 10.1371/journal.pone.0068910

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Corrigendum: A Quantitative Electroencephalography Study on Cochlear Implant-Induced Cortical Changes in Single-Sided Deafness with Tinnitus

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In the published article, there was an error in affiliation 1. Instead of "Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Hospital, Seoul, South Korea", it should be "Department of Otorhinolaryngology-Head and Neck Surgery, Seoul National University Bundang Hospital, Seongnam, South Korea". The authors apologize for this error and state that this does not change the scientific conclusions of the article in any way.

The original article has been updated.

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Clinical Validation of a New Tinnitus Assessment Technology

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Current clinical assessment of tinnitus relies mainly on self-report. Psychoacoustic assessment of tinnitus pitch and loudness are recommended but methods yield variable results. Herein, we investigated the proposition that a previously validated fixed laboratory-based method (Touchscreen) and a newly developed clinically relevant portable prototype (Stand-alone) yield comparable results in the assessment of psychoacoustic tinnitus pitch and loudness. Participants with tinnitus [$N = 15$, 7 with normal hearing and 8 with hearing loss (HL)] and participants simulating tinnitus (simulators, $N = 15$) were instructed to rate the likeness of pure tones (250—16 kHz) to their tinnitus pitch and match their loudness using both methods presented in a counterbalanced order. Results indicate that simulators rated their "tinnitus" at lower frequencies and at louder levels (~10 dB) compared to tinnitus participants. Tinnitus subgroups (with vs. without HL) differed in their predominant tinnitus pitch (i.e., lower in the tinnitus with HL subgroups), but not in their loudness matching in decibel SL. Loudness at the predominant pitch was identified as a factor yielding significant sensitivity and specificity in discriminating between the two groups of participants. Importantly, despite differences in the devices' physical presentations, likeness and loudness ratings were globally consistent between the two methods and, moreover, highly reproducible from one method to the other in both groups. All in all, both methods yielded robust tinnitus data in less than 12 min, with the Stand-alone having the advantage of not being dependent of learning effects, being user-friendly, and being adapted to the audiogram of each patient to further reduce testing time.

Keywords: tinnitus, psychoacoustic test tool, loudness, pitch, hearing loss

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INTRODUCTION

Tinnitus is a common health condition that affects from 11.9 to 30.3% of the general population and its prevalence increases with increasing age (1, 2). Tinnitus can interfere with daily life and is associated with significant psychological distress, anxiety, and other health-related issues (1). Individuals with tinnitus seeking clinical services are at a clear disadvantage compared to others because there are no established guidelines for the clinical assessment and intervention for tinnitus. At the basis of clinical service is assessment, which confirms diagnosis, as well as determines and monitors intervention, and yet, current clinical assessments of tinnitus mostly rely on patients' self-report.

Tinnitus can be characterized by its psychoacoustic properties (pitch and loudness), which pertain to the auditory domain, and by its associated distress, which pertains to the psychological

domain. Some clinical settings assess psychoacoustic measurements of pitch and loudness. Classical forced-choice paradigm and the method of adjustment are the preferred methods used (3). However, the procedure is passive, with clinicians controlling the stimulus parameters presented to the patient (3). These techniques are usually mastered by highly skilled clinicians and yet do not provide stable measurements of the tinnitus percept within a session or between sessions over time (3). This lack of reliability compromises tinnitus assessments such that clinical trials often have to rely on visual analog scales or tinnitus questionnaires as main treatment outcomes (4, 5). These outcomes, however, are highly unsatisfactory because some therapeutic interventions (e.g., transcranial magnetic stimulation of the auditory cortex, deep brain stimulation, noise-notched music) are targeted primarily at decreasing the psychoacoustic loudness of tinnitus, which in turn would supposedly decrease the associated psychological distress. However, the precise relationship between loudness and distress is unknown and usually statistical correlations are not very high (6). Therefore, the best tinnitus assessment should include reliable measures of both of these aspects.

Recently, an active method allowing the patient to control parameters using a Touchscreen has shown good test–retest reliability for tinnitus predominant pitch and loudness matching over several months (6, 7). Moreover, psychoacoustic tinnitus loudness matches were higher for simulators than for participants with tinnitus and was a better predictor for specificity (e.g., correctly detecting simulators) than predominant pitch (4). A similar method specifically designed for clinical settings could be very useful for health-care professionals to assess the progression of the tinnitus over several months in individual patients and assess treatment' efficacy with confidence. High levels of sensitivity and specificity of the method are interesting features that would be valuable in medicolegal cases of tinnitus. For these purposes, a new Stand-alone prototype (hereafter, Stand-alone) was developed from the first laboratory device (hereafter, Touchscreen), in order to capture the main tinnitus characteristics—likeness and loudness ratings—but in a format that is more suited for clinical purposes.

The aim of the present study was, therefore, to compare performance of the two methods, including participants simulating having tinnitus. As previously reported, the tinnitus likeness and most importantly the loudness should be significantly different between tinnitus participants and simulators for both methods. Likewise, loudness should be a better predictor of tinnitus presence compared to tinnitus predominant pitch, again for both methods.

As time efficiency is a major issue for clinicians, testing time for the two devices was assessed and compared. We predicted that the modification made to the Stand-alone device should provide a more efficient testing time than the Touchscreen method for patients with hearing loss (HL). Finally, the effect of presenting two pure tones instead of the standard three pure tones was assessed, as it could also potentially reduce testing time.

A second objective was to compare two tinnitus subtypes. In particular, tinnitus with and without HL has been shown to engage two different types of brain structures (8, 9). In addition, differences in tinnitus spectrum have been noted across studies between tinnitus patients with and without HL mostly in the very high frequency region (6, 10). From a clinical standpoint, these two groups are also very different in term of assessment and therapeutic approach. For instance, hearing assessment for tinnitus patients with HL is usually longer than for patients without HL, and thus, any saved time in the assessment of tinnitus would be a valuable asset for a clinical device. In that respect, tinnitus subgroups were further examined on the basis of this criterion.

MATERIALS AND METHODS

Participants

Two groups of participants who had not participated in our previous studies using the Touchscreen method were recruited through word of mouth or advertisements in local newspapers. Tinnitus participants had to have chronic bilateral tinnitus, not have complete deafness in one or both ears, and be in good health. Health was further verified by questions on possible diseases (e.g., neurological disease), conditions (e.g., uncontrolled hypertension), and medication. This group consisted of 15 adults (9M, 6F) with a mean age of 45 years (min: 23; max: 69). Simulator participants had to have had a previous experience of transient tinnitus (less than a day) more than 3 months before the experiment so that they could rely on this past experience to fake tinnitus. Most participants reported having previous experience of transient tinnitus after loud sound exposure such as a music concert, a very frequent phenomenon (10). This group consisted of 15 adults (7M, 8F) with a mean age of 32 years (min: 21; max: 62). They were instructed to simulate this sound perception with the intention of convincing the experimenter that they had tinnitus. None of the participants reported otologic condition other than HL and none of the participants were smokers.

Tinnitus Subtypes

The normal hearing (NH) Tinnitus subgroup ($N = 7$) had hearing thresholds ≤25 dB hearing loss (HL) at all frequencies from 250 to 8 kHz in both ears. The HL tinnitus subgroup ($N = 8$) had hearing thresholds >25 dB HL at least at one frequency between 250 and 8 kHz in either ear.

Hearing Assessment

An otoscopy was performed in order to rule out earwax compaction and outer ear pathology. Standard hearing detection thresholds were assessed in each ear monaurally from 0.25 to 8 kHz in half-octave steps by a clinical audiologist using the standard modified Hughson–Westlake up–down procedure (5) with an AC-40 clinical audiometer and ER-3A insert earphones in a sound-proof booth (ANSI S3.6-2004 standard norms). Very-high frequency thresholds (9–16 kHz) were also assessed monaurally in each ear using Sennheiser HDA-200 supra-aural headphones.

Tinnitus Matching Method

Tinnitus assessment was run with both the Touchscreen and the Stand-alone in each participant in a counterbalanced order. Both methods used 3-s pure tones ranging from 0.25 to 16 kHz presented three times in a pseudorandom order such that no

two identical frequencies were presented in a row. Participants were first asked to rate the likeness of the tone to their tinnitus pitch on a Likert-type scale in which 0 = "does not match my tinnitus at all" and 10 = "perfectly matches my tinnitus." During the same trial, they had to match the loudness of the tone—that is, the sound level at which that specific frequency contributed to their tinnitus—by moving a visual gauge (Touchscreen) or a potentiometer (Stand-alone) that increased and decreased the sound level by 1 dB steps. Participants were allowed to play each pure tone as many times as needed for both methods. The sound was presented binaurally using closed DT 770 Pro/250 dynamic headphones (Beyerdynamic, Heilbronn, Germany) for the Touchscreen method and HDA-300 Sennheiser (Sennheiser electronic GmbH & Co. KG, Germany) headphones for the Stand-alone device. The main difference between the two methods (see **Figure 1**) is that the Stand-alone uses standard buttons for playing sounds and rating likeness and a potentiometer for loudness matching. The design of the Stand-alone maximizes ergonomics and comfort to improve its use by older patients.

In addition, in order to make the method more time-efficient, especially for participants with some degree of HL, an additional step was implemented in the Stand-alone method. The audiogram of each participant was taken into account such that (1) frequencies for which a threshold could not be found (i.e., higher than the limits of the audiometer) were not presented and (2) levels of the frequencies presented were always beyond threshold. This information was added to the device just before the task began.

Calibration

Headphones were calibrated before each session with a SoundPro SE/DL sound level meter using a QE-4170 microphone model (Quest Technologies, Oconomowoc, WI, USA) and an EC-9A 2cc ear coupler (Quest Electronics, Oconomowoc, WI, USA).

Procedure

All participants were first assessed with the hearing evaluation. Then, they were tested with one of the two matching methods (Touchscreen or Stand-alone) in a counterbalanced order. They were next tested with the other method, both taking place in the same sound attenuated room. Participants were asked to provide repeatable tinnitus matching responses to the best of their ability. Simulators were instructed to use their past experience of tinnitus to convince the experimenter that they had tinnitus but were not instructed to use any particular method to provide consistent responses during matching. The time required to perform the tinnitus-matching task for each device was assessed using a conventional chronometer. The experimenter started the chronometer as soon as the participant press the « play » button for the first time, that is, after the instruction was given for each device individually. The study was approved by the ethics committee of Université de Montréal and was conducted with the understanding and written consent of each participant.

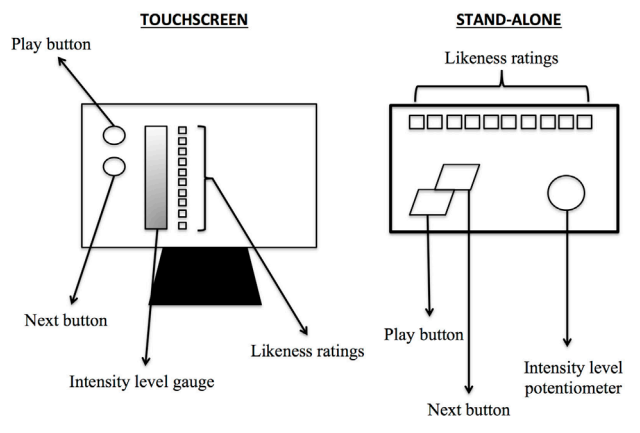

FIGURE 1 | Schematization of the Touchscreen and the Stand-alone devices used in the experiment.

Analyses

In all analyses below, the two groups (Tinnitus vs. Simulators) were first compared, and in a second step, the Tinnitus subgroups (NH vs. HL) were compared. Hearing thresholds were analyzed with a 2 × (2 × 16) ANOVA with Group (first, Tinnitus vs. Simulators; second, Tinnitus NH vs. HL) as a between-subject factors and ear (right, left) and frequency (250–16 kHz) as within-subject factors. Similar analyses were run on likeness ratings and loudness matching but with Methods (Touchscreen vs. Stand-alone) rather than ear as a within-subject factor. Product-to-moment Pearson correlations and paired-sample *T*-tests were run on these data to compare the two methods. The first and second predominant pitches and their corresponding loudness were extracted from the tinnitus spectrum of each participant and compared between groups and subgroups as described above.

Sensitivity and specificity analyses were run for each method (Touchscreen, Stand-alone) using logistic regressions taking group as the dependent factor putting the first predominant pitch and its corresponding loudness match as independent predictors.

To investigate potential learning effects in using devices, testing time was analyzed using a 2 × (2 × 2) ANOVA with Group [(1) Tinnitus vs. Simulators; (2) Tinnitus NH vs. HL] as between-subject factors and order of presentation (Touchscreen first vs. Stand-alone first) and Method (Touchscreen vs. Stand-alone) as within-subject factors. Finally, comparisons were made between using all three instances of pure tones and the first two instances only.

RESULTS

Hearing Thresholds

Hearing thresholds of all 16 frequencies did not differ across ears (all *p*s >0.05) except at 12.5 kHz ($p = 0.001$) and, therefore, were averaged. When considering two groups (Tinnitus, Simulators), the two-way interaction between frequency and group was significant, $F(15, 420) = 3.32$, $p < 0.001$. Thresholds for the Tinnitus group were higher from 3 to 14 kHz, all *p*s <0.05 and only marginally higher at 16 kHz, $p = 0.07$. When considering only the tinnitus subgroups NH vs. HL, the same pattern emerged, $F(15, 195) = 23.59$, $p < 0.001$. Thresholds for the HL subgroup were

higher from 2 to 16 kHz, all *ps* <0.04. **Figure 2** displays the best thresholds for the three groups for Tinnitus and Simulator group (**Figure 2A**) and Tinnitus subgroups NH and HL (**Figure 2B**).

Tinnitus Pitch Matching

On likeness ratings, there was no significant main effect of Methods or any interaction involving this factor (all *ps* >0.05), suggesting that the two methods work similarly (see **Table 1**). Paired-sample *T*-tests revealed only one significant difference at 9 kHz with a seven mean difference in the ratings between the two methods (see **Table 1**). Overall, correlations between the two methods were very high (mean $r = 0.74$, min: 0.53, max: 0.95). **Figure 2A** shows the likeness ratings for the Tinnitus and Simulator groups with merged Methods. There was a two-way interaction between Group and Frequency, $F(15, 420) = 1.70$, $p < 0.05$. Simulators' ratings were higher compared to Tinnitus' for frequencies 250–3 kHz irrespective of Methods, all *ps* <0.05.

For the Tinnitus subgroups, there was no effect of Methods or interaction involving this factor (all *ps* >0.05). **Figure 2B** shows the likeness ratings for the two subgroups NH and HL tinnitus. There was a significant interaction between frequency and subgroups, $F(15, 195) = 5.16$, $p < 0.001$. Ratings were higher for frequencies 14 and 16 kHz for the NH subgroup ($p < 0.001$) and marginally significant for the 12.5 kHz ($p = 0.05$). None of the other frequency differed (all *ps* >0.14). There was also a main effect of subgroup, $F(1, 13) = 7.02$, $p = 0.02$, with NH tinnitus subgroup rating their tinnitus with higher scores than the HL subgroup (means of 4 and 3, respectively).

When considering the first and the second predominant pitch, that is, the frequencies with the first and the second highest ratings in each individual participant, the Tinnitus group did not differ from the Simulator group (see **Table 2**). Interestingly, the Tinnitus subgroups differed from one another in terms of the first predominant pitch, with a higher predominant pitch for the NH subgroup compared to the HL subgroup (see **Table 2**).

Tinnitus Loudness Matching

On loudness ratings, there was no main effect of Methods or any interaction between Methods and Groups (all *ps* >0.10), again suggesting that both methods work similarly. However, there was a two-way interaction between Methods and Frequency, $F(15, 420) = 2.43$, $p = 0.002$: loudness matches with the Touchscreen were significantly lower for 2, 3, and 4 kHz and higher for 9 kHz (see **Table 1**). Overall, correlations between the two methods were again very high (mean $r = 0.65$, min: 0.26, max: 0.88). The expected group effect was significant $F(1, 28) = 7.32$, $p = 0.011$, with higher loudness matches for the Simulator compared to the Tinnitus group (mean of 17.7 and 6.1 dB SL, respectively) (see **Figure 3**). For the Tinnitus subgroups, there was no effect of Methods or interaction involving this factor (all *ps* >0.05). There was no main effect or interaction involving subgroups (means were: 7.97 and 4.41 dB SL for the NH and HL tinnitus, respectively).

When considering the loudness associated with the first and the second predominant pitches, the Tinnitus and Simulator groups differed significantly (see **Table 2**). The two Tinnitus subgroups did not differ on loudness for the two predominant frequencies.

Testing Time

Figure 4 displays testing times for the two methods according to order of presentation. Analyses revealed learning effects depending on the method used, as supported by a significant interaction between Method and Order of presentation, $F(1, 26) = 28.02$, $p < 0.001$: for the Stand-alone method, the order of presentation did not matter (testing times of 9 min 22 s, n.s.) whereas when the Touchscreen method was presented first, it took significantly

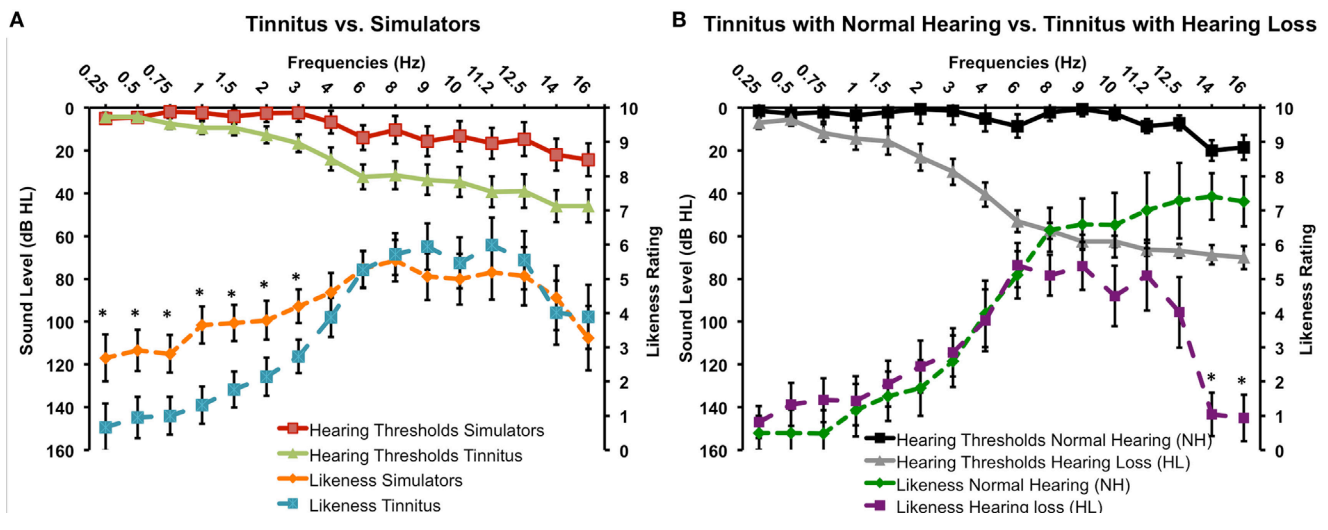

FIGURE 2 | Best hearing thresholds and tinnitus likeness ratings for the Tinnitus and Simulator groups (A) and the two Tinnitus subgroups with normal hearing and with hearing loss (B) (SEM).

TABLE 1 | Mean differences and product-to-moment Pearson correlations between the two methods (Touchscreen vs. Stand-alone) and the two groups (Simulators and Tinnitus groups merged) for likeness ratings and loudness matching at each frequency.

	Likeness ratings				Loudness matching			
Frequency (kHz)	**Mean difference**	***p*-Value**	***r***	***p*-Value**	**Mean difference (dB)**	***p*-Value**	***r***	***p*-Value**
0.25	0.1	0.70	0.95	<0.001	−0.4	0.76	0.88	<0.001
0.5	0.2	0.59	0.76	<0.001	−0.4	0.88	0.78	<0.001
0.75	−0.2	0.33	0.85	<0.001	−2.3	0.49	0.70	<0.001
1	0.3	0.33	0.79	<0.001	−1.9	0.56	0.71	<0.001
1.5	−0.4	0.27	0.66	<0.001	−5.0	0.15	0.67	<0.001
2	−0.3	0.43	0.66	<0.001	**−8.4**	**0.05**	0.56	0.001
3	−0.4	0.21	0.64	<0.001	**−14.0**	**<0.001**	0.74	<0.001
4	−0.4	0.37	0.62	<0.001	**−7.5**	**0.05**	0.69	<0.001
6	−0.3	0.46	0.53	0.003	−5.3	0.07	0.68	<0.001
8	0.2	0.64	0.65	<0.001	5.9	0.07	0.65	<0.001
9	**0.7**	**0.04**	0.81	<0.001	**9.9**	**0.01**	0.56	0.001
10	0.1	0.87	0.81	<0.001	4.4	0.11	0.73	<0.001
11.2	0.1	0.71	0.87	<0.001	1.0	0.72	0.61	<0.001
12.5	0.4	0.37	0.76	<0.001	−7.7	0.07	0.44	0.014
14	**1.0**	**0.04**	0.76	<0.001	1.6	0.55	0.67	<0.001
16	0.9	0.09	0.76	<0.001	0.0	1.00	0.26	0.164
Grand mean	0.1		0.74		−1.9		0.65	

Significant differences between the two methods are in bold.

TABLE 2 | Comparisons between the first and second predominant pitches of the tinnitus spectrum (in kHz) and their corresponding loudness (in dB SL) for the two methods.

	Tinnitus	**Simulators**	***p*-Value**	**Tinnitus with normal hearing**	**Tinnitus with hearing loss**	***p*-Value**
Touchscreen						
First predominant pitch (kHz)	11.37	8.98	n.s.	14.64	8.5	**=0.001**
Second predominant pitch (kHz)	9.06	9.86	n.s.	11.03	8.84	n.s.
Loudness predominant pitch (SL)	−1.9	20	**=0.015**	−10	5.2	n.s.
Loudness second predominant pitch (SL)	6.1	22	**=0.046**	6.3	5.9	n.s.
Stand-alone						
First predominant pitch (kHz)	11.09	8.39	n.s.	14	8.55	**=0.02**
Second predominant pitch (kHz)	10.01	7.06	n.s.	12.21	8.08	**=0.04**
Loudness predominant pitch (SL)	10.8	24.92	**=0.02**	5.75	15.2	n.s.
Loudness second predominant pitch (SL)	9.18	27.48	**=0.002**	5.7	12.23	n.s.

Significant differences between the two methods are in bold.
n.s., not significant.

more time than when it was presented after the Stand-alone method (12 min 44 s vs. 8 min 32 s, $p = 0.009$). There was also a main effect of Method, with overall testing time shorter for the Stand-alone compared to the Touchscreen, $F(1, 26) = 10{,}37$, $p = 0.003$ (testing time Stand-alone = 10 min 46 s; range = 4 min 30 s to 20 min 01 s; testing time Touchscreen = 9 min 21 s; range: 5 min 23 s and 16 min 48 s). Finally, there was also a main effect of group, with the Simulator group being faster than the Tinnitus group, $F(1, 26) = 7.24$, $p = 0.011$ (8 min 23 s vs. 11 min 36 s for the two groups, respectively).

Analyses were also run to examine testing time differences between methods for the tinnitus subtypes (with or without HL) with the implemented audiogram. The two-way interaction between Method (Touchscreen, Stand-alone) and subgroup (without, with HL) with order of presentation as a co-variable was significant, $F(1, 12) = 7.03$, $p = 0.021$. For the subgroup without HL, testing time did not differ between methods (11 min 5 s vs. 10 min 41 s for the Touchscreen and Stand-alone, respectively, $p = 0.75$) whereas for the subgroup HL, testing time was—marginally—shorter with the Stand-alone (13 min 48 s vs. 10 min 54 s for the Touchscreen and Stand-alone, respectively, $p = 0.052$). All but one tinnitus participant with HL took less time with the Stand-alone device (see **Figure 5**).

Two vs. Three Instances Presentation

The impact of presenting the two first vs. three instances of each frequency on the likeness ratings and loudness matching was also examined (see **Table 3**). Only two significant differences were found, one at 9 kHz for the likeness ratings (mean difference = 0.27) and one at 12.5 kHz for the loudness matches (mean difference = 3.4 dB SL). The analysis between the Tinnitus and Simulator groups was rerun on likeness and loudness matches. Similar results were obtained: for pitch matching, the frequency by groups interaction was marginally significant $F(15, 420) = 1.62$, $p = 0.066$ and *post hoc* tests revealed significant differences in ratings between the two groups for the frequencies 0.25–3 kHz, with higher ratings for the Simulators. For the loudness matching, the Frequency by Methods interaction was significant $F(15, 420) = 2.51$, $p = 0.001$, similar to the three instances results. The main group effect was also significant $F(1, 28) = 7.14$, $p = 0.012$,

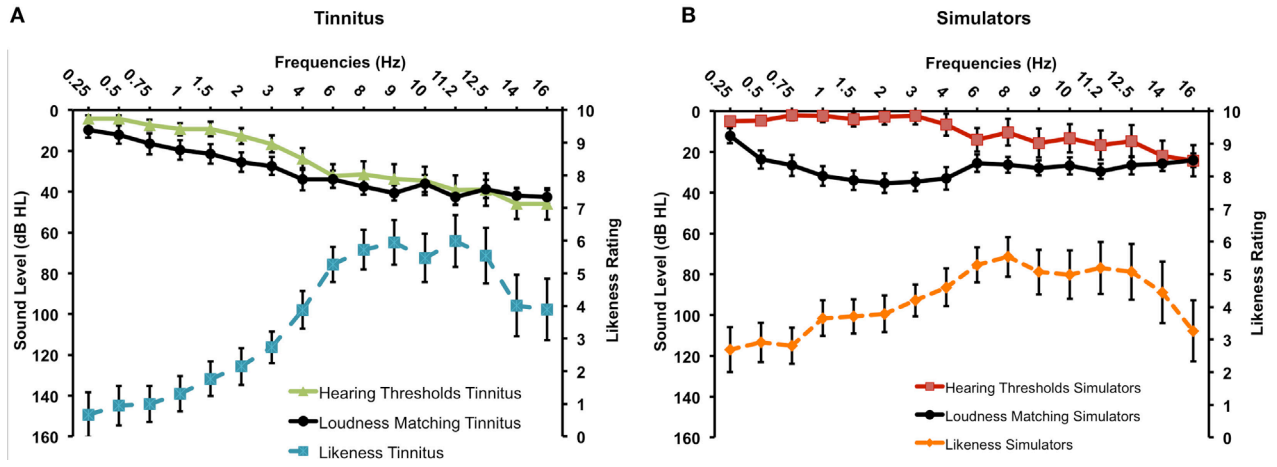

FIGURE 3 | Tinnitus spectrum (likeness ratings and loudness matches) for the Tinnitus (A) and the Simulator (B) groups (SEM).

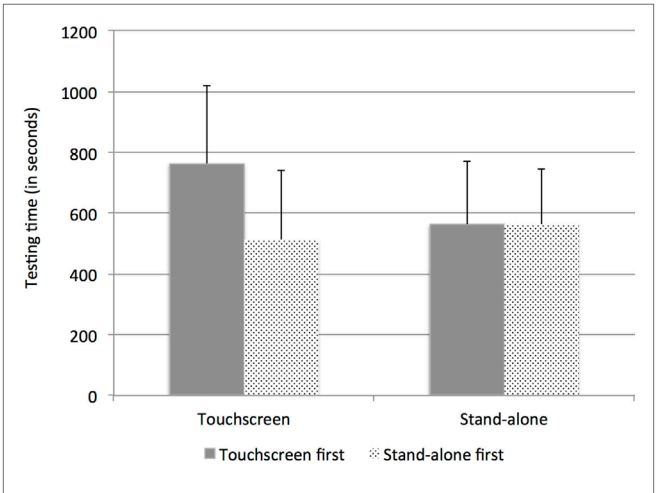

FIGURE 4 | Testing time (SD) for the two methods and the two groups according to the order of presentation (Touchscreen or Stand-alone first).

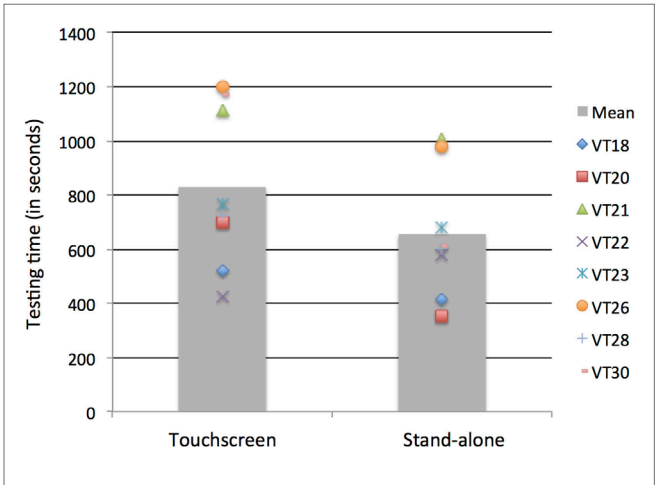

FIGURE 5 | Testing time for the subgroup Tinnitus with hearing loss using the Touchscreen or Stand-alone device. Individual data are shown.

with Simulators having higher loudness matches (mean: 18 dB SL) than the Tinnitus participants (mean: 6.8 dB SL).

Predominant Pitch vs. Loudness as Predictors of Having Tinnitus

The logistic regression model taking tinnitus predominant pitch and loudness (in dB SL) at the tinnitus predominant pitch as predictor variables for the likelihood of having tinnitus was significant for the Touchscreen, $\chi^2(2) = 6.855$, $p = 0.032$. The model explained 27.2% (Nagelkerke R^2) of the variance and correctly classified 70.0% of cases, with 73.3% sensitivity (i.e., correctly classifying tinnitus participants in the Tinnitus group) and 67.7% specificity (i.e., correctly classifying Simulators in the Simulator group). This model only included loudness as a significant predictor ($p = 0.062$), and not pitch ($p = 0.90$): lower loudness values were associated with greater likelihood of having tinnitus. A very similar pattern was found for the Stand-alone, $\chi^2(2) = 7.261$, $p = 0.026$. The model explained 28.7% (Nagelkerke R^2) of the variance and correctly classified 73.3% of cases, with 73.3% sensitivity and 73.3% specificity. Likewise, the model only included loudness as a significant predictor ($p = 0.064$), and not pitch ($p = 0.48$): lower loudness values were associated with greater likelihood of having tinnitus. Running the same analyses taking only the first two instances rather than three yielded basically the same pattern of results.

DISCUSSION

The two methods examined here performed very similarly for participants with tinnitus as well as for participants simulating having tinnitus. Indeed, both methods produced replicable tinnitus spectrum, with similar likeness frequency ratings and very

TABLE 3 | Mean differences and product-to-moment Pearson correlations between two methods instances (three vs. two) for likeness ratings and loudness matching at each frequency for merged Methods.

	Likeness ratings				Loudness matching			
Frequency (kHz)	**Mean difference**	***p*-Value**	***r***	***p*-Value**	**Mean difference (dB)**	***p*-Value**	***r***	***p*-Value**
0.25	0.02	0.61	0.99	<0.001	−0.37	0.44	0.98	<0.001
0.5	0.05	0.41	0.99	<0.001	−0.30	0.59	0.99	<0.001
0.75	0.06	0.28	0.99	<0.001	0.002	1.0	0.99	<0.001
1	−0.07	0.12	0.99	<0.001	−0.11	0.80	0.99	<0.001
1.5	−0.09	0.45	0.96	<0.001	−0.76	0.47	0.96	<0.001
2	0.07	0.40	0.98	<0.001	1.24	0.06	0.98	<0.001
3	0.04	0.75	0.94	<0.001	−0.67	0.56	0.96	<0.001
4	0.11	0.30	0.96	<0.001	−1.73	0.25	0.94	<0.001
6	0.04	0.74	0.94	<0.001	1.03	0.17	0.98	<0.001
8	0.11	0.24	0.98	<0.001	−0.39	0.71	0.94	<0.001
9	**0.27**	**0.004**	0.98	<0.001	0.76	0.14	0.99	<0.001
10	0.08	0.40	0.98	<0.001	−1.40	0.15	0.96	<0.001
11.2	-0.01	0.97	0.99	<0.001	−0.17	0.77	0.99	<0.001
12.5	0.07	0.46	0.99	<0.001	**−3.42**	0.04	0.88	<0.001
14	0.04	0.56	0.99	<0.001	−0.67	0.10	0.99	<0.001
16	0.05	0.61	0.99	<0.001	−1.18	0.29	0.89	<0.001
Grand mean	0.05		0.98		−0.51		0.96	

Significant mean differences between the two methods are in bold.

few differences in loudness matches. Both methods had highly correlated likeness ratings and loudness matches. Our study confirms that tinnitus spectrum, including frequency and loudness, can be quickly, yet robustly measured in the laboratory as well as in the clinic. Time efficiency is a major issue for clinicians and much research efforts are dedicated to the development of automated tests that are reliable and can be run while recruiting minimal clinician's participation (11–14). Studies have endeavored to reduce test time in almost every domain of audiology including electrophysiology (13), conventional audiometry (12), speech audiometry (15), hearing screening (11), and ototoxicity monitoring (14). Our test fits perfectly in this movement by offering a testing time less than 12 min to get robust tinnitus data. While the participant is performing the task, clinicians' expertise can be used for other purposes, such as writing reports, planning therapy, or consulting colleagues. Considering time efficiency, the Stand-alone device seems to be a better choice than the Touchscreen device for several reasons. First, the Stand-alone device has shown similar testing time independently of the order of presentation, which suggest that this device is so "user-friendly" and so easy to use, that pre-training is not necessary to improve time efficiency. In addition, the implementation of the audiogram of each patient reduces further the testing time and that is not dependent on learning effects such that it can be used confidently to measure therapeutic success or evolution of patients. This new feature might also be of particular interest to clinicians currently using the « conventional » pitch and loudness matching procedure with the audiometer. Indeed, the conventional method requires high skills from clinicians who need to consider, before each sound presentation, the frequency, the presentation level, and the degree of HL while focusing on the feedback provided by the patient during the tinnitus evaluation. These downfalls are potentially avoided with the proposed patient-directed methods. In addition, and despite the small number of participants tested in this study, the test offers good sensitivity and specificity (70% and above), lending itself for medicolegal purposes.

Presenting two pure tones instead of the standard three pure tones yielded essentially the same results. These results suggest that it could be possible to reduce testing time even more by using only two instances instead of three. This would mean that a tinnitus evaluation of pitch and loudness matching could be obtained in about 6–7 min instead of 9–10 min. Since the comparison between three and two instances was made from the same data, however, the current analysis should be taken cautiously: significant differences would mean that the third instance has a value that would be remote from the mean of the two first instances. A within-subject study design comparing two different testing sessions, one using three instances and the other one using two instances is needed before concluding on the validity of reducing the instances of presentation to two. Until further research is carried out, we believe that, for a test under 10 min, the safest solution for now would be to keep three instances.

As reported previously (6), Simulators rated lower frequencies as more similar to their tinnitus than Tinnitus participants, and overall they "matched" their tinnitus about 10 dB louder than the Tinnitus group. When considering the first and second predominant pitches of the tinnitus spectrum, only loudness matches differed between Tinnitus and Simulator participants.

Regarding tinnitus subgroups, both methods again produced comparable results, but given our small sample size, these results are tentative. Tinnitus participants with NH and HL differed mainly on the predominant pitch of their tinnitus, with—unsurprisingly—a lower predominant pitch for tinnitus participants with HL compared to those with NH. Combined with previous studies from other laboratories that have reported similar findings on likeness ratings (15–18), our study strengthens the evidence that tinnitus spectrum is mirroring the HL. Overall, the tinnitus spectrums of both tinnitus subgroups were almost

identical with the exception of two very high frequencies: 14 and 16 kHz. Such differences have been noted previously, sometimes as a decline when looking at heterogeneous tinnitus groups with a wide range of HL (10) or as an increase in ratings when looking at tinnitus groups with NH (7). In the present study, hearing thresholds at those high frequencies were too elevated in the HL subgroup to be tested efficiently with either method. Considering that both subgroups (NH or HL) displayed very similar ratings for all frequencies with the exception of those two, it can be presumed that they are probably part of the tinnitus spectrum for the HL group as well, but it was not possible to assess them successfully. These results also suggest that simulators are different in their low-frequency likeness ratings from both tinnitus subgroups, either with or without HL. In addition, our study brings new information about tinnitus subgroups, in that they did not differ from one another in their loudness ratings when expressed in decibel above thresholds (dB SL). Loudness matching, an original asset of our methods compared to previous studies, is particularly important given loudness—not frequency—is the most important predictor of the presence of tinnitus. Therefore, our findings suggest that the effect of recruitment on tinnitus loudness matching did not affect significantly the results obtained by the HL subgroups. Yet, the mean loudness matching for the NH group was slightly higher (~8 dB SL) than for the HL group (~4 dB SL), which could be attributed to loudness recruitment. Most importantly, both loudness measures were lower than the Simulator group (~18 dB SL), yielding again confidence that the methods can be used with tinnitus patients displaying different types of HL. From the current data, if loudness recruitment does have an effect on tinnitus loudness matching, this aspect seems to be clinically irrelevant. Further studies with larger groups should help answering this question.

AUTHOR CONTRIBUTIONS

SH and PF designed the study and analyzed the data. SH wrote the manuscript and both authors finalized and approved the manuscript.

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REFERENCES

1. Shargorodsky J, Curhan GC, Farwell WR. Prevalence and characteristics of tinnitus among US adults. *Am J Med* (2010) 123(8):711–8. doi:10.1016/j.amjmed.2010.02.015
2. McCormack A, Edmondson-Jones M, Somerset S, Hall D. A systematic review of the reporting of tinnitus prevalence and severity. *Hear Res* (2016) 337:70–9. doi:10.1016/j.heares.2016.05.009
3. Henry JA, Zaugg TL, Schechter MA. Clinical guide for audiologic tinnitus management I: assessment. *Am J Audiol* (2005) 14(1):21–48. doi:10.1044/1059-0889(2005/005)
4. Hall DA, Haider H, Kikidis D, Mielczarek M, Mazurek B, Szczepek AJ, et al. Toward a global consensus on outcome measures for clinical trials in tinnitus: report from the First International Meeting of the COMiT Initiative, November 14, 2014, Amsterdam, The Netherlands. *Trends Hear* (2015) 19. doi:10.1177/2331216515580272
5. Langguth B, Goodey R, Azevedo A, Bjorne A, Cacace A, Crocetti A, et al. Consensus for tinnitus patient assessment and treatment outcome measurement: Tinnitus Research Initiative meeting, Regensburg, July 2006. *Prog Brain Res* (2007) 166:525–36. doi:10.1016/S0079-6123(07)66050-6
6. Basile CÉ, Fournier P, Hutchins S, Hébert S. Psychoacoustic assessment to improve tinnitus diagnosis. *PLoS One* (2013) 8(12):e82995. doi:10.1371/journal.pone.0082995
7. Fournier P, Hébert S. Gap detection deficits in humans with tinnitus as assessed with the acoustic startle paradigm: does tinnitus fill in the gap? *Hear Res* (2013) 295:16–23. doi:10.1016/j.heares.2012.05.011
8. De Ridder D, Vanneste S, Freeman W. The Bayesian brain: phantom percepts resolve sensory uncertainty. *Neurosci Biobehav Rev* (2014) 44:4–15. doi:10.1016/j.neubiorev.2012.04.001
9. Vanneste S, De Ridder D. Deafferentation-based pathophysiological differences in phantom sound: tinnitus with and without hearing loss. *Neuroimage* (2016) 129:80–94. doi:10.1016/j.neuroimage.2015.12.002
10. Degeest S, Corthals P, Vinck B, Keppler H. Prevalence and characteristics of tinnitus after leisure noise exposure in young adults. *Noise Health* (2014) 16(68):26–33. doi:10.4103/1463-1741.127850
11. Hagerman B. Efficiency of speech audiometry and other tests. *Br J Audiol* (1993) 27(6):423–5. doi:10.3109/03005369309076719
12. Driscoll C, Kei J, McPherson B. Outcomes of transient evoked otoacoustic emission testing in 6-year-old school children: a comparison with pure tone screening and tympanometry. *Int J Pediatr Otorhinolaryngol* (2001) 57(1):67–76. doi:10.1016/S0165-5876(00)00445-6
13. Mahomed-Asmail F, Swanepoel de W, Eikelboom RH. Diagnostic hearing assessment in schools: validity and time efficiency of automated audiometry. *J Am Acad Audiol* (2016) 27(1):42–8. doi:10.3766/jaaa.15041
14. Vander Werff KR. Accuracy and time efficiency of two ASSR analysis methods using clinical test protocols. *J Am Acad Audiol* (2009) 20(7):433–52. doi:10.3766/jaaa.20.7.5
15. Moffat G, Adjout K, Gallego S, Thai-Van H, Collet L, Noreña AJ. Effects of hearing aid fitting on the perceptual characteristics of tinnitus. *Hear Res* (2009) 254(1–2):82–91. doi:10.1016/j.heares.2009.04.016
16. Roberts LE, Moffat G, Baumann M, Ward LM, Bosnyak DJ. Residual inhibition functions overlap tinnitus spectra and the region of auditory threshold shift. *J Assoc Res Otolaryngol* (2008) 9(4):417–35. doi:10.1007/s10162-008-0136-9
17. Norena A, Micheyl C, Chéry-Croze S, Collet L. Psychoacoustic characterization of the tinnitus spectrum: implications for the underlying mechanisms of tinnitus. *Audiol Neurootol* (2002) 7(6):358–69. doi:10.1159/000066156
18. Heijneman KM, de Kleine E, Wiersinga-Post E, van Dijk P. Can the tinnitus spectrum identify tinnitus subgroups? *Noise Health* (2013) 15(63):101–6. doi:10.4103/1463-1741.110290

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Validation of Online Versions of Tinnitus Questionnaires Translated into Swedish

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Background: Due to the lack of objective measures for assessing tinnitus, its clinical evaluation largely relies on the use of questionnaires and psychoacoustic tests. A global assessment of tinnitus burden would largely benefit from holistic approaches that not only incorporate measures of tinnitus but also take into account associated fears, emotional aspects (stress, anxiety, and depression), and quality of life. In Sweden, only a few instruments are available for assessing tinnitus, and the existing tools lack validation. Therefore, we translated a set of questionnaires into Swedish and evaluated their reliability and validity in a group of tinnitus subjects.

Methods: We translated the English versions of the Tinnitus Functional Index (TFI), the Fear of Tinnitus Questionnaire (FTQ), the Tinnitus Catastrophizing Scale (TCS), the Perceived Stress Questionnaire (PSQ-30), and the Tinnitus Sample Case History Questionnaire (TSCHQ) into Swedish. These translations were delivered via the internet with the already existing Swedish versions of the Tinnitus Handicap Inventory (THI), the Hospital Anxiety and Depression Scale (HADS), the Hyperacusis Questionnaire (HQ), and the World Health Organization Quality of Life questionnaire (WHOQoL-BREF). Psychometric properties were evaluated by means of internal consistency [Cronbach's alpha (α)] and test–retest reliability across a 9-week interval [Intraclass Correlation Coefficient (ICC), Cohen's kappa] in order to establish construct as well as clinical validity using a sample of 260 subjects from a population-based cohort.

Results: Internal consistency was acceptable for all questionnaires ($\alpha > 0.7$) with the exception of the "social relationships" subscale of the WHOQoL-BREF. Test–retest reliability was generally acceptable (ICC > 0.70, Cohens kappa > 0.60) for the tinnitus-related questionnaires, except for the TFI "sense of control" subscale and 15 items of the TSCHQ. Spearmen rank correlations showed that almost all questionnaires on tinnitus are significantly related, indicating that these questionnaires measure different aspects of the same construct. The data supported good clinical validity of the tinnitus-related questionnaires.

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Conclusion: Our results suggest that most Swedish adaptations of the questionnaires are suitable for clinical and research settings and should facilitate the assessment of treatment outcomes using a more holistic approach by including measures of tinnitus fears, emotional burden, and quality of life.

Keywords: tinnitus, questionnaires, anxiety, depression, stress, hyperacusis, quality of life, TFI

INTRODUCTION

Tinnitus is the perception of one or more sounds despite the physical absence of such sound(s) (Chan, 2009). This condition is chronically experienced by a large portion of the population (>15%) and severely debilitating for about 1–2% of the population, affecting sleep, concentration, and productivity at work (Dobie, 2003; Heller, 2003). Tinnitus is associated with a higher risk of receiving disability pension (Friberg et al., 2012) and perceived as an enormous socioeconomic burden (Cederroth et al., 2013). In the Netherlands, tinnitus-related costs have been estimated to be € 6.8 billion per year (Maes et al., 2013). The prevalence of tinnitus is age-dependent, peaking in the seventh decade of life (Nondahl et al., 2002; Gopinath et al., 2010a,b; Shargorodsky et al., 2010; Park B. et al., 2014; Park K. H. et al., 2014). Tinnitus remains a clinical enigma because of the lack of effective treatments for stopping phantom tinnitus perception (Chan, 2009). Presently, tinnitus assessment relies on self-report questionnaires and subjective psychoacoustic measures (Langguth et al., 2007). Tinnitus heterogeneity varies in its phenotypes and may be objective (emitted by the ear itself and perceivable by an external observer) or subjective (only perceived by the patient), chronic or occasional, pulsatile or non-pulsatile, noise or tonal, constant or intermittent, and unilateral or bilateral (Langguth et al., 2013). Tinnitus may present with a high number of etiologies (e.g., noise exposure, stress, or physical trauma) and a multitude of co-morbidities (e.g., hypertension or diabetes; Langguth et al., 2013). The large variety in tinnitus profiles is thought to partly responsible for the lack of success in clinical treatment trials (Tunkel et al., 2014). Thus, tools need to be urgently identified for reliably assessing tinnitus and enabling the classification of patient subgroups according to a defined set of characteristics.

Several efforts have been made to establish a consensus for patient assessment and outcome measurement (Langguth et al., 2007; Landgrebe et al., 2012; Zeman et al., 2012, 2014). Nevertheless, a recent systematic review has shown that more than 100 instruments are used for primary outcome measures in clinical trials (Hall et al., 2016), evincing that there is still no agreement on how to assess tinnitus. For this reason, a working group of the Cooperation in Science and Technology (COST) action TINNET, a European Tinnitus research Network (www.tinnet.tinnitusresearch.net), is currently standardizing assessment methods and defining a core set of domains and instruments (Hall et al., 2015).

In Sweden, national guidelines on the management and treatment of tinnitus are lacking, and clinics in the different counties rely on local recommendations. The only questionnaires recommended are the Tinnitus Handicap Inventory (THI) and the Hospital Anxiety and Depression Scale (HADS). However, the Swedish versions of these questionnaires lack validity. Thus, the number of patients with tinnitus in Sweden receiving appropriate care is rather small when compared to the large capacities of other European clinics (Karolinska Hospital in Stockholm Sweden, $n = 70$ patients per year vs. the Tinnitus Clinic at the Charité in Berlin, $n = 3000$ new patients per year; or in the Adelante Tinnitus Expert Center in Maastricht, Netherlands, $n = 700$ newly referred patients per year), even when considering the population size of the respective cities. We selected a number of additional questionnaires (for instance, the Tinnitus Sample Case History Questionnaire (TSCHQ), Tinnitus Functional Index (TFI), Fear of Tinnitus Questionnaire (FTQ), Tinnitus Catastrophizing Scale (TCS), and Perceived Stress Questionnaire (PSQ-30) according to recommendations given in a consensus meeting (Langguth et al., 2007) or because of their successful application in clinical trials on tinnitus (Cima et al., 2012). Each of the questionnaires was translated into Swedish. A set of validated questionnaires would not only enable Swedish clinics to assess the burden of tinnitus in a wider context but also other aspects such as measures of tinnitus fears, emotional burden, and quality of life.

MATERIALS AND METHODS

Subjects

Patients with tinnitus were identified in the fifth wave of the Swedish Longitudinal Occupational Survey of Health (SLOSH). All patients aged between 18 and 85 years who had previously agreed to be contacted ($n = 620$) were invited to join STOP and participate in an online survey. Additionally, 319 participants were recruited through flyers. Two hundred and seventy one subjects registered with STOP (http://stop.ki.se) gave their written informed consent to participate in the survey. After excluding participants without tinnitus and incomplete test–retest data, a total sample size of 260 subjects was achieved. The project was approved by the local ethics committee "*Regionala etikprövningsnämnden*" in Stockholm (2014/1998-31/4). The database project and the server were coordinated and located at the Department of Physiology and Pharmacology of the Karolinska Institutet, Sweden.

Selection of Questionnaires

Based on a consensus meeting in 2006, Langguth et al. (2007) recommended the use of several questionnaires such as the TSCHQ (Landgrebe et al., 2010), the THI (Newman et al., 1996, 1998), the Tinnitus-Beeinträchtigungs-Fragebogen (TBF-12; Greimel et al., 1999), the Major Depression Inventory (MDI; Bech and Wermuth, 1998), and the World Health

Organization-Quality of life questionnaire (WHO, 1998). These questionnaires have been used in a large number of studies, albeit preferentially in Europe (Hall et al., 2016).

The TSCHQ was designed to assess the most important tinnitus characteristics and the tinnitus history of patients (Landgrebe et al., 2010). Tinnitus-related impairment in daily life is typically assessed with the THI (Newman et al., 1996, 1998). The TFI (Meikle et al., 2012; Henry et al., 2016) has been proposed as a more recent questionnaire with very high internal consistency of 0.97 and test–retest reliability of 0.78. We favored the TFI over the TBF-12 because of its high responsiveness to treatment-related changes.

In a randomized controlled trial on cognitive behavioral therapy (CBT) that included 245 patients with tinnitus, Cima et al. (2012) reported the successful and valid use of various questionnaires developed for assessing tinnitus-related emotional affects. Tinnitus-specific emotional reactivity and cognitions were evaluated with the TCS and the FTQ (Cima et al., 2011). The TCS is used for assessing cognitive misinterpretations of tinnitus sounds and the FTQ for measuring tinnitus-related fears (Cima et al., 2011). Both questionnaires showed excellent internal consistency values (TCS: Cronbach's alpha = ·0.94; FTQ: Cronbach's alpha = 0.82). Moreover, Cima et al. (2011) evaluated negative emotional affects with the HADS that also showed good reliability (Cronbach's alpha = 0.71–0.90; Spinhoven et al., 1997). The HADS is used for evaluating both depression and anxiety and has been previously tested on the Swedish tinnitus population (Andersson et al., 2003). Therefore, we decided to replace the MDI recommended in the 2006 consensus meeting (Langguth et al., 2007) that only evaluates depression and used the HADS instead. Stress is widely evaluated with the PSQ-30 showing an internal consistency of $0.80 < \alpha < 0.86$ (Levenstein et al., 1993). The combination of HADS and PSQ-30 allows the distinct evaluation of stress, anxiety and depression.

No Hyperacusis Questionnaire (HQ) was suggested in the initial recommendation (Langguth et al., 2007). However, because about 40–55% of patients with tinnitus experience this condition (Baguley, 2003; Schecklmann et al., 2014), we also considered the HQ (Khalfa et al., 2002), which had been validated in a group of tinnitus patients showing an internal consistency of $\alpha = 0.88$ (Fackrell et al., 2015). A Swedish version was developed with an internal consistency of $\alpha = 0.92$, albeit tested on people with Williams Syndrome (Blomberg et al., 2006).

The Health Utilities Index (HUI)—validated for assessing quality of life of patients with tinnitus (Maes et al., 2011)—was used as a primary outcome measure to evaluate the efficacy of specialized CBT on quality of life (Cima et al., 2012). However, a quality of life questionnaire developed by the WHO has also been shown to be suitable for patients with tinnitus (Zeman et al., 2014). The World Health Organization Quality of Life Scale (WHOQoL-BREF), which is a shorter version of the long questionnaire (WHO, 1998), is already available in many different languages and appears to be more appropriate for world-wide use than the HUI.

Permission to translate the questionnaires into Swedish was obtained from all developers of source language questionnaires: B. Langguth and W. Schlee (TSCHQ), J. A. Henry (TFI), R. R. L. Cima (FTQ and TCS), S. Levenstein (PSQ-30). For the TFI translation, as the reproduction in whole or in part is prohibited without the written consent of Oregon Health & Science University (OHSU), a license was obtained from OHSU, who agreed on the above procedure and authorized the validation of the translated TFI questionnaire. For further use in the clinics in Sweden, additional agreements will be needed.

Translation

No clear guidelines exist on how to translate questionnaires (Epstein et al., 2015), in particular when cultural adaptations are required as in the case of translations from English into Swedish. Since the objective of our translations was to find a functional equivalent but not a literal formulation of the original versions, we relied on a procedure called TRAPD (translation, review, adjudication, pre-testing, and documentation) developed by Harkness (2003). This procedure includes translators as well as a team reviewing the translations and presenting the final version (Harkness, 2003). The original English versions of the questionnaires were thus translated into Swedish by three native Swedish speakers (whose mother tongue was the target language, who were fluent in English and country residents with experience in the target culture). All translators were briefed on the background of the project before the translation. First, all translators worked independently and then in a team to produce one single reconciled forward translation. This forward translation was then reviewed and discussed by a multidisciplinary committee from our clinic that included a doctor, an ENT specialist, an audiologist, a psychologist, two researchers, and a statistician to provide an additional level of quality control. All members of the reviewing committee agreed on the final version. Some of the questions and responses were slightly modified in order to produce fully comprehensible items in the Swedish language. Backward translation was conducted by a blinded native Swedish and fluent English speaker, with no knowledge of the original questionnaire. The backward-translated version was evaluated by the project leader and the translator and used as a tool to ensure that the meaning of the items was not altered (conceptual accuracy), rather than as a measure of translation accuracy. The Swedish versions of the questionnaires are available upon request.

Online Survey

Before field-testing from October 2015 to January 2016, we carried out a pilot test of these online surveys on a small group of respondents ($n = 6$) in order to detect any flaws in routing, layout, comprehension, length, software use (different browsers and mobile devices), and data transfer to the server. After giving written consent, patients were invited to participate in a secure online survey that included sociodemographic variables as well as the following questionnaires: the TSCHQ (Landgrebe et al., 2010), THI (Newman et al., 1996, 1998), TFI (Henry et al., 2016), FTQ (Cima et al., 2011), TCS (Cima et al., 2011), HQ (Khalfa et al., 2002), PSQ-30 (Levenstein et al., 1993), HADS

(Andersson et al., 2003), and WHOQoL-BREF (1998). **Table 1** presents an overview of the questionnaires: number of items as well as total and subscale scores. Scores were based on the scoring guideline of each questionnaire. Participants had to complete the questionnaires twice. The median time interval between initial and subsequent assessment was 70 days (Q1 = 66, Q3 = 71, range = 16–94 days). We performed no interventions between the test and re-test sessions.

Statistical Analyses

The sample and the questionnaire values underwent descriptive analysis [frequencies (*n*), percentages (%), means (*m*), standard

TABLE 1 | Questionnaire overview.

Questionnaire	Number of items	Total score	Subscale scores	
TSCHQ	35	–	–	
THI[a]	25	Range = 0–100; categories: 1. 0–16: Negligible 2. 18–36: Light weight 3. 38–56: Moderate weight 4. 58–76: Severe weight 5. 78–100: Catastrophic	–	
TFI[a]	25	Range = 0–100; Categories: 1. 0–17: Not a problem 2. 18–31: Small problem 3. 32–53: Moderate problem 4. 54–72: Big problem 5. 73–100: Very big problem	Range = 0–100 1. Intrusive 2. Sense of control 3. Cognitive 4. Sleep	5. Auditory 6. Relaxation 7. Quality of life 8. Emotional
FTQ[a]	17	Range = 0–17	–	
TCS[a]	13	Range = 0–52	–	
HQ[a]	14	Range = 0–42; Categories: 1. ≤28 = No hyperacusis 2. >28 = Hyperacusis	3. Attentional (range = 0–12) 4. Emotional (range = 0–18) 5. Social (range = 0–12)	
PSQ-30[a]	30	Range = 0–1; Categories: 1. <0.34 = Low stress level 2. 0.34–0.46 = Moderate stress level 3. >0.46 = High stress level	–	
HADS[a]	14	–	Range = 0–21 1. Anxiety 2. Depression Categories: 1. 0–7 = Normal 2. 8–10 = Borderline 3. 11–21 = Abnormal	
WHOQoL-BREF[b]	26	–	Range = 4–20 1. Physical health 2. Psychological 3. Social relationships 4. Environment	Range: 1–5 single items 5. Overall quality of life 6. Overall health

TSCHQ, Tinnitus Sample Case History Questionnaire; THI, Tinnitus Handicap Inventory; TFI, Tinnitus Functional Index; FTQ, Fear of Tinnitus Questionnaire; TCS, Tinnitus Catastrophizing Scale; HQ, Hyperacusis Questionnaire; PSQ-30, Perceived Stress Questionnaire; HADS, Hospital Anxiety and Depression Scale; WHOQoL-BREF, World Health Organization Quality of Life Scale (short version).

[a] *Higher score, higher impairment.*

[b] *Higher score, higher quality of life.*

deviations (*SD*), medians (med), and percentiles (Q1, first quartile/25th percentile; Q3, third quartile/75th percentile)]. Mann-Whitney *U*-tests were used to examine gender differences in tinnitus-related questionnaire values and Spearman's rank correlations to assess the relation between age and tinnitus-related questionnaire values.

A range of standardly-used analyses were carried out to assess the psychometric properties of tinnitus-related questionnaires. Cronbach's alpha coefficient was used to assess the internal consistency of multi-item scales based on correlations between items on the same test or subscale and to show the extent to which several items proposed to measure the same construct result in similar scores. Coefficients >0.70 are considered acceptable (Cohen, 1960; Grouven et al., 2007; see also, http://www.rehabmeasures.org/rehabweb/rhstats.aspx). Test–retest reliability is used to evaluate how stable patients respond over time. The consistency of tinnitus-related data was assessed by Cohen's kappa coefficient for categorical variables and Intraclass Correlation Coefficient (ICC) for metric variables. ICCs > 0.70 and Cohen's kappa > 0.60 are considered acceptable (Cohen, 1960; Grouven et al., 2007; see also, http://www.rehabmeasures.org/rehabweb/rhstats.aspx). Because construct validity indicates whether instruments measure the same theoretical concept, it was used to assess inter-scale correlations [Spearman's rank correlation coefficient (ρ)] within and between tinnitus-related questionnaires. Correlation coefficients ≥0.40 indicate that questionnaires or subscales measure the same aspects of tinnitus (convergent validity), whereas correlation coefficients between <0.40 indicate that questionnaires or subscales measure different aspects (discriminant validity; algebraic signs are omitted; Hays and Hayashi, 1990). Known-group comparisons were used to evaluate the clinical validity of the tinnitus-related questionnaires. The statistical significance of group differences in tinnitus occurrence, onset, and manifestation was tested with Mann-Whitney *U*-tests.

The significance level was set at $p \leq 0.050$. The software package SPSS for Windows, Version 23, was used for all statistical analyses.

RESULTS

Subject Characteristics

Sociodemographic Data

Two hundred and sixty Swedish subjects (52.3% men) were included in the study. The median age was 62.40 years (Q1 = 56.00, Q3 = 68.00, ranging from 21 to 87 years).

Tinnitus-Related Data

The majority of participants reported experiencing continuous tinnitus (86.9%). 64.6% of subjects perceived a gradual onset of tinnitus, and 65.8% described tinnitus of (very) high frequency. Most subjects reported constant tinnitus manifestation over time (73.8%) and that they had consulted with a clinician because of the condition (61.5%). The median time since the onset of tinnitus was 15.00 years (Q1 = 6.00, Q3 = 25.00, ranging from 0 to 55 years, missing information: $n = 30$). Subjects related the initial onset to the following reasons: loud blast of sound ($n = 41$, 15.8%), stress ($n = 37$, 14.2%), change in hearing ($n = 30$, 11.5%), head trauma ($n = 2$, 0.9%), whiplash ($n = 5$, 1.9%), and others ($n = 51$, 19.6%). 36.2% ($n = 94$) could not specify any specific reason. Tinnitus was reduced by music or environmental sounds in 53.5% of subjects and intensified by loud noise in 51.2% and by stress in 53.8%. Medication had no influence on tinnitus in 91.9% of subjects. On a scale from 0 to 100, the median loudness of tinnitus was 60.00 (Q1 = 35.00, Q3 = 75.00, $n = 259$), the median percentage of total awake time being aware of tinnitus was 60.00 (Q1 = 25.00, Q3 = 100.00, $n = 225$), and the median percentage of total awake time being annoyed or distressed of tinnitus was 20.00 (Q1 = 10.00, Q3 = 50.00, $n = 226$). **Table 2** presents both tinnitus-related and additional clinical data (TSCHQ).

Questionnaire Data

The median scores and quartiles of the test and re-test sessions are presented in **Table 3**. At the initial assessment, the THI was 24.00 (Q1 = 14.00, Q3 = 38.00), and 10.8% of subjects described their tinnitus as *severe* to *catastrophic*. The average TFI score was 24.0 (Q1 = 14.00, Q3 = 38.00), and 16.9% of subjects described to have a big or a very big problem. Stress was evaluated by means of the PSQ (median = 0.27, Q1 = 0.16, Q3 = 0.39), and 16.2% of subjects scored high stress levels. Anxiety was measured with the HADS (median = 2.0, Q1 = 1.0, Q3 = 5.0), in which 10% of subjects showed abnormally high scores. Depression, also evaluated with the HADS (median = 4.0, Q1 = 2.0, Q3 = 8.0), showed abnormally high scores in 4.6% of subjects. The median TCS-value was 11.5 (Q1 = 5.0, Q3 = 20.0), and that of the FTQ was 4.0 (Q1 = 3.0, Q3 = 6.0); however, no subscale is available for determining severity levels. The HQ showed that 17.7% of subjects had hyperacusis according to a >28 cut-off value. Quality of life was measured with the WHOQoL subscales for physical (median = 16.0, Q1 = 13.7, Q3 = 17.7), psychological (median = 16.0, Q1 = 14.0, Q3 = 17.3), social (median = 14.7, Q1 = 13.3, Q3 = 16.0), and environmental (median = 16.5, Q1 = 15.0, Q3 = 8.0) relationships. Age was significantly associated with tinnitus-related questionnaire scores, with exception of the THI "intrusive" subscale score, the HQ "emotional" subscale score, and the TCS total score (**Table 4**). Correlation coefficients were small to moderate in size. In general, the older the subjects, the fewer were the impairments and the better the quality of life reported. Women tended to have more impairments and less quality of life than men (**Table 4**). Significant differences were found for 17 out of 25 values.

Psychometric Properties

Internal Consistency

Cronbach's alpha for multi-item scales ranged from 0.69 to 0.97 (see **Table 4**). Thus, internal consistency was acceptable, except for the WHOQoL-BREF subscale "social relationships" that fell short of reaching the conventional cut-off value of $\alpha \leq 0.70$.

Test–Retest Reliability

ICC ranged between 0.68 and 0.90 (**Table 4**) and Cohen's kappa from 0.34 to 0.93 (**Table 2**). Test–retest reliability was acceptable, except for the subscale "sense of control" of the TFI and for 15 items of the TSCHQ. Critical items of the TSCHQ were: 3b

TABLE 2 | Tinnitus-related data and test–retest reliability assessed with the Tinnitus Sample Case History Questionnaire (TSCHQ).

	Initial assessment		Test–retest reliability
	n	%	Cohen's kappa/Intra-class correlation coefficient
Onset of tinnitus (exact date)	–	–	0.34
Relation of initial onset of tinnitus (etiology)			0.51
Loud blast of sound	41	15.8	
Stress	37	14.2	
Change in hearing	30	11.5	
Head trauma	2	0.8	
Whiplash	5	1.9	
Other	51	19.6	
Do not know	94	36.2	
Tinnitus occurrence			0.73
Occasionally	34	13.1	
Permanently	226	86.9	
Time of day of tinnitus emergence			0.50
When awakening	20	7.7	
In the morning	8	3.1	
Around noon	24	9.2	
In the afternoon	16	6.2	
In the evening	39	15.0	
Before sleeping	21	8.1	
Do not know	132	50.8	
Perceiving the onset of tinnitus			0.78
Gradual	168	64.6	
Abrupt	92	35.4	
Pulsation of tinnitus			0.69
Yes, with heartbeat	33	12.7	
Yes, different from heartbeat	12	4.6	
No	215	82.7	
Location of tinnitus			0.62
Right ear	25	9.6	
Left ear	35	13.5	
Both ears, worse in right ear	37	14.2	
Both ears, worse in left ear	38	14.6	
Both ears equally	79	30.4	
Inside the head	43	16.5	
Elsewhere	3	1.2	
Tinnitus manifestation over time			0.55
Intermittent	68	26.2	
Constant	192	73.8	
Loudness of tinnitus (median, Q1/Q3)	60	35/75	0.83
Tinnitus loudness variation from day to day			0.58
Yes	173	66.5	
No	87	33.5	
Percentage of total awake time of tinnitus awareness (median, Q1/Q3)	60	25/100	0.71
Percentage of total awake time being distressed by tinnitus (median, Q1/Q3)	20	10/50	0.78
Sound of tinnitus			0.56
Tone	102	39.2	
Noise	108	41.5	
Crickets	36	13.8	
Other	14	5.4	
Pitch of tinnitus			0.47
Low frequency	15	5.8	
Medium frequency	74	28.5	
High frequency	120	46.2	
Very high frequency	51	19.6	
Reduction of tinnitus by music or environmental sounds			0.44
Yes	139	53.5	
No	65	25.0	
Do not know	56	21.5	
Worsening of tinnitus by loud noise			0.54
Yes	133	51.2	
No	82	31.5	
Don't know	45	17.3	
Tinnitus affected by head movement or touch			0.66
Yes	54	20.8	
No	206	79.2	
Tinnitus affected by nap			0.60
Yes, worsening of tinnitus	7	2.7	
Yes, reducing of tinnitus	34	13.1	
No, no effect	219	84.2	
Tinnitus affected by sleep at night			0.35
Yes	49	18.8	
No	103	39.6	
Do not know	108	41.5	
Tinnitus affected by stress			0.70
Yes, worsening of tinnitus	139	53.5	
Yes, reducing of tinnitus	2	0.8	
No, no effect	119	45.8	
Tinnitus affected by medication			0.67
Yes	21	8.1	
No	239	91.9	
Contacted a clinician due to tinnitus			0.69
Yes	160	61.5	

TABLE 2 | Continued

	Initial assessment		Test–retest reliability
	n	%	Cohen's kappa/Intra-class correlation coefficient
No	100	38.5	
Number of different tinnitus treatments			0.61
0	196	75.4	
1	18	63.9	
2–4	28	10.8	
5 or more	18	6.9	
Tinnitus occurrence in family			0.74
Yes	187	71.9	
No	73	28.1	
Comorbidity			0.83
Yes	129	49.6	
No	131	50.4	
Medication			0.87
Yes	166	63.8	
No	94	36.2	
Currently under treatment for psychiatric problems			0.73
Yes	16	6.2	
No	244	93.8	
Hearing problem			0.74
Yes	220	84.6	
No	40	15.4	
Hearing aids			0.93
Yes, on both ears	74	28.5	
Yes, on the right ear	8	3.1	
Yes, on the left ear	9	3.5	
No	169	65.0	
Problems tolerating sounds			0.41
Never	41	15.8	
Rarely	44	16.9	
Sometimes	100	38.5	
Usually	49	18.8	
Always	26	10.0	
Sounds cause pain or physical discomfort			0.46
Yes	115	44.2	
No	124	47.2	
Do not know	21	8.1	
Headache			0.77
Yes	56	21.5	
No	204	78.5	
Vertigo or dizziness			0.56
Yes	72	27.7	
No	188	72.3	
Temporomandibular disorder			0.67
Yes	29	11.2	
No	231	88.8	
Neck pain			0.82
Yes	72	27.7	
No	188	72.3	
Other pain syndromes			0.65
Yes	71	27.3	
No	189	72.7	

Test–retest reliability was assessed by Cohen's kappa coefficient for categorical variables and intra-class correlation coefficient for metric variables. ICCs > 0.70 and Cohen's kappa > 0.60 are considered acceptable. Descriptive data of Tinnitus Sample Case History Questionnaire is presented at initial assessment.

(the time of day tinnitus starts), 5 (initial onset), 7 (etiology), 10 (intermittent or constant), 11 (varying loudness), 14 (sound of tinnitus), 15 (pitch of tinnitus), 18 (different treatments), 19 (reduction by ambient sounds), 20 (tinnitus worse by noise), 22 (nap effects), 23 (tinnitus affected by night sleep), 28 (problems tolerating sounds), 29 (pain induced by noise), and 31 (vertigo or dizziness).

Construct Validity

Spearmen's rank correlations showed that almost all tinnitus-related questionnaires were significantly related (mainly $p < 0.001$), with the exception of the correlation between the "auditory" subscale of the TFI and the "social relationships" subscale of WHOQoL-BREF ($\rho = -0.12$, $p = 0.053$). 55% ($n = 165$) of 300 correlations yielded coefficients of $\geq$0.40, and 10.3% ($n = 31$) substantial coefficients of $\geq$0.70. These findings indicated that the questionnaires measured different aspects of the same construct. In general, higher correlation coefficients were observed between total and subscale scores of the THI, TFI, HQ, and HADS. WHOQoL-BREF showed correlation coefficients of $\geq$0.40 mainly within its subscales but not with other tinnitus-related questionnaires. **Table 5** summarizes the correlation coefficients.

Clinical Validity

Subjects with a more severe clinical condition (permanent tinnitus, abrupt onset, and constant manifestation of tinnitus) tended to report more tinnitus-related impairments than subjects with a less severe clinical condition (occasional tinnitus, gradual onset, and intermittent manifestation of tinnitus). **Table 6** summarizes the results of the Mann-Whitney *U*-tests.

DISCUSSION

Overall, the Swedish versions of the tinnitus-specific questionnaires showed good internal consistency, test–retest reliability, construct, as well as clinical validity. Internal consistency was excellent ($\alpha > 0.90$) for the THI, TFI, TCS,

TABLE 3 | Descriptive analyses of tinnitus-related questionnaires.

N = 260		Initial assessment		Subsequent assessment	
Questionnaire		**med (Q1, Q3)**	**Range**	**med (Q1, Q3)**	**Range**
THI[b,c]					
Sum score		24.0 (14.0, 38.0)	0–90	22.0 (12.0, 36.0)	0–94
		n	**%**	***n***	**%**
Categories	Negligible	82	31.5	97	37.5
	Light weight	110	42.3	99	38.2
	Moderate weight	40	15.4	42	16.2
	Severe weight	20	7.7	14	5.4
	Catastrophic	8	3.1	7	2.7
TFI[b,c]					
Sum score		24.0 (14.0, 38.0)	0–90	22.0 (12.0, 36.0)	0–94
		n	**%**	***n***	**%**
Categories	No problem	78	30.0	84	32.4
	Small problem	57	21.9	63	24.3
	Moderate problem	81	31.2	69	26.6
	Big problem	34	13.1	28	10.8
	Very big problem	10	3.8	15	5.8
Subscales	Intrusive	43.3 (23.3, 63.3)	0–100	43.3 (23.3, 60.0)	0–100
	Sense of control	43.3 (16.7, 66.7)	0–100	43.3 (20.0, 66.7)	0–100
	Cognitive	20.0 (6.7, 43.3)	0–93	16.7 (3.3, 40.0)	0–100
	Sleep	13.3 (0.0, 43.3)	0–100	13.3 (0.0, 40.0)	0–100
	Auditory	43.3 (16.7, 69.2)	0–100	36.7 (20.0, 66.7)	0–100
	Relaxation	23.3 (10.0, 50.0)	0–100	20.0 (6.7, 50.0)	0–100
	Quality of life	20.0 (2.5, 42.5)	0–93	17.5 (2.5, 40.0)	0–100
	Emotional	6.7 (0.0, 16.7)	0–67	6.7 (0.0, 16.7)	0–67
FTQ[b]					
Sum score		4.0 (3.0, 6.0)	1–14	4.0 (3.0, 6.0)	1–17
TCS[b,c]					
Sum score		11.5 (5.0, 20.0)	0–45	10.0 (4.0, 19.0)	0–52
PSQ-30[b,c]					
PSQ index		0.27 (0.16, 0.39)	0.00–0.82	0.24 (0.13–0.39)	0.00–0.81
		n	**%**	***n***	**%**
Categories	Low stress level	163	62.7	171	65.8
	Moderate stress level	55	21.2	45	17.3
	High stress level	42	16.2	43	16.5
HQ[b,c]					
Sum score		17.0 (10.0, 25.0)	0–39	17.0 (10.0, 25.0)	0–39
		n	**%**	***n***	**%**
Categories	No hyperacusis	214	82.3	214	82.3
	Hyperacusis	46	17.7	46	17.7
Subscales	Attentional	5.0 (2.0, 7.0)	0–12	5.0 (2.0, 7.0)	0–12
	Emotional	7.0 (4.0, 11.0)	0–18	7.0 (4.0, 77.0)	0–18
	Social	5.0 (2.0, 8.0)	0–12	5.0 (2.0, 8.0)	0–12
HADS[b]					
Subscales	Anxiety	4.0 (2.0, 8.0)	0–17	4.0 (2.0, 7.0)	0–18
	Depression	2.0 (1.0, 5.0)	0–15	2.0 (1.0, 5.0)	0–18

(Continued)

TABLE 3 | Continued

N = 260		Initial assessment		Subsequent assessment	
Questionnaire		**med (Q1, Q3)**	**Range**	**med (Q1, Q3)**	**Range**
		n	**%**	***n***	**%**
THI[b,c]					
Sum score		24.0 (14.0, 38.0)	0–90	22.0 (12.0, 36.0)	0–94
		n	**%**	***n***	**%**
Categories anxiety	Normal	193	74.2	199	76.5
	Borderline	41	15.8	37	14.2
	Abnormal	26	10.0	24	9.2
Categories depression	Normal	227	87.3	226	86.9
	Borderline	21	8.1	23	8.8
	Abnormal	12	4.6	11	4.2
WHOQoL-BREF[a]					
Subscales	Physical health[C]	16.0 (13.7, 17.7)	5–20	16.0 (13.7, 17.7)	6–20
	Psychological	16.0 (14.0, 17.3)	7–20	16.0 (14.0, 17.3)	7–20
	Social relationships	14.7 (13.3, 16.0)	5–20	14.7 (13.3, 16.0)	5–20
	Environment	16.5 (15.0, 18.0)	10–20	16.5 (15.0, 18.0)	10–20
	Overall quality of life	4.0 (4.0, 5.0)	1–5	4.0 (4.0, 5.0)	1–5
	Overall health	4.0 (3.0, 4.0)	1–5	4.0 (3.0, 4.0)	1–5

THI, Tinnitus Handicap Inventory; TFI, Tinnitus Functional Index; FTQ, Fear of Tinnitus Questionnaire; TCS, Tinnitus Catastrophizing Scale; PSQ-30, Perceived Stress Questionnaire; HQ, Hyperacusis Questionnaire; HADS, Hospital Anxiety and Depression Scale; WHOQoL-BREF, World Health Organization Quality of Life Scale (short version).

[a] *Higher score, higher quality of life.*

[b] *Higher score, higher impairment.*

[c] *n = 259 at subsequent assessment.*

HQ, and PSQ-30, good for the HADS ($0.80 \leq \alpha \leq 0.90$), and acceptable for the FTQ ($0.70 \leq \alpha \leq 0.80$). However, the subscale "social relationships" of the WHOQoL-BREF showed low internal consistency that fell short of reaching the conventional cut-off value of $\alpha \leq 0.70$.

Test–retest reliability was acceptable, except for the subscale sense of control of the TFI (ICC = 0.68) and for 15 items of the TSCHQ that includes descriptive data about tinnitus. The comparison of TSCHQ scores with previously published descriptive analyses by the Tinnitus Research Initiative (Schecklmann et al., 2014) showed very similar prevalence for specific items. For instance, gradual perception of tinnitus at its onset was reported by 64.6% of subjects in STOP vs. 50% in the TRI. Similarly, high-frequency perceptions were reported by 65.8% of subjects in STOP vs. 72% in the TRI. 73.8% of subjects in the STOP reported constant tinnitus in comparison to 84% in the TRI. Cohen's kappa coefficients of several TSCHQ items (e.g., first tinnitus experience, manifestation of tinnitus over time, or suffering from headaches) were below the cut-off value of $k > 0.60$. However, this result does not mean that the questionnaire is not reliable *per-se*. The low kappa values may reflect (a) variables that differ in time or are fluctuating, (b) variables that are not accurately remembered, (c) that subjects did not understand the item, and (d) how reliably or conscientiously subjects respond to questionnaires. Nonetheless, this finding suggests that caution should be taken in the interpretation of some items of the TSCHQ. The test–retest reliability of the TSCHQ should also be investigated in a different sample in order to find out whether the phrasing of specific questions should be modified—this could also apply to the original English version of the TSCHQ.

Backward translation is often conducted to ensure the reliability of the forward translation, however, we found no guideline on how to score the reliability of a backward translation. We considered one-word change in the translated version, as a meaningful difference when comparing to the original version. Using this criterion, we found that in the case of English-Swedish translations, near 60% of backward-translated items from the TSCHQ and the TFI differed from the original version. With shorter sentences, as those found in the PSQ-30, this number went down to 40%. Importantly, of all backward-translated items in which a change from the original version was observed, only 6% of them had potentially altered meaning. Verification of the Swedish items helped confirming that they were culturally adapted and thus appropriate for testing. The low score for the subscale sense of control of the TFI (ICC = 0.68) could potentially derive from translation failures. The verb "*to cope*" in English has the equivalent "*att hantera*" in Swedish, but which has additional meanings such as "*to handle*" or "*to manage.*" Such differences, when evaluating the "sense of control" could alter the test–retest reliability. Potentially, this variability in the test–retest sessions might not necessarily occur in a more severe group of individuals such as those recruited in clinics, which is not the case with the STOP cohort (population based).

TABLE 4 | Internal consistency and test–retest reliability of tinnitus-related questionnaires and relations with age and sex.

N = 260	Internal consistency	Test–retest reliability		Age	Sex		
Questionnaire	**Cronbach's α**	**ICC (95% CI)**	***F* (*df*)**	**Spearmen's rank correlation**	**Men med (Q1, Q3)**	**Women med (Q1, Q3)**	***U*-test**
THI total[b,c]	**0.93**	**0.90 (0.87, 0.92)**	**18.665 (258)****	**−0.26****	**22.0 (12.0, 37.5)**	**26.0 (16.0, 39.5)**	**7165.0***
TFI total[b,c]	**0.97**	**0.88 (0.85, 0.90)**	**15.314 (258)****	**−0.22****	**24.6 (12.4, 40.0)**	**34.8 (14.9, 20.1)**	**7066.0***
Intrusive	0.84	0.82 (0.78, 0.86)	10.097 (258)**	−0.09	41.7 (23.3, 63.3)	43.3 (24.2, 63.3)	7912.5
Sense of control	0.81	0.68 (0.61, 0.74)	5.256 (258)**	−0.12*	43.3 (16.7, 66.7)	41.7 (16.7, 65.8)	8322.0
Cognitive	0.95	0.81 (0.76, 0.85)	9.494 (258)**	−0.23**	16.7 (6.7, 36.7)	25.0 (6.7, 55.8)	7003.0*
Sleep	0.95	0.79 (0.74, 0.83)	8.510 (258)**	−0.13*	10.0 (0.0, 35.8)	20 (0.0, 50.0)	7000.0*
Auditory	0.95	0.76 (0.70, 0.81)	7.255 (258)**	−0.15*	33.3 (16.7, 63.3)	46.7 (20.0, 75.8)	7451.0
Relaxation	0.94	0.79 (0.74, 0.83)	8.493 (258)**	−0.24**	20.0 (6.7, 40.0)	30.0 (10.0, 60.0)	6962.5*
Quality of life	0.91	0.87 (0.84, 0.90)	14.569 (258)**	−0.21**	12.5 (2.5, 35.0)	23.8 (5.0, 46.9)	6919.0*
Emotional	0.88	0.81 (0.77, 0.85)	9.581 (258)**	−0.24**	3.3 (0.0, 13.3)	6.7 (0.0, 20.0)	7534.0
FTQ total[b]	**0.71**	**0.74 (0.68, 0.79)**	**6.638 (259)****	**−0.23****	**4.0 (3.0, 6.0)**	**4.0 (3.0, 6.0)**	**8136.0**
TCS total[b,c]	**0.93**	**0.82 (0.77, 0.86)**	**10.315 (258)****	**−0.07**	**11.0 (5.0, 19.0)**	**13.0 (5.0, 21.8)**	**7857.5**
PSQ-30 total[b,c]	**0.94**	**0.88 (0.85, 0.91)**	**16.624 (258)****	**−0.41****	**23 (0.13, .34)**	**0.3 (0.2, 0.4)**	**6358.5****
HQ total[b,c]	**0.90**	**0.88 (0.85, 0.91)**	**16.141 (258)****	**−0.18***	**13.0 (8.0, 20.0)**	**22.0 (14.0, 28.8)**	**5165.5****
Attentional	0.75	0.73 (0.67, 0.78)	6.388 (258)**	−0.22**	4.0 (1.0, 6.0)	6.0 (3.0, 9.0)	5337.0**
Emotional	0.83	0.86 (0.82, 0.89)	13.199 (258)**	−0.11	6.0 (3.3, 10.0)	9.0 (5.0, 13.0)	6313.5**
Social	0.82	0.83 (0.78, 0.86)	10.426 (258)**	−0.18*	4.0 (2.0, 6.0)	7.0 (4.0, 9.0)	5046.5**
HADS[b]							
Anxiety	0.85	0.85 (0.81, 0.88)	12.278 (259)**	−0.21**	4.0 (2.0, 6.0)	5.0 (3.0, 9.0)	6860.5*
Depression	0.83	0.85 (0.81, 0.88)	12.505 (259)**	−0.32**	2.0 (1.0, 4.8)	2.5 (1.0, 6.0)	7457.0
WHOQoL-BREF[a]							
Physical health[c]	0.84	0.88 (0.85, 0.91)	15.628 (258)**	0.18*	16.0 (14.1, 17.7)	15.4 (12.6, 17.1)	6900.5*
Psychological	0.84	0.87 (0.84, 0.90)	14.721 (259)**	0.27**	16.7 (14.7, 17.3)	15.3 (13.3, 16.7)	6261.0**
Social relationships	0.69[d]	0.78 (0.73, 0.83)	8.192 (259)**	0.21**	14.7 (12.3, 16.0)	14.7 (13.3, 16.0)	8414.5
Environment	0.78	0.80 (0.75, 0.84)	9.020 (259)**	0.26**	16.5 (15.5, 18.0)	16.0 (15.0, 17.5)	6733.0*
Overall quality of life	–	0.74 (0.69, 0.80)	6.876 (259)**	0.25**	4.0 (4.0, 5.0)	4.0 (4.0, 5.0)	7091.0*
Overall health	–	0.71 (0.65, 0.77)	5.920 (259)**	0.21**	4.0 (3.0, 4.0)	3.0 (2.0, 4.0)	6849.5*

THI, Tinnitus Handicap Inventory; TFI, Tinnitus Functional Index; FTQ, Fear of Tinnitus Questionnaire; TCS, Tinnitus Catastrophizing Scale; PSQ-30, Perceived Stress Questionnaire; HQ, Hyperacusis Questionnaire; HADS, Hospital Anxiety and Depression Scale; WHOQoL-BREF, World Health Organization Quality of Life Scale (short version).

***Correlation is significant at the 0.001 level. *Correlation is significant at the 0.05 level.*

[a]higher score, higher quality of life.

[b]higher score, higher impairment.

[c]n = 259 at subsequent assessment.

[d]Internal consistency of domain social relationship (WHOQoL-BREF) increases to α = 0.73 without item number 21. However, the item-total correlation between item 21 and its subscale is r = 0.42, which is acceptable.

Indeed, it is possible that the low values obtained for some of the items of the TSCHQ are due to the fact that the population tested within the STOP includes participants from the general population and not clinical (outpatient) individuals. When comparing the scores of STOP participants with the scores obtained in other studies, we observed that the scores of the different questionnaires were lower than normal. Because most studies failed to report median values, we compared the mean values. Our average THI score was 28.34 in comparison to the range of 40–55 found in the literature (Kaldo et al., 2007; Westin et al., 2011; Albu and Chirtes, 2014; Jasper et al., 2014). The TFI average was 31.74 vs. 40.6 (Fackrell et al., 2016), so that the overall score was lower than that typically found in the literature. Similarly, the anxiety level of 5.12 measured with the HADS was lower than that reported in other studies (6.2–8.7; Kaldo et al., 2007; Westin et al., 2011; Albu and Chirtes, 2014; Jasper et al., 2014). The average of 3.44 for depression in the STOP cohort was also lower than the range of 4.05–6.5 described in the literature (Kaldo et al., 2007; Westin et al., 2011; Albu and Chirtes, 2014; Jasper et al., 2014). The average values of 13.86 of tinnitus-specific cognitions measured with the TCS were almost two times less than the baseline score of 21.11 in the study by Cima et al. (2012). Fear-reactivity as measured by the FTQ was 4.57 in the STOP cohort vs. 7.25 (Cima et al., 2012) at baseline. This finding may be due to the fact that all patients in the Cima trial had severely irritating tinnitus at baseline with an average THI score of 38.96 (*SD* 22.88; Cima et al., 2012) in contrast to our study sample who had significantly less severe tinnitus with an average score of 28.34. The STOP values were more comparable with the values

TABLE 5 | Convergent validity: spearmen's rank correlations between tinnitus-related questionnaires.

	THI total	TFI total	TFI intrusive	TFI sense of control	TFI cognitive	TFI sleep	TFI auditory	TFI relaxation	TFI quality of life	TFI emotional	FTQ total	TCS total	HQ total	HQ attentional	HQ emotional	HQ social	PSQ-30 total	HADS anxiety	HADS depression	WHOQoL physical health	WHOQoL psychological	WHOQoL social relationships	WHOQoL environment	WHOQoL overall quality of life	WHOQoL overall health
THI total	–																								
TFI total	0.85**	–																							
TFI intrusive	0.70**	0.85**	–																						
TFI sense of control	0.67**	0.77**	0.68**	–																					
TFI cognitive	0.78**	0.89**	0.71**	0.66**	–																				
TFI sleep	0.60**	0.73**	0.58**	0.48**	0.67**	–																			
TFI auditory	0.61**	0.73**	0.61**	0.45**	0.55**	0.39**	–																		
TFI relaxation	0.71**	0.86**	0.71**	0.65**	0.81**	0.67**	0.48**	–																	
TFI quality of life	0.79**	0.89**	0.66**	0.59**	0.77**	0.56**	0.74**	0.71**	–																
TFI emotional	0.75**	0.79**	0.65**	0.58**	0.71**	0.57**	0.50**	0.69**	0.72**	–															
FTQ total	0.55**	0.57**	0.47**	0.46**	0.52**	0.39**	0.39**	0.51**	0.49**	0.54**	–														
TCS total	0.68**	0.66**	0.59**	0.61**	0.62**	0.52**	0.37**	0.63**	0.54**	0.63**	0.65**	–													
HQ total	0.57**	0.56**	0.38**	0.32**	0.56**	0.42**	0.43**	0.52**	0.59**	0.50**	0.31**	0.37**	–												
HQ attentional	0.40**	0.43**	0.27**	0.23**	0.47**	0.31**	0.28**	0.44**	0.44**	0.38**	0.22**	0.29**	0.87**	–											
HQ emotional	0.55**	0.55**	0.39**	0.33**	0.47**	0.37**	0.51**	0.44**	0.61**	0.46**	0.32**	0.33**	0.87**	0.61**	–										
HQ social	0.52**	0.49**	0.32**	0.29**	0.55**	0.42**	0.30**	0.48**	0.46**	0.47**	0.26**	0.36**	0.87**	0.70**	0.61**	–									
PSQ–30 total	0.47**	0.44**	0.23**	0.33**	0.48**	0.33**	0.25**	0.47**	0.43**	0.43**	0.26**	0.34**	0.49**	0.46**	0.32**	0.52**	–								
HADS anxiety	0.40**	0.35**	0.19**	0.22**	0.42**	0.30**	0.20**	0.40**	0.33**	0.39**	0.28**	0.39**	0.45**	0.40**	0.31**	0.48**	0.75**	–							
HADS depression	0.42**	0.40**	0.19**	0.29**	0.44**	0.29**	0.23**	0.39**	0.43**	0.39**	0.22**	0.27**	0.43**	0.37**	0.36**	0.41**	0.71**	0.65**	–						
WHOQoL physical health	–0.41**	–0.45**	–0.27**	–0.32**	–0.46**	–0.41**	–0.22**	–0.44**	–0.43**	–0.43**	–0.22**	–0.30**	–0.44**	–0.38**	–0.34**	–0.45**	–0.64**	–0.53**	–0.60**	–					
WHOQoL psychological	–0.37**	–0.34**	–0.16*	–0.24**	–0.41**	–0.26**	–0.13*	–0.37**	–0.33**	–0.35**	–0.25**	–0.30**	–0.37**	–0.35**	–0.26**	–0.38**	–0.70**	–0.68**	–0.71**	0.66**	–				
WHOQoL social relationships	–0.30**	–0.28**	–0.15*	–0.22**	–0.27**	–0.20**	–0.12	–0.32**	–0.30**	–0.29**	–0.12	–0.23**	–0.27**	–0.26**	–0.20**	–0.27**	–0.50**	–0.42**	–0.50**	0.46**	0.57**	–			
WHOQoL environment	–0.31**	–0.33**	–0.15*	–0.23**	–0.36**	–0.26**	–0.18**	–0.34**	–0.37**	–0.28**	–0.18**	–0.20**	–0.34**	–0.28**	–0.31**	–0.31**	–0.55**	–0.49**	–0.53**	0.64**	0.61**	0.43**	–		
WHOQoL overall quality of life	–0.38**	–0.38**	–0.23**	–0.25**	–0.39**	–0.35**	–0.16**	–0.40**	–0.39**	–0.41**	–0.24**	–0.28**	–0.41**	–0.39**	–0.34**	–0.35**	–0.63**	–0.57**	–0.60**	0.64**	0.65**	0.50**	0.61**	–	
WHOQoL overall health	–0.34**	–0.33**	–0.22**	–0.23**	–0.32**	–0.31**	–0.13*	–0.34**	–0.30**	–0.34**	–0.14*	–0.23**	–0.37**	–0.30**	–0.30**	–0.37**	–0.51**	–0.43**	–0.48**	0.72**	0.55**	0.37**	0.47**	0.56**	–

THI, Tinnitus Handicap Inventory; TFI, Tinnitus Functional Index; FTQ, Fear of Tinnitus Questionnaire; TCS, Tinnitus Catastrophizing Scale; PSQ-30, Perceived Stress Questionnaire, HQ, Hyperacusis Questionnaire; HADS, Hospital Anxiety and Depression Scale; WHOQoL-BRE, World Health Organization Quality of Life Scale (short version).

***Correlation is significant at the 0.01 level (2-tailed). *Correlation is significant at the 0.05 level (2-tailed).*

TABLE 6 | Clinical validity of tinnitus-related questionnaires.

N = 260	Tinnitus occurrence			Tinnitus onset			Tinnitus manifestation		
Questionnaire	**Occasionally *n* = 34 med (Q1, Q3)**	**Permanently *n* = 226 med (Q1, Q3)**	***U*-test**	**Gradual *n* = 168 med (Q1, Q3)**	**Abrupt *n* = 92 med (Q1, Q3)**	**U-Test**	**Intermittent *n* = 68 med (Q1, Q3)**	**Constant *n* = 192 med (Q1, Q3)**	**U-test**
THI total[b]	**17.0 (6.0, 26.0)**	**26.0 (16.0, 42.0)**	**2239.5****	**23.0 (12.0, 36.0)**	**28.0 (16.0, 46.0)**	**6691.0**	**20.0 (10.0, 31.5)**	**26.0 (16.0, 42.0)**	**5256.0***
TFI total[b]	**11.0 (4.7, 25.4)**	**32.4 (16.3, 46.4)**	**1948.0****	**29.2 (12.1, 42.6)**	**34.4 (14.0, 50.3)**	**6738.0**	**18.2 (9.8, 38.9)**	**32.6 (17.7, 46.7)**	**4883.0***
Intrusive	20.0 (12.5, 37.5)	46.7 (26.7, 66.7)	1757.5**	43.3 (23.3, 63.3)	46.7 (23.3, 65.8)	7367.5	26.7 (14.2, 56.7)	46.7(26.7, 66.7)	4476.0**
Sense of control	11.7 (3.3, 40.8)	46.7 (20.0, 66.7)	2233.5**	40.0 (13.3, 63.3)	48.3 (24.2, 70.0)	6506.5*	36.7 (10.0, 62.5)	46.7 (20.0, 66.7)	5576.5
Cognitive	6.7 (0.0, 26.7)	20.0 (6.7, 43.3)	2708.0*	16.7 (6.7, 39.2)	23.3 (10.0, 55.8)	6671.0	10.0 (6.7, 30.0)	23.3 (6.7, 46.7)	5476.0*
Sleep	6.7 (0.0, 21.7)	16.7 (0.0, 44.2)	2970.5*	10.0 (0.0, 40.0)	16.7 (0.8, 50.0)	6741.0	8.3 (0.0, 39.2)	16.7 (0.0, 43.3)	5801.0
Auditory	10.0 (3.3, 36.7)	46.7 (22.5, 73.3)	1878.5**	46.7 (20.0, 66.7)	35.0 (10.8, 70.0)	7214.0	30.0 (10.0, 55.8)	50.0 (20.0, 73.3)	4746.5**
Relaxation	10.0 (0.0, 23.3)	26.7 (10.0, 53.3)	2426.0**	20.0 (4.2, 46.7)	33.3 (10.0, 60.0)	6370.5*	18.3 (3.3, 32.5)	26.7 (10.0, 56.7)	5278.0*
Quality of life	2.5 (0.0, 20.6)	22.5 (5.0, 47.5)	2197.0**	20.0 (2.5, 42.5)	22.5 (3.1, 47.5)	7245.0	11.3 (2.5, 26.9)	22.5 (5.0, 47.5)	5205.0*
Emotional	1.7 (0.0, 4.2)	6.7 (0.0, 16.7)	2415.5**	6.7 (0.0, 13.3)	6.7 (0.8, 26.7)	6412.0*	3.3 (0.0, 10.0)	6.7 (0.0, 20.0)	5314.5*
FTQ total[b]	**4.0 (2.8, 4.0)**	**4.0 (3.0, 6.0)**	**2921.0***	**4.0 (3.0, 6.0)**	**4.0 (3.0, 6.0)**	**7036.0**	**4.0 (3.0, 6.0)**	**4.0 (3.0, 6.0)**	**5913.5**
TCS total[b]	**8.0 (2.0, 16.0)**	**12.0 (6.0, 21.0)**	**2860.5***	**11.0 (6.0, 19.0)**	**13.5 (5.0, 22.0)**	**7184.5**	**10.0 (4.0, 16.0)**	**12.5 (6.0, 21.0)**	**5344.5***
PSQ-30 total[b]	**0.2 (0.2,0.4)**	**0.3 (0.2,0.4)**	**3735.0**	**0.3 (0.2,0.4)**	**0.3 (0.1,0.4)**	**6874.0**	**0.3 (0.2,0.4)**	**0.3 (0.2,0.4)**	**6370.5**
HQ total[b]	**14.5 (8.0, 22.5)**	**17.0 (10.0, 25.0)**	**3422.0**	**15.0 (8.3, 24.0)**	**20.0 (12.0, 27.8)**	**6204.5***	**15.0 (9.3, 21.8)**	**17.5 (10.0, 26.0)**	**5681.5**
Attentional	4.5 (2.8, 7.0)	5.0 (2.0, 8.0)	3817.0	4.0 (2.0, 6.8)	6.0 (3.0, 8.0)	6154.5*	4.0 (2.0, 6.0)	5.0 (2.0, 8.0)	5458.5*
Emotional	5.0 (3.0, 9.3)	7.0 (4.0, 11.0)	3084.0	6.0 (4.0, 10.0)	8.0 (5.0, 12.0)	6522.0*	6.0 (4.0, 10.0)	7.0 (4.0, 11.8)	5738.5
Social	5.0 (2.0, 7.5)	5.0 (2.0, 8.0)	3641.5	4.0 (2.0, 7.8)	6.0 (3.0, 9.0)	6409.5*	5.0 (2.0, 7.0)	5.0 (2.0, 8.8)	6204.5
HADS[b]									
Anxiety	4.0 (2.0, 8.0)	4.0 (2.0, 8.0)	3766.0	4.0 (2.0, 7.0)	5.0 (2.0, 8.0)	6486.5*	4.0 (2.0, 7.0)	5.0 (2.0, 8.0)	6085.5
Depression	1.0 (1.0, 4.0)	2.0 (1.0, 5.0)	3223.5	2.0 (1.0, 4.0)	3.0 (1.0, 6.8)	6501.0*	2.0 (1.0, 5.0)	2.0 (1.0, 5.0)	6475.5
WHOQoL-BREF[a]									
Physical health	17.1 (14.1, 18.3)	15.4 (13.7, 17.7)	3284.5	16.6 (13.7, 17.7)	14.9 (13.1, 17.1)	6326.5*	16.0 (13.3, 17.7)	15.4 (13.7, 17.7)	6277.0
Psychological	16.0 (14.0, 17.3)	16.0 (14.0, 17.3)	3734.5	16.0 (14.7, 17.3)	15.3 (13.3, 16.7)	6169.5*	16.0 (13.5, 17.3)	16.0 (14.0, 17.3)	6468.5
Social relationships	14.7 (12.0, 16.0)	14.7 (13.3, 16.0)	3820.0	14.7 (13.3, 16.0)	14.7 (12.3, 16.0)	7113.5	14.7 (12.0, 16.0)	14.7 (13.3, 16.0)	6034.5
Environment	16.5 (15.5, 17.1)	16.5 (15.0, 18.0)	3834.5	16.5 (15.1, 18.0)	16.0 (15.0, 17.5)	7009.0	16.5 (15.0, 18.0)	16.5 (15.0, 18.0)	6247.0
Overall quality of life	4.0 (4.0, 5.0)	4.0 (4.0, 5.0)	3638.0	4.0 (4.0, 5.0)	4.0 (3.0, 5.0)	5965.0*	4.0 (4.0, 5.0)	4.0 (4.0, 5.0)	6338.5
Overall health	4.0 (3.0, 4.0)	4.0 (3.0, 4.0)	3763.5	4.0 (3.0, 4.0)	3.0 (2.0, 4.0)	6606.5*	4.0 (3.0, 4.0)	4.0 (3.0, 4.0)	6436.0

THI, Tinnitus Handicap Inventory; TFI, Tinnitus Functional Index; FTQ, Fear of Tinnitus Questionnaire; TCS, Tinnitus Catastrophizing Scale; PSQ-30, Perceived Stress Questionnaire; HQ, Hyperacusis Questionnaire; HADS, Hospital Anxiety and Depression Scale; WHOQoL-BREF, World Health Organization Quality of Life Scale (short version).

***Correlation is significant at the 0.001 level. *Correlation is significant at the 0.05 level.*

[a]Higher score, higher quality of life.

[b]Higher score, higher impairment.

Summary of Mann-Whitney U-Tests in relation to occurrence (occasional vs. permanent), onset (gradual vs. abrupt), and manifestation (intermittent vs. constant).

for the 12 month follow-up trial by Cima et al. (2012) that were 11.73 for the TCS and 4.20 for the FTQ. Our study participants only seemed to be mildly affected by tinnitus compared to the RCT population. The average scores for quality of life were very similar to those reported in the literature (Abbott et al., 2009; Kreuzer et al., 2014; Schecklmann et al., 2014). Most published studies involved patients with tinnitus recruited in clinical centers or from medical registries, whereas the subjects recruited in the initial phase of the STOP were representative of the general population that may include individuals diagnosed and not diagnosed with tinnitus. As a consequence, this difference may potentially result in lower severity scores for all questionnaires. These findings emphasize the need of testing these questionnaires in a group of outpatients from clinics in Sweden.

Interestingly, the hyperacusis scores of the HQ were very similar to those found in the literature (Fackrell et al., 2015). However, using the criterion of >28 of Khalfa et al. (2002), we would obtain a proportion of 17.7% of subjects with hyperacusis, but this percentage is well below the reported 40–55% typically found in the tinnitus population (Baguley, 2003; Schecklmann et al., 2014). Indeed, reevaluation of the cut-off threshold has recently been recommended (Fackrell et al., 2015).

The potential to distribute questionnaires online has large benefits over paper versions, both in research and in clinical settings, because large data sets can be created with minimal administrative efforts. Moreover, the use of online questionnaires may precede anamnesis and audiological assessment to allow a more focused discussion at the clinic. Distributing the HADS and HQ questionnaires over the internet has proved successful and validated against pen and paper (Andersson et al., 2002, 2003; Thorén et al., 2012). The internal consistency and reliability of the online questionnaires tested here suggests that they could be used in paper versions in clinics that do not yet have the IT infrastructure to implement web-based versions.

CONCLUSIONS

This study shows the likely suitability of the Swedish versions of the THI, the TFI, the TCS, the FTQ, the HQ, the PSQ-30, the HADS, and the WHOQoL-BREF for measuring outcome in a clinical and research setting. The reliability and validity of these questionnaires translated into Swedish are comparable with that of the original English language versions. Some items of the TSCHQ may have to be removed or rewritten to further improve the reliability of this questionnaire. Additional research may be required to evaluate the sensitivity of each questionnaire in longitudinal studies and their usefulness for measuring treatment outcomes.

AUTHOR CONTRIBUTIONS

BL, WS, BC, RC, and CC designed the study. EI, RH, VP, CL, NE, and CC provided a consensus agreement on the final translated questionnaires. NE and CC developed the web-survey, coordinated the recruitment of subjects, and collected the data. KM and CC analyzed the data. CC, WS, and KM drafted the initial version of the manuscript. All authors contributed to the final version of the manuscript.

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REFERENCES

Abbott, J. A., Kaldo, V., Klein, B., Austin, D., Hamilton, C., Piterman, L., et al. (2009). A cluster randomised trial of an internet-based intervention program for tinnitus distress in an industrial setting. *Cogn. Behav. Ther.* 38, 162–173. doi: 10.1080/16506070902763174

Albu, S., and Chirtes, F. (2014). Intratympanic dexamethasone plus melatonin versus melatonin only in the treatment of unilateral acute idiopathic tinnitus. *Am. J. Otolaryngol.* 35, 617–622. doi: 10.1016/j.amjoto.2014.06.009

Andersson, G., Kaldo-Sandström, V., Ström, L., and Strömgren, T. (2003). Internet administration of the Hospital Anxiety and Depression Scale in a sample of tinnitus patients. *J. Psychosom. Res.* 55, 259–262. doi: 10.1016/S0022-3999(02)00575-5

Andersson, G., Lindvall, N., Hursti, T., and Carlbring, P. (2002). Hypersensitivity to sound (hyperacusis): a prevalence study conducted via the Internet and post. *Int. J. Audiol.* 41, 545–554. doi: 10.3109/14992020209056075

Baguley, D. M. (2003). Hyperacusis. *J. R. Soc. Med.* 96, 582–585. doi: 10.1258/jrsm.96.12.582

Bech, P., and Wermuth, L. (1998). Applicability and validity of the Major Depression Inventory in patients with Parkinson's Disease. *Nord. J. Psychiatry* 52, 305–309. doi: 10.1080/08039489850149741

Blomberg, S., Rosander, M., and Andersson, G. (2006). Fears, hyperacusis and musicality in Williams syndrome. *Res. Dev. Disabil.* 27, 668–680. doi: 10.1016/j.ridd.2005.09.002

Cederroth, C. R., Canlon, B., and Langguth, B. (2013). Hearing loss and tinnitus–are funders and industry listening? *Nat. Biotechnol.* 31, 972–974. doi: 10.1038/nbt.2736

Chan, Y. (2009). Tinnitus: etiology, classification, characteristics, and treatment. *Discov. Med.* 8, 133–136.

Cima, R. F., Crombez, G., and Vlaeyen, J. W. (2011). Catastrophizing and fear of tinnitus predict quality of life in patients with chronic tinnitus. *Ear Hear.* 32, 634–641. doi: 10.1097/AUD.0b013e3182 1106dd

Cima, R. F., Maes, I. H., Joore, M. A., Scheyen, D. J., El Refaie, A., Baguley, D. M., et al. (2012). Specialised treatment based on cognitive behaviour therapy versus usual care for tinnitus: a randomised controlled trial. *Lancet* 379, 1951–1959. doi: 10.1016/S0140-6736(12)60469-3

Cohen, J. (1960). A coefficient of agreement for nominal scales. *Educ. Psychol. Meas.* 20, 37–46. doi: 10.1177/0013164460020 00104

Dobie, R. A. (2003). Depression and tinnitus. *Otolaryngol. Clin. North Am.* 36, 383–388. doi: 10.1016/S0030-6665(02)00168-8

Epstein, J., Santo, R. M., and Guillemin, F. (2015). A review of guidelines for cross-cultural adaptation of questionnaires could not bring out a consensus. *J. Clin. Epidemiol.* 68, 435–441. doi: 10.1016/j.jclinepi.2014.11.021

Fackrell, K., Fearnley, C., Hoare, D. J., and Sereda, M. (2015). Hyperacusis Questionnaire as a tool for measuring hypersensitivity to sound in a tinnitus research population. *Biomed Res. Int.* 2015:290425. doi: 10.1155/2015/290425

Fackrell, K., Hall, D. A., Barry, J. G., and Hoare, D. J. (2016). Psychometric properties of the Tinnitus Functional Index (TFI): assessment in a UK research volunteer population. *Hear. Res.* 335, 220–235. doi: 10.1016/j.heares.2015.09.009

Friberg, E., Jansson, C., Mittendorfer-Rutz, E., Rosenhall, U., and Alexanderson, K. (2012). Sickness absence due to otoaudiological diagnoses and risk of disability pension: a nationwide Swedish prospective cohort study. *PLoS ONE* 7:e29966. doi: 10.1371/journal.pone.0029966

Gopinath, B., McMahon, C. M., Rochtchina, E., Karpa, M. J., and Mitchell, P. (2010a). Incidence, persistence, and progression of tinnitus symptoms in older adults: the Blue Mountains Hearing Study. *Ear Hear.* 31, 407–412. doi: 10.1097/AUD.0b013e3181cdb2a2

Gopinath, B., McMahon, C. M., Rochtchina, E., Karpa, M. J., and Mitchell, P. (2010b). Risk factors and impacts of incident tinnitus in older adults. *Ann. Epidemiol.* 20, 129–135. doi: 10.1016/j.annepidem.2009.09.002

Greimel, K. V., Leibetseder, M., Unterrainer, J., and Albegger, K. (1999). [Can tinnitus be measured? Methods for assessment of tinnitus-specific disability and presentation of the Tinnitus Disability Questionnaire]. *HNO* 47, 196–201. doi: 10.1007/s001060050382

Grouven, U., Bender, R., Ziegler, A., and Lange, S. (2007). [The kappa coefficient]. *Dtsch. Med. Wochenschr.* 132(Suppl. 1), e65–e68. doi: 10.1055/s-2007-959046

Hall, D. A., Haider, H., Kikidis, D., Mielczarek, M., Mazurek, B., Szczepek, A. J., et al. (2015). Toward a global consensus on outcome measures for clinical trials in tinnitus: report from the First International Meeting of the COMiT Initiative, November 14, 2014, Amsterdam, The Netherlands. *Trends Hear.* 19, 270–289. doi: 10.1177/2331216515580272

Hall, D., Haider, H., Szczepek, A. J., Lau, P., Rabau, S., Jones-Diette, J., et al. (2016). Systematic review of outcome domains and instruments used in clinical trials of tinnitus treatments in adults. *Trials* 17, 1–19. doi: 10.1186/s13063-016-1399-9

Harkness, J. A. (2003). "Questionnaire translation," in *Cross-Cultural Survey Methods*, eds F. van de Vijver and P. Mohler (Hoboken, NJ: John Wiley & Sons).

Hays, R. D., and Hayashi, T. (1990). Beyond internal consistency reliability: rationale and user's guide for Multitrait Analysis Program on the microcomputer. *Behav. Res. Methods Instrum. Comput.* 22, 167–175. doi: 10.3758/BF03203140

Heller, A. J. (2003). Classification and epidemiology of tinnitus. *Otolaryngol. Clin. North Am.* 36, 239–248. doi: 10.1016/S0030-6665(02)00160-3

Henry, J. A., Griest, S., Thielman, E., McMillan, G., Kaelin, C., and Carlson, K. F. (2016). Tinnitus functional index: development, validation, outcomes research, and clinical application. *Hear. Res.* 334, 58–64. doi: 10.1016/j.heares.2015.06.004

Jasper, K., Weise, C., Conrad, I., Andersson, G., Hiller, W., and Kleinstäuber, M. (2014). Internet-based guided self-help versus group cognitive behavioral therapy for chronic tinnitus: a randomized controlled trial. *Psychother. Psychosom.* 83, 234–246. doi: 10.1159/000360705

Kaldo, V., Cars, S., Rahnert, M., Larsen, H. C., and Andersson, G. (2007). Use of a self-help book with weekly therapist contact to reduce tinnitus distress: a randomized controlled trial. *J. Psychosom. Res.* 63, 195–202. doi: 10.1016/j.jpsychores.2007.04.007

Khalfa, S., Dubal, S., Veuillet, E., Perez-Diaz, F., Jouvent, R., and Collet, L. (2002). Psychometric normalization of a Hyperacusis Questionnaire. *J. Otorhinolaryngol. Relat. Spec.* 64, 436–442. doi: 10.1159/000067570

Kreuzer, P. M., Landgrebe, M., Resch, M., Husser, O., Schecklmann, M., Geisreiter, F., et al. (2014). Feasibility, safety and efficacy of transcutaneous vagus nerve stimulation in chronic tinnitus: an open pilot study. *Brain Stimul.* 7, 740–747. doi: 10.1016/j.brs.2014.05.003

Landgrebe, M., Azevedo, A., Baguley, D., Bauer, C., Cacace, A., Coelho, C., et al. (2012). Methodological aspects of clinical trials in tinnitus: a proposal for an international standard. *J. Psychosom. Res.* 73, 112–121. doi: 10.1016/j.jpsychores.2012.05.002

Landgrebe, M., Zeman, F., Koller, M., Eberl, Y., Mohr, M., Reiter, J., et al. (2010). The Tinnitus Research Initiative (TRI) database: a new approach for delineation of tinnitus subtypes and generation of predictors for treatment outcome. *BMC Med. Inform. Decis. Mak.* 10:42. doi: 10.1186/1472-6947-10-42

Langguth, B., Goodey, R., Azevedo, A., Bjorne, A., Cacace, A., Crocetti, A., et al. (2007). Consensus for tinnitus patient assessment and treatment outcome measurement: Tinnitus Research Initiative meeting, Regensburg, July 2006. *Prog. Brain Res.* 166, 525–536. doi: 10.1016/S0079-6123(07)66050-6

Langguth, B., Kreuzer, P. M., Kleinjung, T., and De Ridder, D. (2013). Tinnitus: causes and clinical management. *Lancet Neurol.* 12, 920–930. doi: 10.1016/S1474-4422(13)70160-1

Levenstein, S., Prantera, C., Varvo, V., Scribano, M. L., Berto, E., Luzi, C., et al. (1993). Development of the perceived stress questionnaire: a new tool for psychosomatic research. *J. Psychosom. Res.* 37, 19–32. doi: 10.1016/0022-3999(93)90120-5

Maes, I. H., Cima, R. F., Vlaeyen, J. W., Anteunis, L. J., and Joore, M. A. (2013). Tinnitus: a cost study. *Ear Hear.* 34, 508–514. doi: 10.1097/AUD.0b013e31827d113a

Maes, I. H., Joore, M. A., Cima, R. F., Vlaeyen, J. W., and Anteunis, L. J. (2011). Assessment of health state in patients with tinnitus: a comparison of the EQ-5D and HUI mark III. *Ear Hear.* 32, 428–435. doi: 10.1097/AUD.0b013e3181fdf09f

Meikle, M. B., Henry, J. A., Griest, S. E., Stewart, B. J., Abrams, H. B., McArdle, R., et al. (2012). The tinnitus functional index: development of a new clinical measure for chronic, intrusive tinnitus. *Ear Hear.* 33, 153–176. doi: 10.1097/AUD.0b013e31822f67c0

Newman, C. W., Jacobson, G. P., and Spitzer, J. B. (1996). Development of the Tinnitus Handicap Inventory. *Arch. Otolaryngol. Head Neck Surg.* 122, 143–148. doi: 10.1001/archotol.1996.01890140029007

Newman, C. W., Sandridge, S. A., and Jacobson, G. P. (1998). Psychometric adequacy of the Tinnitus Handicap Inventory (THI) for evaluating treatment outcome. *J. Am. Acad. Audiol.* 9, 153–160.

Nondahl, D. M., Cruickshanks, K. J., Wiley, T. L., Klein, R., Klein, B. E., and Tweed, T. S. (2002). Prevalence and 5-year incidence of tinnitus among older adults: the epidemiology of hearing loss study. *J. Am. Acad. Audiol.* 13, 323–331.

Park, B., Choi, H. G., Lee, H. J., An, S. Y., Kim, S. W., Lee, J. S., et al. (2014). Analysis of the prevalence of and risk factors for tinnitus in a young population. *Otol. Neurotol.* 35, 1218–1222. doi: 10.1097/mao.0000000000000472

Park, K. H., Lee, S. H., Koo, J. W., Park, H. Y., Lee, K. Y., Choi, Y. S., et al. (2014). Prevalence and associated factors of tinnitus: data from the Korean National Health and Nutrition Examination Survey 2009–2011. *J. Epidemiol.* 24, 417–426. doi: 10.2188/jea.JE20140024

Schecklmann, M., Landgrebe, M., Langguth, B., and TRI Database Study Group. (2014). Phenotypic characteristics of hyperacusis in tinnitus. *PLoS ONE* 9:e86944. doi: 10.1371/journal.pone.0086944

Shargorodsky, J., Curhan, G. C., and Farwell, W. R. (2010). Prevalence and characteristics of tinnitus among US adults. *Am. J. Med.* 123, 711–718. doi: 10.1016/j.amjmed.2010.02.015

Spinhoven, P., Ormel, J., Sloekers, P. P., Kempen, G. I., Speckens, A. E., and Van Hemert, A. M. (1997). A validation study of the Hospital Anxiety and Depression Scale (HADS) in different groups of Dutch subjects. *Psychol. Med.* 27, 363–370. doi: 10.1017/S0033291796004382

Thorén, E. S., Andersson, G., and Lunner, T. (2012). The use of research questionnaires with hearing impaired adults: online vs. paper-and-pencil administration. *BMC Ear Nose Throat Disord.* 12:12. doi: 10.1186/1472-6815-12-12

Tunkel, D. E., Bauer, C. A., Sun, G. H., Rosenfeld, R. M., Chandrasekhar, S. S., Cunningham, E. R. Jr., et al. (2014). Clinical practice guideline: tinnitus. *Otolaryngol. Head Neck Surg.* 151, S1–S40. doi: 10.1177/0194599814547475

Westin, V. Z., Schulin, M., Hesser, H., Karlsson, M., Noe, R. Z., Olofsson, U., et al. (2011). Acceptance and commitment therapy versus tinnitus retraining therapy in the treatment of tinnitus: a randomised controlled trial. *Behav. Res. Ther.* 49, 737–747. doi: 10.1016/j.brat.2011.08.001

WHO (1998). Development of the World Health Organization WHOQOL-BREF quality of life assessment. The WHOQOL Group. *Psychol. Med.* 28, 551–558. doi: 10.1017/S0033291798006667

Zeman, F., Koller, M., Langguth, B., Landgrebe, M., and Tinnitus Research Initiative database study. (2014). Which tinnitus-related aspects are relevant for quality of life and depression: results from a large international multicentre sample. *Health Qual. Life Outcomes* 12:7. doi: 10.1186/1477-7525-12-7

Zeman, F., Koller, M., Schecklmann, M., Langguth, B., Landgrebe, M., and TRI database study group. (2012). Tinnitus assessment by means of standardized self-report questionnaires: psychometric properties of the Tinnitus Questionnaire (TQ), the Tinnitus Handicap Inventory (THI), and their short versions in an international and multi-lingual sample. *Health Qual. Life Outcomes* 10:128. doi: 10.1186/1477-7525-10-128

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Validation of the Italian Tinnitus Questionnaire Short Form (TQ 12-I) as a Brief Test for the Assessment of Tinnitus-Related Distress: Results of a Cross-Sectional Multicenter-Study

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Moschen R, Fioretti A, Eibenstein A, Natalini E, Cuda D, Chiarella G, Rumpold G and Riedl D (2018) Validation of the Italian Tinnitus Questionnaire Short Form (TQ 12-I) as a Brief Test for the Assessment of Tinnitus-Related Distress: Results of a Cross-Sectional Multicenter-Study. Front. Psychol. 9:65. doi: 10.3389/fpsyg.2018.00065

Objectives: The use of reliable and valid psychometric tools to assess subjectively experienced distress due to tinnitus is broadly recommended. The purpose of the study was the validation of the Italian version of Tinnitus Questionnaire 12 item short form (TQ 12-I) as a brief test for the assessment of patient reported tinnitus-related distress.

Design: Cross-sectional multicenter questionnaire study.

Setting: Tinnitus Center, European Hospital (Rome), the Department of Otorhinolaryngology, "Guglielmo da Saliceto" Hospital (Piacenza), and the Department of Audiology and Phoniatry, "Mater Domini" University Hospital (Catanzaro).

Participants: One hundred and forty-three outpatients with tinnitus treated at one of the participating medical centers.

Main Outcome Measures: Tinnitus Questionnaire Short Form (TQ 12-I), compared to the Tinnitus Handicap Inventory (THI), Brief Symptom Inventory (BSI), and Short Form (SF-36) Health Survey.

Results: Our factor analysis revealed a two-factor solution (health anxiety, cognitive distress), accounting for 53.5% of the variance. Good internal consistency for the total score ($\alpha = 0.86$) and both factors ($\alpha = 0.79$–0.87) was found. Moderate correlations with the THI ($r = 0.65$, $p < 0.001$) indicated good convergent validity. Tinnitus distress was further correlated to increased psychological distress ($r = 0.31$, $p < 0.001$) and reduced emotional well-being ($r = -0.34$, $p < 0.001$).

Conclusion: The study clearly showed that the TQ 12-I is a reliable and valid instrument to assess tinnitus-related distress which can be used in clinical practice as well as for research.

Keywords: Tinnitus Questionnaire, TQ 12, validation, tinnitus, tinnitus distress

INTRODUCTION

Tinnitus is defined as a subjective acoustic perception in the absence of any external source (Hallam et al., 1984). Epidemiological studies reported a prevalence of 10–16% for chronic tinnitus in the adult population that increases with age (Davis and El Refaie, 2000; Shargorodsky et al., 2010; McCormack et al., 2014). The majority of people with tinnitus do not suffer from it strongly. Yet, about 0.5–3% of the general population develop severe distress and experience impairment in everyday life, sleep, mood, concentration and daily work (Davis and El Refaie, 2000; Gopinath et al., 2010). Because the subjectively experienced distress due to tinnitus cannot sufficiently be explained by psychoacoustic parameters (e.g., tinnitus loudness) (Henry and Meikle, 2000), psychological factors like depression, anxiety or catastrophizing have been assumed to be adjuvant to explain tinnitus distress (Milerová et al., 2013; Weise et al., 2013; McKenna et al., 2014).

National and international guidelines recommend using psychometrically and clinically validated questionnaires for the assessment of tinnitus severity (Langguth et al., 2007; AWMF, 2015). In order to be applicable during routine clinical practice a questionnaire for the assessment of tinnitus severity should not only be psychometrically and clinically validated but also quickly administered to minimize the patients' burden. Several reliable and internationally validated tools for the assessment of tinnitus severity have been developed. One of the most commonly used tools is the Tinnitus Questionnaire (TQ) (Goebel and Hiller, 1998), which assesses different aspects of tinnitus related distress (emotional and cognitive distress, intrusiveness, auditory perceptual difficulties, sleep disturbance and somatic complaints). The TQ has been translated and validated in several different languages, including German, Dutch, French, and Chinese. To enable a faster but equally reliable assessment of tinnitus related distress the TQ 12 was initially developed by Hiller and Goebel (2004). The TQ 12 was validated in German (Hiller and Goebel, 2004), Portuguese (Cerejeira et al., 2009), Greek (Panagiotopoulos et al., 2015), Chinese (Kam, 2012), Dutch (Vanneste et al., 2011), and Arabic (El Beaino and Eter, 2017). Good psychometric properties were reported for all of the translated versions of the TQ 12: the psychometric analyses across various validations showed good reliability (internal consistency: α = 0.86–0.90; retest-reliability: k = 0.89–0.91) and high correlation with the TQ total score (r = 0.90–0.93) (Hiller and Goebel, 2004; Kam, 2012; Zeman et al., 2012; Panagiotopoulos et al., 2015). The TQ 12 total score allows the classification of the patients as compensated (0–7), moderately distressed (8–12), severely distressed (13–18), and most severely distressed (19–24) (Hiller and Goebel, 2004). Yet, conflicting evidence was found for the factorial structure: most of the validations presumed a single global factor, whereas Panagiotopoulos et al. (2015) reported a three factor solution (Distress, Health pre-occupation, and Depression). So far, neither the TQ nor the TQ 12 have been validated in Italian. The aim of the present study was to evaluate the reliability, validity and factorial structure of the Italian TQ 12-I.

MATERIALS AND METHODS

Sample and Setting

The sample of this cross-sectional multi-center study consisted of 143 outpatients, which were include from the following healthcare institutions: Tinnitus Center, European Hospital (Rome), the Department of Otorhinolaryngology, "Guglielmo da Saliceto" Hospital (Piacenza), and the Department of Experimental and Clinical Medicine, Unit of Audiology and Phoniatrics, University "Magna Graecia" (Catanzaro). Patients were included in the study if they (a) had tinnitus for at least 3 months, (b) were older than 18 years, (c) spoke Italian fluently and (d) had no apparent cognitive impairment. The patients completed the questionnaires as part of the routine clinical practice. Written informed consent was obtained by all patients.

Measures

Tinnitus Sample Case History (TSCH)

Sociodemographic and clinical data were assessed with the Italian version of the TSCH. It was developed by the Tinnitus Research Initiative as in attempt to standardize the assessment of sociodemographic and clinical data in tinnitus research (Langguth et al., 2007). The questionnaire consists of 35 items on background (i.e., age, gender), tinnitus history (i.e., loudness, pitch, percentage of awake time aware of tinnitus, hyperacusis) and related conditions (i.e., hearing impairment, noise annoyance, vertigo/dizziness).

Tinnitus Questionnaire (TQ 12)

The TQ 12 consists of 12 items, which can be scored on a three point ordinal Likert Scale ranging from 0 to 2. The total score has a range of 0–24 points with higher values indicating higher tinnitus distress. The items of the TQ 12 correlated highly with the TQ total score, were reliable and showed good responsiveness (Hiller and Goebel, 2004). The Italian translation of the English TQ 12 was provided by an Italian native speaker, fluent in the source language and expert in the medical field of tinnitus. This forward translation was checked by a second independent person with the same level of expertise.

Tinnitus Handicap Inventory (THI)

The Tinnitus Handicap Inventory (Newman et al., 1996) is a one of the most commonly used questionnaires to assess tinnitus distress. The THI consists of 25 items that can be divided in a functional, emotional and catastrophic scale. Patients can score the frequency/intensity of these symptoms on a three point ordinal Likert Scale ranging from 0 to 4. The total score ranges from 0 to 100 points with higher values indicating higher tinnitus distress The total score the THI can be graduated in five grades of tinnitus severity: slight (0–16), mild (18–36), moderate (38–56), severe handicap (58–76), and catastrophic (78–100) (Zeman et al., 2014). Good reliability (α = 0.94) and validity were reported for the total score of the Italian THI version (Passi et al., 2008; Salviati et al., 2013).

Brief Symptom Inventory (BSI)

Mental health symptoms and psychological distress were assessed with the Brief Symptom Inventory (BSI; Derogatis, 1993). The BSI consists of 53 items, which can be divided into nine subscales and three scales to capture global psychological distress. Good reliability and validity for the subscales and total score were reported (De Leo et al., 1993).

Short Form (36) Health Survey

The health status was measured with the Short Form (36) Health Survey (SF-36) (Ware and Sherbourne, 1992), a broadly used, well established instrument to assess the Health-Related Quality of Life (HRQOL). The SF-36 consists of 36 items which can be divided into eight subscales and a physical and psychological total score. The subscale's internal consistencies were tested in nine samples, showing a Cronbach's α between 0.77 and 0.93. Good validity has been reported in several studies (Apolone and Mosconi, 1998).

Statistical Analysis

Psychometric values (means, standard deviations, item-total correlations) of the items are presented. The floor and ceiling effect, defined as the highest and lowest 15% of the scale were also calculated. A factor analyses (maximum likelihood, direct oblimin) with a fixed number of factors was conducted to investigate the three-factor solution proposed by Panagiotopoulos et al. (2015). Based on the results of this initial analysis an additional exploratory factor analysis was calculated. Scree plots and Eigenvalues were used to determine the ideal number of factors. Reliability was evaluated by calculating Cronbach's α (internal consistency) and item-total correlations. The convergent validity was evaluated by two-sided Pearson correlations with the THI and the discriminant validity was examined by correlations with BSI and SF-36 scores. To test if the proposed TQ 12-I severity grades differentiated well enough between different levels of tinnitus distress, analyses of variances (ANOVAs) with Bonferroni-corrected *post hoc* analyses were calculated. Statistical analyses were conducted using IBM SPSS (v.22).

RESULTS

A total sample of 146 patients were included in the study, of which 46.5% were recruited in Piacenza, 36.1% in Rome, and 17.4% in Catanzaro. The sample had a mean age of 53.6 years and 54% were men. The mean tinnitus duration was 7.5 years and a large proportion of the sample reported tinnitus in both ears (41.0%). More than half of the sample (57.5%) had subjective hearing problems and about a third (36.3%) reported physical discomfort because of surrounding sounds (hyperacusis). About 21% of the sample had undergone several or many treatments due to their tinnitus. Based on the THI total score patients' tinnitus distress was graded as follows: 19.9% very mild, 26.2% mild, 31.9% moderate, 15.6% severe, and 6.4% catastrophic. About 5% of the sample reported to be currently under treatment for psychiatric disorders. For details see **Table 1**.

Psychometric Values

The mean, standard deviation and item-total correlation of each item are presented in **Table 2**. The mean TQ 12-I total score was 10.8 (*SD*: 5.8). No floor or ceiling effect was found.

We initially tested the proposed factor solution of Panagiotopoulos et al. (2015) by factor analysis (main component analysis and maximum likelihood). Bartlett's test of sphericity [χ^2 (66) = 827.9, $p < 0.001$] was significant and the Kaiser–Meyer–Olkin measure verified the sampling adequacy for the analysis (KMO = 0.85). Initial Eigenvalues indicated that the first three factors explained 41.7%, 19.1%, and 7.9% of the variance, respectively. The three-factor-solution explained 58.4% of the variance, the diagonals of the anti-image correlation matrix were between 0.69 and 0.93.

TABLE 1 | Clinical properties of the sample.

TN loudness: 0–100 (*SD*)	53.2 (28.9)
Missing	10 (6.8%)
Duration: years (*SD*)	7.5 (10.3)
Family history of tinnitus complaints	37 (25.3%)
Missing	1 (0.7%)
Subjective hearing problem	84 (57.5%)
Missing	1 (0.7%)
Hearing aid	19 (13.7%)
Missing	1 (0.7%)
Hyperacusis	53 (36.3%)
Missing	1 (0.7%)
Tinnitus loudness varies during the day:	
Yes	96 (65.8%)
No	48 (32.9%)
Missing	2 (1.4%)
Pulsatile tinnitus	41 (27.5%)
If yes: with heartbeat	15 (38.5%)
If yes: different from heartbeat	24 (61.5%)
Location	
Right ear	30 (20.5%)
Left ear	36 (24.7%)
Both ears	60 (41.0%)
Inside the head	11 (7.5%)
Missing	9 (6.2%)
Pitch	
Very high frequency	20 (13.7%)
High frequency	58 (39.7%)
Medium frequency	49 (33.6%)
Low frequency	12 (8.2%)
Missing	7 (4.8%)
Number of treatments for tinnitus	
None	78 (53.4%)
One	37 (25.3%)
Several	23 (15.8%)
Many	6 (4.1%)
Missing	2 (1.4%)

TABLE 2 | Mean, standard deviations, and variance of the TQ 12-I.

	Mean	*SD*	Item-total correlation
1. I am aware of the noises from the moment I get up to the moment I sleep	1.34	0.75	0.14
2. Because of the noises I worry that there is something seriously wrong with my body	0.61	0.80	0.50
3. If the noises continue my life will not be worth living	0.48	0.80	0.44
4. I am more irritable with my family and friends because of the noises	0.82	0.72	0.66
5. I worry that the noises might damage my physical health	0.74	0.76	0.61
6. I find it harder to relax because of the noises	0.99	0.74	0.65
7. My noises are often so bad that I cannot ignore them	1.14	0.75	0.57
8. It takes me longer to get to sleep because of the noises	0.99	0.82	0.53
9. I am more liable to feel low because of the noises	0.90	0.78	0.71
10. I often think about whether the noises will ever go away	1.19	0.80	0.32
11. I am a victim of my noises	0.73	0.82	0.62
12. The noises have affected my concentration	0.92	0.76	0.70

Item-total correlations were corrected for the individual contribution of each item.

The factorial structure was examined with oblique rotation (oblimin), but we were not able to replicate the proposed three-factor-solution. Furthermore, the scree plot and the Eigenvalues supported a two-factor-solution (**Figure 1**). Therefore we also calculated an exploratory factor analysis (maximum likelihood) without a predefined number of factors. The results clearly supported the two-factor solution, explaining 53.5% of the variance. **Table 3** shows the factor loadings after rotation. Based on these results the remaining calculations were conducted for the two-factor solution. The content analysis for naming the extracted factors was independently conducted by two researchers, differences were resolved by consensus (reconciliation process). The items clustered on the same component suggest that factor 1 represents 'health anxiety,' while factor 2 represents 'cognitive distress' related to tinnitus.

Good internal consistency was found for the total score ($\alpha = 0.86$) as well as for factor 1 'health anxiety' ($\alpha = 0.87$) and factor 2 'cognitive distress' ($\alpha = 0.79$). The item-total correlations were acceptable for all items, except item 1. Also, the Cronbach's α would be slightly higher for the total scale if item 1 was excluded ($\alpha = 0.87$), but not for factor 2.

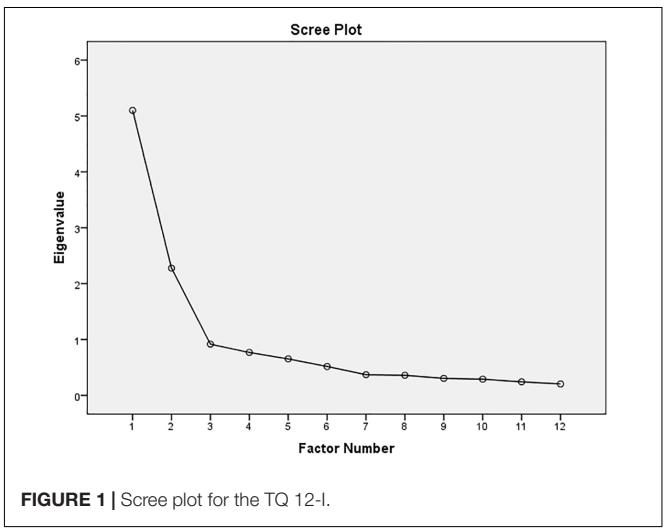

FIGURE 1 | Scree plot for the TQ 12-I.

Validity Analysis

The TQ 12-I correlated moderately with the THI-total score and its subscales, which indicated good convergent validity. Furthermore elevated tinnitus distress measured by the TQ 12-I total score was correlated positively with higher global psychological distress (BSI total score) and negatively with emotional well-being (SF-36 emotional score). Details are presented in **Table 4**. The correlations with the BSI and SF-36 were significantly lower than with the THI, which indicates good discriminant validity. No significant correlation was found between tinnitus distress and physical well-being.

We found no correlation between the TQ 12-I total score and age ($p = 0.57$), gender ($p = 0.23$) or symptom duration ($p = 0.72$). Yet, patients who had higher scores on the TQ 12-I also reported a subjectively louder ear noise ($r = 0.27$, $p = 0.002$). The presence of subjective hearing problems were more present in patients with higher age ($p = 0.04$). In our sample patients with subjective hearing problems did not differ in their perceived tinnitus loudness from patients without subjective hearing problems ($p = 0.41$).

According to the proposed cut-off values by Hiller and Goebel (2004) for the German TQ 12 31.9% of the present sample reported no clinically relevant tinnitus distress (1–7 points), 27.0% were moderately distressed (8–12 points), 32.6% were severely distressed (13–18 points) and 8.5% were most severely distressed (>19 points). The ANOVA showed significant differences between the TQ 12-I severity groups regarding the THI-total score ($F = 31.86$, $p < 0.001$). *Post hoc* analyses revealed significant differences between all severity groups, except severely and most severely distressed patients (**Figure 2**).

DISCUSSION

For use in busy clinical practice, a questionnaire for the assessment of tinnitus severity should not only be

TABLE 3 | Results of the factor analysis (Pattern Matrix).

	Factor	
	Health anxiety	**Cognitive distress**
1. I am aware of the noises from the moment I get up to the moment I sleep	−0.188	**0.534**
2. Because of the noises I worry that there is something seriously wrong with my body	**0.765**	0.054
3. If the noises continue my life will not be worth living	**0.827**	−0.025
4. I am more irritable with my family and friends because of the noises	**0.654**	0.490
5. I worry that the noises might damage my physical health	**0.751**	0.232
6. I find it harder to relax because of the noises	0.409	**0.776**
7. My noises are often so bad that I cannot ignore them	0.217	**0.812**
8. It takes me longer to get to sleep because of the noises	0.414	**0.508**
9. I am more liable to feel low because of the noises	**0.639**	0.508
10. I often think about whether the noises will ever go away	0.080	**0.460**
11. I am a victim of my noises	**0.700**	0.311
12. The noises have affected my concentration	0.531	**0.715**

Extraction Method: Maximum Likelihood; Rotation Method: Oblimin with Kaiser Normalization; bold values indicate which factor the item was assigned.

TABLE 4 | Correlations between TQ 12-I, THI, BSI, and SF-36.

	TQ 12-I total score	TQ 12-I health anxiety	TQ 12-I cognitive distress
THI total score	0.65***	0.44***	0.66***
THI functional subscale	0.60***	0.39***	0.64***
THI emotional subscale	0.58***	0.42***	0.56***
THI catastrophic subscale	0.59***	0.41***	0.61***
BSI total score	0.31***	0.21**	0.32***
SF-36 – physical subscale	−0.14	−0.07	−0.17
SF-36 – emotional subscale	−0.34***	−0.22**	−0.36***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, two-tailed*

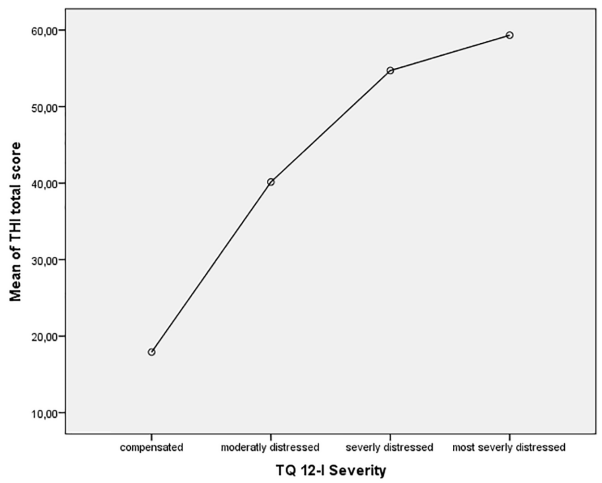

FIGURE 2 | Comparison of TQ 12-I severity groups with THI total score.

psychometrically and clinically validated, but also quickly to use to minimize the patients' burden. For the TQ 12 good psychometric and clinical values have been reported in several languages (Hiller and Goebel, 2004; Cerejeira et al., 2009; Vanneste et al., 2011; Kam, 2012; Zeman et al., 2012; Panagiotopoulos et al., 2015). Thus far the psychometric properties of the Italian translation of the TQ 12 have not been investigated. The aim of the present study therefore, was to evaluate the Italian short version of the Tinnitus Questionnaire, the TQ 12-I, in a multicenter study in which 143 patients with tinnitus complaints from three study centers participated. The results of our study showed that the TQ 12-I is a reliable and valid measure for assessing tinnitus-related distress and its severity.

The psychometric evaluation of the individual items showed no floor- or ceiling effects, which indicates that all items are well suited to discriminate between low and high levels of tinnitus distress. We found item-total correlations from acceptable to good (values higher than 0.3) for all items, except for item 1 ('I am aware of the noises from the moment I get up to the moment I sleep'), which had a value of 0.14. This was somehow surprising since item 1 had good item-total correlations (values of 0.3–0.6) in all other versions of the TQ 12 (Hiller and Goebel, 2004; Cerejeira et al., 2009; Vanneste et al., 2011; Kam, 2012; Panagiotopoulos et al., 2015). The translation of the items was independently checked by native-speakers in the course of translation and confirmed by further native speaking researches in the course of the data interpretation. Because of the good psychometric properties in previous studies and the high content validity of the item, we recommend to retain the item in the questionnaire.

It has been pointed out that the factorial structure of the TQ is a weak spot of the otherwise well suited questionnaire. Thus far, only one study has investigated the factorial structure of the TQ 12 (Panagiotopoulos et al., 2015). In that study, with three hundred and one adult Greek patients, the authors reported a three-factor solution, which could not be replicated in our sample. An additional exploratory factor analysis showed that a two-factor solution was better suited for the patients in our sample. Apart from intercultural differences, a possible explanation for the conflicting findings might be the comparatively low mean TQ 12 score in the Greek validation

study compared to our study and other validation studies (Hiller and Goebel, 2004; Cerejeira et al., 2009; Kam, 2012).

The two factors were named 'health anxiety' and 'cognitive distress,' based on a thorough content analysis. The factor **'health anxiety'** included questions that depicted tinnitus-related anxiety, for example catastrophic assumptions regarding one's own future life if the tinnitus persisted (item 3 'If the noises continue my life will not be worth living'), fears about an underlying severe physical illness (item 2: 'Because of my noises I worry that there is something seriously wrong with my body') or about threatening consequences of tinnitus (item 5: 'I worry that the noises might damage my physical health'). Additionally, the questions also cover the influence of the tinnitus on the patients' mood (item 9: 'I am more liable to feel low because of the noises') and feelings of helplessness or resignation toward tinnitus (item 11: 'I am a victim of my noises').

The factor **'cognitive distress'** includes questions that address experienced impairments in daily life, such as difficulties to distract oneself from the tinnitus (item 7: 'My noises are often so bad that I cannot ignore them'), to relax or to concentrate (item 6: 'I find it harder to relax because of the tinnitus'; item 12: 'The noises have affected my concentration'), or to fall asleep (item 8: 'It takes me longer to get to sleep because of the noises').

Several studies showed a close association of distressing tinnitus with anxiety, depressive symptoms and sleep disturbances (Wallhausser-Franke et al., 2012; Cronlein et al., 2016; Ziai et al., 2017). The TQ 12-I captures aspects of tinnitus-related anxiety, catastrophic worrying about its causes and consequences as well as impairments in daily life, mood and functions. Due to its easy and intelligible wording the TQ 12-I can be administered to patients with differing educational backgrounds. The identification and reduction of tinnitus-associated emotional and cognitive distress through patient education, reassurance and demystification of tinnitus promote the habituation process of tinnitus (Moschen et al., 2015). The TQ 12-I provides indications for a subsequent differentiated assessment of comorbid disorders by validated psychopathology questionnaires (Wallhausser-Franke et al., 2012) and may serve for the assessment of therapeutic outcomes.

In our study, the total score of the TQ 12-I had a good internal consistency of $\alpha = 0.86$, which was comparable with previous studies (Hiller and Goebel, 2004; Cerejeira et al., 2009; Vanneste et al., 2011; Kam, 2012; Zeman et al., 2012; Panagiotopoulos et al., 2015). The internal consistency of the two subscales was also good (health anxiety: $\alpha = 0.87$; cognitive distress: $\alpha = 0.80$). The previously problematic item 1 did not lower the factor's Cronbach α.

We furthermore investigated the validity of the TQ 12-I and its subscales through correlations with tinnitus distress (THI), general psychological distress (BSI), and quality of life (SF-36). As expected, the correlation between the two tinnitus related questionnaires was strong and highly significant, which indicated good convergent validity. Higher tinnitus distress as measured by the TQ 12-I was correlated with higher psychological distress and with lower emotional quality of life. These findings are in accordance with a large body of previous research (Shargorodsky et al., 2010; Milerová et al., 2013; Riedl et al., 2015). The two factors also showed significant correlations with the THI and its subscales as well as psychological distress and emotional quality of life.

In their initial development of the TQ 12 Hiller and Goebel (2004) proposed four grades of tinnitus severity, based on the total score of the TQ 12. To increase the usability of the TQ 12-I in a clinical context we have evaluated the severity grades for the TQ 12-I. Our analysis confirmed the proposed cut-offs, although the differences between the 'severely distress' and 'most severely distressed' groups were not statistically significant. This might be caused by the comparatively small size of the group with the most severely distressed patients ($n = 12$). The results indicate, that the proposed cut-off values may be used for the Italian TQ 12-I.

Limitations

In our sample the group of highly distressed patients was comparatively small, although the mean score was comparable to, or even higher than in previous validation studies (Hiller and Goebel, 2004; Vanneste et al., 2011; Panagiotopoulos et al., 2015). The small sample size may have influenced the results of the factor analysis. The proposed factorial structure should be further validated with confirmatory factor analysis in an international sample. In addition, we did not evaluate divergent validity of tinnitus severity with other comorbidity like hearing loss. The cross-sectional design of the present study did not allow us to evaluate the sensitivity to change (responsiveness) or the retest-reliability of the TQ 12-I. Previous studies have shown promising results regarding retest-reliability (Kam, 2012; Panagiotopoulos et al., 2015) and responsiveness (Hiller and Goebel, 2004; Zeman et al., 2012) of the TQ 12.

CONCLUSION

The present study showed that the TQ 12-I is a reliable and valid tool to assess the severity of tinnitus distress. The items of the TQ 12-I can be used in everyday clinical practice and research and permit a compact, quick and economical assessment of the most important aspects of subjective tinnitus distress. The provided cutoffs facilitate the interpretation of individual scores and provide indications for a multidisciplinary therapeutic approach.

ETHICS STATEMENT

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study was performed in three ENT centers in Italy, namely Rome, Catanzaro, and Piacenza. Heads of these centers (AE, GC, and DC) agreed on the procedures and provided access to tinnitus patients. Participation was

voluntary and every patient gave written consent before filling out the questionnaires.

AUTHOR CONTRIBUTIONS

RM and AF designed the study; GC collected the data at the ENT center in Catanzaro; AF, EN, and AE collected the data at the Tinnitus Center in Rome; DC collected the data at the ENT center in Piacenza; AF and EN consulted on the redaction of the manuscript at all stages and contributed to the revision of the manuscript; RM, GR, and DR analyzed the data, wrote the paper and commented on the manuscript at all stages; DR provided the statistical expertise and revised the manuscript at several stages.

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REFERENCES

Apolone, G., and Mosconi, P. (1998). The Italian SF-36 Health Survey: translation, validation and norming. *J. Clin. Epidemiol.* 51, 1025–1036. doi: 10.1016/S0895-4356(98)00094-8

AWMF (2015). *Treatment of Chronic Tinnitus. S3 – Guideline of the German Society of Otolaryngoly*. Available at: http://www.awmf.org/uploads/tx_szleitlinien/017-064l_S3_Chronischer_Tinnitus_2015-02.pdf

Cerejeira, R., Cerejeira, J., Paiva, S., Goncalves, P., Firmino, H., Quartilho, M., et al. (2009). The Portuguese version of Mini-Tinnitus Questionnaire: brief screening test for assessment of tinnitus-induced stress. *Otol. Neurotol.* 30, 112–115. doi: 10.1097/MAO.0b013e31818de749

Cronlein, T., Langguth, B., Pregler, M., Kreuzer, P. M., Wetter, T. C., and Schecklmann, M. (2016). Insomnia in patients with chronic tinnitus: cognitive and emotional distress as moderator variables. *J. Psychosom. Res.* 83, 65–68. doi: 10.1016/j.jpsychores.2016.03.001

Davis, A., and El Refaie, A. (2000). "Epidemiology of tinnitus," in *Tinnitus Handbook*, ed. R. S. Tyler (San Diego, CA: Singular), 1–21.

De Leo, D., Frisoni, G. B., Rozzini, R., and Trabucchi, M. (1993). Italian community norms for the brief symptom inventory in the elderly. *Br. J. Clin. Psychol.* 32(Pt 2), 209–213. doi: 10.1111/j.2044-8260.1993.tb01045.x

Derogatis, L. R. (1993). *Brief Symptom Inventory (BSI): Administration, Scoring and Procedures Manual*. Minneapolis, MN: Pearson.

El Beaino, M., and Eter, E. (2017). Arabic validation of the tinnitus handicap inventory and the mini-tinnitus questionnaire on 100 adult patients. *Clin. Otolaryngol.* doi: 10.1111/coa.12980 [Epub ahead of print].

Goebel, G., and Hiller, W. (1998). *Tinnitus-Fragebogen (TF). Ein Instrument zur Erfassung von Belastung und Schweregrad bei Tinnitus*. Göttingen: Hogrefe.

Gopinath, B., Mcmahon, C. M., Rochtchina, E., Krapa, M. J., and Mitchell, P. (2010). Incidence, persistence and progression of tinnitus symptoms in older adults: the blue mountains hearing study. *Ear Hear.* 31, 407–412. doi: 10.1097/AUD.0b013e3181cdb2a2

Hallam, R. S., Rachman, S., and Hinchcliffe, R. (1984). "Psychological aspects of tinnitus," in *Contributions to Medical Psychology*, ed. S. Rachman (Oxford: Pergamon), 31–53.

Henry, J. A., and Meikle, M. B. (2000). Psychoacoustic measures of tinnitus. *J. Am. Acad. Audiol.* 11, 138–155.

Hiller, W., and Goebel, G. (2004). Rapid assessment of tinnitus-related psychological distress using the Mini-TQ. *Int. J. Audiol.* 43, 600–604. doi: 10.1080/14992020400050077

Kam, A. C. (2012). A screening tool for tinnitus-related distress - the Chinese version of mini tinnitus questionnaire: our experience in one hundred and fourteen adult patients. *Clin. Otolaryngol.* 37, 234–237. doi: 10.1111/j.1749-4486.2012.02479.x

Langguth, B., Goodey, R., Azevedo, A., Bjorne, A., Cacace, A., Crocetti, A., et al. (2007). Consensus for tinnitus patient assessment and treatment outcome measurement: tinnitus research initiative meeting, Regensburg, July 2006. *Prog. Brain Res.* 166, 525–536. doi: 10.1016/S0079-6123(07)66050-6

McCormack, A., Edmondson-Jones, M., Fortnum, H., Dawes, P., Middleton, H., Munro, K. J., et al. (2014). The prevalence of tinnitus and the relationship with neuroticism in a middle-aged UK population. *J. Psychosom. Res.* 76, 56–60. doi: 10.1016/j.jpsychores.2013.08.018

McKenna, L., Handscomb, L., Hoare, D. J., and Hall, D. A. (2014). A scientific cognitive-behavioral model of tinnitus: novel conceptualizations of tinnitus distress. *Front. Neurol.* 5:196. doi: 10.3389/fneur.2014.00196

Milerová, J., Anders, M., Dvořák, T., Sand, P. G., Königer, S., and Langguth, B. (2013). The influence of psychological factors on tinnitus severity. *Gen. Hosp. Psychiatry* 35, 412–416. doi: 10.1016/j.genhosppsych.2013.02.008

Moschen, R., Riedl, D., Schmidt, A., Kumnig, M., Bliem, H. R., and Rumpold, G. (2015). The development of Acceptance of chronic tinnitus in the course of a cognitive-behavioral group therapy. *Z. Psychosom. Med. Psychother.* 61, 237–245. doi: 10.13109/zptm.2015.61.3.238

Newman, C. W., Jacobson, G. P., and Spitzer, J. B. (1996). Development of the Tinnitus Handicap Inventory. *Arch. Otolaryngol. Head Neck Surg.* 122, 143–148. doi: 10.1001/archotol.1996.01890140029007

Panagiotopoulos, G., Galanakis, M., Varvogli, L., Chrousos, G., and Darviri, C. (2015). Validation of the Greek version of mini tinnitus questionnaire as a brief screening test for assessment of tinnitus-related distress: our experience in 301 adult patients. *Clin. Otolaryngol.* 40, 363–369. doi: 10.1111/coa.12383

Passi, S., Ralli, G., Capparelli, E., Mammone, A., Scacciatelli, D., and Cianfrone, G. (2008). The THI questionnaire: psychometric data for reliability and validity of the Italian version. *Int. Tinnitus J.* 14, 26–33.

Riedl, D., Rumpold, G., Schmidt, A., Zorowka, P. G., Bliem, H. R., and Moschen, R. (2015). The influence of tinnitus acceptance on the quality of life and psychological distress in patients with chronic tinnitus. *Noise Health* 17, 374–381. doi: 10.4103/1463-1741.165068

Salviati, M., Macrì, F., Terlizzi, S., Melcore, C., Provenzano, A., Capparelli, E., et al. (2013). The tinnitus handicap inventory as a screening test for psychiatric comorbidity in patients with tinnitus. *Psychosomatics* 54, 248–256. doi: 10.1016/j.psym.2012.05.007

Shargorodsky, J., Curhan, G. C., and Farwell, W. R. (2010). Prevalence and characteristics of tinnitus among US adults. *Am. J. Med.* 123, 711–718. doi: 10.1016/j.amjmed.2010.02.015

Vanneste, S., Plazier, M., Van Der Loo, E., Ost, J., Meeus, O., Van De Heyning, P., et al. (2011). Validation of the Mini-TQ in a Dutch-speaking population: a rapid assessment for tinnitus-related distress. *B-ENT* 7, 31–36.

Wallhausser-Franke, E., Brade, J., Balkenhol, T., D'amelio, R., Seegmuller, A., and Delb, W. (2012). Tinnitus: distinguishing between subjectively perceived loudness and tinnitus-related distress. *PLOS ONE* 7:e34583. doi: 10.1371/journal.pone.0034583

Ware, J. E. Jr., and Sherbourne, C. D. (1992). The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med. Care* 30, 473–483. doi: 10.1097/00005650-199206000-00002

Weise, C., Hesser, H., Andersson, G., Nyenhuis, N., and Zastrutzki, S. (2013). The role of catastrophizing in recent onset tinnitus: its nature and association with tinnitus distress and medical utilization. *Int. J. Audiol.* 52, 177–188. doi: 10.3109/14992027.2012.752111

Zeman, F., Koller, M., Langguth, B., and Landgrebe, M. (2014). Which tinnitus-related aspects are relevant for quality of life and depression: results from

a large international multicentre sample. *Health Qual. Life Outcomes* 12:7. doi: 10.1186/1477-7525-12-7

Zeman, F., Koller, M., Schecklmann, M., Langguth, B., and Landgrebe, M. (2012). Tinnitus assessment by means of standardized self-report questionnaires: psychometric properties of the Tinnitus Questionnaire (TQ), the Tinnitus Handicap Inventory (THI), and their short versions in an international and multi-lingual sample. *Health Qual. Life Outcomes* 10:128. doi: 10.1186/1477-7525-10-128

Ziai, K., Moshtaghi, O., Mahboubi, H., and Djalilian, H. R. (2017). Tinnitus patients suffering from anxiety and depression: a review. *Int. Tinnitus J.* 21, 68–73. doi: 10.5935/0946-5448.20170013

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Polish Translation and Validation of the Tinnitus Handicap Inventory and the Tinnitus Functional Index

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Objective: The need for validated measures enabling clinicians to classify tinnitus patients according to the severity of tinnitus and screen the progress of therapies in our country led us to translate into Polish and to validate two tinnitus questionnaires, namely the Tinnitus Handicap Inventory (THI) and the Tinnitus Functional Index (TFI).

Design: The original English versions of the questionnaires were translated into Polish and translated back to English by three independent translators. These versions were then finalized by the authors into a Polish THI (THI-Pl) and a Polish TFI (TFI-Pl). Participants from three laryngological centers in Poland anonymously answered the THI-Pl ($N = 98$) and the TFI-Pl ($N = 108$) in addition to the Polish versions of the Center for Epidemiologic Studies Depression Scale as a measure of self-perceived level of depression, and the Satisfaction With Life Scale to assess self-perceived quality of life. Both were used to determine discriminant validity. Two Visual Analog Scales were used to measure tinnitus annoyance and tinnitus loudness in order to determine convergent validity.

Results: Similar to the original version of the THI, the THI-Pl showed a high internal consistency (Cronbach's $\alpha = 0.93$). The exploratory factor analysis revealed that the questionnaire has a three-factorial structure that does not correspond to the original division for functional, catastrophic, and emotional subscales. Convergent and discriminant validities were confirmed. The TFI-Pl showed high internal consistency (Cronbach's $\alpha = 0.96$) with the reliability ranging from 0.82 to 0.95 for its different subscales. Factor analysis confirmed an eight-factorial structure with factors assigning all items to appropriate subscales reported in the original version of the questionnaire. Discriminant and convergent validities were also confirmed for the TFI-Pl.

Conclusion: We translated and validated the Polish versions of the THI and the TFI to make them suitable for clinical use in Poland.

Keywords: Tinnitus Handicap Inventory, THI, Tinnitus Functional Index, TFI, Polish, adaptation

INTRODUCTION

Tinnitus ("ringing in the ears") is described as the perception of sound without any external stimulation. Chronic tinnitus is a common condition, affecting around 10% of the general population, and for some people this condition is debilitating (Langguth et al., 2011). As for the Polish adult population, Skarżyński et al. (2000) estimated that 20% experienced tinnitus lasting more than 5 min, around 5% were affected by chronic tinnitus, and for 4% (almost 1.6 million adults) tinnitus caused severe annoyance. Moreover, Polish children are affected by this problem as well. The study by Raj-Koziak et al. (2011) reports that more than 5% of 55201 7-year-old children tested reported their perception of tinnitus to be often or very often.

Tinnitus can seriously affect quality of life and, in extreme cases, even lead to suicide (Jastreboff and Hazell, 2004). Among tinnitus related comorbidities we can distinguish anxiety (Udupi et al., 2013; Kehrle et al., 2016), depression (Langguth et al., 2011), or sleep disorders (Crönlein et al., 2016). Tinnitus may be closely linked to hearing loss (Martines et al., 2010; Schecklmann et al., 2012). Different therapeutic approaches offer the use of hearing aids, tinnitus maskers, or tinnitus instruments that combine both (Vernon and Meikle, 2003); counseling sessions (e.g., cognitive behavior therapy, see Cima et al., 2014); counseling combined with the use of sound generators [e.g., Tinnitus Retraining Therapy (TRT), see Jastreboff and Hazel, 1993]; relaxation techniques (e.g., Mindfulness Based Stress Reduction, see Roland et al., 2015); neuromodulation (e.g., the Acoustic Coordinated Reset Neuromodulation, see Tass et al., 2012); or brain stimulation (e.g., Repetitive Transcranial Magnetic Stimulation Treatment, see Folmer et al., 2015). There are many possible options for treatment; however, none of these provides immediate and constant relief for tinnitus (for further details see, e.g., Baguley et al., 2013; Maldonado Fernández et al., 2015).

At the moment, no valid and standardized questionnaire for the assessment of tinnitus is available in Poland. As of yet, clinicians in Poland rely on self-made, non-validated translations of the original version of the questionnaire, which limits the reliability of patient assessment, but also prevents the comparison of therapeutic outcomes with other clinics using validated instruments.

We decided to translate and validate the Tinnitus Handicap Inventory (THI) and the Tinnitus Functional Index (TFI) developed by Newman et al. (1996) and Meikle et al. (2012), respectively. Our choice of the THI was based on two observations: firstly, it is a well-known questionnaire that has been extensively used in clinical and scientific practice to measure tinnitus distress and to test potential reduction of the THI score as a consequence of applied therapy (see, e.g., Landgrebe et al., 2010; Shekhawat et al., 2013; Roland et al., 2015; Wilson et al., 2015; Wise et al., 2015; Zobay et al., 2015). It was determined that a clinically meaningful change is achieved with a reduction of 20 or more points of the total score of the THI (Newman et al., 1996, 1998; Fackrell et al., 2014). Secondly, the THI is integrated into the Tinnitus Research Initiative database, which contains standardized data collected from different tinnitus research centers and countries (Landgrebe et al., 2010). A validated Polish version of the tool would enable clinicians not only to classify tinnitus patients according to severity, but would also create a possibility for Polish scientists to contribute to international research projects.

Our second choice—the TFI—was based on its unique feature; precisely, the possibility to evaluate therapeutic outcomes (Meikle et al., 2012). In addition, this questionnaire may be used by clinicians and researchers to classify patients according to tinnitus severity. Nevertheless, our goal was to provide to Polish ENT centers a tool which enables the evaluation of the effectiveness of applied treatments (see, e.g., Krings et al., 2014; Folmer et al., 2015; Overdevest et al., 2015; Roland et al., 2015; Wilson et al., 2015; Fackrell et al., 2016).

MATERIALS AND METHODS

Participants

The study was performed in three clinics in Polish cities—namely Poznań, Gdańsk and Łódź —where paper versions of the questionnaires were filled out anonymously by 98 patients (for the THI-Pl) and 108 patients (for the TFI-PI) who reported tinnitus as either a primary complaint or secondary complaint after hearing loss (defined by hearing loss exceeding 25 dB HL for at least one of the frequencies tested in the audiological pre-interview). Ten of 108 patients tested with the TFI-Pl questionnaire did not fill out the THI-Pl. Participation was voluntary, all participants gave oral consent before filling out the questionnaire, and data was stored and analyzed completely anonymously. The sample was heterogeneous and included a large variety of patients visiting our clinics. This range included, for example, patients with different degrees of hearing loss and self-reported tinnitus severity visiting the ENT doctor for the first time, but also those who had already undergone particular treatments and patients currently in the therapeutic process (Poznań: mainly TRT or electrostimulation; Gdańsk: TRT; Łódź: TRT, counseling sessions). A study information sheet was provided and all volunteers were informed about the aim of the study as well as the estimated time for completing the questionnaires. The participants filled out the questionnaires while waiting for their consultation with an ENT specialist. Descriptive measures of patients who filled in the THI-Pl and TFI-Pl questionnaires are shown in Supplementary Tables S1 and S2, respectively.

Tinnitus Handicap Inventory

The THI (Newman et al., 1996) is a self-reported measure consisting of 25 items divided into three subscales: functional (11 items measuring the functional aspects of tinnitus such as mental, social/occupational, and physical functioning), catastrophic (five items reflecting catastrophic responses to tinnitus, including depression, and sleep disturbance), and emotional (nine items representing affective responses to tinnitus). There are three possible answers to each item (and 25 items in total): "yes" (four points), "sometimes" (two points), and "no" (zero points). Scores are calculated for the THI total scale (range 0–100 points) as well as for the three subscales: functional (THIf), catastrophic (THIc),

and emotional (THIe)—ranges 0–44, 0–20, and 0–36 points, respectively). It is noteworthy that some researchers proposed a unifactorial structure of the questionnaire, with no division for subscales (Baguley and Andersson, 2003; Fackrell et al., 2014).

The THI has been widely validated and translated, for example, into Danish (Zachariae et al., 2000), Korean (Kim et al., 2002), Italian (Monzani et al., 2008), Chinese (Kam et al., 2009), German (Kleinstäuber et al., 2015), Persian (Jalali et al., 2015), and Russian (Oron et al., 2015).

Tinnitus Functional Index

The TFI (Meikle et al., 2012) is a self-report measure used for the evaluation of tinnitus severity, measuring clinically important changes. It consists of 25 items divided into 8 subscales addressing different domains of tinnitus severity: intrusive (three items, TFIint), sense of control (three items, TFIsoc), cognitive (three items, TFIcog), sleep (three items, TFIsleep), auditory (three items, TFIaud), relaxation (three items, TFIrelax), quality of life (four items, TFIqol) and emotional (three items, TFIem). The TFI-Pl uses a 10-point scale in the range 0–10 or 0–100%. The scores are calculated for the total scale and all subscales (range 0–100 each). Detailed scoring instructions are provided by the authors (Meikle et al., 2012).

The TFI is currently translated and validated by scientists from Holland (Rabau et al., 2014) and England (Fackrell et al., 2016).

Translation Procedure

The original versions of the questionnaires were translated into Polish by the authors fluent in English. These authors agreed to the translated version being further forwarded to three independent translators who performed translations back into English. Two of these translators were native English speakers and one was an English teacher who studied English philology. They were unfamiliar with the original version of the questionnaire. After comparing the original and the translated versions, the authors constructed the final versions of the THI-Pl and TFI-Pl (see "Supplementary Material 1" which displays the Polish versions of the THI and information on how to obtain the TFI), based on a simple frequency criterion. More precisely, when discrepancies between the three translations provided were observed, the authors chose the version preferred by two of the three translators. Other forms of discrepancies were not observed. The questionnaires were pre-tested by the authors themselves and with hospital staff who volunteered for this task.

Additional Measures

Participants were additionally asked to complete the Polish version of The Centre for Epidemiological Studies Depression Scale (CES-D; Radloff, 1977; Polish adaptation: Dojka et al., 2003; Kaniasty, 2003; Ziarko et al., 2013) and the Polish version of The Satisfaction With Life Scale (SWLS; Diener et al., 1985; Pavot and Diener, 1993; Polish adaptation: Juczyński, 2001). We also used two versions of the Visual Analog Scale as a measure of self-perceived tinnitus annoyance and tinnitus loudness.

The CES-D is a tool which is free of charge and widely used in clinical practice to estimate the number and intensity of depressive symptoms after the diagnosis of depression (Ziarko et al., 2013). It consists of 25 items divided into four subscales measuring: (1) depressive affect (depressive mood), (2) lack of positive affect (well-being), (3) somatic symptoms and inhibition of activity (somatic symptoms), and (4) attitude to other people (intrapersonal affect) (Radloff, 1977; Ziarko et al., 2013). The structure of the Polish version of the tool is internally compliant with the theoretical assumptions and its temporal stability was confirmed in the longitudinal studies (Ziarko et al., 2013).

The SWLS assesses self-perceived quality of life. The tool includes five statements with which patients have to express their degree of agreement (1–"I totally disagree," 7–"I totally agree"). The total score of the Polish and original version of this tool is a general indicator of a participant's satisfaction with their life (Juczyński, 2001).

The Visual Analog Scales (VAS; assessing self-perceived tinnitus anxiety and tinnitus loudness) were chosen, because they are considered to be valid and effective tools for measuring reductions in tinnitus severity in people with chronic tinnitus (Adamchic et al., 2012). The Visual Analog Scale was used in order to ascertain the severity of tinnitus (tinnitus annoyance and tinnitus loudness). The VAS scales consisted of two 10 cm lines with marked endpoints. There were two faces drawn: a smiling one indicating lack of annoyance or no perception of tinnitus (painted under the left endpoint of a line) and a sad one indicating extreme annoyance or extremely loud tinnitus (painted under the right endpoint of a line).

Statistical Analysis

Statistical analyses of data were performed with the Statistical Package for Social Sciences (v22; SPSS, Inc., Chicago, IL, USA). Since the majority of our samples were non-Gaussian (indicated by the Shapiro–Wilk test), we relied mainly on non-parametric techniques. More specifically, whenever the normality assumption was rejected, we used the Wilcoxon–Mann–Whitney for comparisons of two (unpaired) groups. Alternatively, the *t*-test was preferred for Gaussian samples. Similarly, the Kruskal–Wallis test served to compare larger numbers of groups when the normality hypothesis was rejected. For the Gaussian sample, we used a one-way ANOVA. Spearman's rank correlation coefficient (rho) was used for measuring associations. Internal consistency was evaluated by measuring Cronbach's alpha coefficient in order to assess questionnaire reliability and the item-total correlation. The Kaiser-Meyer-Olkin (KMO) Measure of Sampling Adequacy and Bartlett's Test of Sphericity were assessed to assure that our data was eligible for our exploratory factor analysis. Correlations between the factors were calculated, and factor analysis was carried out using the oblique Oblimin rotation. In addition, two orthogonal rotations (Varimax, Quartimax) were investigated. For increased robustness, we selected the Unweighted Least Squares method as an estimation technique. Confirmatory factor analysis (CFA) was performed using IBM SPSS AMOS 22. Statistical significance was set for p-values smaller than 0.05.

In order to determine the discriminant validity, we asked participants to complete the Polish version of The Centre for Epidemiological Studies Depression Scale (CES-D; Radloff, 1977; Polish adaptation: Dojka et al., 2003; Kaniasty, 2003; Ziarko et al., 2013) and the Polish version of The Satisfaction With Life

Scale (SWLS; Diener et al., 1985; Pavot and Diener, 1993; Polish adaptation: Juczyński, 2001). We assumed that discriminant validity would be confirmed when at most moderate ($\rho < 0.6$) correlations between the THI and TFI total score and CES-D and SWLS scores were observed (Newman et al., 1996; Fackrell et al., 2014).

To access the convergent validity, we used two versions of the Visual Analog Scale as a measure of the self-perceived tinnitus annoyance and tinnitus loudness. We assumed that convergent validity would be confirmed when at least strong correlations ($\rho > 0.6$) between the THI and TFI total score and VAS annoyance and VAS loudness scores were observed.

Five TFI-Pl questionnaires from two female and three male respondents with seven or more omitted responses were removed from the analysis, according to the recommendation by Meikle et al. (2012). We did not have to exclude any THI-Pl questionnaires. Thus, the final number of questionnaires retained was $N = 98$ and $N = 103$ for the THI and TFI analysis, respectively. Note that our THI-Pl sample contained a total of 35 missing observations, and the TFI-Pl sample contained a total of 22 missing observations (after the exclusion of five participants), resulting from questions skipped by responders. Since this constitutes only ∼1% of the data, we applied a mean-replacement for reliability analysis as well as a factor analysis.

RESULTS

The Polish version of the THI

Descriptive Statistics, Intergroup Differences, and Correlations

The average age of patients who filled in the THI-Pl questionnaire was 51.72 years ($SD = 13.06$) and the mean duration of tinnitus was 6.56 years ($SD = 11.64$). Responses given to particular items of the THI-Pl are presented in Supplementary Table S3. **Table 1** shows average scores obtained by participants for the THI-Pl, VAS scales, CES-D, and SWLS. **Table 2** summarizes correlations between the different scales. The highest correlations were found for the THI total score and VAS annoyance. The relation between the THI total score (or its subscales) and gender was not significant, as shown by the Wilcoxon–Mann–Whitney test. In addition, the Kruskal–Wallis test did not reveal any significant differences for THI total score (or its subscales) resulting from variations in tinnitus pitch, localization, or character. The Wilcoxon–Mann–Whitney test showed significant influence of hearing loss on the total THI score ($p = 0.044$). Participants subject to hearing loss obtained higher scores than normal hearing patients (41.6 vs. 33.4). There were no significant correlations between the THI-Pl and age or duration of tinnitus.

Convergent validity was confirmed by strong positive Spearman's correlations for the total THI score and the VAS annoyance and loudness scales. Discriminant validity was assessed by weak negative correlations ($\rho < 0.4$) for the SWLS and moderate positive correlations ($\rho < 0.6$) for the CES-D total score.

Internal Consistency

The THI-Pl has the same high internal consistency reliability as the original version of the questionnaire ($\alpha = 0.93$; from Newman et al., 1996, which serves as a reference for all statistical

TABLE 1 | Average scores and their standard deviations for THI-Pl and additional measures obtained from the Polish participants.

	Scale							
	THI-Pl	**THIf**	**THIc**	**THIe**	**VASa**	**VASl**	**CES-D**	**SWLS**
Mean	38.6	17.7	8.8	12.1	48.9	50.0	15.6	21.5
SD	22.9	10.3	5.1	9.5	26.3	26.2	10.6	5.8

THI, Tinnitus Handicap Inventory; Pl, Polish; f, functional; c, catastrophic; e, emotional; VAS, Visual Analog Scale; a, annoyance; l, loudness; SWLS, The Satisfaction With Life Scale; CES-D, The Centre for Epidemiological Studies Depression Scale.

TABLE 2 | Correlations (Spearman's rho) between the THI-Pl and other measures used in the adaptation procedure.

Measure	**VASa**	**VASl**	**CES-D**	**SWLS**
THI-Pl	0.799***	0.610***	0.528***	−0.300**

****$p < 0.001$; **$p < 0.01$; THI, Tinnitus Handicap Inventory; Pl, Polish; VAS, Visual Analog Scale; a, annoyance; l, loudness; CES-D, The Centre for Epidemiological Studies Depression Scale; SWLS, The Satisfaction With Life Scale.*

TABLE 3 | Corrected item total correlation for all items of the THI-Pl.

Scale/Item	**Corrected item-total correlation**
F 1	0.706
F 2	0.303
E 3	0.514
F 4	0.514
C 5	0.673
E 6	0.668
F 7	0.405
C 8	0.481
F 9	0.720
E 10	0.682
C 11	0.486
F 12	0.720
F 13	0.627
E 14	0.711
F 15	0.536
E 16	0.694
E 17	0.628
F 18	0.495
C 19	0.246
F 20	0.674
E 21	0.713
E 22	0.743
C 23	0.632
F 24	0.266
E 25	0.748

F, functional; E, emotional; C, catastrophic.

measures in the following as well, if not stated otherwise). **Table 3** presents the corrected item total correlation. Further details concerning Cronbach's alpha coefficient if an item is deleted are included in Supplementary Table S4. Items 2, 19, and 24 had the lowest possible corrected item total correlation, with values lower than 0.4–0.30, 0.25, and 0.27, respectively. The analysis revealed that the removal of these items would only slightly elevate Cronbach's alpha coefficient from $\alpha = 0.933$ to $\alpha = 0.935$. We therefore decided to preserve the original number of items in the questionnaire. For the other 22 items, the corrected item total correlation was high ($r = 0.6$). When compared to the original version of questionnaire, internal consistency reliability was slightly lower for the functional subscale ($\alpha = 0.83$ vs. $\alpha = 0.86$) and slightly higher for the catastrophic ($\alpha = 0.70$ vs. $\alpha = 0.68$) and emotional ($\alpha = 0.90$ vs. $\alpha = 0.87$) subscales. Supplementary Table S5 lists the reliability coefficients of the THI-Pl and other adaptations. The last two columns report the average internal consistency reliability of all adaptations excluding the Polish and the original English version and the sample standard deviation. Cronbach's alpha determined for the Polish adaptation fell within the estimated 95% quantile of all questionnaires as presented in Supplementary Table S5.

Factor Analysis

We started with an exploratory factor analysis, assuming the original structure of the questionnaire (Newman et al., 1996) with three factors. All factors were moderately correlated with each other, as can be seen in the lower part of **Table 4**, supporting the choice of an oblique rotation. The first main factor with an eigenvalue of 10.2 explained 41.0% of the variance; the second with an eigenvalue of 1.9–7.5% and the third with an eigenvalue of 1.4–5.5% together explained 54.1% of the total variance. Supplementary Figure S1 shows a scree plot of factors with corresponding eigenvalues. On the one hand, the first factor is dominant, which at first glance supports a unifactorial questionnaire structure. **Table 4** represents the rotated factor loadings of the described solution and the factor correlations. Investigation of the factor loadings and their corresponding eigenvalues led us to three conclusions. Firstly, three factors contribute to the loadings of the 25 items, but these factors do not correspond to the subscales of the questionnaire. Except for three items, all of these loadings can be considered important as they have values greater than 0.4 (Floyd and Widaman, 1995; Baguley and Andersson, 2003). In addition, the remaining items carry loadings higher than 0.27 and two of these are greater than 0.3. Secondly, of the first factor loads on 15 items, six belong to the emotional subscale, four to the catastrophic subscale, and five to the functional one. In our opinion, these items may be considered to be referring to the impact of tinnitus on everyday functioning and the emotional state of the patient. Five questions are loaded by the second factor, four of which originally belonged to the functional and one to the emotional subscale. These items could be viewed as describing the aspect of "helplessness" resulting from the perception of tinnitus. The third factor also loads five items, two belonging to the functional, two to the emotional, and one to the catastrophic subscale. Four of these questions refer either to relationships with other people or to social activities. Item number 12 concerns satisfaction with life ("Does your tinnitus make it difficult for you to enjoy life?") and, in our belief, can be linked with satisfaction from social relations. Taking into consideration loadings greater than 0.3, only three items (5, 22, and 23) are double-loaded. Two of them—item 5 ("Because of your tinnitus, do you feel desperate?") and item 23 ("Do you feel that you can no longer cope with your tinnitus?")—are additionally loaded by the third factor, which seems to be appropriate in the context of "helplessness." The question "Does your tinnitus make you feel anxious?" (item 22) is loaded by the third factor, but also by the first one. Since this item refers to the emotional state of the patient, an observation of double-loading seems to be justified.

Thirdly, the first factor alone explains less than half of the total variance. Taking this and the factor loadings of the second and

TABLE 4 | Rotated factor loading matrices of the predefined-factor models.

	Factor		
Scale/Item	**1**	**2**	**3**
	10.25	**1.88**	**1.38**
E 16	**0.672**		
F 15	**0.641**		
F 20	**0.637**		
F 7	**0.627**		
C 8	**0.534**		
F 18	**0.516**		
C 23	**0.511**		0.359
E 25	**0.494**		
F 1	**0.494**		
E 10	**0.477**		
E 14	**0.465**		
E 6	**0.447**		
C 19	**0.435**		
C 5	**0.425**		0.355
E 3	**0.280**		
E 17		**−0.886**	
F 13		**−0.744**	
F 9		**−0.586**	
F 2		**−0.472**	
F 12		**−0.452**	
E 21			**0.699**
F 24			**0.556**
E 22	0.464		**0.473**
F 4			**0.319**
C 11			**0.310**
	Correlations		
Factor 1	1.000	−0.433	0.317
Factor 2	−0.433	1.000	−0.442
Factor 3	0.317	−0.442	1.000

Eigenvalues are presented below the names of factors. Lower part of the table shows correlations between the factors. Loadings > 0.30 displayed (with exception of Item 3). Loadings assigned to particular factors in bold; F, functional; E, emotional; C, catastrophic.

third factors into account, it may be advisable to consider at least a two- or even three-factorial structure for attaining approximately 50% explained variation.

Moreover, we also investigated the effect of using orthogonal rotations for exploratory purposes (Supplementary Table S6 reports the results). Then the results changed substantially: on the one hand, the Varimax solution suggests a structure similar to that obtained by the Oblimin solution. On the other hand, the Quartimax rotation leads to one main factor carrying the highest load for 22 of the 25 items, suggesting a unifactorial structure.

Lastly, we carried out a CFA for investigating if we can confirm the original three-factorial structure suggested by Newman et al. (1996). Some of the values of our fit indices were not too far from those obtained by Kleinstäuber et al. (2015); for example, Kleinstäuber et al. (2015) obtained an RMSEA of 0.060, whereas our corresponding value equals 0.084. However, the overall results did not permit us to conclude an acceptable fit with the data, which is also in line with Kleinstäuber et al. (2015). Supplementary Table S14 presents details of the estimation results. Due to our comparably low sample size and the resulting lower reliability of all χ^2-based statistics, we refrained from investigating further modifications of our factor model.

The Polish version of the TFI

Descriptive Statistics, Intergroup Differences, and Correlations

Supplementary Table S7 presents responses to particular items of the TFI-Pl. **Table 5** shows the average scores obtained by participants for the TFI-Pl, VAS scales, CES-D, and SWLS. **Table 6** summarizes the correlations between the different scales (correlations between THI-Pl and TFI-Pl are included). Lastly, Supplementary Table S8 presents more details on correlations of all THI and TFI scales with other measures.

TABLE 5 | Average scores and their standard deviations for TFI-Pl, the original version of the TFI (values in brackets), and additional measures obtained from the Polish participants.

Scale	Mean	*SD*
TFI	46.7 (54.4)	22.5 (24.7)
TFIint	53.1 (67.8)	25.9 (24.3)
TFIsoc	64.0 (64.7)	27.0 (25.0)
TFIcog	40.2 (48.0)	25.0 (29.0)
TFIsleep	42.6 (51.5)	31.5 (35.3)
TFIaud	40.0 (53.1)	29.3 (30.2)
TFIrelax	50.5 (59.9)	28.9 (30.3)
TFIqol	36.4 (46.3)	29.4 (29.9)
TFIem	45.6 (47.2)	29.0 (31.8)
VASa	4.8	2.6
VASl	4.9	2.6
CES-D	15.2	10.3
SWLS	21.8	5.8

TFI, Tinnitus Functional Index; int, intrusive; soc, sence of control; cog, cognitive; aud, auditory; relax, relaxation; qol, quality of life; em, emotional; VAS, Visual Analog Scale; a, annoyance; l, loudness; CES-D, The Centre for Epidemiological Studies Depression Scale; SWLS, The Satisfaction With Life Scale.

We did not discover any impact of gender or hearing loss on the TFI-Pl score or its subscales (all p-values from t-test were non-significant). The Wilcoxon–Mann–Whitney test showed significant influence of hearing loss presence on the scores obtained in the auditory, quality of life, and emotional subscales ($p = 0.017$, $p = 0.020$, and $p = 0.024$, respectively). The group with participants subject to hearing loss obtained higher scores than normal hearing patients. In addition, the Kruskal–Wallis test revealed significant differences for the quality of life ($p = 0.03$) and emotional ($p = 0.049$) subscales and tinnitus pitch as well as for the sense of control subscale and tinnitus characteristics (tonal vs. non-tonal tinnitus). The highest scores in both the quality of life and emotional subscales were obtained by participants whose tinnitus pitch corresponded to a sound of 125 Hz; the lowest score corresponded to 500 Hz. The lowest sense of control was reported for patients experiencing a mixed type of tinnitus. A one-way ANOVA did not confirm any significant differences for the TFI-Pl total score (or its subscales) resulting from variations in tinnitus localization or character. Significant differences were observed for the relaxation subscale and tinnitus pitch ($p = 0.012$). A tinnitus pitch of 125 Hz interfered with relaxation particularly strongly, whereas 250 Hz corresponded to the lowest score obtained on the relaxation subscale. Weak but significant correlations were found between the auditory subscale and age or duration of tinnitus.

Convergent validity was confirmed with strong positive Spearman's correlations for the total TFI-Pl score and VAS loudness scale and very strong correlations with the VAS annoyance scale. Moreover, strong correlations were found between the THI-Pl and TFI-Pl. Discriminant validity was shown with no significant correlations for the SWLS and weak positive correlations for the CES-D. The lowest correlations were found for the TFI auditory subscale.

Internal Consistency

The TFI-Pl has almost the same high internal consistency reliability as the original version of the questionnaire ($\alpha = 0.96$ vs. 0.97). Consistency reliability of the TFI subscales ranged from 0.83 (sense of control subscale) to 0.95 (emotional subscale). **Table 7** shows the corrected-item total correlation; Supplementary Table S9 section contains additional information about Cronbach's alpha when a particular item is deleted. Supplementary Table S10 section presents Cronbach's alpha coefficients for the TFI total and all subscales for the TFI-Pl and other adapted versions.

TABLE 6 | Correlations (Spearman's rho) for TFI-Pl and other measures used in the adaptation procedure.

Measure	THI-Pl	VASa	VASl	CES-D	SWLS
TFI-Pl	782***	0.837***	0.761***	0.361***	−0.223*

****$p < 0.001$; *$p < 0.05$; TFI, Tinnitus Functional Index; THI, Tinnitus Handicap Inventory; Pl, Polish; VAS, Visual Analog Scale; a, annoyance; l, loudness; CES-D, The Centre for Epidemiological Studies Depression Scale; SWLS, The Satisfaction With Life Scale.*

Factor Analysis

Similar to the THI, we carried out an exploratory factor analysis based on the oblique Oblimin rotation, since several factors showed moderate correlation (see lower part of **Table 8**). The resulting eigenvalues greater than or equal 1 indicated that five factors are sufficient for explaining the total variance (the fifth factor had an eigenvalue of 0.999). The first factor explained 52.7% of variance, the second 10.8%, the third 5.7%, and the two remaining 5.2% and 4.0%, respectively. The following three factors had eigenvalues of lower than 1 (0.87, 0.67, and 0.51), explaining altogether 8.2% of variance. Meikle et al. (2012) obtained similar results in the factor analysis of their first prototype of the TFI with 43 items, where the eigenvalues of factors five to eight were also lower than 1. However, these authors considered eight factors meaningful and decided to retain all of them. They then performed further confirmatory analysis of a second prototype as well as the final version of the TFI with a specified number (8) of factors. In their analysis of the final version, 79.5% of the total variance was explained, whereas in our case eight factors are explained 86.7%. It may be noted that we performed our factor analysis using the Unweighted Least Squares method with oblique Oblimin rotation, since factors were correlated. The Kaiser-Meyer-Olkin (KMO) Measure of Sampling Adequacy was high (KMO = 0.903) and Bartlett's Test of Sphericity was statistically significant ($p < 0.001$).

Table 8 presents the pattern matrix of an eight-factor solution. All factors contribute to the loadings of the 25 items and correspond to the respective subscales of the questionnaire. Except for three items, all loadings have values greater than 0.5. The remaining items (TFI 20, TFI 19, and TFI 2) carry loadings higher than 0.3 and are loaded by two factors. The decision to assign item TFI 2 to the intrusive subscale seems arbitrary, since it is equally loaded (0.305) by the factor referring to the sense of control subscale. However, assignment of this item to any other factor would leave only two items remaining in the subscale, which is not recommended (Meikle et al., 2012).

In summary, our investigation of the factor loadings and their corresponding eigenvalues led to the conclusion that the original structure of the questionnaire should be replicated.

For exploratory purposes, we also investigated the results of a factor analysis with orthogonal rotations (Supplementary Tables S12 and S13 show the results). The loadings obtained from the Varimax solution suggest a seven-factorial structure; for the Quartimax solution, the corresponding number reduces to five.

Moreover, we carried out a CFA for investigating if we can confirm the original eight-factorial structure suggested by Meikle et al. (2012). The results, on the one hand, were not fully satisfactory in terms of fit indices and thus did not permit us to conclude an acceptable fit with the data (Supplementary Table S14 presents details on the estimation results). On the other hand, some of these values were not much lower than those obtained by Fackrell et al. (2016); e.g., TLI (0.915 vs. 0.939) or RMSEA (0.084 vs. 0.064). Obviously, the same limitations caused by our comparably low sample size apply.

TABLE 7 | Corrected item total correlation for all items of the TFI-Pl.

Item	Corrected item-total correlation
1	0.585
2	0.736
3	0.666
4	0.374
5	0.669
6	0.717
7	0.747
8	0.740
9	0.772
10	0.609
11	0.655
12	0.642
13	0.604
14	0.487
15	0.599
16	0.739
17	0.715
18	0.728
19	0.769
20	0.833
21	0.780
22	0.721
23	0.784
24	0.798
25	0.798

DISCUSSION

Objectives

The main goal of our study was to provide Polish clinicians and researchers with validated translations of the THI and the TFI questionnaires that could facilitate the classification of tinnitus patients according to severity and potential evaluation of therapeutic outcomes. The results of the presented work show that valid and reliable Polish versions of the THI and TFI questionnaires were constructed. The THI-Pl and TFI-Pl are satisfactory in terms of construct and criterion validity. They may be used by Polish clinicians working with people who have tinnitus and by Polish scientists working on international scientific research reports. Moreover, the TFI-Pl provides a decent psychometric tool and may be used by Polish clinicians working with patients suffering from tinnitus by enabling the detection of treatment-related changes. Polish scientists working in the tinnitus field may consider scores and subscores to identify factors potentially influencing results obtained during their research. For the THI-Pl one may focus stronger on the total score in clinical and scientific reports.

Limitations

We would also like to address two issues, which could be considered as limitations of our study. The size of our sample was not very large (98 patients for the THI-Pl, 108 patients for the TFI-Pl), and that created natural limits, for instance, for performing the CFA which could be of potential interest

for some professional researchers working on adaptations or construction of questionnaires. On the other hand, only four among the 19 adaptations of THI cited in this paper were based on larger amounts of questionnaires (Brazilian/Portuguese–180, French–174, German–373, Japanese–182; Shinden et al., 2002; Ferreira et al., 2005; Schmidt et al., 2006; Ghulyan-Bédikian et al., 2010; Kleinstäuber et al., 2015, respectively), and only the German version of the THI included a presentation of the CFA results. In that study the CFA was justified by a sufficient number of patients. Six studies recruited a similar number of participants (Chinese–114, Persian–102, Italian–100, Korean–111, Turkish–110, and Arabic–100; Kim et al., 2002; Aksoy et al., 2007; Monzani et al., 2008; Kam et al., 2009; Jalali et al., 2015; Barake et al., 2016), and the remaining nine studies tested much fewer tinnitus patients. As far as the TFI is concerned, both the Dutch and English adaptations recruited more participants (263 and 294; Fackrell et al., 2014; Rabau et al., 2014, respectively). In the first case, the authors performed an EFA; in the second a CFA. Our aim was to adapt already existing tools in a manner that would enable the comparison of the THI-Pl and the TFI-Pl with other language versions. We believe that our choice of sample size and factor analysis has good justification in light of the mentioned literature. Future work will be needed to

TABLE 8 | Rotated factor loading matrix of the predefined-factor model.

	Factor							
	1	2	3	4	5	6	7	8
Item	13.2	2.7	1.4	1.3	1.0	0.9	0.7	0.5
21	**0.793**							
22	**0.713**							
20	**0.399**					0.305		
19	**0.315**					0.305		
14		**−0.993**						
13		**−0.828**						
15		**−0.763**						
7			**0.842**					
8			**0.807**					
9			**0.642**					
11				**−0.913**				
12				**−0.790**				
10				**−0.684**				
1					**0.995**			
3					**0.537**			
23						**0.789**		
25						**0.720**		
24						**0.685**		
5							**0.901**	
4							**0.670**	
6							**0.507**	
2					**0.305**		0.305	
17								**0.961**
18								**0.735**
16								**0.734**
	Correlations							
Factor 1	1.000	−0.609	0.511	−0.331	0.326	0.604	0.250	0.531
Factor 2	−0.609	1.000	−0.485	0.172	−0.286	−0.397	−0.149	−0.245
Factor 3	0.511	−0.485	1.000	−0.491	0.385	0.447	0.471	0.543
Factor 4	−0.331	0.172	−0.491	1.000	−0.437	−0.328	−0.470	−0.565
Factor 5	0.326	−0.286	0.385	−0.437	1.000	0.353	0.492	0.422
Factor 6	0.604	−0.397	0.447	−0.328	0.353	1.000	0.411	0.492
Factor 7	0.250	−0.149	0.471	−0.470	0.492	0.411	1.000	0.402
Factor 8	0.531	−0.245	0.543	−0.565	0.422	0.492	0.402	1.000

Eigenvalues are presented below the names of factors. Lower part of the table shows correlations between the factors. Loadings > 0.30 displayed. Loadings assigned to particular factors in bold.

assess the test-retest reliability, which, when confirmed would add important value to Polish versions of the THI and TFI questionnaires.

The Polish version of the THI

Conclusions from the THI-Pl factor analysis are ambiguous since the scree-plot shows a clear dominance of the first factor and limited contribution of the second and third factors in terms of explained variation, which supports a unifactorial structure. On the other hand, multiple factor loadings above the threshold of 0.4 attributed to the second and third factors suggest that a three-factorial structure should not be discarded. Therefore, our results are between those of Newman et al. (1996) and the more recent findings suggesting a unifactorial structure (Baguley and Andersson, 2003; Fackrell et al., 2014). Consequently, one could eventually consider the current subscale division or alternatively simply treat the THI-Pl as a homogenous tool.

Lastly, one aspect is particularly noteworthy in the context of the factor analysis results. Since all factors are moderately correlated with each other, an oblique rotation seems appropriate. However, ignoring the correlation and using the orthogonal Quartimax rotation may lead to substantially different results, with loadings clearly supporting a unifactorial structure. It may be possible that related phenomena may be, in part, responsible for varying results on the factorial structure obtained by different studies.

High correlations between the THI subscales observed in the original version of the questionnaire were also observed in the Polish THI adaptation. Similarly, as far as the total and subscale scores are concerned, the THI-Pl has high internal reliability. Convergent validity was confirmed by strong correlations with the VAS scale accessing tinnitus severity annoyance and the VAS loudness scale. Discriminant validity was assessed by moderate associations between the THI and the CES-D measuring the self-perceived level of depression, and weak associations for the SWLS scale measuring the self-perceived quality of life. Thus, our expectations concerning convergent and discriminant validity were confirmed.

The Polish version of the TFI

During the development of the first version of the TFI containing 43 items, Meikle et al. (2012) reported four factors with eigenvalues higher or equal to 1 when performing the factor analysis. Nevertheless, they decided to preserve eight subscales in the TFI and continued further analysis on a second prototype and the final version of the questionnaire with a specified number (8) of factors. For statistical purposes only—i.e., considering factors with eigenvalues greater or equal to one—we decided to perform an additional factor analysis with five factors. This approach was also motivated by the scree plot (Supplementary Figure S2), which does not support an eight-factorial structure, but rather suggests three to four factors, thus encouraging the exclusion of factors with eigenvalues smaller than 1. Moreover, the five factors contributed to 78.4% of the explained variation, which can be considered satisfactory. Note that for the sake of comparability with our eight-factor solution, we chose a similar setting (Unweighted Least Squares, oblique Oblimin rotation). In the five-factorial solution the first and main factor loaded 10 items, and the second, third, fourth, and fifth factors loaded five, four, three, and three items, respectively. The relaxation, quality of life, and emotional subscales were assigned to one factor, and the intrusive subscale was separated into sense of control (two items) and sleep (one item) subscales. This observation is in line with concerns described by Meikle et al. (2012). These authors state that the intrusive, quality of life, and emotional subscales may reflect only a general tinnitus severity factor or both general and specific factors. Our conclusions from five- and eight-factorial solutions seem to confirm this reflection. The rotated factor matrix of this solution is available in Supplementary Table S11. Furthermore, for the TFI-Pl both orthogonal transformations investigated suggest factorial structures with a lower number of factors. This suggests, in particular, that the popular Varimax rotation should be used with care for analyzing the TFI-Pl (and potentially the TFI in other languages).

The TFI-Pl presents excellent internal consistency reliability and is satisfactory in terms of convergent and discriminant validity. As for the factor analysis results, an eight-factorial solution assigns all items to appropriate subscales for both the TFI-Pl and the original version of the TFI.

Finally, we would like to emphasize the importance of the fact that Polish versions of tinnitus questionnaires will provide Polish researchers with the possibility to become active within international scientific networks. In light of the complex nature of tinnitus, the collaboration between different communities seems to be of very great importance. Using the THI-Pl and TFI-Pl, Polish scientists will not only be able to compare and report results of their studies dedicated to tinnitus, but also become valued contributors on international scientific platforms.

ETHICS STATEMENT

The study was performed in three ENT centers in Poland, namely Poznan, Gdansk, and Lodz. Heads of these centers (MD Eugeniusz Szymiec, MD Wiesława Klemens and MD Piotr Kotylo) agreed on the procedures and provided access to tinnitus patients. Participation was voluntary and completely anonymous, all procedures were non-intrusive. Every patient gave oral consent before filling out the questionnaire. Patients visiting one of the three ENT centers in Poland were informed about the aim of the study as well as the estimated time for completing the questionnaires. Once a patient agreed to volunteer, a study information sheet was provided.

AUTHOR CONTRIBUTIONS

MW translated the questionnaire, designed and performed experiments, analyzed data and wrote the paper; ES, AL-M, and MM performed experiments at the ENT center in Poznań; WK performed experiments at the ENT Private Practice in Gdynia; PK performed experiments at the ENT center in Łódź; WS consulted on the redaction of the manuscript at all stages, and contributed to the revision of the manuscript; AP commented on

the manuscript at all stages; JB provided statistical expertise and revised the manuscript at several stages.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpsyg.2016.01871/full#supplementary-material

REFERENCES

Adamchic, I., Langguth, B., Hauptmann, C., and Tass, P. A. (2012). Psychometric evaluation of visual analog scale for the assessment of chronic tinnitus. *Am. J. Audiol.* 21, 215–225. doi: 10.1044/1059-0889(2012/12-0010)

Aksoy, S., Firat, Y., and Alpar, R. (2007). The Tinnitus Handicap Inventory: a study of validity and reliability. *Int. Tinnitus J.* 3, 94–98.

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Baguley, D. M., and Andersson, G. (2003). Factor analysis of the Tinnitus Handicap Inventory. *Am. J. Audiol.* 12, 31–34. doi: 10.1044/1059-0889(2003/007)

Barake, R., Rizk, S. A., Ziade, G., Zaytoun, G., and Bassim, M. (2016). Adaptation of the arabic version of the Tinnitus Handicap Inventory. *Otolaryngol. Head Neck Surg.* 154, 508–512. doi: 10.1177/0194599815621551

Cima, R. F., Andersson, G., Schmidt, C. J., and Henry, J. A. (2014). Cognitive-behavioral treatments for tinnitus: a review of the literature. *J. Am. Acad. Audiol.* 25, 29–61. doi: 10.3766/jaaa.25.1.4

Crönlein, T., Langguth, B., Pregler, M., Kreuzer, P. M., Wetter, T. C., and Schecklmann, M. (2016). Insomnia in patients with chronic tinnitus: cognitive and emotional distress as moderator variables. *J. Psychosom. Res.* 83, 65–68. doi: 10.1016/j.jpsychores.2016.03.001

Diener, E., Emmons, R. A., Larsen, R. J., and Griffin, S. (1985). The satisfaction with life scale. *J. Pers. Assess.* 49, 71–75. doi: 10.1207/s15327752jpa4901_13

Dojka, E., Gorkiewicz, M., and Pajak, A. (2003). Psychometric value of CES-D scale for the assessment of depression in polish population. *Psychiatr. Pol.* 37, 281–292.

Fackrell, K., Hall, D. A., Barry, J., and Hoare, D. J. (2014). "Tools for tinnitus measurement: development and validity of questionnaires to assess handicap and treatment effects," in *Tinnitus: Causes Treatment and Short & Long-Term Health Effects*, eds F. Signorelli and F. Turjman (New York, NY: Nova Science Publishers Inc), 13–60.

Fackrell, K., Hall, D. A., Barry, J. G., and Hoare, D. J. (2016). Psychometric properties of the Tinnitus Functional Index (TFI): assessment in a UK research volunteer population. *Hear. Res.* 335, 220–235. doi: 10.1016/j.heares.2015.09.009

Ferreira, F. E., Cunha, F., Onishi, E. T., Barreiro, F., and Gananca, F. (2005). Tinnitus Handicap Inventory: cross-cultural adaptation to Brazilian Portuguese. *Pro Fono* 17, 303–310. doi: 10.1590/S0104-56872005000300004

Floyd, F. J., and Widaman, K. F. (1995). Factor analysis in the development and refinement of clinical assessment instruments. *Psychol. Assess.* 7, 286–299. doi: 10.1037/1040-3590.7.3.286

Folmer, R. L., Theodoroff, S. M., Casiana, L., Shi, Y., Griest, S., and Vachhani, J. (2015). Repetitive transcranial magnetic stimulation treatment for chronic tinnitus: a randomized clinical trial. *JAMA Otolaryngol. Head Neck Surg.* 141, 716–722. doi: 10.1001/jamaoto.2015.1219

Ghulyan-Bédikian, V., Paolino, M., Giorgetti-D'Esclercs, F., and Paolino, F. (2010). Propriétés psychométriques d'une version francaise du Tinnitus Handicap Inventory [Psychometric properties of a French adaptation of the Tinnitus Handicap Inventory]. *L'Encéphale.* 36, 390–396.

Jalali, M. M., Soleimani, R., Fallahi, M., Aghajanpour, M., and Elahi, M. (2015). Psychometric properties of the persian version of the Tinnitus Handicap Inventory (THI-P). *Iran J. Otorhinolaryngol.* 27, 83–94.

Jastreboff, P. J., and Hazel, J. W. P. (1993). A neurophysiological approach to tinnitus. Clinical implications. *Br. J. Audiol.* 27, 7–17. doi: 10.3109/03005369309077884

Jastreboff, P. J., and Hazell, J. W. P. (2004). *Tinnitus Retraining Therapy: Implementing the Neurophysiological Model*. Cambridge: Cambridge University Press.

Juczyński, Z. (2001). *Narzędzia Pomiaru w Promocji i Psychologii Zdrowia [Measurement Tools in Health Promotion and Health Psychology]*. Warszawa: Pracownia Testów Psychologicznych Polskiego Towarzystwa Psychologicznego.

Kam, A. C. S., Cheung, A. P. P., Chan, P. Y. B., Leung, E. K. S., Wong, T. K. C., Van Hasselt, C. A., et al. (2009). Psychometric properties of the Chinese (Cantonese) Tinnitus Handicap Inventory. *Clin. Otolaryngol.* 34, 309–315. doi: 10.1111/j.1749-4486.2009.01946.x

Kaniasty, K. (2003). *Klęska Żywiołowa czy Katastrofa Społeczna? Psychospołeczne Konsekwencje Polskiej Powodzi 1997 Roku [Natural or Social Catastrophy? Psychosocial Consequences of 1997 Flood in Poland]*. Gdańsk: Gdańskie Wydawnictwo Psychologiczne.

Kehrle, H. M., Sampaio, A. L., Granjeiro, R. C., Oliveira, T. S., and Oliveira, C. A. (2016). Tinnitus annoyance in normal-hearing individuals correlation with depression and anxiety. *Ann. Otol. Rhinol. Laryngol.* 125, 185–194. doi: 10.1177/0003489415606445

Kim, J. H., Lee, S. Y., Kim, C. H., Lim, S. L., Shin, J., Chung, W. H., et al. (2002). Reliability and validity of a Korean adaptation of the Tinnitus Handicap Inventory. *Korean J. Otolaryngol.* 45, 328–334.

Kleinstäuber, M., Frank, I., and Weise, C. (2015). A confirmatory factor analytic validation of the Tinnitus Handicap Inventory. *J. Psychosom. Res.* 78, 277–284. doi: 10.1016/j.jpsychores.2014.12.001

Krings, J. G., Wineland, A., Kallogjeri, D., Rodebaugh, T. L., Nicklaus, J., Lenze, E. J., et al. (2014). A novel treatment for tinnitus and tinnitus-related cognitive difficulties using computer-based cognitive training and D-Cycloserine. *JAMA Otolaryngol. Head Neck Surg.* 141, 18–26. doi: 10.1001/jamaoto.2014.2669

Landgrebe, M., Zeman, F., Koller, M., and Eberl, Y. (2010). The Tinnitus Research Initiative (TRI) database: a new approach for delineation of tinnitus subtypes and generation of predictors for treatment outcome. *BMC Med. Inform. Decis. Mak.* 10:42. doi: 10.1186/1472-6947-10-42

Langguth, B., Landgrebe, M., Kleinjung, T., Sand, G. P., and Hajak, G. (2011). Tinnitus and depression. *World J. Biol. Psychiatry* 12, 489–500. doi: 10.3109/15622975.2011.575178

Maldonado Fernández, M., Shin, J., Scherer, R. W., and Murdin, L. (2015). Interventions for tinnitus in adults: an overview of systematic reviews. *Cochrane Database Syst. Rev.* 2015:CD011795. doi: 10.1002/14651858.CD011795

Martines, F., Bentivegna, D., Martines, E., Sciacca, V., and Martinciglio, G. (2010). Characteristics of tinnitus with or without hearing loss: clinical observations in Sicilian tinnitus patients. *Auris Nasus Larynx* 37, 685–693. doi: 10.1016/j.anl.2010.03.008

Meikle, M. B., Henry, J. A., Griest, S. E., Stewart, B. J., Abrams, H. B., McArdle, R., et al. (2012). The Tinnitus Functional Index: development of a new clinical measure for chronic intrusive tinnitus. *Ear Hear.* 33, 153–176. doi: 10.1097/AUD.0b013e31822f67c0

Monzani, D., Genovese, E., Marrara, A., Gherpelli, C., Pingani, L., Forghieri, M., et al. (2008). Validity of the Italian adaptation of the Tinnitus Handicap Inventory; focus on quality of life and psychological distress in tinnitus-sufferers. *Acta Otorhinolaryngol. Ital.* 28, 126–134.

Newman, C. W., Jacobson, G. P., and Spitzer, J. B. (1996). Development of the Tinnitus Handicap Inventory. *Arch Otolayrngol. Head Neck Surg.* 122, 143–148. doi: 10.1001/archotol.1996.01890140029007

Newman, C. W., Sandridge, S. A., and Jacobson, G. P. (1998). Psychometric adequacy of the Tinnitus Handicap Inventory (THI) for evaluating treatment outcome. *J. Am. Acad. Audiol.* 9, 153–160.

Oron, Y., Sergeeva, N., Kazlak, M., Barbalat, I., Spevak, S., Lopatin, A. S., et al. (2015). Russian adaptation of the Tinnitus Handicap Inventory. *Int. J. Audiol.* 54, 485–489. doi: 10.3109/14992027.2014.996823

Overdevest, J. B., Pross, S. E., and Cheung, S. W. (2015). Tinnitus following treatment for sporadic acoustic neuroma. *Laryngoscope* 126, 1639–1643. doi: 10.1002/lary.25672

Pavot, W., and Diener, E. (1993). Review of the satisfaction with life scale. *Psychol. Assess.* 5, 164–172. doi: 10.1037/1040-3590.5.2.164

Rabau, S., Wouters, K., and Van de Heyning, P. (2014). Validation and translation of the dutch tinnitus functional index. *B-ENT.* 10, 251–258.

Radloff, L. S. (1977). The CES-D Scale: a selfreport depression scale for research in the general population. *Appl. Psychol. Meas.* 1, 385–401. doi: 10.1177/014662167700100306

Raj-Koziak, D., Kochanek, K., Piłka, A., Bartnik, G., Fabijańska, A., and Skarżyński, H. (2011). Ocena częstości występowania szumów usznych u dzieci z prawidłowym wynikiem badania przesiewowego słuchu [Tinnitus in children with normal results of hearing screening test]. *Otolaryngologia* 10, 171–175.

Roland, L. T., Lenze, E. J., Hardin, F. M., Kallogjeri, D., Nicklaus, J., Wineland, A. M., et al. (2015). Effects of mindfulness based stress reduction therapy on subjective bother and neural connectivity in chronic tinnitus. *Otolaryngol. Head Neck Surg.* 152, 919–926. doi: 10.1177/0194599815571556

Schecklmann, M., Vielsmeier, V., Steffens, T., Landgrebe, M., Langguth, B., and Kleinjung, T. (2012). Relationship between audiometric slope and tinnitus pitch in tinnitus patients: insights into the mechanisms of tinnitus generation. *PLoS ONE* 7:e34878. doi: 10.1371/journal.pone.0034878

Schmidt, L. P., Teixeria, V. N., Dall'Igna, C., Dallagnol, D., and Smith, M. M. (2006). Brazilian Portuguese language version of the "Tinnitus Handicap Inventory"; validity and reproducibility. *Rev. Bras. Otorrinolaringol.* 72, 808–810.

Shekhawat, G. S., Searchfield, G. D., Kobayashi, K., and Stinear, C. M. (2013). Prescription of hearing-aid output for tinnitus relief. *Int. J. Audiol.* 52, 617–625. doi: 10.3109/14992027.2013.799787

Shinden, S., Ogawa, K., Inoue, Y., Tazoe, M., and Asano, K. (2002). Tinnitus annoyance and difficulty in activities of daily life evaluated by the Tinnitus Handicap Inventory (THI). *Audiol. Japan.* 45, 685–691. doi: 10.4295/audiology.45.685

Skarżyński, H., Rogowski, M., Bartnik, G., and Fabijańska, A. (2000). Organization of Tinnitus Management in Poland. *Acta Otolaryngol.* 120, 225–226. doi: 10.1080/000164800750000973

Tass, A. P., Adamchic, I., Freund, H. J., von Stackelberg, T., and Hauptmann, C. (2012). Counteracting tinnitus by acoustic coordinated reset neuromodulation. *Restor. Neurol. Neurosci.* 30, 137–159. doi: 10.3233/RNN-2012-10218

Udupi, V. A., Uppunda, A. K., Mohan, K. M., Alex, J., and Mahendra, M. H. (2013). The relationship of perceived severity of tinnitus with depression, anxiety, hearing status, age and gender in individuals with tinnitus. *Int. Tinnitus J.* 18, 29–34. doi: 10.5935/0946-5448.20130005

Vernon, J. A., and Meikle, M. B. (2003). Tinnitus: clinical measurement. *Otolaryngol. Clin. North Am.* 36, 293–305. doi: 10.1016/S0030-6665(02)00162-7

Wilson, M. B., Kallogjeri, D., Joplin, C. N., Gorman, M. D., Krings, J. G., Lenze, E. J., et al. (2015). Ecological momentary assessment of tinnitus using smartphone technology. A pilot study. *Otolaryngol. Head Neck Surg.* 152, 897–903. doi: 10.1177/0194599815569692

Wise, K., Kobayashi, K., and Searchfield, G. D. (2015). Feasibility study of a game integrating assessment and therapy of tinnitus. *J. Neurosci. Methods* 15, 1–7. doi: 10.1016/j.jneumeth.2015.04.002

Zachariae, R., Mirz, F., Johansen, L. V., Andersen, S. E., Bjerring, P., and Pedersen, C. B. (2000). Reliability and validity of a Danish adaptation of the Tinnitus Handicap Inventory. *Scand. Audiol.* 29, 37–43. doi: 10.1080/010503900424589

Ziarko, M., Kaczmarek, ŁD., and Haładziński, P. (2013). Polish version of Center for Epidemiological Studies Depression Scale (CES-D): results of a preliminary study on the psychometric properties of the scale. *Curr. Issues Personal. Psychol.* 1, 51–61. doi: 10.5114/cipp.2013.40637

Zobay, O., Palmer, A. R., Hall, D. A., Sereda, M., and Adjamian, P. (2015). Source space estimation of oscillatory power and brain connectivity in tinnitus. *PLoS ONE* 10:e0120123. doi: 10.1371/journal.pone.0120123

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Assessing Auditory Processing Deficits in Tinnitus and Hearing Impaired Patients with the Auditory Behavior Questionnaire

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Background and Purpose: Auditory processing disorders (APD), tinnitus and hearing loss (HL) are typical issues reported by patients in audiologic clinics. These auditory impairments can be concomitant or mutually excluding. APD are not necessarily accompanied by significant HL, whereas many adults exhibit peripheral HL and typical cognitive deficits often associated with APD. Since HL, tinnitus and APD affects to several parts of the ascending auditory pathway from the periphery to the auditory cortex, there could be some interrelationship between them. For instance, tinnitus has been reported to degrade the auditory localization capacity. Tinnitus is believed to be triggered by deafferentation of normal peripheral input to the central auditory system. This peripheral deficit can be accompanied by HL or not, since a type of permanent cochlear damage (thus deafferentation) without an elevation of hearing thresholds might persist. Therefore, a combined study of APD, tinnitus and HL on the same cohort of patients can be audiologically relevant and worthy.

Methods: Statistical analysis is applied to a cohort of 305 patients attending an audiology clinic in Madrid (Spain). This group of patients is first categorized in four subgroups, namely, HLTG (with tinnitus and HL), NHLTG (with tinnitus and without HL), HLNTG (with HL but no tinnitus), and NHLNTG (neither tinnitus nor HL). The statistical variables include Age, Average Auditory Threshold (ATT), for assessing HL, Tinnitus Handicap Inventory (THI), for measuring tinnitus, and a new 25-item Auditory Behavior Questionnaire (ABQ), for scoring APD. Factor analysis is applied to arrange these items into 4 subscales. The internal consistency reliability of this ABQ is confirmed by calculating Cronbach's coefficients α. The test-retest reliability is assessed by the intraclass correlation coefficients, *ICC*. Statistical techniques applied to the data set include descriptive analysis of variables and Spearman rank correlations (ρ) between them.

Results: Overall reliability of ABQ is confirmed by an α value of 0.89 and by an *ICC* of 0.91. Regarding the internal consistency reliability, the four subscales prove a fairly good consistency with α coefficients above 0.7. Average values of statistical variables show significantly lower age of patients with tinnitus and no HL, which can provide a cue of noise overexposure of this segment of population. These younger patients show also

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decreased ABQ and similar THI in comparison with patients in the other subgroups. A strong correlation ($\rho = 0.63$) was found between AAT and Age for the HLNTG subgroup. For the HLTG subgroup, a moderate correlation ($\rho = 0.44$) was found between ABQ and THI.

Conclusion: The utilized questionnaire (ABQ), together with AAT and THI, can help to study comorbid hearing impairments in patients regularly attending an audiological clinic.

Keywords: auditory processing disorder, tinnitus, hearing loss, questionnaires

INTRODUCTION

The auditory system transmits sounds from the environment to the auditory cortex where they are processed to produce a perception. The sound signal, a vibroacoustic wave, is transduced into an electrical train of pulses at the synapses between the hair cells of the Corti organ and the auditory nerve. This interface is a powerful device able to transmit signals from the periphery to the auditory pathway spanning 12 decades in amplitude (120 dB) and 3 decades in frequency (20–20 kHz) (Knipper et al., 2013). This mechano-electrical transduction of sound waves into a train of electrical spikes is completed within 1–4 ms with standard deviation of roughly 0.8 ms, which is even lesser than the corresponding constant time of mammalian visual cells (Kopp-Scheinpflug and Tempel, 2015). Auditory signals are thus reliably transmitted along large diameter axons and across highly specialized synapses through the afferent auditory pathway.

Acoustic signals (coded as electrical spike trains) spread from the auditory nerve to the higher central auditory system through the brainstem, an intricate network of neurons with soma grouped into the ascending auditory nuclei. The auditory brainstem involves many sophisticated auditory processing including frequency analysis, sound localization, temporal integration and discrimination, binaural cues for spatial analysis, multisensorial processing, and others. Processing mechanisms for higher order patterns of sound are carried out in the primary auditory cortex (Griffiths, 2002). Any abnormal deviation of this rather sophisticated sound transduction, coding, and processing system arises auditory impairments, including hearing loss (HL), tinnitus and auditory processing disorders (APD).

Peripheral deficits afford HL or hypoacusis. The prevalence of HL has, due to aging of the population, doubled over the past 30 years (Knipper et al., 2013). HL is usually measured as an elevation of hearing thresholds expressed in dB. However, recent studies have revealed a type of permanent cochlear damage, without an elevation of hearing thresholds (Weisz et al., 2006; Schaette and McAlpine, 2011). This subtle damage should be linked to a permanent and progressive degeneration of auditory fibers that occurs in association with damage of the inner hair cell synapse (Knipper et al., 2013).

Tinnitus is the medical term for the auditory perception of sounds in the absence of any external source. As an auditory phantom perception, it seems to be the correlate of maladaptive attempts of the brain to reorganize due to distorted sensory input (Kleinjung et al., 2009). This notion is confirmed by the finding that HL is the most important risk factor for developing tinnitus and that most people with sudden unilateral deafness experience tinnitus. In general, there is increasing evidence that tinnitus is related to alterations of neuronal functioning in the central auditory system which compensates for diminished input by upregulating its responsiveness in sub-cortical and cortical networks (Eggermont, 2012). This auditory percept presents in a great variety: a rustling, whistling, ringing, murmuring or humming sound (neural sounds) which can come in high or low tones, be loud or soft and be continuous or interrupted. Tinnitus is an uncomfortable symptom affecting severely the quality of life of adults (Holm et al., 2005). This sound sensation may cause many audiological, cognitive and neurological issues ranging from hearing and attention deficits to anxiety, annoyance, irritability, disturbed sleep patterns, and depression (Zeng et al., 2011; Zhang, 2013). Tinnitus as such is not an abnormal sensation. Most people will experience tinnitus after a couple of minutes in a silent anechoic room. Tinnitus that occurs every day for more than 5 min is reported by 10–15% of the population, and for 1–2% it affects their quality of life considerably (Van de Heyning et al., 2007; Hall et al., 2015).

APD refers to difficulties in the auditory mechanisms underlying the following abilities of the auditory system: sound localization and lateralization, auditory discrimination, auditory pattern recognition, temporal aspect of audition, and auditory performance in competing, or with degraded, acoustic signals (ASHA-American Speech-Language-Hearing Association, 2005). Therefore, it is a disorder associated with the impaired capability of the auditory system to process complex sound signals, especially in degraded or noisy scenarios (Griffiths, 2002). It may be associated with difficulties in listening, speech understanding, language development, and learning (Jerger and Musiek, 2000). The prevalence of APD is 2–7% for school-aged children (with a ratio of 2:1 in boys with respect to girls), and 10–20% in the elderly (Skarzynski et al., 2015). APD is not necessarily accompanied by a significant increase in the pure tone hearing thresholds, especially in young people. Furthermore, increased audiometric thresholds cannot fully account for the difficulty that elderly listeners experience in processing speech in noise (George et al., 2007). These concomitant problems make the diagnosis of APD more difficult. In normal hearing patients, APD can be diagnosed using screening tests including competing words, competing sentences, dichotic listening, speech understanding in noise, filtered speech, and phonemic synthesis (Skarzynski et al., 2015; Weihing et al., 2015). More recently, speech-evoked auditory brainstem responses (Kopp-Scheinpflug and Tempel, 2015; Rocha-Muniz et al., 2016) and mismatch negativity (MMN)

(Rocha-Muniz et al., 2015) have been proposed to objectively detect APD.

Since HL, tinnitus and APD are associated with pathologies (or alterations) at different locations on the ascending auditory pathway, it can be hypothesized that these might be interrelated. The main goal of this work is to present a correlational analysis to assess associations between HL, tinnitus and APD in a sample of patients exhibiting these hearing disorders. Furthermore, a new measure for assessing APD, the Auditory Behavior Questionnaire is introduced.

MATERIALS AND METHODS

Participants

The study sample consists of a cohort of 305 patients, 174 men (age = 44 ± 14 years) and 131 women (47 ± 14 years), attending an audiologic clinic in Madrid (Spain) between 2011 and 2014.

Firstly we performed *post-hoc* comparisons in the basis of HL obtaining two groups: NHLG composed of subjects with normal hearing, and HLG formed with subjects with hearing loss. The threshold for being included in these groups was calculated as the average of the tonal audiometric thresholds (AAT) for 0.25, 0.5, 1, 2, 4, and 8 kHz frequency bands (George et al., 2007; Savastano, 2008):

$$AAT = \frac{1}{6}\sum_{n=1}^{6} HL\left(f_n\right) \quad (1)$$

where f_n are the octave band frequencies between 250 Hz and 8 kHz. Individuals with average threshold under (over) 25 dB were included in the NHLG (HLG). According with this criterion, 195 patients were found to fit into the HLG and 110 into the NHLG.

Then, subjects were classified as suffering of tinnitus (182 patients) or not (123 patients). All tinnitus sufferers were asked to fill in the THI questionnaire (Spanish version proposed and validated by Herraiz et al., 2001). From the 182 tinnitus sufferers, 53 belonged to the NHLG and the other 129 were included in the HLG. From the 123 non tinnitus patients, 57 belonged to the NHLG and the other 66 were included in the HLG. The categorizing of patients is summarized in **Table 1**.

TABLE 1 | Categorizing of patients.

Normal Hearing Group (NHLG)		Hearing Loss Group (HLG)	
110		195	
Tinnitus (NHLTG)	**Non tinnitus (NHLNTG)**	**Tinnitus (HLTG)**	**Non tinnitus (HLNTG)**
53	57	129	66

Measures

All patients referring hypoacusis, tinnitus, hyperacusis, APD, acoustic distortion, aural pressure, acoustic trauma, or otalgy were selected for this study. All of them were subjected to audiological and ENT explorations. Audiological exploration was carried out into an audiological cabin, using a two-channel clinic audiometer AC40 from Interacoustics, including tonal audiometry, logoaudiometry, discomfort threshold, and distortion products otoacoustic emissions (DPOAEs). ENT exploration consisted of otoscopy, rhinoscopy, and faringoscopy. Besides AAT and THI, all patients were asked to fill in the Auditory Behavior Questionnaire described in the following Section.

The Auditory Behavior Questionnaire

A large number of validated questionnaires have been proposed for quantifying tinnitus distress, disability or handicap. The patient responses to these questionnaires are summed resulting in a final score, which is then used to rate their tinnitus severity. A percentage of tinnitus patients also report listening difficulties typically related with the auditory processing disorder. Although, some of these questionnaires include auditory perceptual difficulties (e.g., the Tinnitus Functional Index, Meikle et al., 2012; Henry et al., 2016) as one of the assessed subscales, none of them was designed to deal with the auditory processing issues undergone by tinnitus patients. In this work a new 25-item questionnaire, the Auditory Behavior Questionnaire (ABQ), is used to assess the auditory processing difficulties associated to hearing impaired subjects. This questionnaire can be useful to complement current measures of auditory processing deficits, like speech-in-noise tests, in tinnitus sufferers (Gilles et al., 2016).

In a first stage, the questionnaire was based on 114 items, including questions about the auditory functions that could be altered as a consequence of the APD. A pre-test pilot study helped to choose the correct extension of the questions, the resistance or rejection degree to some of them and the time needed for completion. This pilot study was led by a consulting panel, consisting of three ENT specialists with substantial experience with hearing impaired patients, one audiologist with direct experience with APD, and one psychologist expert in development of questionnaires.

In a second stage, the 114 initial items were reduced to 25 final items. The main criteria for choosing the 25 final items from these initial 114 were the descriptive analysis and the repetition frequency of each one. The opinion of patients was also taken into account, asking them to rank the items with which they felt more identified. The final 25 items were those that got a greater punctuation from patients. All items were related with the altered processing functions reported by the ASHA-American Speech-Language-Hearing Association (2005), as well as with the alteration of cognitive functions as attention, memory and auditory comprehension. Furthermore, a 25-item questionnaire should facilitate the inter-comparison with a well-established 25-item questionnaire for tinnitus, the THI. The original version in Spanish of the ABQ questionnaire is shown in Figure S1. For understanding purposes to non-Spanish readers, an English adaptation of the items (non-tested and non-validated) is provided in Figure S2. Factor analysis was applied for reliably grouping these items into 4 subscales.

Scoring of the ABQ

Likewise as with THI, each patient response of the ABQ is rated as 0 (no), 2 (sometimes), or 4 (yes). Therefore, the total score of the ABQ, the sum of the individual responses, ranges between 0 and 100. Auditory processing handicap is then rated as slight (ABQ≤28), moderate (29≤ABQ≤58) or severe (ABQ≥59).

Statistical Analyses

Firstly, a factor analysis is applied for categorizing the 25 items into 4 subscales. Then, the internal consistency of each subscale and the full questionnaire is assessed by the Cronbach's alpha coefficient (α). α values greater than 0.7 are considered to provide acceptable internal consistency (Müller et al., 2016). Test-retest allowed checking the reliability of the questionnaire over the time by the intraclass correlation coefficient, *ICC*.

Age, ABQ score, AAT score (as calculated by Equation 1), and THI score (Spanish version) will be the statistical variables for this analysis. A descriptive analysis will be carried out for each of the subgroups defined in **Table 1**.

Finally, Spearman rank correlation analysis will be applied to paired variables for each subgroup. Spearman rank correlation will be used to identify and test the strength of relationships between these variables. Positive Spearman correlation coefficients (ρ) between x and y variables denote that both variables increase monotonically, and vice-versa, a negative correlation coefficient indicates that when x increases y decreases monotonically. The correlation between the variables is considered to be very weak for $|\rho| \leq 0.2$, weak for $0.2 < |\rho| \leq 0.4$, moderate for $0.4 < |\rho| \leq 0.6$, strong for $0.6 < |\rho| \leq 0.8$ and very strong for $|\rho| > 0.8$. Omitting algebraic signs, when comparing different questionnaires for the same construct, $\rho \geq 0.4$ denotes that both measure the same construct (convergent validity), whilst $\rho < 0.4$ signifies that both measure different aspects (discriminant validity) (Müller et al., 2016).

Data sets will be analyzed with MATLAB and R, with significance level $p \leq 0.05$.

RESULTS

Factor Analysis

The 25 questions were aimed to identify those aspects of the auditory processing that can produce some kind of disablement related to the auditory processing capabilities, such as the attentional and memory capacity or selective attention, the auditory discrimination capability, the time aspects required for the correct comprehension of the sound message, the comprehension and integration of information, including discrimination and multisensory integration, the ability to structure thoughts and to coordinate auditory process with non-verbal auditory information, and the ability to assess space orientation features. A factor analysis was carried out to identify the underlying subscales.

Table 2 summarizes the correlation values between the ABQ items, arranged in a way that stands out the similarities. It can be seen that there are a subset of items with higher correlation values, thus a pattern can be envisaged that allows discerning three or four significant groups of items. A scree plot, **Figure 1**, shows that there are 5 eigenvalues greater than 1. On the other hand, a parallel analysis suggests 3 as the number of factors to be retained in the analysis. A comparison between models with 3, 4, and 5 factors evidenced that the 5-factor model provided latent factors with only 2 items that, in turn, were closely related to items from other factors. In addition, some parameters (as the Root Mean Square Error of Approximation, RMSEA) proved a poor performance of the 3-factor model. Therefore, the 4-factor model based on principal axis factoring with oblique rotation was selected. This model gave a $\chi^2 = 283.25$, with 206 degrees of freedom, and a RMSEA = 0.061, what seems adequate to our case (Hutchinson and Olmos, 1998).

Table 3 summarizes the loadings of the items as a function of the factors. The 4 subscales resulting from this analysis have then been defined as:

- Auditory Discrimination (AD): consists of 8 items and is expected to assess attention, memory, auditory discrimination, and the time aspects required for the correct comprehension of the sound message.
- Multisensorial Integration (MI): consists of 9 items supposed to evaluate comprehension and integration of information, as well as discrimination and multisensorial integration.
- Concentration Capacity (CC): consists of 5 items expected to pick up the selective attention and difficulties to concentrate in sound environments.
- Understanding Capacity (UC): consists of 3 items that would allow measuring the ability to understand speech in noisy and reverberant environments.

The resulting model is shown in **Figure 2**. The English version of the final questionnaire (originally in Spanish), defined according to this model, is shown in Figure S2. Notice that this English version consists of a translation by the authors and has not been rigorously tested nor validated (Müller et al., 2016). Users interested in using this version should request permission to use to the first author (idiges8@gmail.com).

Reliability

Table 4 summarizes the results of the reliability tests. Third column shows the results for the internal consistency reliability tests that turns out to have an α above 0.75 for all the subscales, except for "Concentration Capacity" ($\alpha = 0.69$) which is yet acceptable.

Fourth column of **Table 4** shows the values of the *ICC* resulting of applying the test-retest to 35 patients. As it can be seen test-retest reliability evidences a good consistency for all the subscales.

Descriptive Analysis of Variables

Some relevant statistical parameters of the variables (Age, ABQ, AAT, and THI) for each subgroup are summarized in **Table 5** and **Figures 3–6**.

A significant difference in age was found between the median of patients with or without HL, with a confidence level of 95%, regardless they suffer from tinnitus or not, **Figure 3**. The median ages of patients in NHLTG and NHLNTG are 11 and 15.5 years lower than HLTG and HLNTG, respectively. Both NHLTG and

TABLE 2 | Correlation values between the questionnaire items ordered to show compact groups of values.

	1	2	7	5	9	6	12	10	11	3	4	14	16	18	19	17	20	21	22	23	8	13	24	15	25
1	–																								
2	0.67	–																							
7	0.25	0.32	–																						
5	0.32	0.46	0.34	–																					
9	0.36	0.49	0.35	0.62	–																				
6	0.37	0.47	0.36	0.52	0.48	–																			
12	0.33	0.52	0.36	0.51	0.56	0.61	–																		
10	0.03	0.23	0.26	0.20	0.21	0.18	0.22	–																	
11	0.38	0.54	0.39	0.49	0.43	0.49	0.54	0.26	–																
3	0.34	0.55	0.29	0.41	0.31	0.40	0.39	0.25	0.53	–															
4	0.35	0.59	0.27	0.43	0.38	0.43	0.45	0.31	0.55	0.83	–														
14	0.25	0.42	0.28	0.36	0.38	0.38	0.45	0.24	0.38	0.39	0.40	–													
16	0.18	0.39	0.25	0.39	0.38	0.36	0.45	0.24	0.42	0.46	0.50	0.44	–												
18	0.12	0.19	0.30	0.15	0.23	0.13	0.18	0.14	0.17	0.19	0.12	0.07	0.14	–											
19	0.04	0.11	0.13	0.18	0.12	0.07	0.18	0.08	0.11	0.15	0.15	0.08	0.06	0.38	–										
17	0.05	0.13	0.25	0.04	0.08	0.20	0.24	0.12	0.12	0.17	0.13	0.11	0.09	0.35	0.26	–									
20	0.09	0.18	0.29	0.24	0.17	0.19	0.25	0.11	0.18	0.24	0.19	0.16	0.19	0.28	0.35	0.28	–								
21	0.03	0.09	0.16	0.14	0.13	0.15	0.15	0.10	0.14	0.18	0.19	0.11	0.21	0.33	0.30	0.33	0.40	–							
22	0.06	0.12	0.27	0.17	0.17	0.19	0.26	0.15	0.11	0.14	0.20	0.16	0.15	0.27	0.36	0.32	0.40	0.46	–						
23	0.10	0.07	0.28	0.10	0.15	0.11	0.12	0.21	0.17	0.10	0.06	0.12	0.04	0.34	0.26	0.28	0.21	0.20	0.29	–					
8	0.20	0.25	0.37	0.18	0.18	0.09	0.15	0.23	0.28	0.24	0.19	0.17	0.17	0.31	0.19	0.17	0.15	0.16	0.18	0.39	–				
13	0.20	0.21	0.31	0.17	0.18	0.17	0.20	0.20	0.20	0.17	0.19	0.20	0.17	0.23	0.18	0.25	0.18	0.14	0.21	0.41	0.53	–			
24	0.14	0.24	0.31	0.27	0.23	0.16	0.25	0.20	0.20	0.20	0.20	0.30	0.17	0.19	0.28	0.16	0.35	0.19	0.24	0.31	0.18	0.25	–		
15	0.19	0.18	0.28	0.11	0.16	0.22	0.20	0.11	0.22	0.26	0.24	0.23	0.29	0.32	0.21	0.30	0.32	0.39	0.22	0.24	0.28	0.24	0.22	–	
25	0.07	0.20	0.25	0.13	0.13	0.22	0.15	0.17	0.19	0.24	0.24	0.19	0.24	0.27	0.15	0.19	0.19	0.24	0.20	0.26	0.29	0.17	0.23	0.40	–

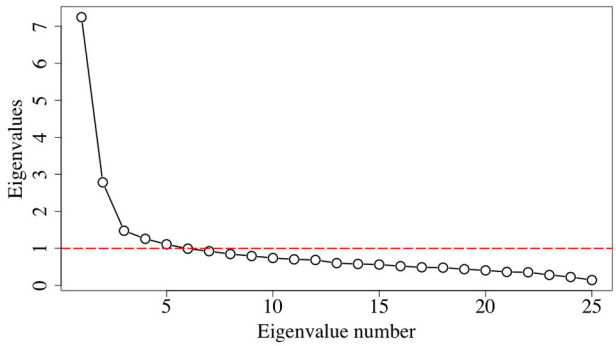

FIGURE 1 | Scree plot of the test correlation matrix.

TABLE 3 | Loadings of the items for the resulting factors.

ID	AD	MI	CC	UC
9	0.86			
12	0.85			
5	0.8			
6	0.75			
2	0.57			0.27
11	0.51			0.24
1	0.46			
14	0.45			
21		0.77		
20		0.65		
22		0.65		
19		0.54		
17		0.48		
18		0.41	0.25	
15		0.41		
24		0.27		
25		0.24	0.23	
8			0.89	
13			0.71	
23			0.59	
7	0.34		0.35	
10			0.23	
4				0.82
3				0.81
16	0.34			0.34

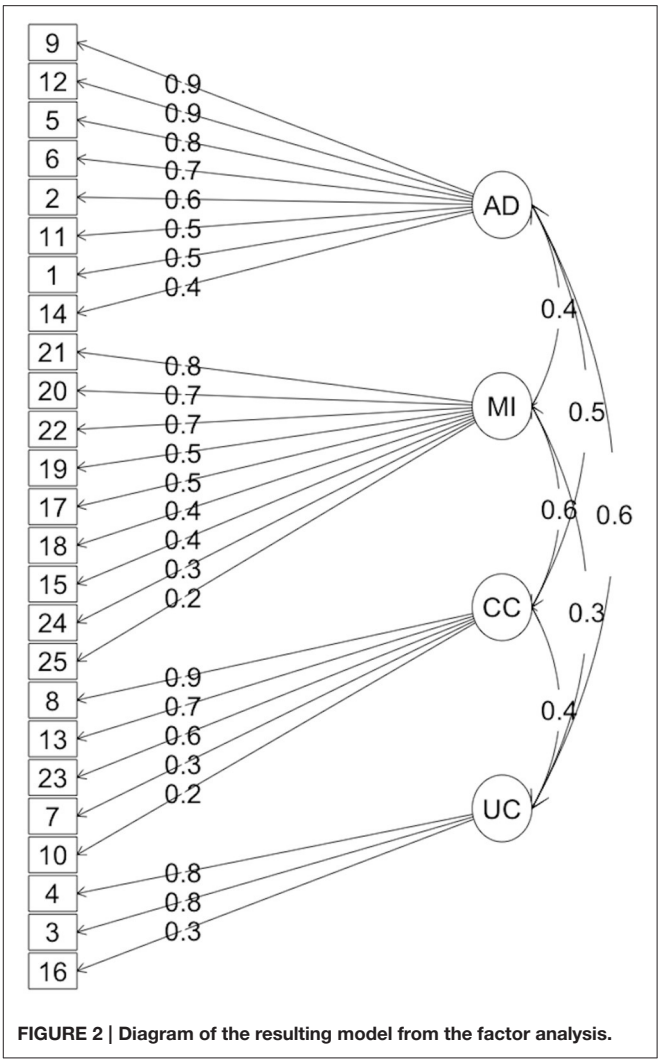

FIGURE 2 | Diagram of the resulting model from the factor analysis.

TABLE 4 | Initial and test-retest Cronbach's alpha for each subscale of the ABQ.

Subscale	No. of Items	$\alpha_{initial}(N = 310)$	$ICC_{test\text{-}retest}(N = 35)$
Overall	25	0.89	0.91
AD	8	0.87	0.77
MI	9	0.78	0.88
CC	5	0.69	0.83
UC	3	0.81	0.75

NHLNTG also have a smaller variability as compared to the HLTG and HLNTG. Thus, in average, patients without HL are younger than patients with HL.

Regarding the ABQ, and considering the median values for all the subgroups, patients exhibit a moderate processing disorder (29<ABQ≤58) in the HLNTG (thus, with HL and without tinnitus), and a slight processing disorder (ABQ<28) in the HLTG, NHLTG, and NHLNTG. The skewness of the ABQ for the different subgroups is much greater for the NHLTG than for the rest of the subgroups, so that the relative amount of cases with high ABQ scores for this subgroup is greater than in the other subgroups. Furthermore, AAT distribution is more asymmetric than the rest of variables. Also, AAT and THI values of patients in the different subgroups do not show significant differences, **Figures 5**, **6**.

Rank Correlations

Table 6 summarizes the Spearman rank correlation coefficients between paired statistical variables. As it can be seen, AAT

TABLE 5 | Descriptive analysis for the variables of the four subgroups.

	Age				ABQ				AAT		THI	
	HLTG	HLNTG	NHLTG	NHLNTG	HLTG	HLNTG	NHLTG	NHLNTG	HLTG	HLNTG	HLTG	NHLTG
N	129	66	53	57	129	66	53	57	129	66	129	53
Median	49	52.5	38	37	28	35	14	28	40	40	44	44
SD	12.7	15.6	9.2	11.0	19.6	19.5	13.8	16.7	22.1	22.0	25.4	22.1
Skewness	0.081	−0.23	0.49	0.013	0.44	0.17	1.032	0.451	1.29	1.087	0.11	−0.11

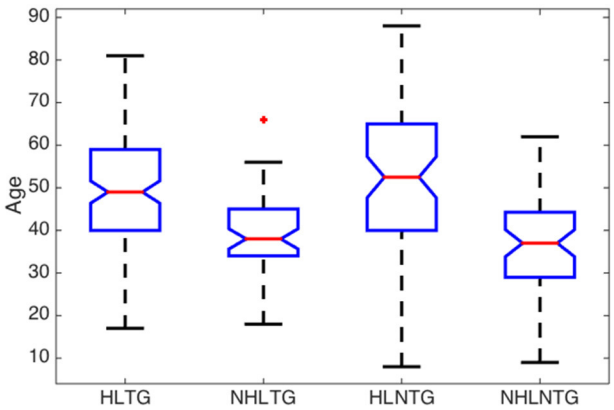

FIGURE 3 | Boxplot of descriptive analysis of age by subgroups.

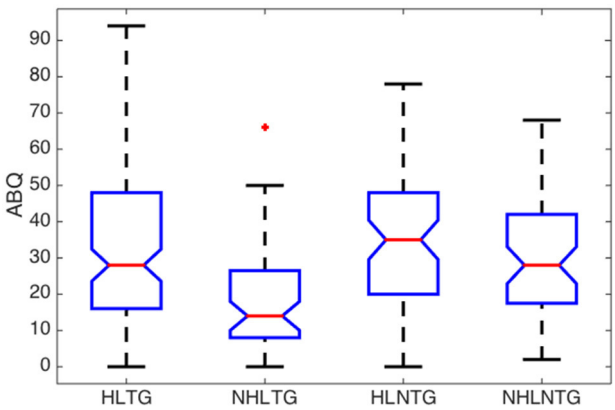

FIGURE 4 | Boxplot of descriptive analysis of ABQ by subgroups.

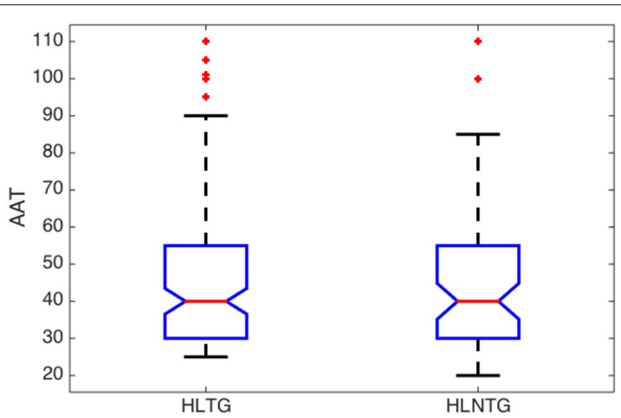

FIGURE 5 | Boxplot of descriptive analysis of AAT by subgroups.

exhibits a strong monotonic increasing relationship with age for patients in the HLNTG subgroup, and a weak relationship in the HLTG subgroup. Thus, HL seems to increase strongly with age for patients without tinnitus, and weakly for patients with tinnitus. ABQ shows a weak monotonically decreasing relationship with age for patients in the NHLTG subgroup. Furthermore, the relationship between ABQ and THI is moderate for the HLTG subgroup, and weak for the NHLTG subgroup. Hence, ABQ increases moderately or weakly with THI in patients without or with HL, respectively. The interrelationship of ABQ with AAT is weak for both HLTG and HLNTG subgroups. All the other paired variables reveal a very weak interrelationship ($|\rho| < 0.2$).

Table 7 shows the expected inter-relationships between the analyzed hearing impairments as well as their variation with age. The usually reported increasing HL with age is confirmed in our results of **Table 6**. Furthermore, the rank correlation coefficient is almost double for patients without tinnitus (HLNTG) in comparison to patients with tinnitus (HLTG). For illustrating better some of these inter-relationships, **Figures 7**, **8** show the scatter plots of AAT vs. Age, for the HLTG and HLNTG subgroups, and ABQ vs. THI, for the HLTG and NHLTG subgroups, respectively.

DISCUSSION

The descriptive analysis of the statistical variables has provided remarkable results. Firstly, it was found that the average age of patients without HL is significantly lower that the corresponding average age of patients with HL, see **Figure 3**. This is consistent with the fact that younger people begins to experience auditory troubles even though HL has not been developed yet. Many authors attribute these troubles to noise overexposure (Emmerich et al., 2002; Gilles et al., 2016). According to Liberman and Liberman (2015), diffuse loss of internal hair cells (IHC), or the auditory nerve fibers they innervate, has to exceed 80–90% before auditory thresholds increase significantly. Secondly, for patients with tinnitus, the average ABQ score is significantly lower (half) without HL than

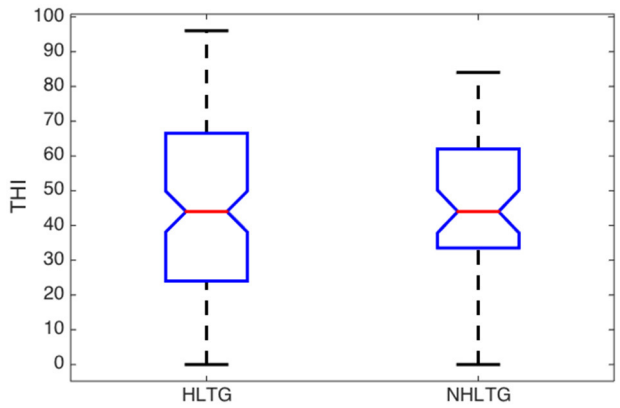

FIGURE 6 | Boxplot of descriptive analysis of THI by subgroups.

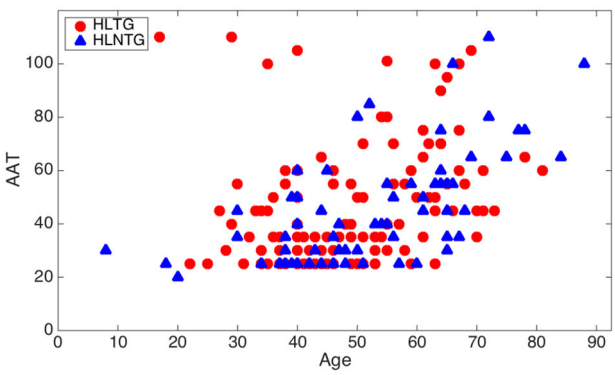

FIGURE 7 | Scatter plot of AAT vs. age for the HLTG and HLNTG subgroups.

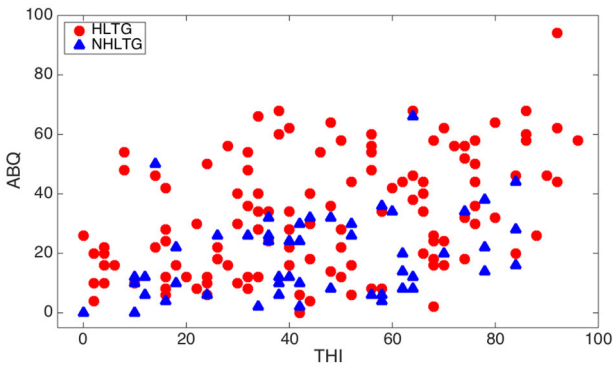

FIGURE 8 | Scatter plot of ABQ vs. THI for the HLTG and NHLTG subgroups.

TABLE 6 | Spearman rank correlation coefficients between variables for each subgroup.

	Age	ABQ	AAT	THI
ABQ	0.13 (HLTG)			
	0.13 (HLNTG)			
	−0.31 (NHLTG)	1		
	0.06 (NHLNTG)			
AAT	0.34 (HLTG)	0.31 (HLTG)	1	
	0.63 (HLNTG)	0.21 (HLNTG)		
THI	−0.06 (HLTG)	0.44 (HLTG)	−0.02 (HLTG)	1
	−0.17 (NHLTG)	0.34 (NHLTG)		

TABLE 7 | Expected inter-relationship between Age, HL, Tinnitus, and APD.

	Age	APD	HL	Tinnitus
APD	⇑		⇑	⇑
HL	⇑	≈		≈
Tinnitus	⇑	≈	⇑	

with HL (see **Table 5**). In other words, HL seems to weaken the auditory processing capabilities of subjects with tinnitus. Hyvärinen et al. (2016) found that tinnitus may degrade auditory localization ability, although this effect can also be due to the associated levels of HL. And thirdly, we have not found differences between the average THI score in patients with or without HL (see **Figure 6**). In principle, this could contradict the finding that subjective discomfort seems to be higher in tinnitus patients with HL than in those without HL (Ganz Sanchez et al., 2005; Savastano, 2008). Nevertheless, if we take into consideration that, in our patients cohort, the average age of subjects in the NHLTG subgroup is 11 years lower than those in the HLTG subgroup, this result reinforce the idea that tinnitus in young people can be triggered by damage in the IHC-auditory nerve synapses (Liberman and Liberman, 2015).

The prevalence of APD is expected to increase with age (Skarzynski et al., 2015). Our results provided a weak monotonic increasing dependence of ABQ with age for subjects with HL (HLTG and HLNTG), see **Table 6**. For patients with normal hearing, this dependence is decreasing for patients with tinnitus (NHLTG) and practically plane for patients without tinnitus (NHLNTG). Thus, the expected increasing dependence of ABQ with age is not confirmed in patients with normal hearing.

APD is also expected to increase with HL (George et al., 2007). However, our results provided a weak correlation of ABQ with AAT, regardless the patients are suffering of tinnitus or not, see **Table 6**.

The increasing interdependency between APD and tinnitus has been reported by some authors. Newman et al. (1994) investigated the relationship between psychoacoustic judgements, speech understanding ability and self-perceived handicap in tinnitus and hearing impaired subjects. Hyvärinen et al. (2016) reported that tinnitus may degrade auditory localization ability, although this effect is, for the most part, due to the associated levels of HL. Gilles et al. (2016) showed that young people with noise induced tinnitus, but normal hearing thresholds, proved impaired speech-in-noise performance. Jain and Sahoo (2014) found that tinnitus has an effect on certain aspects of auditory processing like temporal resolution, speech

perception in noise and frequency discrimination in individuals with normal hearing. Our results confirm a monotonically increasing inter-relationship between ABQ and THI, moderate for patients with concomitant HL (HLTG), and weak for patients with normal hearing (NHLTG).

We have not found significant correlation of THI score with age, which does not contradict the generalized idea that the incidence of tinnitus increases with aging, since THI measures negative reactions of tinnitus, not its prevalence.

Most patients of tinnitus have a related HL, attributable to aging, noise exposure, or chronic otitis media (Holm et al., 2005). Although, HL is an important risk factor for tinnitus, this can occur independently from broad increase of hearing thresholds. Normal hearing thresholds can also be accompanied by impaired function of efferent fibers that project from the brainstem to the cochlea (Schaette and McAlpine, 2011). We have not found a significant correlation factor between THI and AAT, see **Table 6**. Therefore, our results do not support the generalized belief that tinnitus increases with HL. Again, this result reinforces the model of tinnitus triggered by cochlear synaptopathy, which can occur due to noise overexposure (Liberman and Liberman, 2015; Gilles et al., 2016).

The subjective discomfort has been reported to be higher in tinnitus patients with HL than in those without HL (Ganz Sanchez et al., 2005; Savastano, 2008). However, we have found the same average THI in patients with HL (HLTG) and normal hearing (NHLTG), see **Table 5**.

Limitations

Since audiological assessment of subjects was obtained at a single point of time, the study presented here is cross-sectional in itself. Thus, although the results have been interpreted as an estimation of co-occurring hearing impairments in a cohort, they should not be given a prospective significance. The current study, as applied in this work, has been used to analyse comorbidities between hearing impairments, but cannot discriminate between causes and effects. It is also worth mentioning that the above discussed results and interpretations have the known limitations of behavior science studies. Namely, correlational analysis is able to assess the direction and strength of inter-relationships between paired variables but does not provide causal links between them (Stangor, 2011). Thus, the correlational analysis applied in this work has demonstrated that some of the variables are associated, as discussed above, but this relationship could be due to another external variable. Furthermore, although factor analysis results suggest the existence of four latent variables, the need of using oblique rotation, together with the value of the loadings of some of the items, suggests the existence of interactions between them that would require further in-depth studies. It should be considered that the analysis reported here was based on descriptive analysis which has not controlled for the effects of other explanatory and confounding variables such as age, sex, AAT, THI, or type of work experience, and environmental (noise levels) or biological factors. Also, we are currently collecting relevant data, firstly, to carry out statistical diagnostic on ABQ in predicting HL, and secondly, to allow control for other variables in the statistical analysis.

CONCLUSIONS

An inter-relationship study between hearing loss, tinnitus and auditory processing disorder in 305 patients attending an audiological clinic has been carried out in this work. Such audiological disorders have been measured by the average auditory threshold, tinnitus handicap inventory and auditory behavior questionnaire, respectively. The results of this study have confirmed the expected monotonically increasing dependency of auditory behavior questionnaire score and average auditory threshold with age, as well as auditory behavior questionnaire score with average auditory threshold and tinnitus handicap inventory score. However our results, unlike those previously reported by others, show that tinnitus handicap inventory score does not increase with either age or average auditory threshold.

ETHICS STATEMENT

The study presented in this manuscript contains a post-analysis of tests and questionnaires from a dataset that did not require approval from a medical ethical board. All the patients attending an audiological clinic (ACURE) were subjected to standard audiological tests and asked to fill the questionnaires. Thus, the analyses we include in this manuscript resulted from a post-processing of these data, paying special attention to keep all patients de-identified. Anyway, the study would be approved by an ethical committee if it had been submitted.

AUTHOR CONTRIBUTIONS

ID designed the ABQ. FS and PC analyzed the data. PC and FS drafted the initial and final versions of the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnins.2017.00187/full#supplementary-material

REFERENCES

ASHA-American Speech-Language-Hearing Association (2005). *(Central) Auditory Processing Disorders*. Technical Report.

Eggermont, J. J. (2012). *The Neuroscience of Tinnitus*. London: Oxford University Press.

Emmerich, E., Richter, F., Hagner, H., Giessler, F., Gehrlein, S., and Dieroff, H.-G. (2002). Effects of discotheque music on audiometric results and central acoustic evoked neuromagnetic responses. *Int. Tinnitus J.* 8, 13–19.

Ganz Sanchez, T., Torres de Madeiros, I. R., Dias levy, C. P., Oiticica Ramalho, J. R., and Ferreira Bento, R. (2005). Tinnitus in normally hearing patients:

clinical aspects and repercussions. *Rev. Bras. Otorrinolaringol.* 71, 427–431. doi: 10.1590/S0034-72992005000400005

George, E. L. J., Zekveld, A. A., Kramer, S. E., Goverts, S. T., Festen, J. M., and Houtgast, T. (2007). Auditory and nonauditory factos affecting speech reception in noise by older listeners. *J. Acoust. Soc. Am.* 121, 2362–2375. doi: 10.1121/1.2642072

Gilles, A., Schlee, W., Rabau, S., Wouters, K., Fransen, E., and Van de Heyning, P. (2016). Decreased speech-in-noise understanding in young adults with tinnitus. *Frontiers Neurosci.* 10:288. doi: 10.3389/fnins.2016.00288

Griffiths, T. D. (2002). Central auditory processing disorders. *Curr. Opin. Neurol.* 15, 31–33. doi: 10.1097/00019052-200202000-00006

Hall, D. A., Haider, H., Kikidis, D., Mielczarek, M., Mazurek, B., Szczepek, A. J., et al. (2015). Toward a global consensus on outcome measures for clinical trials in tinnitus: report from the First International Meeting of the COMiT Initiative, November 14, 2014, Amsterdam, The Netherlands. *Trends Hear.* 19:2331216515580272. doi: 10.1177/2331216515580272

Henry, J. A., Griest, S., Thielman, E., McMillan, G., Kaelin, C., and Carlson, K. F. (2016). Tinnitus Functional Index: development, validation, outcomes research, and clinical application. *Hear. Res.* 334, 58–64. doi: 10.1016/j.heares.2015.06.004

Herraiz, C., Hernández Calvín, F. J., Plaza, G., et al. (2001). Evaluación de la incapacidad en los pacientes con acufenos. *Acta Otorrinolaringol. Esp.* 52, 142–145. doi: 10.1016/S0001-6519(01)78247-7

Holm, A. F., Staal, M. J., Mooij, J. J. A., and Albers, F. W. J. (2005). Neurostimulation as a new treatment for severe tinnitus: a pilot study. *Otol. Neurotol.* 26, 425–428. doi: 10.1097/01.mao.0000169784.15083.61

Hutchinson, S. R., and Olmos, A. (1998). Behavior of descriptive fit indexes in confirmatory factor analysis using ordered categorical data. *Struct. Equ. Model.* 5, 344–364. doi: 10.1080/10705519809540111

Hyvärinen, P., Mendonça, C., Santala, O., Pulkki, V., and Aarnisalo, A. A. (2016). Auditory localization by subjects with unilateral tinnitus. *J. Acoust. Soc. Am.* 139, 2280–2289. doi: 10.1121/1.4946897

Jain, C., and Sahoo, J. P. (2014). The effect of tinnitus on some psychoacoustical abilities in individuals with normal hearing sensitivity. *Int. Tinnitus J.* 19, 28–35. doi: 10.5935/0946-5448.20140004

Jerger, J., and Musiek, F. (2000). Report of the consensus conference on the diagnosis of Auditory Processing Disorders in school-aged children. *J. Am. Acad. Audiol.* 11, 467–474.

Kleinjung, T., Steffens, T., Struz, J., and Langguth, B. (2009). Curing tinnitus with a Cochlear Implant in a patient with unilateral sudden deafness: a case report. *Cases J.* 2-7462, 1–3. doi: 10.1186/1757-1626-2-7462

Knipper, M., Van Dijk, P., Nunes, I., Rüttiger, L., and Zimmermann, U. (2013). Advances in the neurobiology of hearing disorders: recent developments regarding the basis of tinnitus and hyperacusis. *Progr. Neurobiol.* 111, 17–33. doi: 10.1016/j.pneurobio.2013.08.002

Kopp-Scheinpflug, C., and Tempel, B. L. (2015). Decreased temporal precision of neuronal signalling as a candidate mechanism of auditory processing disorder. *Hear Res.* 330, 213–220. doi: 10.1016/j.heares.2015.06.014

Liberman, L. D., and Liberman, M. C. (2015). Dynamics of cochlear synaptopathy after acoustic overexposure. *J. Assoc. Res. Otolaryn.* 16, 205–219. doi: 10.1007/s10162-015-0510-3

Meikle, M. B., Henry, J. A., Griest, S. E., Stewart, B. J., Abrams, H. B., McArdle, R., et al. (2012). The Tinnitus Functional Index: development of a new clinical measure for chronic, intrusive tinnitus. *Ear Hear.* 32, 153–176. doi: 10.1097/AUD.0b013e31822f67c0

Müller, K., Edvall, N. K., Idrizbegovic, E., Huhn, R., Cima, R., Persson, V., et al. (2016). Validation of online versions of tinnitus questionnaires translated into Swedish. *Front. Aging Neurosci.* 8:272. doi: 10.3389/fnagi.2016.00272

Newman, C. W., Wharton, J. A., Shivapuja, B. G., and Jacobson, G. P. (1994). Relationships among psychoacoustic judgements, speech understanding ability and self-received handicap in tinnitus subjects. *Audiology* 33, 47–60. doi: 10.3109/00206099409072954

Rocha-Muniz, C. N., Befi-Lopes, D. M., and Schochat, E. (2015). Mismatch negativity in children with specific language impairment and auditory processing disorder. *Braz. J. Otorhinolaryngol.* 81, 408–415. doi: 10.1016/j.bjorl.2014.08.022

Rocha-Muniz, C. N., Filippini, R., Neves-Lobo, I. F., Rabelo, C. M., Morais, A. A., Murphy, C. F. B., et al. (2016). Can speech-evoked Auditory Brainstem Response become a useful tool in clinical practice? *CoDAS* 28, 77–80. doi: 10.1590/2317-1782/20162014231

Savastano, M. (2008). Tinnitus with or without hearing loss: are its characteristics different? *Eur. Arch Otorhinolaryngol.* 265, 1295–1300. doi: 10.1007/s00405-008-0630-z

Schaette, R., and McAlpine, D. (2011). Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J. Neurosci.* 31, 13452–13457. doi: 10.1523/JNEUROSCI.2156-11.2011

Skarzynski, P. H., Wlodarczyk, A. W., Kochanek, K., Pilka, A., Jedrzejczak, W. W., Olszewski, L., et al. (2015). Central auditory processing disorder (CAPD) tests in a school-age hearing screening programme – analysis of 76,429 children. *Ann. Agric. Environ. Med.* 22, 90–95. doi: 10.5604/12321966.1141375

Stangor, C. (2011). *Research Methods for the Behavioral Sciences*. Wadsworth: Cengage Learning.

Van de Heyning, P., Meeus, O., Blaivie, C., Vermeire, K., Boudewyns, A., and De Ridder, D. (2007). Tinnitus: a multidisciplinary clinical approach. *B-ENT*, 3, 3–10.

Weihing, J., Guenette, L., Chermak, G., Brown, M., Ceruti, J., Fitzgerald, K., et al. (2015). Characteristics of pediatric performance of tests battery commonly used in the diagnosis of central auditory processing disorder. *J. Am. Acad. Audiol.* 26, 652–659. doi: 10.3766/jaaa.14108

Weisz, N., Hartmann, T., Dohrmann, K., Schlee, W., and Nore-a, A. (2006). High-frequency tinnitus without hearing loss does not mean absence of deafferentation. *Hear Res.* 222, 108–114. doi: 10.1016/j.heares.2006.09.003

Zeng, F. G., Tang, Q., Dimitrijevic, A., Starr, A., Larki, J., and Blevins, N. H. (2011). Tinnitus suppression by low-rate electric stimulation and its electrophysiological mechanisms. *Hear. Res.* 277, 61–66. doi: 10.1016/j.heares.2011.03.010

Zhang, J. (2013). Auditory cortex stimulation to suppress tinnitus: mechanisms and strategies. *Hear. Res.* 295, 38–57. doi: 10.1016/j.heares.2012.05.007

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Visualization of Global Disease Burden for the Optimization of Patient Management and Treatment

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Background: The assessment and treatment of complex disorders is challenged by the multiple domains and instruments used to evaluate clinical outcome. With the large number of assessment tools typically used in complex disorders comes the challenge of obtaining an integrative view of disease status to further evaluate treatment outcome both at the individual level and at the group level. Radar plots appear as an attractive visual tool to display multivariate data on a two-dimensional graphical illustration. Here, we describe the use of radar plots for the visualization of disease characteristics applied in the context of tinnitus, a complex and heterogeneous condition, the treatment of which has shown mixed success.

Methods: Data from two different cohorts, the Swedish Tinnitus Outreach Project (STOP) and the Tinnitus Research Initiative (TRI) database, were used. STOP is a population-based cohort where cross-sectional data from 1,223 non-tinnitus and 933 tinnitus subjects were analyzed. By contrast, the TRI contained data from 571 patients who underwent various treatments and whose Clinical Global Impression (CGI) score was accessible to infer treatment outcome. In the latter, 34,560 permutations were tested to evaluate whether a particular ordering of the instruments could reflect better the treatment outcome measured with the CGI.

Results: Radar plots confirmed that tinnitus subtypes such as occasional and chronic tinnitus from the STOP cohort could be strikingly different, and helped appreciate a gender bias in tinnitus severity. Radar plots with greater surface areas were consistent with greater burden, and enabled a rapid appreciation of the global distress associated with tinnitus in patients categorized according to tinnitus severity. Permutations in the arrangement of instruments allowed to identify a configuration with minimal variance and maximized surface difference between CGI groups from the TRI database, thus affording a means of optimally evaluating the outcomes in individual patients.

Conclusion: We anticipate such a tool to become a starting point for more sophisticated measures in clinical outcomes, applicable not only in the context of tinnitus but also in other complex diseases where the integration of multiple variables is needed for a comprehensive evaluation of treatment response.

Keywords: diagnostic tests, patient management, value-based decision-making, treatment outcome, disease progression, treatment response, subtyping, gender differences

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INTRODUCTION

Complex health-care conditions can be characterized by a combination of identified and unidentified etiological factors including genetics, environment, and lifestyle (1). Among them are found Alzheimer's disease, schizophrenia, scleroderma, asthma, Parkinson's disease, multiple sclerosis, and osteoporosis. Phenotypic heterogeneity in the expression of etiological factors adds complexity to clinical assessment and management and can underlie a mixed response to the same management strategy. Indeed, a new area of research has emerged to characterize complex disorders as profiles that possess a defined set of characteristics (2). Identifying important parameters for patient profiling is a challenging task, yet it is an important step toward being able to provide personalized treatment and would support efforts to develop new treatments. However, in many complex health-care conditions, this has been hard to achieve. Tinnitus is one such example, with a wide range of problems experienced by those who suffer from this condition (3, 4). Tinnitus is defined as the phantom perception of sounds in the absence of any external stimulus. Diagnosis primarily relies on self-report, yet because the impact of tinnitus can be so varied from one patient to another, how it is best treated in individuals is still unclear (5). For example, some patients can complain primarily from sleeping problems (6), or from impaired cognitive function (7, 8) or from communication disabilities (9). Tinnitus is a highly unmet clinical need and there are still no singularly effective therapies that reliably reduce tinnitus percept or its symptoms (10–13). Inter-subject variability in the severity of different tinnitus complaints at diagnostic assessment and at outcome assessment of treatment-related response poses challenges for clinical research. Researchers would benefit from being able to build up an overall picture of the different independent components (or domains) of this complex condition, in order to meaningfully capture key discriminative features between individuals or treatment-related differences.

Based on the tinnitus psychological model of Dauman and Tyler that clearly distinguishes the mechanisms of tinnitus from the reactions to tinnitus (14), the use of multiple measurement instruments has been proposed to address the challenges in quantifying the different aspects of tinnitus (15, 16). However, different laboratories deal with these measures in various ways. One option is the presentation of scores for each measurement instrument. The rationale of this approach is that even if most of these measurements correlate with each other, they assess slightly different aspects of an individual's tinnitus (17, 18). A further option is the use of statistical methods (namely Principal Components Analysis) to tease apart independent components of the condition in a data-driven way (19). Tyler and colleagues also suggested focusing only on areas that show an impact (20, 21). However, whereas this approach might be useful in clinical practice where a dominant problematic has been found in an individual patient and justifies a primary focus, this should be avoided when performing clinical trials. Indeed, more than a hundred instruments have been used as primary outcome measures in tinnitus clinical trials, which hampers the synthesis of existing evidence (e.g., with meta-analyses) and the delivering of conclusive guidelines for clinical care (22). In order to avoid the random selection of instruments in tinnitus studies and facilitate the comparison and synthesis of clinical data, an approach has been proposed as part of the COMiT initiative (Core Outcome Measures in Tinnitus), which aims to establish an international standard for outcome measurements in clinical trials of tinnitus (23). This approach still uses multiple measurement instruments, but their selection is informed by first identifying in a consensus-manner which tinnitus-related complaints are judged to be the most important ones from the perspective of assessing whether a treatment has been beneficial or not, and then identifying one measurement instrument to assess each relevant complaint (23). This approach thus seeks to tease apart independent components of the condition in a hypothesis-driven way. Independent of which approach is taken, they all require the presentation of multiple measures simultaneously for an individual or group. A graphical visualization approach would facilitate the rapid interpretation and would be more attractive to clinicians and patients than a numerical presentation style. How best to visualize this multiplicity of data and to integrate the complex data patterns into a single clinical interpretation is a challenge that is shared across all approaches described above and that is relevant for many complex disorders.

Any aggregated visualization of data relating to a complex health-care condition should meet a number of requirements to facilitate clinic usage: (i) it should be possible to display measures with different scales (interval, ordinal, etc.), (ii) it should be possible to display individual and group data, with SDs if relevant, (iii) it should be visually appealing, (iv) it should be easy to interpret, and (v) it should be able to represent pre- and post-intervention data.

Here, we introduce a method for the holistic representation of components of health status relevant to a complex multi-attribute condition based on radar plots. Radar plots allow the representation of multivariate data on a two-dimensional graphical illustration and have been suggested as a useful approach for the visualization of multivariate clinical data (24). By selecting tinnitus as a model, we illustrate the usefulness of using radar plots to give a holistic representation of multiple variables used in the assessment of tinnitus. In a first aim, we assess the performance of the radar plots in conveying an ensemble of clinical data from the Swedish Tinnitus Outreach Project (STOP) cohort. In a second aim, we evaluate the performance of the various instruments working together to provide a meaningful overview of treatment outcome using data from the Tinnitus Research Initiative (TRI) database. We propose that this methodology can be applied to any complex clinical disorder where multiple assessment tools are used, for single subjects or group data, cross-sectional or longitudinal data.

METHODS

Participants

Data are reported from two datasets. The first dataset comes from the STOP recruiting participants with or without tinnitus from the Swedish population[1] and the second dataset comes from the TRI database.[2] STOP is a nationwide population cohort with the aims

[1] http://stop.ki.se.
[2] www.tinnitus-database.de.

of identifying tinnitus biomarkers. Free-willing registration was done on a website and after participants provided their informed consent, they were invited to fill an online survey (25). The project was approved by the local ethics committee "Regionala etikprövningsnämnden" in Stockholm (#2014/1998-31/4). The database project and server are coordinated and located at the Department of Physiology and Pharmacology of the Karolinska Institutet, Sweden. For this study, a first cross-sectional set of data collected until the 11th of October 2016 included 311 occasional and 328 constant tinnitus subjects. A second cross-sectional set of data was extracted on the 5th of December 2016. That dataset included 1,223 non-tinnitus control subjects and added 294 constant tinnitus subjects. The socio-demographics of the current STOP dataset are presented in Table S1 in Supplementary Material.The second dataset comes from the TRI database (26). and includes 571 individuals who participated in any clinical treatment study at the University Hospital Regensburg, Germany and had consented to reuse of their anonymized data to address new research questions (34.5% female, 65.5% male, age distribution: 17–87 years, mean age ± SD: 52.3 ± 12.2 years). Collection of data for the TRI database was approved by the Ethics Committee of the University of Regensburg, Germany (#08/046).

Selection of Outcome Domains and Measurement Instruments

A range of health domains were assessed using investigator-administered tests and patient-reported questionnaires. Collectively, they provide an overall clinical impression of global health burden. All domains and associated instruments have been identified from a review of clinical trials of tinnitus treatments in adults (22).

STOP Cohort

In the STOP cohort, 5 domains of tinnitus and associated comorbidities (psychological distress, tinnitus-related worries and fears, emotional affects, hyperacusis, and quality of life) were measured by 14 separate tinnitus instruments, in which adaptation to Swedish was validated in a previous study (25). In a pilot study, of which two subjects are shown in **Figure 1**, auditory values were included as an additional measure of hearing loss. The Tinnitus Handicap Inventory (THI) (27, 28) was used to measure tinnitus-related psychological distress. Participants rated each of the 25 items on a categorical 3-point scale ("yes" = 4/"sometimes" = 2/"no" = 0). The mean global score reflects the sum of all responses with a maximum score of 100 indicating the greatest impact on everyday function. For the purposes of analysis, the THI global score was used since it is considered a unidimensional measure (29). The THI cutoffs were defined previously (30) and are split in five different categories from slight (0–16), mild (18–36), moderate (38–56), severe (58–76), and catastrophic (78–100). For sake of clarity and the rapid interpretation of severity, we combined light and moderate together as well as severe and catastrophic. In the radar plots, three colors helped classifying severity: green (negligible), orange (light/moderate), and red (severe/catastrophic). Three Numerical Rating Scales were taken from the Tinnitus Sample Case History Questionnaire (26). One measured tinnitus loudness (Lo): "Describe the loudness of your tinnitus using a scale from 1 (very faint) to 100 (very loud)." One measured tinnitus awareness (Aw): "What percent of your total awake time, over the last month, have you been aware of your tinnitus?" The third measured tinnitus annoyance (An): "What percent of your total awake time, over the last month, have you been annoyed, distressed, or irritated of your tinnitus?"

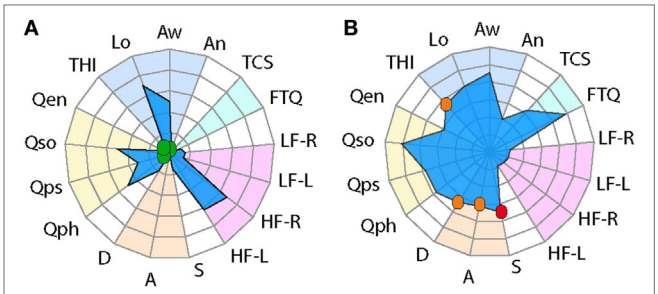

FIGURE 1 | Radar plot profiling for characterizing global health burden in individuals with constant tinnitus. Two tinnitus cases are represented in the radar plots. **(A)** A 72-year-old male subject with high loudness and awareness values displays high-frequency hearing loss but little tinnitus-associated burden. **(B)** A 31-year-old male subject with no hearing loss shows high loudness and awareness scores but with mild/moderate THI scores, moderate depression and anxiety, and severe stress scores. Domains of tinnitus-related burden are grouped in the dark blue region, hearing loss comorbidities in light blue, emotional comorbidities in orange region, and health-related quality of life in yellow. Instruments are labeled as follows: THI, tinnitus-related psychological distress; Lo, tinnitus loudness; Aw, tinnitus awareness; An, tinnitus annoyance; TCS, tinnitus catastrophizing; FTQ, tinnitus fears; LF, low frequency hearing in the left (-L) and right (-R) ears; HF, high-frequency hearing in the left (-L) and right (-R) ears; S, stress; A, anxiety; D, depression scores; Qph, Quality of Life for physical; Qps, psychological, Qso, social; Qen, environment. Color dots illustrate the severity score of those instruments with published severity category boundaries: negligible (green), moderate (orange), and severe (red).

The Fear of Tinnitus Questionnaire measures the worries and fears of patients experiencing tinnitus [FTQ; (31)]. There are 17 items that are rated on a true or false scale. A greater score indicates more extreme fear. Catastrophic cognitive misinterpretations of tinnitus sounds were measured by the Tinnitus Catastrophizing Scale [TCS; (31)]. This is an adapted version of the Pain Catastrophizing Scale in which the word "pain" was substituted by the word "tinnitus" (32). There are 13 items, in which participants indicated the extent to which each statement applies to them using a 5-point scale ("always" = 4 to "not at all" = 0). A greater score indicates more extreme perceptions. TCS has a unidimensional structure and the global score was used (31).

Five important comorbidities are anxiety, depression, stress, hearing loss, and hyperacusis. Measurement instruments for each domain are described as follows: anxiety and depression were measured by the Hospital Anxiety and Depression Scale [HADS; (33)]. HADS comprises 7 items on anxiety (A) and 7 items on depression (D), with each item scored from 0 to 3. Higher scores indicate greater severity, with the maximum score being 21. The HADS cutoffs were defined previously by Zigmond and Snaith (33) and are split in three different categories from normal (0–7, shown in green), borderline (8–10, shown in orange), and abnormal (11–21, shown in red). The Perceived Stress Questionnaire

assesses chronic and acute relationships with stressful events and activities [PSQ-30; (34)]. Thirty items are answered using a 4-point scale, from "almost always" = 4 to "almost never" = 1. The sum of the answers is substracted by 30 and the resulting value is divided by 90, yielding a score between 0.0 and 1.0. Higher scores indicate more severe perceived stress. There is no consensus about the factor structure of the PSQ-30 (34, 35) and so here we used the global score, not any subscale scores. The PSQ-30 cutoffs were defined previously by Levenstein et al. (34, 35) and are split in three different categories from low stress level (<0.34, shown in green), moderate stress level (0.34–0.46, shown in orange), and high stress level (>0.46, shown in red).

Hyperacusis is defined as sound intensities that others would find normal, but are experienced as intolerably loud by affected individuals. This marked intolerance to everyday environmental sounds happens even at moderate levels, in spite of quite often normal hearing thresholds. We measured the condition using the Hyperacusis Questionnaire [HQ; (36)]. The second part of the questionnaire comprises 14 negatively worded items, which are rated on a 4-point scale ("yes, a lot" = 3 to "no" = 0). The total provides the measure of hypersensitivity to sound with higher scores indicating greater sensitivity. The maximum global score is 42 and a global score greater than 28 indicates clinically significant hyperacusis (shown in red), while a global score equal to or less than 28 indicates a negligible problem (shown in green). Again there is no consensus about the factor structure of the HQ (36, 37) and so here we used the global score, not any subscale scores.

For the two tinnitus cases presented in **Figure 1**, hearing loss was reported separately for both low and high frequencies. Hearing was assessed by fixed frequency Bekesy audiometry using a Madsen Astera 2 audiometer and Sennheiser HDA 200 headphone at standard and high audiometric frequencies. Hearing thresholds reported in dB HL (hearing level) were averaged from 0.125 to 6 kHz for lower frequencies. High-frequency thresholds were averaged for frequencies between 8 and 16 kHz. Thresholds were reported separately for left and right ears.

Four domains of health-related quality of life formed the last set of measures [physical health (Qph), psychological (Qps), social relationships (Qso), and environment (Qen)]. We used the WHOQOL-BREF which is a 26-item questionnaire providing a broad reliable measurement with four validated subscale scores (38). Each item has a range of 1–5 and the four domain scores are scaled in a positive direction with higher scores indicating a more positive quality of life. The items on the quality of life must be reversed before scoring.

TRI Database

For the assessment of tinnitus within cases from the TRI database, the WHOQoL-BREF, THI, and five numeric rating scales (0–10) were used (26). Numeric rating scales refer to tinnitus loudness ("How STRONG or LOUD is your tinnitus at present?" Tlou), tinnitus annoyance ("How ANNOYING is your tinnitus at present?" Tann), ability to ignore tinnitus ("How easy is it for you to IGNORE your tinnitus at present?" Tign), tinnitus unpleasantness ("How UNPLEASANT is your tinnitus at present?" Tunp), and the uncomfortable aspect of tinnitus ("How UNCOMFORTABLE is your tinnitus at present, if everything around you is quiet?" Tunc). For assessing perceived treatment-related change, an additional measure was the Clinical Global Impression (CGI) scale (39). This is a single question asked at the end of treatment and requires the patient to give an overall rating of his/her current state compared to the pretreatment baseline. The response is scored on a 7-point scale ("very much better" = 1; "much better" = 2; "minimally better" = 3; "no change" = 4; "minimally worse" = 5; "much worse" = 6; "very much worse" = 7).

Designing the Visualization

A radar plot displays multivariate data in two dimensions. The radar plot comprises a sequence of equi-angular spokes (radii), with each spoke representing one of the measures. The data length of a spoke is proportional to the magnitude of the measurement score, from minimum at the center to maximum at the circumference. A line can then be drawn connecting the data values for each spoke. This gives the plot a star-like appearance and a quantifiable surface area. A patient with high scores across multiple measures is represented by a large surface area (high burden) and conversely a patient with low scores is represented by a small surface area. Evaluation of overall burden takes into account the shape of the radar plot and the size of the plot to address in which domains there are greatest burden experienced. When plots contained two average datasets such as for comparisons of gender or treatment, colors such as blue for men and pink for women, or yellow for pretreatment and orange for posttreatment, were used. Several forms of color coding can be used to facilitate the visualization of the data. Observers do not require any specialized knowledge of the measurement instruments to make an overall judgment about the patient profile. Its interpretation is intuitive. Nevertheless, to support the interpretation of those instruments with published severity category boundaries, we represented negligible problem in green, mild/moderate problems in orange, and severe problems in red. This novel feature of the graphical representation helps the observer to rapidly determine individual burden.

For the STOP dataset, five domains were considered including (i) tinnitus severity (assessed by four instruments, blue background), (ii) tinnitus-related fears (assessed with two questionnaires, light blue background), (iii) hyperacusis (measured with one questionnaire, shown in purple), (iv) emotional comorbidities (assessed by two instruments, orange background), and (v) health-related quality of life (assessed with one questionnaire, yellow background). All scores were adjusted to the same minimum–maximum scale of 0–100. For the TCS, FTQ, and HADS, this was done calculating the percentage of the maximum score (total score/maximum score × 100). The PSQ with a maximum score between 0 and 1 was multiplied by 100. The THI and Numerical rating scales all result in a score between 0 and 100 and were left unmodified. Since the WHOQoL-BREF has higher scores with better life quality, we inversed the scale (100 total score) so that the interpretation of the 0–100 scale was consistent across all measurement instruments. The score from the WHOQoL-BREF was translated into a 0–100 score using the method provided in the WHOQoL user manual using the formula: TRANSFORMED SCORE = (SCORE-4) × (100/16). A value of 100 corresponds to greater severity of negative symptoms. Average hearing threshold values were obtained only in the individual examples and were

left unmodified as the range from 0 dB HL (normal hearing) to >90 dB HL (profound) covers the full range of expected hearing loss (40). For the TRI dataset, a smaller number of domains were available between baseline and follow-up and thus consisted of (i) health-related quality of life (measured with the WHOQoL-BREF), (ii) the tinnitus-related psychological distress (assessed with the THI), and (iii) the individual aspects of tinnitus assessed by the five numeric rating scales.

Statistical Methods

95% confidence intervals were obtained according to the formula: Z^*std/sqrt(n). Group differences were tested by a two-way ANOVA, and multiple comparison tests were mentioned in the legends (Prism version 4.0, GraphPad software). Differences were considered significant if $p < 0.05$.

RESULTS

During a pilot study from the STOP, two tinnitus cases were identified that displayed strikingly different tinnitus-associated burden (**Figure 1**). While both cases displayed relatively high tinnitus loudness and awareness, their radar plot profile indicated a different health burden. Since the THI, the HADS, and the PSQ-30 contain cutoffs for different degrees of severity, these were marked with a color label to grade negligible (green), moderate (orange), or severe (red) scores. A blue surface was used to specify the male gender. A 72-year-old male has a comorbid high-frequency hearing loss, but low scores on tinnitus-related psychological distress (THI), negligible stress (S), anxiety (A), or depression (D) and good quality of life (Qph, Qps, Qso, and Qen) (**Figure 1A**). By contrast, another male, 31 years old, shows good hearing, mild/moderate tinnitus-related psychological distress (THI), moderate stress (S) and anxiety (A), severe depression (D), and poorer quality of life (Qph, Qps, Qso, and Qen) (**Figure 1B**). These two examples capture distinct tinnitus-associated burden across individuals. Based on these findings, we utilized the radar plot as a visualization instrument for assessing cross-sectional data in the STOP cohort.

Cross-sectional Questionnaire-Based Profiling in the STOP Cohort

The two examples provided in **Figure 1** are clearly distinguishable from one another, but how do tinnitus profiles vary in a larger sample? To investigate this question, we analyzed information based on the abovementioned measurement instruments gathered from 639 participants who reported occasional or constant tinnitus in the ongoing STOP study. Since the auditory assessment is still ongoing, these values were not included here. We compared the profiles between occasional and constant tinnitus and took the opportunity to evaluate the differences between men and women (**Figure 2**; **Table 1**). The tinnitus-associated burden denoted by the surface area of each plot appeared greater in constant tinnitus, than in occasional tinnitus (**Figure 2**). Although most scores indicated negligible symptoms in the occasional tinnitus group, scores for the THI and the anxiety subscale of the HADS were mild/moderate in constant tinnitus (**Table 1**). When assessing gender differences for intermittent tinnitus, the overall burden appeared very similar between men and women, with the exception of hyperacusis whereby the plots highlighted a small difference in scores [♀: HQ = 16.60 (15.38–17.82), and ♂: HQ = 12.32 (10.88–13.75), **Figure 2C**]. However, while this difference was statistically significant ($p < 0.0001$), both gender subgroups were below the grading of a clinically meaningful hyperacusis (cutoff = 28). This difference between men and women can be appreciated in the radar plots where an extension of the HQ radii can be seen in women (**Figure 2C**), indicating that the radar plots allow to visually capture subtle changes. For constant tinnitus, many of the tinnitus domains had greater scores in women than in men, including awareness, annoyance, and catastrophic cognitive misinterpretations (**Figure 2F**). Comorbidities were also slightly higher, including hyperacusis and anxiety. Psychological distress (THI) and stress (HADS) were the only instruments showing a shift from the negligible toward the moderate category. Analysis of the data using a two-way ANOVA confirmed that genders differed significantly [$F_{(1, 8,715)} = 193.4$; $p < 0.0001$, **Table 1**]. Again, the visualization of the radar plots merged for men and women enable to observe a greater surface area for women.

Although the radar plots allowed to distinguish contrasting tinnitus-associated burden in constant tinnitus versus occasional as well as subtle differences between genders in both subgroups, we sought to determine whether the radar plots could support the interpretation of those instruments with published severity category boundaries. To do so, an additional set of questionnaire data was collected from 294 subjects with constant tinnitus and added to the previous dataset of 328 for a total of 622 subjects with constant tinnitus. These were classified according to three grades of the THI from slight (range: 0–16, $n = 272$), mild and moderate (range: 18–56, $n = 305$), and severe to catastrophic (range: 58–100, $n = 45$). The HQ, HADS, PSQ-30, and WHOQoL-BREF data from the slight group did not differ from that of non-tinnitus control subjects ($n = 1{,}845$, **Table 2**). Consistently, the radar plots showed no clinically meaningful scores for the slight THI group (**Figure 2G**); however, the mild/moderate THI groups displayed an increased in tinnitus-associated burden with stress scores being *moderate* (**Figure 2H**; **Table 2**), whereas the severe/catastrophic THI group showed much larger surface of the radar plots with *severe* stress and *moderate* anxiety levels (**Figure 2I**; **Table 2**). Hyperacusis scores increased significantly from negligible to mild/moderate and from mild/moderate to severe/catastrophic tinnitus groups, but remained below clinically meaningful hyperacusis values (**Table 2**). Overall, the radar plots facilitated the appreciation of greater tinnitus-associated burden in cross-sectional data from the STOP cohort and suggest that this visualization tool may help in evaluating global tinnitus burden.

Evaluation of Treatment Outcome with the TRI Database

We reasoned that these plots could also be informative with respect to treatment-related response. To investigate this question, we analyzed information from the TRI database that contains information from baseline and posttreatment measures collected from 574 individuals based on three sets of questions

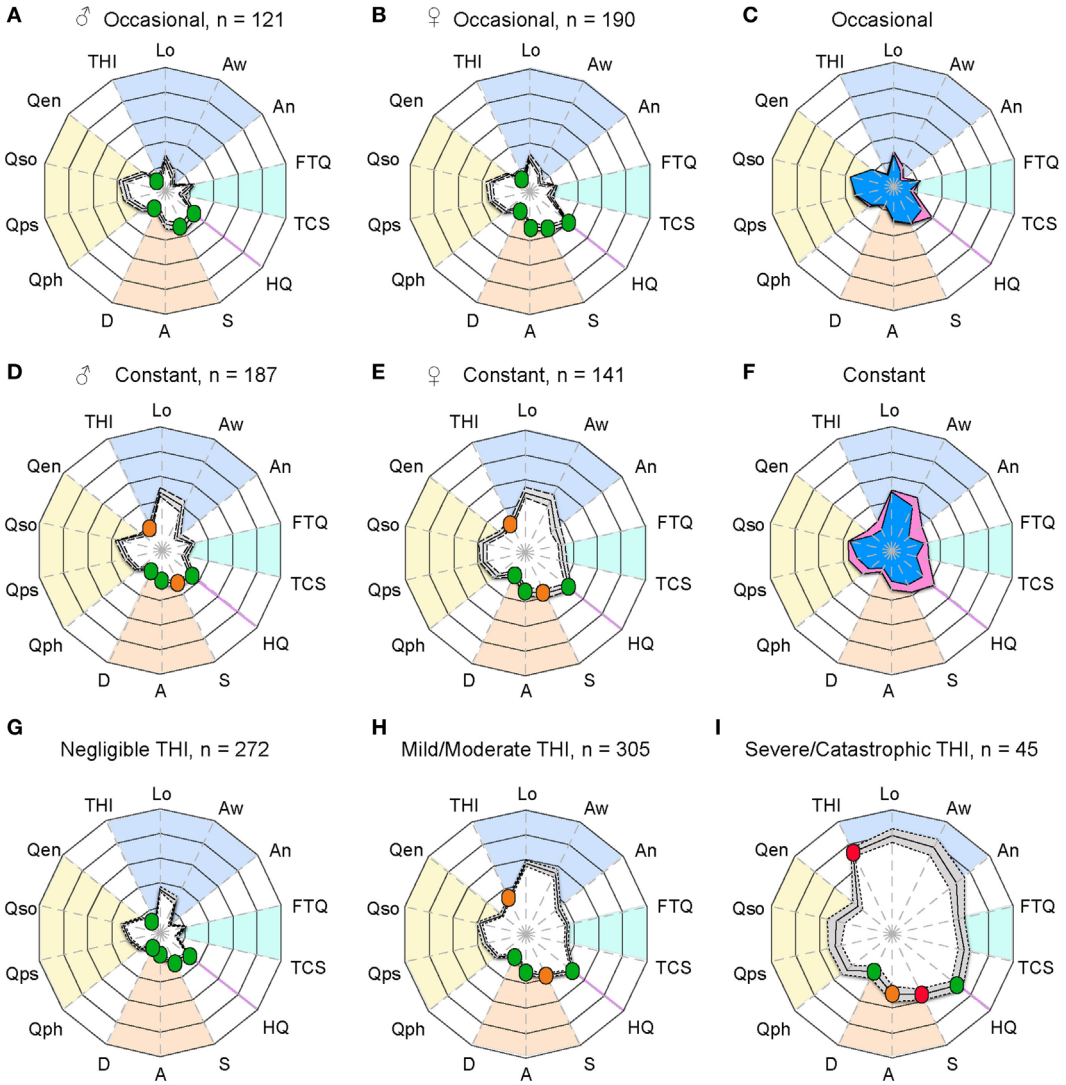

FIGURE 2 | Radar plot profiling that characterizes greater burden in women with tinnitus than in men. Radar plots illustrating the evaluation of global changes in tinnitus burden according to occasional tinnitus for men **(A)** and women **(B)** and constant tinnitus for men **(D)** and women **(E)**. In all four plots, a solid line shows the average, with the 95% confidence intervals represented by the dashed lines. Average tinnitus-associated burden, for men (blue) and women (pink) is shown for occasional **(C)** and constant **(F)** tinnitus. Measures from **(G)** negligible, **(H)** mild/moderate, and **(I)** severe/catastrophic THI groups from the STOP database. The blue background gathers the tinnitus domain assessed with the THI and several numerical rating scales, the light blue represents the tinnitus-associated fears, the hyperacusis domain is marked in purple, emotional affects are in orange, and the yellow background represents the quality of life domain evaluated with the WHOQoL-BREF. The continuous line shows the average scores, and the dashed lines illustrate the 95% confidence intervals. Instruments are labeled as follows: THI, tinnitus-related psychological distress; Lo, tinnitus loudness; Aw, tinnitus awareness; An, tinnitus annoyance; TCS, tinnitus catastrophizing; FTQ, tinnitus fears; HQ, hyperacusis questionnaire; S, stress; A, anxiety; D, depression scores; quality of life for physical (Qph), psychological (Qps), social (Qso), and environment (Qen). Color dots illustrate the severity score of those instruments with published severity category boundaries: negligible (green), moderate (orange), and severe (red).

(THI, WHOQoL-BREF, and tinnitus-related numeric rating scales). The mean age of the group was 53.3 years (SD 12.2) with an average tinnitus duration of 8.4 years (SD 8.87). Women made up 36.7% of the group. These patients were classified into different groups based on the CGI rating (**Table 3**). Only three patients rated their posttreatment state as "very much worse" and as a consequence this category was excluded from analysis ($N = 571$). We note that the size of the "very much better" and "much worse" groups were also rather small ($n = 18$ and $n = 24$, respectively).

We hypothesized that the surface area of the radar plots could be used to evaluate and quantify treatment efficacy. However, for a more sensitive measure of treatment outcome, one has to consider the order of the measures around the circumference since the surface area could be influenced depending on which instruments are situated next to each other. It is thus conceivable that a specific arrangement of the axis makes the radar plot tool more sensitive for displaying clinical changes than other possible arrangements. With the restriction that instruments forming part of the same domain should be displayed in vicinity

TABLE 1 | Raw scores from STOP participants with occasional or constant tinnitus according to gender.

Occasional tinnitus			
	♂ (*N* = 121)	♀ (*N* = 190)	**Two-way ANOVA (*p* value)**
	Average ± CI	**Average ± CI**	
THI	10.35 (8.07–12.63)	11.51 (9.76–13.26)	<0.9999
Lo	25.87 (22.43–29.3)	28.56 (25.62–31.49)	0.228
Aw	13.66 (10.76–16.56)	17.97 (14.8–21.13)	0.6937
An	6.97 (5–8.94)	10.65 (8.09–13.21)	0.8108
FTQ	3.76 (3.37–4.16)	3.61 (3.31–3.92)	>0.9999
TCS	7.02 (5.7–8.33)	8.61 (7.34–9.87)	>0.9999
HQ	12.32 (10.88–13.75)	16.6 (15.38–17.82)	***<0.0001
S	0.33 (0.3–0.36)	0.33 (0.31–0.36)	>0.9999
A	5.7 (5.01–6.39)	5.92 (5.33–6.51)	>0.9999
D	3.32 (2.77–3.86)	3.24 (2.75–3.73)	>0.9999
Qph	16.25 (15.83–16.67)	15.93 (15.53–16.32)	0.4321
Qps	14.98 (14.47–15.5)	14.84 (14.45–15.23)	0.9863
Qso	14.31 (13.77–14.85)	14.6 (14.18–15.02)	>0.9999
Qen	16.45 (16.06–16.84)	16.4 (16.09–16.7)	>0.9999
Constant tinnitus			
	♂ (*N* = 187)	♀ (*N* = 141)	**Two-way ANOVA (*p* value)**
	Average ± CI	**Average ± CI**	
THI	18.73 (16.47–20.99)	26.29 (23.19–29.38)	***0.0001
Lo	47.24 (43.78–50.69)	49.16 (45.05–53.26)	0.0031
Aw	40.23 (35.52–44.94)	47.84 (42.67–53.01)	***<0.0001
An	17.69 (14.48–20.9)	31.32 (26.73–35.9)	***<0.0001
FTQ	4.56 (4.19–4.93)	5.1 (4.66–5.54)	0.7674
TCS	10.55 (9.27–11.83)	16.24 (14.54–17.94)	***<0.0001
HQ	13.75 (12.55–14.96)	18.89 (17.35–20.44)	***<0.0001
S	0.3 (0.28–0.33)	0.37 (0.33–0.4)	**0.0046
A	5.15 (4.61–5.69)	6.54 (5.78–7.29)	***0.0002
D	3.24 (2.8–3.67)	3.94 (3.28–4.61)	0.6755
Qph	16.22 (15.88–16.57)	15.2 (14.71–15.69)	*0.0184
Qps	15.57 (15.23–15.91)	14.44 (13.95–14.92)	**0.0066
Qso	14.49 (14.08–14.89)	14.32 (13.76–14.89)	>0.9999
Qen	16.83 (16.55–17.12)	15.94 (15.54–16.35)	0.1123

See **Figure 1** *for a description of the abbreviations for each measure. Average values and 95% confidence intervals are shown. Pairwise comparisons using Bonferroni tests are reported.*
**p < 0.05.*
***p < 0.001.*
****p < 0.001.*

to each other (e.g., WHOQoL-BREF), we simulated the full set of possible permutations, which allowed a number of 34,560 different variants of the radar plot. For each of these variants, the following steps were repeated:

- Plots for all tinnitus patients were created and the surface area calculated.
- Patients were grouped to the CGI categories (see **Table 3**), the average surface area was calculated for each category to compute the mean distance between groups.
- Patients were grouped to the CGI categories (see **Table 3**) and the variance of the surface area within each category calculated.

The results of these calculations are displayed in **Figure 3A** with the mean surface difference between CGI categories plotted against the mean surface variance within categories. The goal of this step was to select a figure outline that minimized the surface variance within CGI categories and maximized the mean surface difference between CGI categories (marked with a red square). This radar plot configuration appeared as optimal to the set of instruments used within the TRI database. In **Figure 3B**, we show the mean difference in radar plot surface area pre- and post-intervention for each CGI category using the selected radar plot configuration. The CGI category "no change" showed on average no change in the plot's surface, while CGI categories with an improvement show a reduction, while CGI categories with a worsening of the patients' symptoms are characterized by an increase of the plot's surface.

Having determined the optimal configuration of the radar plot to visualize and score treatment-related changes in CGI groups, we plotted the average radar plots of each of the six CGI categories pretreatment and posttreatment (**Figures 4A,B**). All CGI groups in the improvement categories displayed significant reduction in surface [two-way ANOVA; very much better: $F_{(1,\ 374)} = 77.47$, $p < 0.0001$; much better: $F_{(1,\ 1,430)} = 155.3$, $p < 0.0001$, minimally better: $F_{(1,\ 2,860)} = 162.3$, $p < 0.0001$]. With increasing power in the "much better" and "minimally better" groups, significant improvement was detected for all instruments, with the exception of the quality of life domain (Table S2 in Supplementary Material). Conversely, in the "minimally worse" group, significant changes were observed [two-way ANOVA; $F_{(1,\ 506)} = 28.88$, $p < 0.0001$] in particular for the numeric rating scales of loudness, annoyance, ability to ignore, and unpleasantness, while the THI did not detect any worsening ($p > 0.9999$, Table S2 in Supplementary Material). Merging the pretreatment and posttreatment averages on the same radar plot helped appreciating these changes (**Figure 4C**). **Figure 4D** illustrates examples of individuals within each CGI category. As shown in these examples, changes over time can be displayed and appreciated by comparing pretreatment and posttreatment plots.

DISCUSSION

This article describes an innovative visualization method for displaying patient profiles, both to aid clinical assessment and evaluate the effects of treatment-related change, here applied in the context of tinnitus. The method can be adapted to individual patients (e.g., **Figures 1** and **4D**) as well as on a group level (e.g., **Figures 2** and **4A–C**). The present method uses data from a multi-dimensional set of relevant measurement instruments and integrates them into a radar plot. Both total scores on domain-specific questionnaires (e.g., the HQ, HADS, and WHOQoL-BREF) and single-item numeric rating scales as well as a psychoacoustic tests can be incorporated to provide an overview in a single plot. The data representation facilitates the visualization of both individual and group data and gives an understandable representation of burden status in a manner that is accessible to a range of observers. Moreover, the representation is sensitive to changes over time and enables the detection of clinically significant improvements in treatment outcome. Different coloring methods can be used such as in **Figures 1**, **2** and **4** to highlight specific aspects of the data. Usability studies

TABLE 2 | Raw scores from STOP participants according to different THI subgroups.

		THI severity subgroups		
Instruments	**Controls (*N* = 1,223)**	**Negligible (*N* = 272)**	**Mild/moderate (*N* = 305)**	**Severe/catastrophic (*N* = 45)**
THI		8.6 (8–9.2)	31.1 (29.9–32.2)[a]	72.4 (68.9–75.9)[a,b]
VAS Lo		34.9 (32.7–37.2)	56.8 (54.3–59.3)[a]	79.1 (73.9–84.3)[a,b]
VAS Aw		28.8 (25.5–32)	54.6 (51–58.2)[a]	77.3 (70.1–84.5)[a,b]
VAS An		9.8 (8–11.5)	33 (30.1–35.9)[a]	66.2 (58.5–73.9)[a,b]
FTQ		3.6 (3.4–3.8)	5.8 (5.5–6.1)	9.3 (8.6–10.1)[c]
TCS		6.8 (6.2–7.4)	17.4 (16.5–18.3)[a]	30.9 (28.5–33.2)[a,b]
HQ	11.4 (11–11.8)	12 (11.1–12.9)	20.1 (19.2–21)[a]	27.9 (25.5–30.3)[a,b]
S	0.3 (0.3–0.3)	0.3 (0.2–0.3)	0.4 (0.4–0.4)	0.5 (0.5–0.6)
A	4.8 (4.6–5)	4.1 (3.7–4.5)	6.5 (6.1–7)[d]	10.2 (8.9–11.5)[c]
D	2.6 (2.4–2.7)	2.5 (2.2–2.9)	4 (3.6–4.4)	7.1 (5.9–8.4)
Qph	16.9 (16.8–17)	16.7 (16.5–17)	15.3 (15–15.6)	12.5 (11.7–13.4)
Qps	15.7 (15.6–15.9)	16 (15.7–16.2)	14.6 (14.3–14.9)	12.4 (11.6–13.3)
Qso	14.9 (14.8–15.1)	15.1 (14.8–15.4)	13.9 (13.5–14.2)	12.6 (11.4–13.7)
Qen	17 (16.9–17.1)	17 (16.8–17.3)	16.1 (15.8–16.4)	14.3 (13.5–15.1)

See **Figure 1** *for a description of the abbreviations for each measure. Average values and 95% confidence intervals are shown. Multiple comparisons from Holm–Sidak's tests are reported in superscripts, where different letters represent a statistically significant difference.* [a]$p < 0.001$ *compared with negligible THI,* [b]$p < 0.001$ *compared with mild/moderate THI,* [c]$p < 0.01$ *compared with negligible THI,* [d]$p < 0.05$ *compared with negligible THI. Controls did not differ from negligible THI scores.*

TABLE 3 | Categorization of the 571 participants from the Tinnitus Research Initiative database according to their posttreatment CGI rating.

CGI rating	***N***
Very much better	18
Much better	66
Minimally better	131
No change	255
Minimally worse	77
Much worse	24

involving practitioners will be needed to decide which coloring method should be used to optimally display the clinical data.

Saary first proposed radar plots for their use of health-care data (24). Funabiki et al. applied this methodology in the context of the assessment of pervasive developmental disorder and attention-deficit/hyperactivity disorder (41). Similarly, Pierzycki et al. used radar plots to visualize factor structure and test–retest reliability of multiple baseline test scores in the context of tinnitus (19). However, all these studies did not apply this methodology in the context of the evaluation of treatment outcome. To the best of our knowledge, this is the first attempt in the medical area to design a comprehensive visualization tool of integrated measures over time. Here, we show the sensitivity of this tool for the assessment of tinnitus patients at their initial consultation at the clinic and for monitoring their progress under a specific treatment. The methodology presented here suggests that it could be applied for monitoring patients throughout their therapeutic intervention. In our examples, a decrease in the radar plot surface could be interpreted as a clinical improvement, while an increase of the surface could be interpreted as a worsening of clinical symptoms (see **Figure 4D** for individual examples). An important aspect of this use case is the graphical implementation of the minimal clinically important difference (MCID). Meaningful clinical differences need to be highlighted in order to dissociate them from differences that are not of clinical relevance. Further research will be needed to define and implement the MCID in the radar plots.

We also propose that this approach could be applied to other complex disorders where multiple measures are incorporated. We are not stating that the total surface of the area, which can be interpreted as a *global burden* score, can replace the use of a single primary outcome instrument. Rather such visualization tool can be used to appreciate global changes associated with the status of a patient and his responsiveness to a treatment. Although useful for group comparisons, this tool appears to have particular utility in individual patient management strategies. Additionally, specific color-coding methods can be implemented in order to highlight specific aspects of the plot and mark meaningful clinical differences on instruments that have known thresholds of severity. Further work will be needed to develop and standardize a radar plot that is designed to optimally display the clinical status of the tinnitus patients.

An important leverage is the order of the axis used to create the radar plot. As we showed, the selection of the axis order has a strong impact on the surface and surface difference between two time points. Whether instruments need to be grouped according to broad fields such as those used here (e.g., tinnitus, quality of life, emotional burden, and auditory profiles) or instead according to instrument subscales (sleep, cognitive, intrusiveness, relaxation, sense of control, and auditory performance) remains to be established. Furthermore, it is likely that varying the number of data points on the radar plots may also affect the precision and the sensitivity of the tool. The STOP data contain a greater number of instruments than in the TRI data; however, it does not contain treatment outcome data. The TRI data utilized in the present work are limited to (i) the THI, for which validity and reliability has been questioned, (ii) visual analog scales, for which precision is also subject to debate, and (iii) the WHOQoL, which usefulness in the evaluation of treatment outcome is unclear. As a consequence, to evaluate the influence of the number of data points on the precision of the radar plots, studies will have to be performed using a larger set of instruments. This will allow a greater flexibility in evaluating the effects of inclusion

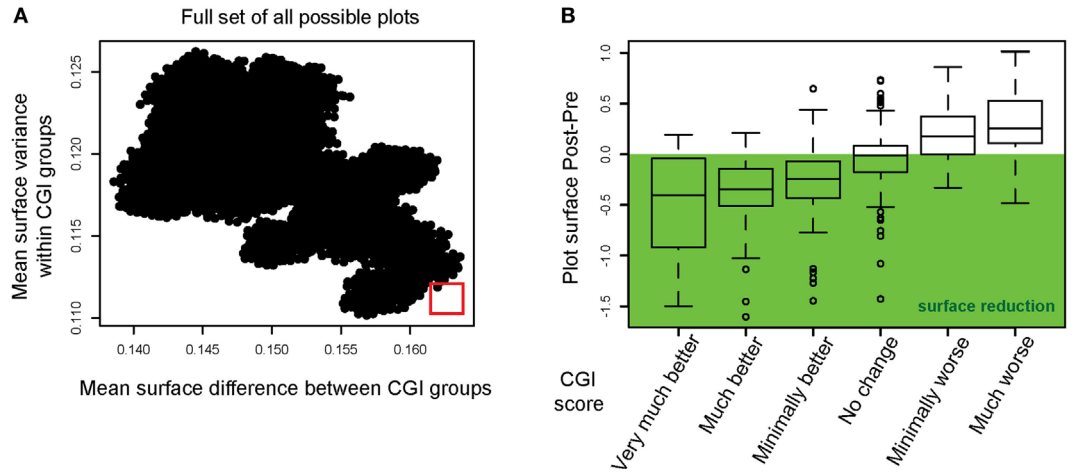

FIGURE 3 | Instrument permutations and alignment of the radar plot with improvements in Clinical Global Impression. **(A)** The full set of all possible radar plots with different surface outlines (i.e., different organization of axes) was simulated. The mean surface difference between CGI groups was calculated for all radar plots (abscissa). The mean surface variance within CGI groups was calculated and plotted on the ordinate. The red square highlights the radar plot outline optimized for maximum difference *between* CGI categories and minimum variance *within* CGI categories. **(B)** Radar plot surface differences (post-intervention minus pre-intervention) are displayed for the optimal outline. The mean surface difference is shown for each CGI category.

or exclusion of specific instruments on the sensitivity of the radar plots in measuring a successful treatment outcome.

The work presented here outlines a framework for this development, which can be used for tinnitus and for other chronic diseases. One important consideration for the optimal use of this visualization method is the selection of measurement instruments. For example, single-item visual analog or numeric rating scales tend to be viewed as inferior because (i) they are more vulnerable to random measurement errors, which are more likely to be eliminated with multiple items, (ii) the reliability statistic "internal consistency" cannot be computed, and (iii) they are more vulnerable to unknown biases in meaning and interpretation. Here, our selection was in part pragmatic since it was constrained by the data that were available in the STOP study and in the TRI database. The present methodology is generic in a sense that it can be adapted to include any measurement scales. Another important leverage will be the selection of instruments that measure unique and independent components of a complex health-care condition since this should maximize discriminability between patient profiles. The selection of the instruments is thus an important influencer of the radar plots' sensitivity to change. The choice of instruments is normally guided by the personal preference of the physician or investigator. However, future synthesis of data originating from multiple centers will require agreement from those heads and other key opinion leaders to use a common set of instruments; something that is challenging given the broad diversity of instruments in current use (22). In this regard, the COMiT initiative is currently in the process of establishing an international consensus-based recommendation of a minimum set of outcome domains and instruments considered critically important for performing clinical trials (23). Such international programs will help defining the core set of measurement instruments. A challenge emerging from such endeavors is to obtain instruments of equivalent reliability and efficiency in different languages and sensitive to culture context (42).

Whether such aggregated visualization of data using radar plots will prove useful to clinicians for the management of complex health-care conditions (e.g., tinnitus) will have to be tested. A preliminary evaluation of the radar plots shows mitigated opinions. Some physicians view it as extremely appealing and practical tool to picture the global status of a patient (tinnitus distress, fear of tinnitus, sensitivity to noise, emotional burden, and quality of life), while others do not foresee in what way the radar plots will enable an optimization of the treatment selection. We predict such opinions will also vary between countries where culture and average socioeconomic status differ, and also where different health-care systems apply (purely social-based health-care system versus insurance-based, clinical versus private practice). Furthermore, some physicians express concerns of exposing tinnitus patients to the long list of questionnaires such as those used in the STOP cohort, which could reinforce the negative thoughts and feelings about tinnitus. The participants in STOP originate from the general population and not from a clinical population, and thus whether such series of questionnaires can used on a clinical group will have to be tested. Future research will also have to evaluate the physician perspective in introducing multiple measures in the assessment of tinnitus burden and provide solid conclusions on the usefulness of such integrated measures in the management and treatment of tinnitus.

We personally view that with the increasing pressure over social health-care systems and the dissatisfaction of patients with regards to the quality of the care, novel methodologies are needed to assess patients at baseline and monitor individual response to a treatment. With the increasing development of value-based health care (43), and the distant monitoring of health conditions, smartphone applications could become routinely used in clinics to monitor patients at distance. In the context of tinnitus, the development of such distant monitoring has been recently initiated by Schlee et al. (44–46). This mobile platform could represent an ideal

setting to collect patient data at different follow-up times, whereas the physician frontend would display an automated radar plot of the patient's status. Our hope is that such a tool would allow an immediate monitoring of treatment progress over time and enable the physician to rapidly readjust a treatment prescription depending on the responsiveness of a patient. Furthermore, the inclusion

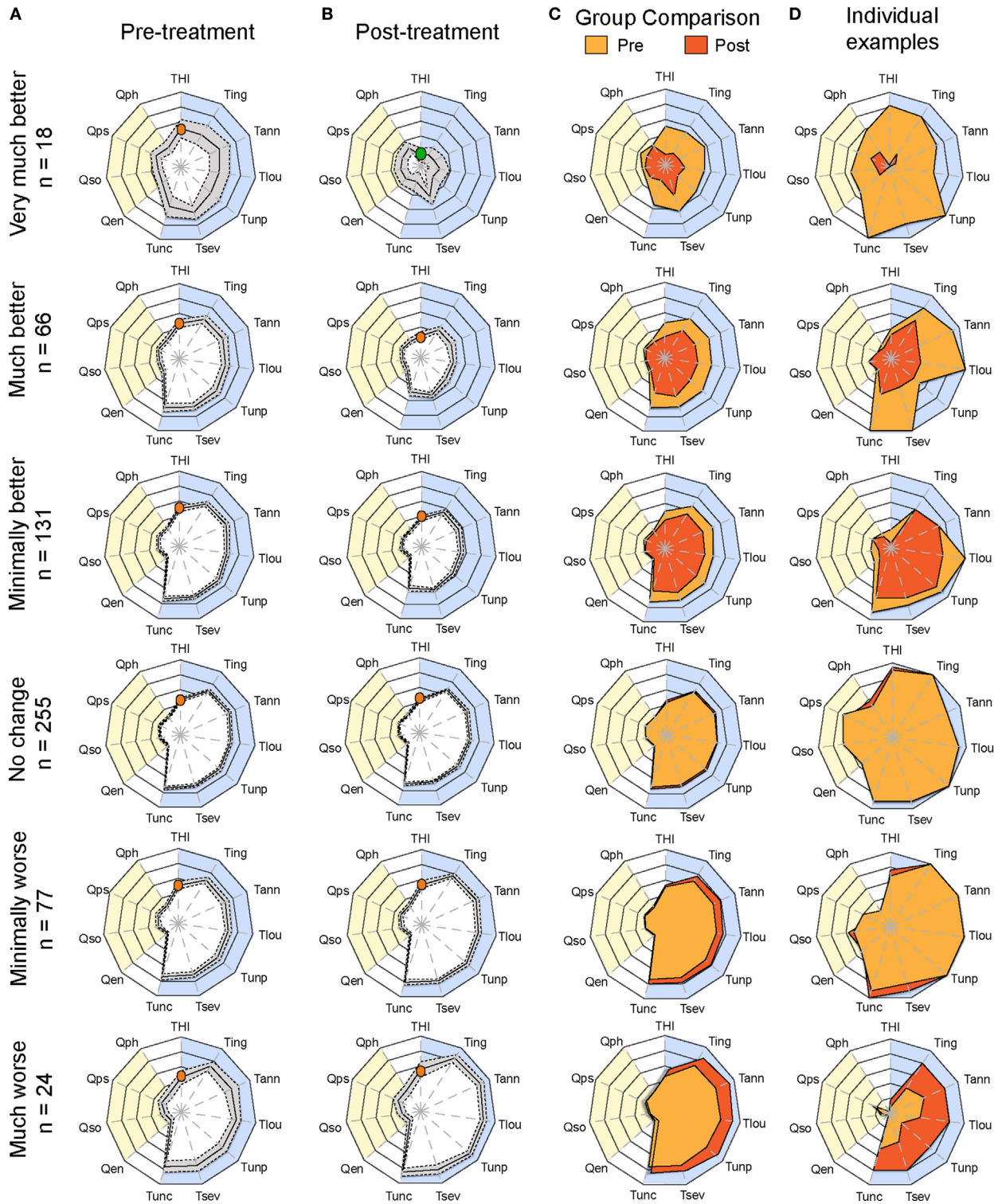

FIGURE 4 | Continued

FIGURE 4 | Continued
Plots of average pretreatment and posttreatment scores based on CGI ratings. Radar plots illustrating the evaluation of global changes in tinnitus burden according to measures from **(A)** baseline (pretreatment) and **(B)** follow-up (posttreatment) during the evaluation of various treatments within the TRI database. Patients were grouped according to Clinical Global Impression scores and their number is shown on the left. The yellow background represents the quality of life domain evaluated with the WHOQoL-BREF and the blue background gathers the tinnitus domain assessed with the THI and several numerical rating scales. The continuous line in the pretreatment and posttreatment shows the average scores, and the dashed lines illustrate the 95% confidence intervals. **(C)** Pretreatment (yellow) and posttreatment (orange) average values are presented on the same plot. The orange dot in the THI scale marks the moderate scores of the group for this instrument, whereas the green shows the progression of the group to negligible THI. Note the reduction of tinnitus-associated burden in the "very much better" group.
(D) Example of individuals within each CGI category to illustrate changes before and after treatment for the same patient. Instruments are labeled as follows: THI, Tinnitus Handicap Inventory; Tign, Tinnitus Numeric Rating Scale (Ignore); Tann, Tinnitus Numeric Rating Scale (Annoyance); Tlou, Tinnitus Numeric Rating Scale (Loudness); Tunp, Tinnitus Numeric Rating Scale (Unpleasant); Tsev, Tinnitus Numeric Rating Scale (Severity); Tunc, Tinnitus Numeric Rating Scale (Uncomfortable); Qen, WHO Quality of Life (environment); Qso, WHO Quality of Life (social relationships); Qps, WHO Quality of Life (psychological); Qph, WHO Quality of Life (physical health). Statistical analyses comparing **(A,B)** are available in Table S2 in Supplementary Material.

of adverse events and side effects would help to rapidly terminate an intervention and address appropriate care. A better recognition of a patient status and its comorbidities will likely improve the priorities and treatment prescription. Overall, this work contributes to novel strategies for high quality care of chronic tinnitus patients and its implementation in the general clinic.

ETHICS STATEMENT

The STOP project was approved by the local ethics committee "Regionala etikprövningsnämnden" in Stockholm (#2014/1998-31/4). Collection of data for the Tinnitus Research Initiative database was approved by the Ethics Committee of the University of Regensburg, Germany (#08/046).

AUTHOR CONTRIBUTIONS

WS, CC, BL, and BC designed the study. NE collected, extracted, and processed the STOP data. WS and CC analyzed the data. DH helped to develop the scientific arguments and contributed to data interpretation. All authors played a role in writing the manuscript and approved the final version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fmed.2017.00086/full#supplementary-material.

REFERENCES

1. Hunter DJ. Gene-environment interactions in human diseases. *Nat Rev Genet* (2005) 6:287–98. doi:10.1038/nrg1578
2. Massa MS, Wang N, Bickerton WL, Demeyere N, Riddoch MJ, Humphreys GW. On the importance of cognitive profiling: a graphical modelling analysis of domain-specific and domain-general deficits after stroke. *Cortex* (2015) 71:190–204. doi:10.1016/j.cortex.2015.06.006
3. Tyler RS, Baker LJ. Difficulties experienced by tinnitus sufferers. *J Speech Hear Disord* (1983) 48:150–4. doi:10.1044/jshd.4802.150
4. Stouffer JL, Tyler RS. Characterization of tinnitus by tinnitus patients. *J Speech Hear Disord* (1990) 55:439–53. doi:10.1044/jshd.5503.439
5. Elgoyhen AB, Langguth B, De Ridder D, Vanneste S. Tinnitus: perspectives from human neuroimaging. *Nat Rev Neurosci* (2015) 16:632–42. doi:10.1038/nrn4003
6. Schecklmann M, Pregler M, Kreuzer PM, Poeppl TB, Lehner A, Cronlein T, et al. Psychophysiological associations between chronic tinnitus and sleep: a cross validation of tinnitus and insomnia questionnaires. *Biomed Res Int* (2015) 2015:461090. doi:10.1155/2015/461090
7. Tegg-Quinn S, Bennett RJ, Eikelboom RH, Baguley DM. The impact of tinnitus upon cognition in adults: a systematic review. *Int J Audiol* (2016) 55:533–40. doi:10.1080/14992027.2016.1185168
8. Trevis KJ, McLachlan NM, Wilson SJ. Cognitive mechanisms in chronic tinnitus: psychological markers of a failure to switch attention. *Front Psychol* (2016) 7:1262. doi:10.3389/fpsyg.2016.01262
9. Gilles A, Schlee W, Rabau S, Wouters K, Fransen E, Van de Heyning P. Decreased speech-in-noise understanding in young adults with tinnitus. *Front Neurosci* (2016) 10:288. doi:10.3389/fnins.2016.00288
10. Hoare DJ, Kowalkowski VL, Kang S, Hall DA. Systematic review and meta-analyses of randomized controlled trials examining tinnitus management. *Laryngoscope* (2011) 121:1555–64. doi:10.1002/lary.21825
11. Baguley D, McFerran D, Hall D. Tinnitus. *Lancet* (2013) 382:1600–7. doi:10.1016/S0140-6736(13)60142-7
12. Cederroth CR, Canlon B, Langguth B. Hearing loss and tinnitus – are funders and industry listening? *Nat Biotechnol* (2013) 31:972–4. doi:10.1038/nbt.2736
13. Langguth B, Kreuzer PM, Kleinjung T, De Ridder D. Tinnitus: causes and clinical management. *Lancet Neurol* (2013) 12:920–30. doi:10.1016/S1474-4422(13)70160-1
14. Dauman R, Tyler RS. *Some Considerations on the Classification of Tinnitus*. Amsterdam: Kugler Publications (1992).
15. Tyler RS. *Neurophysiological Models, Psychological Models, and Treatments for Tinnitus*. New York: Thieme (2006).
16. Langguth B, Goodey R, Azevedo A, Bjorne A, Cacace A, Crocetti A, et al. Consensus for tinnitus patient assessment and treatment outcome measurement: tinnitus research initiative meeting, Regensburg, July 2006. *Prog Brain Res* (2007) 166:525–36. doi:10.1016/S0079-6123(07)66050-6
17. Zeman F, Koller M, Schecklmann M, Langguth B, Landgrebe M; TRI Database Study Group. Tinnitus assessment by means of standardized self-report questionnaires: psychometric properties of the tinnitus questionnaire (TQ), the Tinnitus Handicap Inventory (THI), and their short versions in an

international and multi-lingual sample. *Health Qual Life Outcomes* (2012) 10:128. doi:10.1186/1477-7525-10-128
18. Milerova J, Anders M, Dvorak T, Sand PG, Koniger S, Langguth B. The influence of psychological factors on tinnitus severity. *Gen Hosp Psychiatry* (2013) 35:412–6. doi:10.1016/j.genhosppsych.2013.02.008
19. Pierzycki RH, McNamara AJ, Hoare DJ, Hall DA. Whole scalp resting state EEG of oscillatory brain activity shows no parametric relationship with psychoacoustic and psychosocial assessment of tinnitus: a repeated measures study. *Hear Res* (2016) 331:101–8. doi:10.1016/j.heares.2015.11.003
20. Tyler RS, Noble W, Coelho C. Considerations for the design of clinical trials for tinnitus. *Acta Otolaryngol Suppl* (2006) 126:44–9. doi:10.1080/03655230600895424
21. Tyler RS, Oleson J, Noble W, Coelho C, Ji H. Clinical trials for tinnitus: study populations, designs, measurement variables, and data analysis. *Prog Brain Res* (2007) 166:499–509. doi:10.1016/S0079-6123(07)66048-8
22. Hall DA, Haider H, Szczepek AJ, Lau P, Rabau S, Jones-Diette J, et al. Systematic review of outcome domains and instruments used in clinical trials of tinnitus treatments in adults. *Trials* (2016) 17:270. doi:10.1186/s13063-016-1399-9
23. Hall DA, Haider H, Kikidis D, Mielczarek M, Mazurek B, Szczepek AJ, et al. Toward a global consensus on outcome measures for clinical trials in tinnitus: report from the first International Meeting of the COMiT initiative, November 14, 2014, Amsterdam, The Netherlands. *Trends Hear* (2015) 19: 1–7. doi:10.1177/2331216515580272
24. Saary MJ. Radar plots: a useful way for presenting multivariate health care data. *J Clin Epidemiol* (2008) 61:311–7. doi:10.1016/j.jclinepi.2007.04.021
25. Müller K, Edvall NK, Idrizbegovic E, Huhn R, Cima RFF, Persson V, et al. Validation of online Swedish tinnitus questionnaires. *Front Aging Neurosci* (2016) 8:272. doi:10.3389/fnagi.2016.00272
26. Landgrebe M, Zeman F, Koller M, Eberl Y, Mohr M, Reiter J, et al. The tinnitus research initiative (TRI) database: a new approach for delineation of tinnitus subtypes and generation of predictors for treatment outcome. *BMC Med Inform Decis Mak* (2010) 10:42. doi:10.1186/1472-6947-10-42
27. Newman CW, Jacobson GP, Spitzer JB. Development of the Tinnitus Handicap Inventory. *Arch Otolaryngol Head Neck Surg* (1996) 122:143–8. doi:10.1001/archotol.1996.01890140029007
28. Newman CW, Sandridge SA, Jacobson GP. Psychometric adequacy of the Tinnitus Handicap Inventory (THI) for evaluating treatment outcome. *J Am Acad Audiol* (1998) 9:153–60.
29. Hesser H, Andersson G. Dimensional or categorical approach to tinnitus severity: an item response mixture modeling analysis of tinnitus handicap. *Int J Behav Med* (2014) 21:982–8. doi:10.1007/s12529-013-9375-1
30. McCombe A, Baguley D, Coles R, McKenna L, McKinney C, Windle-Taylor P, et al. Guidelines for the grading of tinnitus severity: the results of a working group commissioned by the British Association of Otolaryngologists, Head and Neck Surgeons, 1999. *Clin Otolaryngol Allied Sci* (2001) 26:388–93. doi:10.1046/j.1365-2273.2001.00490.x
31. Cima RF, Crombez G, Vlaeyen JW. Catastrophizing and fear of tinnitus predict quality of life in patients with chronic tinnitus. *Ear Hear* (2011) 32:634–41. doi:10.1097/AUD.0b013e31821106dd
32. Sullivan MJL, Bishop SC, Pivik J. The Pain Catastrophizing Scale: development and validation. *Psychol Assess* (1995) 7:524–32.
33. Zigmond AS, Snaith RP. The Hospital Anxiety and Depression Scale. *Acta Psychiatr Scand* (1983) 67:361–70. doi:10.1111/j.1600-0447.1983.tb09716.x
34. Levenstein S, Prantera C, Varvo V, Scribano ML, Berto E, Luzi C, et al. Development of the perceived stress questionnaire: a new tool for psychosomatic research. *J Psychosom Res* (1993) 37:19–32. doi:10.1016/0022-3999(93)90120-5
35. Fliege H, Rose M, Arck P, Walter OB, Kocalevent RD, Weber C, et al. The perceived stress questionnaire (PSQ) reconsidered: validation and reference values from different clinical and healthy adult samples. *Psychosom Med* (2005) 67:78–88. doi:10.1097/01.psy.0000151491.80178.78
36. Khalfa S, Dubal S, Veuillet E, Perez-Diaz F, Jouvent R, Collet L. Psychometric normalization of a hyperacusis questionnaire. *ORL J Otorhinolaryngol Relat Spec* (2002) 64:436–42. doi:10.1159/000067570
37. Fackrell K, Fearnley C, Hoare DJ, Sereda M. Hyperacusis questionnaire as a tool for measuring hypersensitivity to sound in a tinnitus research population. *Biomed Res Int* (2015) 2015:290425. doi:10.1155/2015/290425
38. The WHOQOL Group. Development of the World Health Organization WHOQOL-BREF quality of life assessment. *Psychol Med* (1998) 28:551–8. doi:10.1017/S0033291798006667
39. Busner J, Targum SD. The clinical global impressions scale: applying a research tool in clinical practice. *Psychiatry (Edgmont)* (2007) 4:28–37.
40. Clark JG. Uses and abuses of hearing loss classification. *ASHA* (1981) 23:493–500.
41. Funabiki Y, Kawagishi H, Uwatoko T, Yoshimura S, Murai T. Development of a multi-dimensional scale for PDD and ADHD. *Res Dev Disabil* (2011) 32:995–1003. doi:10.1016/j.ridd.2011.01.052
42. Wild D, Eremenco S, Mear I, Martin M, Houchin C, Gawlicki M, et al. Multinational trials-recommendations on the translations required, approaches to using the same language in different countries, and the approaches to support pooling the data: the ISPOR patient-reported outcomes translation and linguistic validation good research practices task force report. *Value Health* (2009) 12:430–40. doi:10.1111/j.1524-4733.2008.00471.x
43. Porter ME. What is value in health care? *N Engl J Med* (2010) 363:2477–81. doi:10.1056/NEJMp1011024
44. Probst T, Pryss R, Langguth B, Schlee W. Emotion dynamics and tinnitus: daily life data from the "TrackYourTinnitus" application. *Sci Rep* (2016) 6:31166. doi:10.1038/srep31166
45. Probst T, Pryss R, Langguth B, Schlee W. Emotional states as mediators between tinnitus loudness and tinnitus distress in daily life: results from the "TrackYourTinnitus" application. *Sci Rep* (2016) 6:20382. doi:10.1038/srep20382
46. Schlee W, Pryss RC, Probst T, Schobel J, Bachmeier A, Reichert M, et al. Measuring the moment-to-moment variability of tinnitus: the TrackYourTinnitus smart phone app. *Front Aging Neurosci* (2016) 8:294. doi:10.3389/fnagi.2016.00294

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REVIEW
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Review of Smart Services for Tinnitus Self-Help, Diagnostics and Treatments

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In the recent years, there has been an increasing interest in the potential of internet- and smartphone-based technologies for the support of tinnitus patients. A broad spectrum of relevant approaches, some in the form of studies, others in the form of market products, have been mentioned in literature. They include auditory treatments, internet-based Cognitive Behavioral Therapy (iCBT), serious games, and questionnaires for tinnitus monitoring. The goal of this study is to highlight the role of existing internet-based and smart technologies for the advancement of tinnitus clinical practice: we consider contributions that refer to treatments and diagnostics, and we include contributions refering to self-help measures. We elaborate on the potential and challenges of such solutions and identify constraints associated to their deployment, such as the demand for familiarity with internet-based services and the need to re-design interactive services so that they fit on the small surface of a smartwatch.

Keywords: tinnitus treatment, tinnitus monitoring, smart technologies, internet-based treatments, iCBT, tinnitus masking, tinnitus, mobile crowd sensing

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1. INTRODUCTION

Tinnitus is defined as the perception of a sound with a lack of an evident external stimulus to that sound. About 10–15% of the general population is affected by tinnitus, whereas for 1–2% the tinnitus is so severe that it directly affects their quality of life, according to Baguley et al. (2013). Modern technologies, including internet-based services and smart devices, are in principle appropriate for reaching and assisting those patients.

In this work, we survey internet- and smartphone-based solutions for tinnitus treatment as well as for everyday monitoring of tinnitus severity. Two earlier reviews have addressed the role of such technologies for tinnitus therapy, namely the works of Nyenhuis et al. (2013) and Greenwell et al. (2016a), which investigate the efficacy of self-help interventions. Since the technologies of relevance in the context of our study lend themselves to self-help measures, there is an overlap between our materials and theirs. As opposed to these reviews however, our aim is not to discern the efficacy of such solutions, but rather to assess the coverage of this technology in scientific literature with respect to treatments, diagnostics and self-help. The secondary aim reported by Greenwell et al. (2016a), namely "identifying what intervention techniques are used within the interventions" (page 83), relates more to our primary goal. However, our emphasis is not on "identify[ing] and describ[ing] the 'active ingredients' of the interventions" (page 83), but rather on identifying the

types of treatment and the types of self-help being offered, the particular challenges that have been addressed in order to make such services as accessible and useful as possible, and the technical limitations that have not been solved yet.

The recent review of Lui et al. (2017) shows some similarities to our approach as well. Lui et al. (2017) study mobile applications for mental health, concentrating on evidence-based apps in the psychotherapeutic context and investigating their efficacy. Tinnitus is not among the conditions they investigate, albeit they discuss apps that support cognitive behavioral therapy (CBT), which belongs to the widespread tinnitus treatments. Accordingly, they do not address the issue of effectiveness of such treatments with respect to tinnitus nor the design of studies involving the use of smartphones and similar technologies among tinnitus patients, as we do here.

2. REVIEW DESIGN

2.1. Specific Aims of the Review

We investigate how internet-based and smart services are taken up clinically for tinnitus diagnostics and treatments, including self-help measures. We aim to identify key findings, key contributors and key challenges.

We solely include scientific articles that report on treatments, diagnostics and self-help for tinnitus, but no gray literature. In addition to the main review, we performed a market overview, on which we report in section 2.3.5. Note that in this market overview it has not been possible to distinguish between services intended exclusively for treatment and those concerning well-being in general.

Internet- or smartphone based treatments for tinnitus can be mainly differentiated into auditory and psychological treatments. *Auditory treatments* encompass (i) environmental sound generators, (ii) tinnitus maskers that generate low-level broad-band noise, (iii) hearing aids, and (iv) devices that combine some of the above, all aiming to reduce or mask the tinnitus percept. *Psychological treatments/interventions*, especially Cognitive Behavioral Therapy (CBT), are also investigated in our search, because earlier studies (e.g., by Martinez-Devesa et al., 2010; Cima et al., 2012) have shown significant effects of CBT on the quality of life and on the depression scores of tinnitus patients. Medications are not considered, since we expect that internet-based and smartphone technologies are used for tinnitus medications in the same way as for medications of other chronical diseases (like diabetes or hypertension), namely to acquire information about the medication, as well as its effects and side-effects, to set reminders about taking the medication and to monitor live signals.

Internet- and smartphone based Diagnostics encompass the use of questionnaires to collect patient assessments and further information, as well as crowdsensing with the help of hardware, e.g., for tinnitus matching. It should be stressed that diagnostics are often linked to treatments. Finally, *Self-help* refers to both helping the patients to cope with tinnitus as well as helping the patients in diagnostics, e.g., by tinnitus matching.

2.2. Approach for the Collection and Selection of Contributions

Our search approach encompasses a broad keyword-based search in Google Scholar using the top-20 results for each keyword, the identification of key authors, a targeted search in selected journals using keywords and key authors, a second search in Google Scholar following the same method as the first one, to update our document collections with more recent publications, and a search for mobile apps released for different smart phones.

2.2.1. Broad Keyword-Based Search in Two Runs

We used Google Scholar to collect articles on the following keywords: "tinnitus smart," "tinnitus smart phone," "tinnitus smartphone," "tinnitus internet based," "tinnitus internet based cbt," "tinnitus icbt," "tinnitus sound therapy smart," "tinnitus mobile," "tinnitus online based," and "tinnitus internet acceptance" (excluding citations and patents). We performed one run on August 1, 2016, and a complementary one on June 11, 2017 to add more recent publications.

To decide which articles we should finally include, we first inspected title and abstract. During this inspection, we excluded articles that had reported on how the internet was used to recruit study participants as well as articles investigating the impact of internet usage (e.g., excessive usage) on the well being of patients. In some cases, it has become necessary to also inspect the contents of the articles themselves.

In the first run, out of the 45 articles that potentially seemed to be relevant, actually, 19 were considered as relevant and were thus included in our study.

The second run delivered 27 new articles that were possibly relevant to this work. Of these 27 articles, 11 were indeed relevant to this review. Moreover, there was another study not being traced by the described search procedure, but being added to the present review as the authors consider it as essential (Henry et al., 2012).

2.2.2. Targeted Search in Scopus and in PubMed Using Keywords

As next step, we performed a more targeted journal search in PubMed, focussing on medical advances, and in Scopus. The search on these platforms was performed on February 27, 2017. For both collections we used the same keywords as before, and we acquired 18, respectively 19 papers we marked as potentially relevant. After inspection and duplicate removal, 26 papers remained, of which 11 were considered to be relevant.

2.2.3. Search for Relevant Mobile Apps

We performed a manual search through the most popular stores for smartphone applications. Apps were searched in the browser version of Google's PlayStore, Apple's AppStore by using their software iTunes, and Microsoft's store included in the Windows 10 Mobile OS, using the keyword "tinnitus." The search run was performed on February 28, 2017. We then inspected the textual descriptions of the apps and removed those not related to tinnitus, e.g., wellness apps, as well as packages composed of multiple apps for different purposes. The results are discussed in section 2.3.5.

2.3. Overview of the Collected Contributions

We have organized the studies we found in the five areas described hereafter. Further, we have used the article collection to draw the co-authorship network depicted in **Figure 1**. The larger the node of an author, the more publications he or she has in the collection. Thick edges indicate authors that publish intensively together. Accordingly, the co-authorship network gives a fast overview of the author teams found in the specific field of this review.

2.3.1. Articles on Psychological Intervention Programs

Internet-based CBT (iCBT) is an approach that delivers Cognitive Behavioral Therapy via the internet. With 28 articles found, this method currently is the best documented and most researched treatment option when it comes to internet-based services. Of these, 15 articles providing an overview of the main findings are discussed in section 3.

Acceptance and Commitment Therapy (ACT) can be delivered in a self-help format via the internet as well (Westin

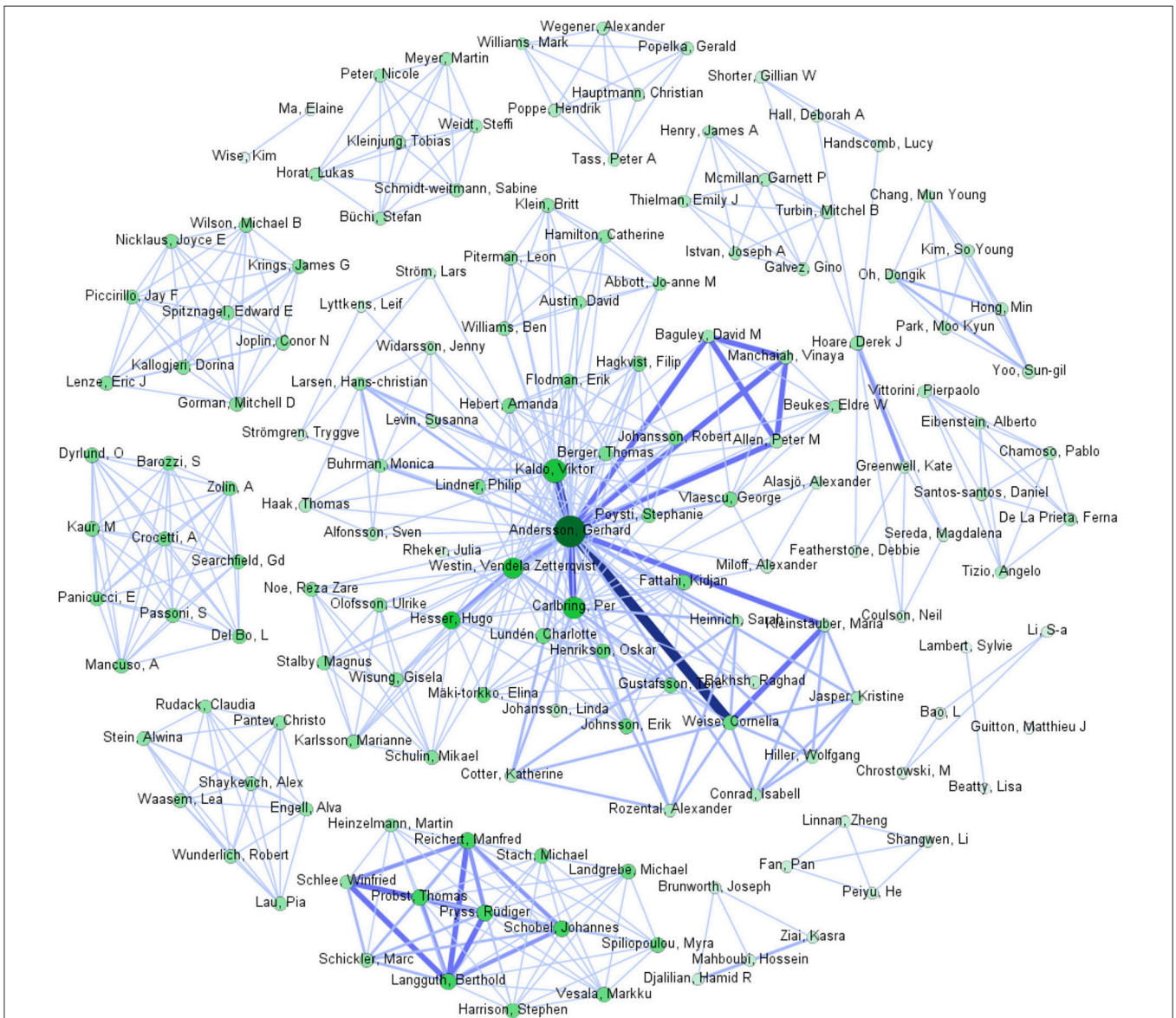

FIGURE 1 | Co-Authorship network: (i) Node size and node color intensity reflect the number of articles that this author wrote and were found relevant for our review; the larger and darker, the higher the number (e.g., Gerhard Andersson has the most works with 21, next is Viktor Kaldo with 7) (ii) Edge thickness and edge color intensity reflect the number of articles co-authored by the nodes linked through the edge; the thicker and darker, the higher the number (e.g., the thickest edge connects Gerhard Andersson and Cornelia Weise, who have co-authored 7 articles). We used the Sci2 Tool [https://sci2.cns.iu.edu/] to extract the network from the bibtex file containing all papers that were identified as relevant. After extracting the network, we used Gephi [https://gephi.org/] (which can be directly called through the Sci2 Tool), to draw the graph. After picking parameters for the colors and size of nodes and edges, we used the layout algorithm "Fruchterman Reingold," which is implemented in Gephi, for achieving a more visually appealing graph-layout.

et al., 2011). One article was found that examined and discussed this in the context of tinnitus treatment.

In another study (Hesser et al., 2012), internet-based ACT (iACT) was compared with iCBT and a moderated online discussion forum. The study revealed substantial improvements for both iCBT and iACT, with no significant difference between the two treatments.

2.3.2. Articles About the Tinnitus E-Programme

The Tinnitus E-Programme (TEP) is an internet-based intervention program that was developed in 2009 and evaluated using the Tinnitus Handicap Inventory (THI) (Newman et al., 1996). The program mainly consists of educational contents (i.e., about tinnitus or "the role of psychological mechanisms in tinnitus" (Greenwell et al., 2015), and relaxation- and attentional-focus exercises. The contents are provided as PDF sheets or mp3 audio files. All of the files are accessible for free on a website created for this intervention program [http://www.tinnituseprogramme.org/]. Two articles dealing with TEP were found: in Greenwell et al. (2015) the program is presented, while in Greenwell et al. (2016b) users' reactions and their daily use of relaxation techniques are reported.

2.3.3. Articles on Smartphone-Delivered Sound Therapy

With technological advances in the development of smartphones, sound therapy is an easily accessible treatment option. There are smartphone applications that claim to reduce tinnitus loudness by the usage of tailor-made notched music (e.g., Tinnitracks [www.tinnitracks.com] or Tinnitus Pro: Music Therapy [www.promedicalaudio.com]) or other forms of sound therapy (Linnan et al., 2015). Two studies provided the patients with tinnitus masking technologies: Yoo et al. (2015) and Mahboubi et al. (2012b).

Another usage of smartphones related to sound therapy are hearing aids that can be controlled via the smartphone via bluetooth. Further, they include volume-control functions as well as tinnitus maskers. An example of this usage is presented in Sauer et al. (2014). A study that further investigates this technology is provided by Barozzi et al. (2016).

2.3.4. Studies About Smartphone-Based Tinnitus Assessment

Diagnosis and assessment of tinnitus are important for a successful treatment. The articles of this category encompass: (1) internet-based hearing tests, namely Mahboubi et al. (2012a), Wunderlich et al. (2015), and Hauptmann et al. (2016), which investigate the potential of internet-based tests in comparison to tests run in the lab; (2) ecological momentary assessments, namely Henry et al. (2012); Pryss et al. (2015a), Schlee et al. (2016); Schickler et al. (2016a), and Wilson et al. (2015); (3) summarizations of the self-measures done by the patients, namely Peter et al. (2016), (4) serious games, namely in Schickler et al. (2016b), and Wise et al. (2015, 2016), (5) a comparison of online vs. paper-based collection of questionnaire data, namely Handscomb et al. (2016) and (6) a study on response times for a web-based emotional Stroop task for tinnitus patients Andersson et al. (2005).

2.3.5. Smartphone Apps Related to Tinnitus

As explained in the last part of section 2.2, three different stores for smartphone applications were searched for tinnitus-related content.

The amount of apps excluded from the initial results vary between the different stores. While there was not much discrepancy between excluded apps from Google's PlayStore (2 of 146 results) and Apple's AppStore (1 of 100 results for iPhone, 4 of 90 results for iPad), Microsoft's Windows 10 Mobile Store displayed a greater number of unrelated applications (58 of 61 results). Most of the irrelevant results in Microsoft's Store included the word "status," which has the same word ending as "tinnitus." This leads to the assumption that search was automatically expanded by similar keywords.

Aside from the amount, we investigated all of the apps in greater detail to count the apps that claim to have a tinnitus masker functionality. This analysis revealed that a large number of apps (104 of 144 in the PlayStore, 66 of 99 in the AppStore for iPhones, 48 of 86 in the AppStore for iPad and 3 of 3 in the Windows 10 Mobile Store) included a masker function in their feature set. Other than maskers, many apps were designed to provide information to the user via news articles, videos or photos. Many apps that were included in the results might be appropriate for tinnitus matching, others require specific kinds of hearing aids, functioning as a control unit for said devices. Very few apps included exercises aiming to treat the tinnitus via hypnosis.

3. DISCUSSION

As shown in section 2.3, internet-based and smartphone-based technologies are used in intervention programs, whereupon iCBT is the most widespread intervention form, as well as for the recording of assessments, whereupon the recordings are often used for diagnostic purposes (e.g., to match the tinnitus frequency) and for self-help support. As discussed below, the new technologies affect (a) the indicators, symptoms and assessments being collected during an intervention or for diagnostic purposes, and (b) the form and intensity of patient involvement.

3.1. Influence of Technologies on the Amount and Form of Collected Patient Information

3.1.1. Assignments and Assessments

Similarly to Lui et al. (2017), we found that CBT and its variants like ACT play a central role among the psychotherapeutic treatments for tinnitus. After the publication of the core article of Andersson and Kaldo (2004) on internet-based CBT (iCBT) for tinnitus, there has been a proliferation of studies on enriching CBT with internet-based functionalities: individual weekly treatment plans are considered by Rheker et al. (2015), homework assignments are suggested by Abbott et al. (2009),

while patient chats are presented by the aforementioned authors and by Hesser et al. (2012).

Next to assignments, internet technologies and smart devices are used to record patient assessments. Peter et al. (2016) propose PRISM (Pictorial Representation of Illness and Self Measure), a two-dimensional pictorial method for assessing the "burden of suffering" of tinnitus patients. PRISM has been designed for interactive self-assessment with help of an iPad. Whilst the aforementioned iCBT studies put emphasis in the *nature and purpose* of the assignments given to the patients (treatment plans, homework assignments), Peter et al. (2016) also elaborate on the modalities for *patient-app-interaction*, i.e., on the utilities for showing and filling the questionnaire.

Ecological Momentary Assessments (EMAs) for tinnitus patients are investigated by Probst et al. (2016a,b); Schlee et al. (2016) and Wilson et al. (2015) with the goal of understanding tinnitus physiology and moment-to-moment evolution. All these studies used smartphone technology. The EMA analyzed by Probst et al. (2016a,b), and Schlee et al. (2016) are recorded with a dedicated mHealth app, i.e., Track Your Tinnitus. Similarly to the study of (Peter et al., 2016) on PRISM, many studies on EMA under Track Your Tinnitus investigate the patient-app-interaction, aiming at usability and minimal cognitive patient effort (Pryss et al., 2015b; Schlee et al., 2016; Schickler et al., 2016a). Among them, Schickler et al. (2016b) investigate the potential of serious games, while Schickler et al. (2016a) focus on smartwatches, which are less obtrusive than smartphones and can further introduce additional functionality, e.g., monitor vital signs. These studies indicate the importance of enhancing personal experience in the interaction between the tinnitus patient and the mechanism recording assessments or delivering tasks.

3.1.2. Patient Recordings for Diagnosis and Intervention

Hauptmann et al. (2016) present a review of mobile apps that are used for measuring tinnitus pitch. The studies of Mahboubi et al. (2012a) and Yoo et al. (2015) investigate tinnitus masking and consider smart technologies with which the patients themselves can identify the tinnitus frequency they are experiencing. Wunderlich et al. (2015) consider ipod-based tinnitus pitch matching. In the study of Mahboubi et al. (2012a), the patients were asked to match their tinnitus frequency using a web-based protocol. Furthermore, they were subsequently provided with a "customized Harmonic Sound Therapy file" Mahboubi et al. (2012b), the patients listened for 1 h[1]. These studies demonstrate the potential of internet-based and smart technologies for tinnitus matching, but also stress the need for a reliable interaction between patient and technology.

[1]Mahboubi et al. (2012a) showed a temporary reduction in tinnitus loudness in 26 of their 32 participants, and in tinnitus annoyance in 27 of 32 participants, leading to the conclusion that internet-based, customized sound therapy can be effective in reducing tinnitus annoyance and loudness.

3.2. Influence of Technologies on the Extent and Form of Patient Participation

Internet-based technologies and smartphones allow for a personalized interaction between patient and eHealth/mHealth application. In the literature we investigated, this led to questions about the role of the therapist and the effect of self-help, and about patient involvement and attrition, as described hereafter.

3.2.1. Therapist Assistance and Self-Help

The role of the therapist has been investigated in the context of iCBT by Kaldo et al. (2008), Hesser et al. (2012), and Rheker et al. (2015) among others, while Jasper et al. (2014) juxtaposed self-help to group-based CBT. A remarkable finding is reported by Rheker et al. (2015), who studied the alternatives of "support-on-demand" and "no-support" and found no differences between the two options.

As opposed to iCBT and similar forms of treatment, self-help apps rely entirely on active patient involvement, whereupon the response rate, once and over a long range of time, is used as indicator of success: Wilson et al. (2015) report a response rate of 79.4% for ECA (889 out of 1120 questionnaires were returned), while Pryss et al. (2015b) report that 90% of the questionnaires filled under Track Your Tinnitus come from 18% of the users and thus, incentives for patient involvement need to be established. Advances in that domain include serious games (Schickler et al., 2016b) and passive forms of crowdsensing, as used for other diseases (e.g., diabetes) to monitor vital signals.

3.2.2. Patient Participation and Attrition

An aspect of substantial interest in the identified literature concerns participation and attrition. Number of participants and percentage of dropouts are used as key indicators of iCBT efficancy: in the studies of Abbott et al. (2009), Hesser et al. (2012), Weise et al. (2012, 2016), Kaldo et al. (2013), Jasper et al. (2014), Beukes et al. (2015, 2016), Rheker et al. (2015), and Heinrich et al. (2016), the number of participants ranges between 44 and 293 (average:113). The drop-outs are depicted in the rightmost column of **Table 1**.

As can be seen in the last two columns of **Table 1**, the variance is substantial. This is in agreement with the findings in the review of Greenwell et al. (2016a, p. 84), where large differences are reported among the surveyed studies as well.

Self-help apps like (Pryss et al., 2015b) and Wilson et al. (2015) do not have an explicit notion of dropout, the patient decides whether or not to use the app. However, patient involvement is not less mission-critical. The observation of Pryss et al. (2015b) that 90% of the Track Your Tinnitus questionnaires come from 18% of the app users indicates a very skewed distribution. However, this does not directly imply patient dissatisfaction; patients would most likely give up the app if they do not need it anymore. Hence, the response rates of self-help apps call for further investigation of the factors modulating attrition.

3.3. Technical Challenges

3.3.1. Platform Diversity

As with many smartphone applications, software for tinnitus monitoring, delivery of information materials, and data

TABLE 1 | Participation and attrition (number of dropouts) in the inspected iCBT studies.

Literature source	#Participants	#drop-outs
Hesser et al., 2012	99	10
Rheker et al., 2015	112	9 in the support-on-demand group (out of 56), 11 in the no-support group (out of 56)
Weise et al., 2012	124	5
Jasper et al., 2014	128	7
Beukes et al., 2016	44 (of which 37 completed the screening questionnaire)	15
Weise et al., 2016	124	5
Heinrich et al., 2016	112	14

collection need to take the platform and application interfaces into account. Compatibility with each smartphone operating system translates into additional costs for software development and maintenance. Interaction with hearing aids, sensors, smartwatches and other devices causes further costs and requires expertise. Furthermore, the user interfaces vary substantially: the design of fill-in forms, especially of those requiring free-text, must be re-considered when input is acquired through smartphones and smartwatches.

3.3.2. Non-obtrusive Data Acquisition

Smart technology is appropriate for the acquisition of data without active user involvement, e.g., from the microphone, camera, gyro-sensors, and accelerometers. Such data can be used for tinnitus monitoring without the need to increase user participation. However, data protection and anonymization of the data needs to be addressed.

For the monitoring of other chronical diseases, such as diabetes, the use of non-obtrusive data acquisition technologies is widespread as reported by Brzan et al. (2016). Tinnitus monitoring is less widespread, but gains momentum, as our findings show. Moreover, the collection of data about sleep, social interaction, and behavioral patterns are relevant as well, as reported e.g., by Wang et al. (2014) and Lane et al. (2011): the findings from such initiatives are also of relevance for the support of tinnitus patients, since tinnitus has been found to ".. [be] engendering the sense of cognitive and emotional reactions" as pointed out by Ghodratitoostani et al. (2016), who also list insomnia among the consequences.

4. SUMMARY, LIMITATIONS AND OUTLOOK

Our review has shown that internet-based and smart technologies are intensively investigated for the potential to support tinnitus patients. The technologies are used during treatment, e.g., to assign tasks to the patients, but also to collect patient assessments, including Ecological Momentary Assessments. The technologies are also used for diagnostic purposes, e.g., for tinnitus matching or to monitor tinnitus evolution over time.

We found that these new technologies influence the amount of collected patient information but also the form of the interaction. There is a thread of studies on internet-based and smart technologies as part of a physician-supervised tinnitus therapy, and a younger thread on self-help solutions for patients. In both threads, there is awareness about the need for dedicated questionnaire designs (in the context of tinnitus monitoring) and to a more limited extent, awareness about the need to cope with diversity of platforms and user interfaces.

The use of internet-based and smart technologies also affects the extent and form of patient participation. We identified studies that investigate the role of therapist assistance and that juxtapose therapy design with and without therapist. The role of patient participation is naturally a central subject in these studies. There are research contributions that simply report on the number of drop-outs from a conducted field study, whereby the variance is high. But the new technologies are also used outside the scope of typical clinical studies: research papers that report on such technology use are mainly concentrating on self-help eHealth/mHealth apps; they measure response rate of the patients rather than drop-outs, and identify also a high variance.

The co-authorship network of **Figure 1** highlights the teams of authors that work intensively on the potential of new technologies for tinnitus. The graph shows that teams build clusters with strong links (thick and intensively colored) among the members, indicating intensive joint publication activity, while there are only few and weak links between clusters. These clusters seems to correspond to the topics being investigated, according to our findings. In particular, the cluster with the strongest links corresponds to the most intensive thread of research; the authors in this cluster work together to investigate the role of internet-based and smart technologies for tinnitus treatment, with emphasis on iCBT. The cluster with the second-strongest links (bottom of the figure) contains authors that work together to understand tinnitus through EMA but at the same time investigate the patient-app-interaction.

A limitation of our review lays in the use of Google Scholar as primary source, whereupon only the top-20 articles were considered. It is possible that relevant articles were overlooked, because they were not in the top-20 positions or because they were not indexed in that query engine. The focus on keyword-based retrieval of scientific works implies that our review did not cover studies on smart technologies for chronical diseases with resemblance to tinnitus nor for comorbidities of tinnitus (like depression), but without explicit reference to the keyword "tinnitus." Research articles that investigate the potential of dedicated devices are underrepresented: studies that consider dedicated hardware next to smart technology, e.g., for tinnitus masking, are covered in our work, but studies that only consider dedicated hardware are not.

Our market overview is biased by our inclusion criterion, namely the keyword "tinnitus": we did not consider mobile apps

that can be used for tinnitus masking and for relaxation but do not refer explicitly to tinnitus. At the same time, our overview did not address the veracity of the texts we acquired. We took the perspective of the non-expert, potential customer for those apps, and did not verify the claims made in the app description. We strongly believe that certification of such apps is necessary to protect patients from misleading statements, but such a task was beyond the scope of our study.

We plan to extend our work by investigating how smart technologies are used in other chronical diseases that show resemblances to tinnitus. The notion of resemblance has to be specified crisply, but our first focus will be on diseases that require patients to fill-in questionnaires frequently (e.g., diabetes) and diseases for which ambient recordings are collected and exploited in a non-obtrusive way (e.g., dementia).

AUTHOR CONTRIBUTIONS

SK and MS created the search strategies. SK created the networks-graphics and drafted the initial manuscript. All authors contributed equally to all other stages of the manuscript development, produced, and approved the manuscript.

REFERENCES

Abbott, J.-A. M., Kaldo, V., Klein, B., Austin, D., Hamilton, C., Piterman, L., et al. (2009). A cluster randomised trial of an internet-based intervention program for tinnitus distress in an industrial setting. *Cogn. Behav. Ther.* 38, 162–173. doi: 10.1080/16506070902763174

Andersson, G., Bakhsh, R., Johansson, L., Kaldo, V., and Carlbring, P. (2005). Stroop facilitation in tinnitus patients: an experiment conducted via the world wide web. *Cyberpsychol. Behav.* 8, 32–38. doi: 10.1089/cpb.2005.8.32

Andersson, G., and Kaldo, V. (2004). Internet-based cognitive behavioral therapy for tinnitus. *J. Clin. Psychol.* 60, 171–178. doi: 10.1002/jclp.10243

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Barozzi, S., Del Bo, L., Crocetti, A., Dyrlund, O., Passoni, S., Zolin, A., et al. (2016). A comparison of nature and technical sounds for tinnitus therapy. *Acta Acust. United Acust.* 102, 540–546. doi: 10.3813/AAA.918971

Beukes, E. W., Manchaiah, V., Allen, P. M., Baguley, D. M., and Andersson, G. (2015). Internet-based cognitive behavioural therapy for adults with tinnitus in the uk: study protocol for a randomised controlled trial. *BMJ Open* 5:e008241. doi: 10.1136/bmjopen-2015-008241

Beukes, E. W., Vlaescu, G., Manchaiah, V., Baguley, D. M., Allen, P. M., Kaldo, V., et al. (2016). Development and technical functionality of an internet-based intervention for tinnitus in the uk. *Int. Intervent.* 6, 6–15. doi: 10.1016/j.invent.2016.08.002

Brzan, P. P., Rotman, E., Pajnkihar, M., and Klanjsek, P. (2016). Mobile applications for control and self management of diabetes: a systematic review. *J. Med. Syst.* 40:210. doi: 10.1007/s10916-016-0564-8

Cima, R. F., Maes, I. H., Joore, M. A., Scheyen, D. J., El Refaie, A., Baguley, D. M., et al. (2012). Specialised treatment based on cognitive behaviour therapy versus usual care for tinnitus: a randomised controlled trial. *Lancet* 379, 1951–1959. doi: 10.1016/S0140-6736(12)60469-3

Ghodratitoostani, I., Zana, Y., Delbem, A. C., Sani, S. S., Ekhtiari, H., and Sanchez, T. G. (2016). Theoretical tinnitus framework: a neurofunctional model. *Front. Neurosci.* 10:370. doi: 10.3389/fnins.2016.00370

Greenwell, K., Featherstone, D., and Hoare, D. J. (2015). The application of intervention coding methodology to describe the tinnitus e-programme, an internet-delivered self-help intervention for tinnitus. *Amer. J. Audiol.* 24, 311–315. doi: 10.1044/2015_AJA-14-0089

Greenwell, K., Sereda, M., Coulson, N., El Refaie, A., and Hoare, D. J. (2016a). A systematic review of techniques and effects of self-help interventions for tinnitus: application of taxonomies from health psychology. *Int. J. Audiol.* 55(Suppl. 3), S79–S89. doi: 10.3109/14992027.2015.1137363

Greenwell, K., Sereda, M., Coulson, N., and Hoare, D. J. (2016b). Understanding user reactions and interactions with an internet-based intervention for tinnitus self-management: mixed-methods process evaluation protocol. *JMIR Res. Protoc.* 5:e49. doi: 10.2196/resprot.5008

Handscomb, L., Hall, D. A., Shorter, G. W., and Hoare, D. J. (2016). Online data collection to evaluate a theoretical cognitive model of tinnitus. *Amer. J. Audiol.* 25, 313–317. doi: 10.1044/2016_AJA-16-0007

Hauptmann, C., Wegener, A., Poppe, H., Williams, M., Popelka, G., and Tass, P. A. (2016). Validation of a mobile device for acoustic coordinated reset neuromodulation tinnitus therapy. *J. Amer. Acad. Audiol.* 27, 720–731. doi: 10.3766/jaaa.15082

Heinrich, S., Rozental, A., Carlbring, P., Andersson, G., Cotter, K., and Weise, C. (2016). Treating tinnitus distress via the internet: a mixed methods approach of what makes patients seek help and stay motivated during internet-based cognitive behavior therapy. *Int. Intervent.* 4, 120–130. doi: 10.1016/j.invent.2016.04.001

Henry, J. A., Galvez, G., Turbin, M. B., Thielman, E. J., McMillan, G. P., and Istvan, J. A. (2012). Pilot study to evaluate ecological momentary assessment of tinnitus. *Ear Hear.* 32:179. doi: 10.1097/AUD.0b013e31822f6740

Hesser, H., Gustafsson, T., Lundén, C., Henrikson, O., Fattahi, K., Johnsson, E., et al. (2012). A randomized controlled trial of internet-delivered cognitive behavior therapy and acceptance and commitment therapy in the treatment of tinnitus. *J. Consult. Clin. Psychol.* 80:649. doi: 10.1037/a0027021

Jasper, K., Weise, C., Conrad, I., Andersson, G., Hiller, W., and Kleinstaeuber, M. (2014). Internet-based guided self-help versus group cognitive behavioral therapy for chronic tinnitus: a randomized controlled trial. *Psychother. Psychosomat.* 83, 234–246. doi: 10.1159/000360705

Kaldo, V., Haak, T., Buhrman, M., Alfonsson, S., Larsen, H.-C., and Andersson, G. (2013). Internet-based cognitive behaviour therapy for tinnitus patients delivered in a regular clinical setting: Outcome and analysis of treatment dropout. *Cogn. Behav. Ther.* 42, 146–158. doi: 10.1080/16506073.2013.769622

Kaldo, V., Levin, S., Widarsson, J., Buhrman, M., Larsen, H.-C., and Andersson, G. (2008). Internet vs. group cognitive-behavioral treatment of distress associated with tinnitus: a randomized controlled trial. *Behav. Ther.* 39, 348–359. doi: 10.1016/j.beth.2007.10.003

Lane, N. D., Mohammod, M., Lin, M., Yang, X., Lu, H., Ali, S., et al. (2011). "Bewell: a smartphone application to monitor, model and promote wellbeing," in *5th International ICST Conference on Pervasive Computing Technologies for Healthcare* (Dublin), 23–26.

Linnan, Z., Peiyu, H., Fan, P., and Shangwen, L. (2015). "Design and implementation of a new tinnitus treatment method based on android," in *2015 IEEE International Conference on Digital Signal Processing (DSP)* (Singapore: IEEE), 263–267.

Lui, J. H., Marcus, D. K., and Barry, C. T. (2017). Evidence-based apps? A review of mental health mobile applications in a psychotherapy context. *Prof. Psychol. Res. Pract.* 48:199. doi: 10.1037/pro0000122

Mahboubi, H., Ziai, K., Brunworth, J., and Djalilian, H. R. (2012a). Accuracy of tinnitus pitch matching using a web-based protocol. *Ann. Otol. Rhinol. Laryngol.* 121, 671–674. doi: 10.1177/000348941212101008

Mahboubi, H., Ziai, K., and Djalilian, H. R. (2012b). Customized web-based sound therapy for tinnitus. *Int. Tinnitus J.* 17, 26–30.

Martinez-Devesa, P., Perera, R., Theodoulou, M., and Waddell, A. (2010). Cognitive behavioural therapy for tinnitus. *Cochr. Library.* 9:CD005233. doi: 10.1002/14651858.CD005233.pub3

Newman, C. W., Jacobson, G. P., and Spitzer, J. B. (1996). Development of the tinnitus handicap inventory. *Arch. Otolaryngol. Head Neck Surg.* 122, 143–148. doi: 10.1001/archotol.1996.01890140029007

Nyenhuis, N., Golm, D., and Kröner-Herwig, B. (2013). A systematic review and meta-analysis on the efficacy of self-help interventions in tinnitus. *Cogn. Behav. Ther.* 42, 159–169. doi: 10.1080/16506073.2013.803496

Peter, N., Kleinjung, T., Horat, L., Schmidt-Weitmann, S., Meyer, M., Büchi, S., et al. (2016). Validation of prism (pictorial representation of illness and self measure) as a novel visual assessment tool for the burden of suffering in tinnitus patients. *Health Qual. Life Outcomes* 14, 1–9. doi: 10.1186/s12955-016-0454-2

Probst, T., Pryss, R., Langguth, B., and Schlee, W. (2016a). Emotion dynamics and tinnitus: daily life data from the "trackyourtinnitus" application. *Sci. Rep.* 6:31166. doi: 10.1038/srep31166

Probst, T., Pryss, R., Langguth, B., and Schlee, W. (2016b). Emotional states as mediators between tinnitus loudness and tinnitus distress in daily life: results from the "trackyourtinnitus" application. *Sci. Rep.* 6:20382 doi: 10.1038/srep20382

Pryss, R., Reichert, M., Langguth, B., and Schlee, W. (2015a). "Mobile crowd sensing in clinical and psychological trials–a case study," in *Computer-Based Medical Systems (CBMS), 2015 IEEE 28th International Symposium on* (Sao Carlos: IEEE), 23–24.

Pryss, R., Reichert, M., Langguth, B., and Schlee, W. (2015b). "Mobile crowd sensing services for tinnitus assessment, therapy, and research," in *Mobile Services (MS), 2015 IEEE International Conference on* (New York, NY: IEEE), 352–359.

Rheker, J., Andersson, G., and Weise, C. (2015). The role of "on demand" therapist guidance vs. no support in the treatment of tinnitus via the internet: a randomized controlled trial. *Int. Intervent.* 2, 189–199. doi: 10.1016/j.invent.2015.03.007

Sauer, G., Dickel, T., and Lotter, T. (2014). "Acoustic wireless control–connecting smart phones to hearing instruments," in *Siemens Whitepaper*. Available online at: https://media.sivantos.com/siemens-website/media/2014/11/2014_11_Acoustic-Wireless-Control.pdf

Schickler, M., Pryss, R., Reichert, M., Heinzelmann, M., Schobel, J., Langguth, B., et al. (2016a). "Using wearables in the context of chronic disorders: results of a pre-study," in *Computer-Based Medical Systems (CBMS), 2016 IEEE 29th International Symposium on* (Dublin; Belfast, UK: IEEE), 68–69.

Schickler, M., Pryss, R., Reichert, M., Schobel, J., Langguth, B., and Schlee, W. (2016b). "Using mobile serious games in the context of chronic disorders: a mobile game concept for the treatment of tinnitus," in *Computer-Based Medical Systems (CBMS), 2016 IEEE 29th International Symposium on* (Dublin; Belfast, UK: IEEE), 343–348.

Schlee, W., Pryss, R. C., Probst, T., Schobel, J., Bachmeier, A., Reichert, M., et al. (2016). Measuring the moment-to-moment variability of tinnitus: the trackyourtinnitus smart phone app. *Front. Aging Neurosci.* 8:294. doi: 10.3389/fnagi.2016.00294

Wang, R., Chen, F., Chen, Z., Li, T., Harari, G., Tignor, S., et al. (2014). "Studentlife: assessing mental health, academic performance and behavioral trends of college students using smartphones," in *Proceedings of the 2014 ACM International Joint Conference on Pervasive and Ubiquitous Computing* (Seattle, WA: ACM), 3–14.

Weise, C., Kleinstäuber, M., and Andersson, G. (2012). "Internet-based cognitive-behavioural treatment of chronic tinnitus," in *12th International Congress of Behavioral Medicine (ICBM), 29 August-1 September 2012* (Budapest).

Weise, C., Kleinstäuber, M., and Andersson, G. (2016). Internet-delivered cognitive-behavior therapy for tinnitus: a randomized controlled trial. *Psychosomat. Med.* 78, 501–510. doi: 10.1097/PSY.0000000000000310

Westin, V. Z., Schulin, M., Hesser, H., Karlsson, M., Noe, R. Z., Olofsson, U. et al. (2011). Acceptance and commitment therapy vs. tinnitus retraining therapy in the treatment of tinnitus: a randomised controlled trial. *Behav. Res. Ther.* 49, 737–747. doi: 10.1016/j.brat.2011.08.001

Wilson, M. B., Kallogjeri, D., Joplin, C. N., Gorman, M. D., Krings, J. G., Lenze, E. J., et al. (2015). Ecological momentary assessment of tinnitus using smartphone technology a pilot study. *Otolaryngol. Head Neck Surg.* 152, 897–903. doi: 10.1177/0194599815569692

Wise, K., Kobayashi, K., Magnusson, J., Welch, D., and Searchfield, G. D. (2016). Randomized controlled trial of a perceptual training game for tinnitus therapy. *Games Health J.* 5, 141–149. doi: 10.1089/g4h.2015.0068

Wise, K., Kobayashi, K., and Searchfield, G. (2015). Feasibility study of a game integrating assessment and therapy of tinnitus. *J. Neurosci. Methods* 249, 1–7. doi: 10.1016/j.jneumeth.2015.04.002

Wunderlich, R., Stein, A., Engell, A., Lau, P., Waasem, L., Shaykevich, A., et al. (2015). Evaluation of ipod-based automated tinnitus pitch matching. *J. Amer. Acad. Audiol.* 26, 205–212. doi: 10.3766/jaaa.26.2.9

Yoo, S.-G., Oh, D., Hong, M., and Park, M. (2015). Study on a user-friendly acoustic therapeutic sound source-based mobile application for tinnitus patients. *Adv. Sci. Lett.* 21, 332–336. doi: 10.1166/asl.2015.5777

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ORIGINAL RESEARCH
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Does Tinnitus Depend on Time-of-Day? An Ecological Momentary Assessment Study with the "TrackYourTinnitus" Application

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Only few previous studies used ecological momentary assessments to explore the time-of-day-dependence of tinnitus. The present study used data from the mobile application "TrackYourTinnitus" to explore whether tinnitus loudness and tinnitus distress fluctuate within a 24-h interval. Multilevel models were performed to account for the nested structure of assessments (level 1: 17,209 daily life assessments) nested within days (level 2: 3,570 days with at least three completed assessments), and days nested within participants (level 3: 350 participants). Results revealed a time-of-day-dependence of tinnitus. In particular, tinnitus was perceived as louder and more distressing during the night and early morning hours (from 12 a.m. to 8 a.m.) than during the upcoming day. Since previous studies suggested that stress (and stress-associated hormones) show a circadian rhythm and this might influence the time-of-day-dependence of tinnitus, we evaluated whether the described results change when statistically controlling for subjectively reported stress-levels. Correcting for subjective stress-levels, however, did not change the result that tinnitus (loudness and distress) was most severe at night and early morning. These results show that time-of-day contributes to the level of both tinnitus loudness and tinnitus distress. Possible implications of our results for the clinical management of tinnitus are that tailoring the timing of therapeutic interventions to the circadian rhythm of individual patients (chronotherapy) might be promising.

Keywords: tinnitus, stress, circadian fluctuation, time-of-day, ecological momentary assessment

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INTRODUCTION

Tinnitus, the phantom perception of sound (Baguley et al., 2013; Langguth et al., 2013), is perceived by 5.1% up to 42.7% of the population according to a recent review including 39 studies from 16 countries (McCormack et al., 2016). These percentages depend on age (with older persons showing higher prevalence), gender (with male persons showing higher prevalence), and the definition of

tinnitus used in the epidemiological study (McCormack et al., 2016). Prevalence rates of tinnitus also have increased over the years (Nondahl et al., 2012; Martinez et al., 2015), and one can speculate about the reasons for this apparent increase. On a neuronal basis, auditory-limbic interactions play a central role in the development of (chronic) tinnitus (Rauschecker et al., 2010; Leaver et al., 2011). In many cases, tinnitus is associated with psychological distress and incapacity for work (Bhatt et al., 2016) resulting in high socio-economic costs (Maes et al., 2013). Cognitive-behavioral therapy (CBT) has proven to have the potential to reduce the burden of tinnitus for the individual (Hesser et al., 2011) as well as the society/economy (Maes et al., 2014). But not all tinnitus patients reach clinically relevant improvements with CBT (e.g., Jasper et al., 2014). Other therapeutic approaches, including pharmacological therapy (Langguth and Elgoyhen, 2012), auditory stimulation (Hobson et al., 2012) or brain stimulation (Langguth and De Ridder, 2013), have only revealed small and inconsistent effects in subgroups of patients. It is assumed that an important reason for the poor treatment response in clinical trials is the heterogeneity of tinnitus (Landgrebe et al., 2012; Baguley et al., 2013), both across patients (inter-individual heterogeneity) as well as within patients over time (intra-individual heterogeneity, see Dauman et al., 2015).

Therefore, it is important to understand the factors that contribute to the heterogeneity of tinnitus. Psychological variables, such as fear-related cognition (e.g., Cima et al., 2011; Kleinstäuber et al., 2013), an accepting stance toward tinnitus (e.g., Weise et al., 2013; Riedl et al., 2015), emotions (e.g., Probst et al., 2016a,b), and avoidance/safety behaviors (e.g., Hesser and Andersson, 2009; Kleinstäuber et al., 2013), have been demonstrated to account for the heterogeneity of tinnitus in several studies (see also the "scientific cognitive-behavioral model of tinnitus"; McKenna et al., 2014). Moreover, recent neuroscience studies imply that the moment-to-moment variability of tinnitus is related to brain oscillatory patterns like the alpha power in temporal regions (Schlee et al., 2014) and time-of-day (Basinou et al., 2017); circadian fluctuations have been shown, for example, in auditory pathway structures related to tinnitus like the cochlea (Meltser et al., 2014) and the inferior colliculus (Park et al., 2016). Another hint for a link between tinnitus and circadian rhythms is provided by findings of reduced tinnitus severity after intake of melatonin (e.g., Pirodda et al., 2010; Ajayi et al., 2014; Miroddi et al., 2015). Furthermore, pain, which shares many similarities with tinnitus (De Ridder et al., 2011; Rauschecker et al., 2015), has been found to underlie circadian variations (e.g., Strian et al., 1989; Gilron and Ghasemlou, 2014; Buttgereit et al., 2015). Furthermore, depression, which overlaps in its pathophysiology with tinnitus (Langguth et al., 2011), is characterized by changes in circadian rhythm (e.g., Germain and Kupfer, 2008; Wirz-Justice, 2008).

The question of whether tinnitus varies systematically over the course of the day, however, has not yet been studied systematically. The limited possibilities of traditional assessment methods to routinely track symptoms in the daily routine have made it rather difficult to validly study whether tinnitus shows time-of-day-dependence. To overcome these limitations of traditional assessment methods, newer technological developments can be used to electronically gather valid daily life data (ecological momentary assessments, EMA; Trull and Ebner-Priemer, 2013, 2014; Adams et al., 2017). Henry et al. (2012), for example, used personal digital assistants (PDA) in a 2-week pilot study with 24 participants to obtain EMA during a 12-h interval (8 a.m.–8 p.m.) and showed that the scores of the 10-item screening version of the Tinnitus Handicap Inventory (THI-S; Newman et al., 2008) were not significantly different between 3-h time blocks (8 a.m.–11 a.m., 11 a.m.–2 p.m., 2 p.m.–5 p.m., and 5 p.m.–8 p.m.). Although Henry et al. (2012) failed to demonstrate time-of-day-dependence of tinnitus, a 2-week pilot smartphone-based study with 20 participants on fluctuations of tinnitus within an 11-h interval (9 a.m.–8 p.m.) suggested that tinnitus does vary within a single day (Wilson et al., 2015). But Wilson et al. (2015) did not report at which time-of-day the participants rated their tinnitus as more or less severe. In another diary study, Flor et al. (2004) reported tinnitus being worst at the beginning of a day, thus supporting the time-of-day-dependence of tinnitus. To our knowledge there are only these three studies with ambivalent results that addressed the time-of-day-dependence of tinnitus in daily life with EMA (Flor et al., 2004; Henry et al., 2012; Wilson et al., 2015).

The current study used EMA from the "TrackYourTinnitus" (TYT) mobile application (Pryss et al., 2015a,b; Schlee et al., 2016) to explore whether tinnitus fluctuates within a 24-h interval (night and upcoming day). Tinnitus is operationalized by two questions in TYT, one question is on tinnitus loudness and the other question on tinnitus distress. Prior research suggested that an assessment of both tinnitus loudness and tinnitus distress is necessary for a comprehensive assessment, since tinnitus loudness and tinnitus distress are only moderately correlated (e.g., Hiller and Goebel, 2007; Wallhäusser-Franke et al., 2012) and processed in different but interconnected brain areas (e.g., Leaver et al., 2012; Ueyama et al., 2013; De Ridder et al., 2014; Vanneste et al., 2014). Therefore, it appears possible that tinnitus loudness and tinnitus distress show either similar or different ups and downs within a 24-h interval. Accordingly, the present study investigated the time-of-day-dependence of tinnitus loudness as well as of tinnitus distress. Moreover, stress is known to be associated with tinnitus (e.g., Hébert et al., 2004; Hébert and Lupien, 2007, 2009; Alsalman et al., 2016) and stress-related hormones like cortisol and adrenocorticotropic hormone (ACTH) underlie circadian rhythms (e.g., Dickmeis, 2009; Lightman and Conway-Campbell, 2010; Conway-Campbell et al., 2012), which could influence the potential time-of-day-dependence of tinnitus (loudness and distress). Thus, we also explored whether the stress-level as assessed with TYT depends on time-of-day and whether the 24-h fluctuations of tinnitus loudness and tinnitus distress change when taking the stress-level into account.

MATERIALS AND METHODS

The material and the methods were approved by the Ethics Committee of the University Clinic of Regensburg and were

carried out in accordance with the approved guidelines. Information that the TYT data will be used for scientific analyses is included in the mobile applications of "TrackYourTinnitus" as well as on the "TrackYourTinnitus" website and, therefore, the TYT users were informed that the data will be used for scientific purposes. Written consent, however, was not possible to obtain given the nature of the study. The study participants were anonymized.

"TrackYourTinnitus" Platform

The TYT platform (www.trackyourtinnitus.org, Pryss et al., 2015a,b) consists of a website for registration, two mobile applications (for iOS and Android), and a MySQL database as a central repository for the data collected. Users can either use TYT whenever they want or they can set a user-defined schedule to receive random notifications. For the study at hand, only these notification-triggered assessments were investigated. At each of these notifications, the users are asked to rate their tinnitus and other tinnitus-related variables (e.g., subjective stress-level). Although the attention might be directed toward the tinnitus by such notifications, Henry et al. (2012) and Schlee et al. (2016) found that repeatedly rating tinnitus and associated variables in daily life does not have detrimental effects. The present study investigated the following variables the users were asked to rate at each notification: "Current tinnitus loudness" (subjective rating of current tinnitus loudness on a visual analog scale [VAS], including a zero value for moments without loudness: min: 0; max: 1), "current tinnitus distress" (subjective rating of current tinnitus distress on a VAS including a zero value for moments without distress: min: 0; max: 1), and "current stress-level" (subjective rating of current stress-level on a VAS: min: 0; max: 1). Moreover, the timestamps of the assessments were used to explore the time-of-day-dependence of tinnitus. In TYT, the timestamps represent the local time of the time zone a given user is in when providing the assessments.

The data set used for the current study was exported in June 2016. After excluding the self-initiated assessments and the assessments given within the 15 min after the last assessment (for the inter-assessment interval of 15 min see also Pryss et al., 2015b), we had access to 25,863 notification-triggered assessments. For the present study, we only included the 25,092 assessments without missing values in any of the three target variables. Furthermore, as we were interested in within-day variations, we only considered data from days with at least three completed assessments, resulting in a total number of 17,209 assessments.

Sample

The final sample consisted of 350 participants. Two-hundred and fifty three participants (72.2%) were male, 94 (26.9%) were female, and 3 did not indicate their gender. On average, participants were 45.4 ($SD = 12.1$) years old (17 participants did not report their age). The median number of years since onset of tinnitus was 5.4, ranging from 0 to 61.8 years. According to participants, onset of tinnitus was related to loud blast of sound ($n = 48$), whiplash ($n = 9$), change in hearing ($n = 38$), stress ($n = 99$), head trauma ($n = 12$), and other causes ($n = 141$) (3 participants did not report events related to onset of tinnitus). The median number of days per participant (with at least three assessments) was 11, ranging from 1 to 415 days. This corresponds to a total number of 3,570 days. The median number of assessments per day was 4, ranging from 3 (the minimum requirement to be included in this study) to 18 assessments.

Statistical Analyses

To test our hypotheses, we used multilevel modeling (MLM; Raudenbush and Bryk, 2002; Singer and Willett, 2003). MLM is ideally suited to address the nested structure of our data, with assessments (Level 1) nested in days (Level 2), and days nested in participants (Level 3). First, we estimated two MLMs predicting tinnitus loudness and tinnitus distress from time-of-day, respectively. Time-of-day was dummy-coded using five binary variables indicating whether the assessment was in the early morning (T1, from 4 a.m. to 8 a.m.), in the late morning (T2, from 8 a.m. to 12 p.m.), in the afternoon (T3, from 12 p.m. to 4 p.m.), in the early evening (T4, from 4 p.m. to 8 p.m.), or in the late evening (T5, from 8 p.m. to 12 a.m.). Assessments during the night (from 12 a.m. to 4 a.m.) were defined as the reference group. The full three-level MLM with random intercepts at Level 2 and 3 and random slopes at Level 3 (Model I) is summarized below:

$$
\begin{aligned}
\text{Level 1:}\quad & y_{ijk} = \pi_{0jk} + \pi_{1jk}(T1) + \pi_{2jk}(T2) + \pi_{3jk}(T3) \\
& \quad + \pi_{4jk}(T4) + \pi_{5jk}(T5) + e_{ijk} \\
\text{Level 2:}\quad & \pi_{0jk} = \beta_{00k} + r_{0jk} \\
& \pi_{1jk} = \beta_{10k} \\
& \pi_{2jk} = \beta_{20k} \\
& \pi_{3jk} = \beta_{30k} \\
& \pi_{4jk} = \beta_{40k} \\
& \pi_{5jk} = \beta_{50k} \\
\text{Level 3:}\quad & \beta_{00k} = \gamma_{000} + u_{00k} \\
& \beta_{10k} = \gamma_{100} + u_{10k} \\
& \beta_{20k} = \gamma_{200} + u_{20k} \\
& \beta_{30k} = \gamma_{300} + u_{30k} \\
& \beta_{40k} = \gamma_{400} + u_{40k} \\
& \beta_{50k} = \gamma_{500} + u_{50k}
\end{aligned}
$$

The model decomposes the amount of tinnitus loudness/distress (y) of participant k on day j at assessment i into a series of fixed and random effects. The fixed effect γ_{000} represents the expected (population) tinnitus loudness/distress during the night before an average day of an average participant. The fixed effects γ_{100}, γ_{200}, γ_{300}, γ_{400}, and γ_{500} represent the expected change in tinnitus loudness/distress from night to early and late morning, afternoon and early and late evening, respectively. The random effects at Level 3, u_{00k}, u_{10k}, u_{20k}, u_{30k}, u_{40k}, and u_{50k}, indicate that the level of tinnitus loudness/distress at night as well as its later change during the day may differ between participants. The random intercept at Level 2, r_{0jk}, indicates that the baseline level of tinnitus loudness/distress may differ between days within participants. We assumed random effects to be multivariate normally distributed within levels, and residuals to be independent and identically distributed across levels.

Second, we estimated the same MLM for stress-level as the dependent variable (y). Moreover, we estimated two further MLMs predicting tinnitus loudness and tinnitus distress from time-of-day, this time including stress-level as an additional predictor. As stress-level varied across all three levels, we decomposed its variance into three separate mean-centered variables capturing variation of stress within days (S1), variation of stress within participants across days (S2), and variation of stress across participants (S3) prior to estimating the MLMs. The full three-level MLM with random intercepts at Level 2 and 3 and random slopes at Level 3 (Model II) is summarized below:

$$
\begin{aligned}
\text{Level 1:}\quad & y_{ijk} = \pi_{0jk} + \pi_{1jk}(T1) + \pi_{2jk}(T2) + \pi_{3jk}(T3) \\
& \quad + \pi_{4jk}(T4) + \pi_{5jk}(T5) + \pi_{6jk}(S1) + e_{ijk} \\
\text{Level 2:}\quad & \pi_{0jk} = \beta_{00k} + \beta_{01k}(S2) + r_{0jk} \\
& \pi_{1jk} = \beta_{10k} \\
& \pi_{2jk} = \beta_{20k} \\
& \pi_{3jk} = \beta_{30k} \\
& \pi_{4jk} = \beta_{40k} \\
& \pi_{5jk} = \beta_{50k} \\
& \pi_{6jk} = \beta_{60k} \\
\text{Level 3:}\quad & \beta_{00k} = \gamma_{000} + \gamma_{001}(S3) + u_{00k} \\
& \beta_{01k} = \gamma_{010} + u_{01k} \\
& \beta_{10k} = \gamma_{100} + u_{10k} \\
& \beta_{20k} = \gamma_{200} + u_{20k} \\
& \beta_{30k} = \gamma_{300} + u_{30k} \\
& \beta_{40k} = \gamma_{400} + u_{40k} \\
& \beta_{50k} = \gamma_{500} + u_{50k} \\
& \beta_{60k} = \gamma_{600} + u_{60k}
\end{aligned}
$$

The newly defined fixed effects, γ_{001}, γ_{010}, and γ_{600}, represent the expected between-participant, between-day, and within-day effect of stress on tinnitus loudness/distress after controlling for time-of-day. The remaining fixed effects, γ_{000}, γ_{100}, γ_{200}, γ_{300}, γ_{400}, and γ_{500}, represent the expected level of tinnitus loudness/distress at night as well as its later change during the day after controlling for the influence of stress. The newly defined random effects, u_{01k} and u_{60k}, indicate that the between- and within-day effects of stress may differ between participants. Due to model identification issues, we restricted the covariances between random effects of stress and the remaining random effects in the model to be zero.

All models were estimated using full maximum likelihood estimation. Analyses were conducted with the package "lme4" (Bates et al., 2015) of the statistical platform R (R Core Team, 2015). We used Satterthwaite's approximations to derive p-values for fixed effects. Pairwise comparisons between the six distinct timeframes were explored using the Tukey Honest Significant Difference method as implemented in the package "multcomp" (Hothorn et al., 2008). Finally, we quantified the effect size of time-of-day on tinnitus loudness/distress by means of a pseudo R^2 statistic. This statistic can be computed by subtracting the residual variance $Var(e_{ijk})$ of Model I from the residual variance of an intercept-only model without any predictors, divided by this latter residual variance. It represents the relative amount of variance in tinnitus loudness/distress within days that is explained by time-of-day (i.e., by the five variables T1–T5).

RESULTS

In total, 186 assessments (1.1%) were completed at night, 460 (2.7%) were completed in the early morning, 4,200 (24.4%) were completed in the late morning, 4,941 (28.7%) in the afternoon, 4,724 (27.5%) in the early evening, and 2,698 (15.7%) were completed in the late evening (see **Figure 1**). **Table 1** summarizes the estimated fixed effects of all MLMs (standard deviations and correlations of random effects can be found in the Supplementary Material). Results suggest that tinnitus was louder and more distressing during the night and early morning hours than during all other timeframes of the day (see Models I in the first and third columns of **Table 1**). Tukey's post-hoc tests revealed that differences between late morning, afternoon, and early evening were non-significant (see **Table 2**). However, tinnitus was significantly louder in the late evening compared to the afternoon and early evening. This pattern of results is visualized in **Figures 2A,B**. The pseudo R^2 statistics revealed that time-of-day explained 20.6% of the within-day variance of tinnitus loudness, and 13.0% of the within-day variance of tinnitus distress.

Next, we tested whether time-of-day influences the subjective stress-level (see Model I in the right hand column of **Table 1** and **Figure 2C**). Tukey's post-hoc tests suggested that stress-level increased from morning to afternoon, decreased from afternoon to evening, and did not differ compared to the night (see **Table 2**). Time-of-day explained 7.8% of the within-day variance of stress.

Finally, the models predicting tinnitus loudness and tinnitus distress from time-of-day and stress-level revealed that the stress-level had incremental effects across all three levels (see Models II in **Table 1**): Tinnitus was louder and more distressing when the level of stress was higher at a specific time-of-day compared to other times-of-day, when it was higher during a whole day compared to other days, and when it was higher during the whole assessment period for a given participant (compared to other participants). Nevertheless, the effects of time-of-day on tinnitus loudness and tinnitus distress

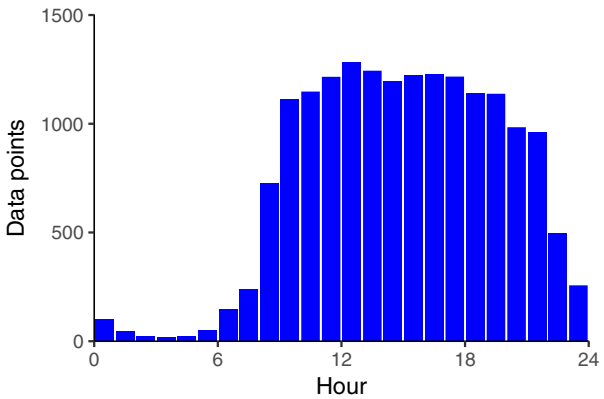

FIGURE 1 | Number of data points per hour.

TABLE 1 | Estimated fixed effects (and their standard errors) for five MLMs.

		Tinnitus loudness		Tinnitus distress		Stress-level
		Model I	Model II	Model I	Model II	Model I
Intercept (night)	γ_{000}	0.529*** (0.021)	0.521*** (0.018)	0.435*** (0.021)	0.423*** (0.016)	0.303*** (0.020)
Early morning (vs. night) effect	γ_{100}	−0.017 (0.021)	−0.015 (0.019)	−0.015 (0.022)	−0.014 (0.018)	−0.015 (0.020)
Late morning (vs. night) effect	γ_{200}	−0.089*** (0.019)	−0.086*** (0.017)	−0.083*** (0.020)	−0.077*** (0.016)	0.009 (0.019)
Afternoon (vs. night) effect	γ_{300}	−0.098*** (0.019)	−0.099*** (0.017)	−0.083*** (0.019)	−0.083*** (0.016)	0.023 (0.018)
Early evening (vs. night) effect	γ_{400}	−0.093*** (0.019)	−0.092*** (0.017)	−0.080*** (0.020)	−0.074*** (0.015)	0.006 (0.018)
Late evening (vs. night) effect	γ_{500}	−0.071*** (0.019)	−0.062*** (0.016)	−0.066*** (0.019)	−0.052*** (0.014)	−0.023 (0.018)
Within-day effect of stress	γ_{600}		0.287*** (0.020)		0.370*** (0.022)	
Between-day effect of stress	γ_{010}		0.470*** (0.037)		0.585*** (0.035)	
Between-person effect of stress	γ_{001}		0.651*** (0.051)		0.810*** (0.041)	

*Total number of assessments was 17,209. p-values were based on Satterthwaite's approximations. ***$p < 0.001$. night = 12 a.m.–4 a.m. early morning = 4 a.m.–8 a.m. late morning = 8 a.m.–12 p.m. afternoon = 12 p.m.–4 p.m. early evening = 4 p.m.–8 p.m. late evening = 8 p.m.–12 a.m.*

TABLE 2 | Tukey's *post-hoc* tests for five MLMs.

	Tinnitus loudness		Tinnitus distress		Stress-level
	Model I	Model II	Model I	Model II	Model I
Early_morning–night	−0.017 (0.021)	−0.015 (0.019)	−0.015 (0.022)	−0.014 (0.018)	−0.015 (0.020)
Late_morning–night	−0.089*** (0.019)	−0.086*** (0.017)	−0.083*** (0.020)	−0.077*** (0.016)	0.009 (0.019)
Afternoon–night	−0.098*** (0.019)	−0.099*** (0.017)	−0.083*** (0.019)	−0.083*** (0.016)	0.023 (0.018)
Early_evening–night	−0.093*** (0.019)	−0.092*** (0.017)	−0.080*** (0.020)	−0.074*** (0.015)	0.006 (0.018)
Late_evening–night	−0.071** (0.019)	−0.062** (0.016)	−0.066** (0.019)	−0.052** (0.014)	−0.023 (0.018)
Late_morning–early_morning	−0.072*** (0.014)	−0.071*** (0.013)	−0.068*** (0.015)	−0.063*** (0.013)	0.023 (0.012)
Afternoon–early_morning	−0.080*** (0.016)	−0.084*** (0.015)	−0.068*** (0.016)	−0.069*** (0.014)	0.038* (0.012)
Early_evening–early_morning	−0.076*** (0.016)	−0.077*** (0.015)	−0.064*** (0.016)	−0.060*** (0.014)	0.021 (0.013)
Late_evening–early_morning	−0.053** (0.016)	−0.047* (0.015)	−0.050* (0.017)	−0.038* (0.014)	−0.009 (0.012)
Afternoon–late_morning	−0.008 (0.005)	−0.013$^{\#}$ (0.005)	−0.000 (0.005)	−0.006 (0.004)	0.014** (0.004)
Early_evening–late_morning	−0.004 (0.007)	−0.005 (0.006)	0.004 (0.006)	0.003 (0.004)	−0.003 (0.006)
Late_evening–late_morning	0.019 (0.009)	0.025* (0.008)	0.017 (0.008)	0.025** (0.007)	−0.032*** (0.006)
Early_evening–afternoon	0.004 (0.003)	0.007 (0.003)	0.004 (0.004)	0.009* (0.003)	−0.017*** (0.004)
Late_evening–afternoon	0.027** (0.007)	0.037*** (0.007)	0.017 (0.007)	0.031*** (0.006)	−0.046*** (0.005)
Late_evening–early_evening	0.023** (0.006)	0.030*** (0.006)	0.014 (0.006)	0.022*** (0.005)	−0.029*** (0.004)

*Total number of assessments was 17,209. The table presents estimated differences (and their standard errors) for all comparisons between timeframes. p-values were adjusted using the Tukey Honest Significant Difference method. $^{\#}p < 0.10$. *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$. night = 12 a.m.–4 a.m. early morning = 4 a.m.–8 a.m. late morning = 8 a.m.–12 p.m. afternoon = 12 p.m.–4 p.m. early evening = 4 p.m.–8 p.m. late evening = 8 p.m.–12 a.m.*

were still significant (i.e., after controlling for the effects of stress).

DISCUSSION

This study evaluated whether subjective tinnitus loudness and subjective tinnitus distress depend on time-of-day with EMA from the "TrackYourTinnitus" (TYT) mobile application (Pryss et al., 2015a,b; Schlee et al., 2016). Strengths of the present study are that a mobile application was used to obtain EMA of high ecological validity (Trull and Ebner-Priemer, 2013, 2014; Adams et al., 2017), a much higher sampling frequency was applied than in typical clinical studies, and the sample size was much larger than in previous EMA studies on the time-of-day-dependence of tinnitus (Flor et al., 2004; Henry et al., 2012; Wilson et al., 2015).

The main result was that tinnitus (loudness and distress) was rated as more severe during the night and the early morning (from 12 a.m. to 8 a.m.) than during the upcoming day. Interestingly, tinnitus loudness and tinnitus distress showed a very similar time pattern although the neurobiological correlates of loudness and distress differ to a certain degree (e.g., De Ridder et al., 2011; Leaver et al., 2012; Ueyama et al., 2013; Vanneste et al., 2014).

Contrary to the EMA study by Henry et al. (2012), the present investigation found a time-of-day-dependence of tinnitus. The most obvious reason for this discrepancy is that Henry et al. (2012) assessed tinnitus only from 8 a.m. to 8 p.m. and could, therefore, not evaluate tinnitus during the time interval that was related to most severe tinnitus in our study (12 a.m.–8 a.m.). During a time interval (8 a.m.–12 a.m.) that included the 12-h

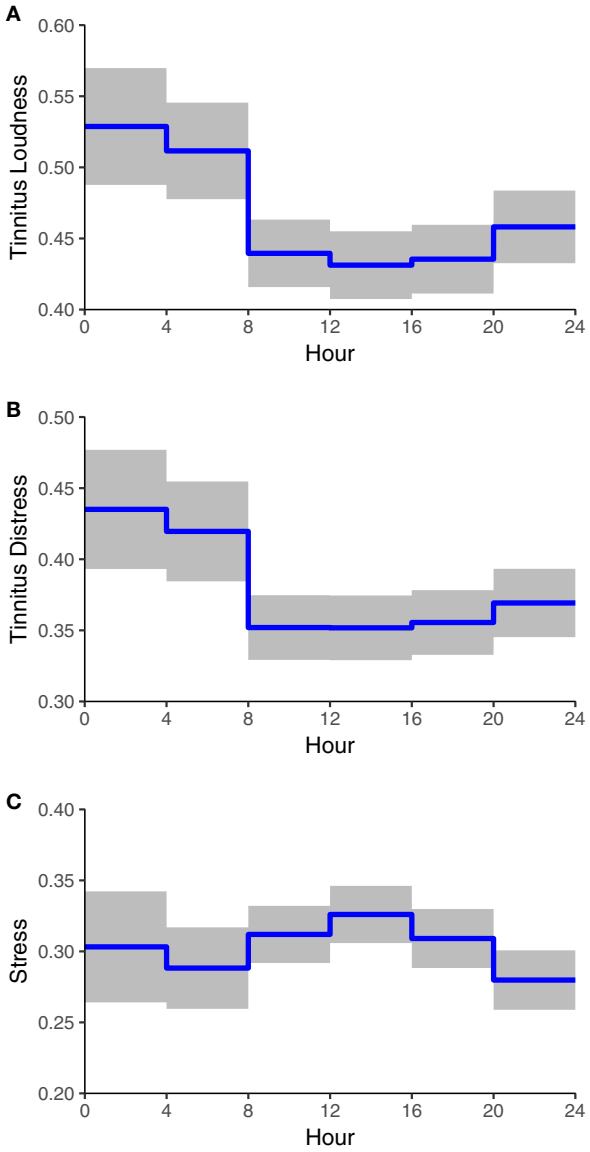

FIGURE 2 | Expected tinnitus loudness **(A)**, tinnitus distress **(B)**, and level of stress **(C)** as a function of time-of-day. The blue lines represent expected values for the average participant on an average day, the gray bands represent 95% confidence intervals.

interval analyzed by Henry et al. (2012) tinnitus did not vary much in our study either (see **Figures 2A,B**). The result that tinnitus was more severe in the early morning hours is in line with a previous diary study (Flor et al., 2004) and fits to the clinical impression of a "morning roar" as it is often anecdotally reported by tinnitus patients. Yet, our result that tinnitus was more severe during the night was not found by Flor et al. (2004). More severe tinnitus (loudness and distress) during the night could at least partially result from the possibility that several assessments at night were given by participants while having sleep disturbances. Sleep disturbances are common among tinnitus patients and their severity correlates positively with measures of tinnitus severity (e.g., Crönlein et al., 2007; Schecklmann et al., 2015; Crönlein et al., 2016). Although we aimed to control for this potential confounder by excluding data from spontaneous ratings and restricting the data analysis to the ratings provided at notifications, the fact that sleep disturbances were not assessed on a daily basis in the present study and, hence, were not available for analysis is a limitation of our study. Future research could explore whether the effect of time-of-day on tinnitus is different between days with and without sleep disturbances. Besides sleep, several other variables are time-of-day dependent and should be considered as potential confounding variables: For example, the environmental sound level depends on time-of-day and tinnitus might be more severe during the night and the early morning, because the environmental sound level in these time intervals cannot mask the tinnitus. Furthermore, a trigger of tinnitus might be (even slight) tension of specific muscles of the craniocervical connection (Bechter et al., 2016), whereby the tension depends mainly from posture, which is influenced by different aspects during the night (e.g., sleeping posture, bed pillow) and during the day. Moreover, the release of stress-related hormones is controlled by a circadian clock (Dickmeis, 2009; Lightman and Conway-Campbell, 2010; Conway-Campbell et al., 2012) and future studies could explore the relationship between circadian tinnitus variability and such stress-related hormones. In the present study, we could only analyze subjective stress-levels with the result that the 24-h course of the subjective stress-level was not parallel to the course of tinnitus (loudness and distress): While tinnitus (loudness and distress) showed their maximum between 12 a.m. and 8 a.m., the maximum of the subjective stress-level was found between 12 p.m. and 4 p.m. The discrepant time curves of tinnitus and subjective stress-level raise further questions, which need to be addressed in future longitudinal studies regarding the tinnitus-stress link. For example, it appears possible that tinnitus and subjective stress interact more in a time-lagged than in an instant manner. Thus, it should be clarified whether increases of subjective stress lead to more severe tinnitus and whether increases of tinnitus severity also lead to more subsequent subjective stress (which again might affect tinnitus severity resulting in a vicious circle). Feedback loops have been described already in research on the pituitary-adrenal system: "positive, delayed, feedforward connection between the pituitary and the adrenals" and "negative feedback of glucocorticoids on ACTH release" (Lightman and Conway-Campbell, 2010, p. 712). It should also be noted in this context that the peaks of our self-reported stress-levels (12 p.m.–4 p.m.) did not correspond to the peaks of cortisol and ACTH releases (4 a.m.–8 a.m.) as illustrated by Lightman and Conway-Campbell (2010). Therefore, the longitudinal associations between tinnitus and self-reported stress might be different from the ones between tinnitus and stress-hormones.

Regarding the observed circadian variability of tinnitus loudness, it is tempting to speculate relations to circadian variations in neuronal sensitivity of peripheral and central auditory structures, which have been identified in recent studies (Park et al., 2016; Basinou et al., 2017). However, tinnitus loudness does not directly reflect neuronal activity in auditory pathways, but also depends on other factors such as attention and emotions. For example, a previous study found that tinnitus becomes louder over time when participants experience

more qualitatively different feelings (Probst et al., 2016b). Therefore, more research is needed to replicate the observed circadian rhythm of tinnitus in large samples, to identify the specific contribution of different factors (e.g., neuronal activity in auditory pathways, attention, and emotion), and to explore whether different persons show a different time-of-day dependence of tinnitus. Moderating variables might, for example, be gender, age (e.g., the time-of-day dependence of tinnitus might be different between men and women or within women before/after the menopause), and the etiology of tinnitus (e.g., the time of time-of-day dependence might be different between noise trauma as etiology and stress as etiology). Future translational and clinical studies with samples large enough to analyze such subgroups are required to explore potential moderators of the time-of-day dependence of tinnitus. These further studies could also measure tinnitus by different assessment methods, because the study at hand only analyzed self-reported tinnitus. For example, by the "gap-in-noise" paradigm (Lowe and Walton, 2015) or psychophysiological measurements such as tinnitus matching. Yet, the reliability and validity of tinnitus matching is an ongoing matter of debate (Hoare et al., 2014; De Ridder et al., 2015). For mobile applications such as TYT, portable methods to assess tinnitus more objectively (see for example, Hébert and Fournier, 2017) that can be integrated in the mobile application are needed. It is necessary to evaluate whether a similar time-of-day dependence of tinnitus can be shown for self-reported tinnitus as well as for more objectively measured tinnitus. Future studies could also compare different statistical approaches and identify the approach most suited to investigate time-of-day dependencies. For example, harmonic regression was performed in an animal study on circadian rhythm (Atger et al., 2015), and an R package for nonparametric circular methods (NPCirc) is available to analyze circular data (Oliveira et al., 2014). These approaches were not performed in the present study due to the nested structure of the data (assessments within days and days within participants).

In summary, the results of our study have implications for both tinnitus research and tinnitus management. Taking time-of-day into account in the study design might be necessary in clinical studies. Yet, the results of our study rely solely on TYT users (who are not representative for clinical tinnitus patients, see Probst et al., 2017) and on one-item questions on tinnitus distress and tinnitus loudness making it difficult to conclude whether the observed differences between night/early morning and the upcoming day are clinically relevant. Future studies using assessment instruments appropriate to distinguish between clinically relevant and clinically irrelevant change (for example the Tinnitus Handicap Inventory (THI), see Zeman et al., 2011) with clinical tinnitus samples are necessary to address this point. Chronobiological aspects should not only be considered in tinnitus research but also in tinnitus treatment. Tailoring the timing of therapeutic interventions to the circadian rhythm of individual tinnitus patients (chronotherapy) might be promising. Further translational and clinical studies are necessary to evaluate the potential of chronotherapy for tinnitus. However, this is a challenging task since "research in the topic is underfunded when compared with other diseases for which the prevalence and cost to society is relatively similar" (Cederroth et al., 2013, p. 972). Nevertheless, the current study might motivate individuals with tinnitus to observe their tinnitus fluctuations and to identify time-intervals during which the access to and the use of tinnitus coping strategies are most crucial.

AUTHOR CONTRIBUTIONS

TP substantially contributed to the design of the study and data preparation, drafted and revised the manuscript. RP substantially contributed to the design of the study, data preparation, conception, implementation and maintenance of the "TrackYourTinnitus" application, and revised the manuscript. BL substantially contributed to the design of the study and revised the manuscript. JR substantially contributed to the design of the study and revised the manuscript. JS substantially contributed to the conception, implementation and maintenance of the "TrackYourTinnitus" application, and revised the manuscript. MR substantially contributed to the conception, implementation and maintenance of the "TrackYourTinnitus" application, and revised the manuscript. MS substantially contributed to the design of the study and revised the manuscript. WS substantially contributed to the design of the study, data preparation, conception and implementation of the "TrackYourTinnitus" application, drafted and revised the manuscript. JZ substantially contributed to the design of the study and performed the statistical analysis, drafted and revised the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnagi.2017.00253/full#supplementary-material

REFERENCES

Adams, W. Z., McClure, E. A., Gray, K. M., Danielson, C. K., Treiber, F. A., and Ruggiero, K. J. (2017). Mobile devices for the remote acquisition of physiological and behavioral biomarkers in psychiatric clinical research. *J. Psychiatr. Res.* 85, 1–14. doi: 10.1016/j.jpsychires.2016.10.019

Ajayi, O. V., Phillips, J. S., Laopaiboon, M., and McFerran, D. (2014). Melatonin for tinnitus. *Cochrane Database Syst. Rev.* 12:CD011435. doi: 10.1002/14651858.CD011435

Alsalman, O. A., Tucker, D., and Vanneste, S. (2016). Salivary stress-related responses in tinnitus: a preliminary study in young male subjects with tinnitus. *Front. Neurosci.* 10:338. doi: 10.3389/fnins.2016.00338

Atger, F., Gobet, C., Marquis, J., Martin, E., Wang, J., Weger, B., et al. (2015). Circadian and feeding rhythms differentially affect rhythmic mRNA transcription and translation in mouse liver. *Proc. Natl. Acad. Sci. U.S.A.* 112, E6579–E6588. doi: 10.1073/pnas.1515308112

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Basinou, V., Park, J.- S., Cederroth, C. R., and Canlon, B: (2017). Circadian regulation of auditory function. *Hear. Res.* 347, 47–55. doi: 10.1016/j.heares.2016.08.018

Bates, D., Maechler, M., Bolker, B, and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. doi: 10.18637/jss.v067.i01

Bechter, K., Wieland, M., and Hamann, G. F. (2016). Chronic cervicogenic tinnitus rapidly resolved by intermittent use of cervical collar. *Front. Psychiatry* 7:43. doi: 10.3389/fpsyt.2016.00043

Bhatt, J. M., Bhattacharyya, N., and Lin, H. W. (2016). Relationships between tinnitus and the prevalence of anxiety and depression. *Laryngoscope* 127, 466–469. doi: 10.1002/lary.26107

Buttgereit, F., Smolen, J. S., Coogan, A. N., and Cajochen, C. (2015). Clocking in: chronobiology in rheumatoid arthritis. *Nat. Rev. Rheumatol.* 11, 349–356. doi: 10.1038/nrrheum.2015.31

Cederroth, C. R., Canlon, B., and Langguth, B. (2013). Hearing loss and tinnitus-are funders and industry listening? *Nat. Biotechnol.* 31, 972–974. doi: 10.1038/nbt.2736

Cima, R. F., Crombez, G., and Vlaeyen, J. W. (2011). Catastrophizing and fear of tinnitus predict quality of life in patients with chronic tinnitus. *Ear. Hear.* 32, 634–641. doi: 10.1097/AUD.0b013e31821106dd

Conway-Campbell, B. L., Pooley, J. R., Hager, G. L., and Lightman, S. L. (2012). Molecular dynamics of ultradian glucocorticoid receptor action. *Mol. Cell. Endocrinol.* 348, 383–393. doi: 10.1016/j.mce.2011.08.014

Crönlein, T., Langguth, B., Geisler, P., and Hajak, G. (2007). Tinnitus and insomnia. *Prog. Brain. Res.* 166, 227–233. doi: 10.1016/S0079-6123(07)66021-X

Crönlein, T., Langguth, B., Pregler, M., Kreuzer, P. M., Wetter, T. C., and Schecklmann, M. (2016). Insomnia in patients with chronic tinnitus: cognitive and emotional distress as moderator variables. *J. Psychosom. Res.* 83, 65–68. doi: 10.1016/j.jpsychores.2016.03.001

Dauman, N., Erlandsson, S., Lundlin, L., and Dauman, R. (2015). Intra-individual variability in tinnitus patients: current thoughts and perspectives. *HNO*. 63, 302–306. doi: 10.1007/s00106-014-2978-2

De Ridder, D., Congedo, M., and Vanneste, S. (2015). The neural correlates of subjectively perceived and passively matched loudness perception in auditory phantom perception. *Brain Behav.* 5: e00331. doi: 10.1002/brb3.331

De Ridder, D., Elgoyhen, A. B., Romo, R., and Langguth, B. (2011). Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8075–8080. doi: 10.1073/pnas.1018466108

De Ridder, D., Vanneste, S., Weisz, N., Londero, A., Schlee, W., Elgoyhen, A. B., et al. (2014). An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci. Biobehav. Rev.* 44, 16–32. doi: 10.1016/j.neubiorev.2013.03.021

Dickmeis, T. (2009). Glucocorticoids and the circadian clock. *J. Endocrinol.* 200, 3–22. doi: 10.1677/JOE-08-0415

Flor, H., Hoffmann, D., Struve, M., and Diesch, E. (2004). Auditory discrimination training for the treatment of tinnitus. *Appl. Psychophysiol. Biofeedback* 29, 113–120. doi: 10.1023/B:APBI.0000026637.77671.f4

Germain, A., and Kupfer, D. J. (2008). Circadian rhythm disturbances in depression. *Hum. Psychopharmacol.* 23, 571–585. doi: 10.1002/hup.964

Gilron, I., and Ghasemlou, N. (2014). Chronobiology of chronic pain: focus on diurnal rhythmicity of neuropathic pain. *Curr. Opin. Support. Palliat. Care* 8, 429–436. doi: 10.1097/SPC.0000000000000085

Hébert, S., and Fournier, P. (2017). Clinical validation of a new tinnitus assessment technology. *Front. Neurol.* 8:38. doi: 10.3389/fneur.2017.00038

Hébert, S., and Lupien, S. J. (2007). The sound of stress: blunted cortisol reactivity to psychosocial stress in tinnitus sufferers. *Neurosci. Lett.* 411, 138–142. doi: 10.1016/j.neulet.2006.10.028

Hébert, S., and Lupien, S. J. (2009). Salivary cortisol levels, subjective stress, and tinnitus intensity in tinnitus sufferers during noise exposure in the laboratory. *Int. J. Hyg. Environ. Health* 212, 37–44. doi: 10.1016/j.ijheh.2007.11.005

Hébert, S., Paiement, P., and Lupien, S. J. (2004). A physiological correlate for the intolerance to both internal and external sounds. *Hear. Res.* 190, 1–9. doi: 10.1016/S0378-5955(04)00021-8

Henry, J. A., Galvez, G., Turbin, M. B., Thielman, E. J., McMillan, G. P., and Istvan, J. A. (2012). Pilot study to evaluate ecological momentary assessment of tinnitus. *Ear. Hear.* 33, 179–290. doi: 10.1097/AUD.0b013e31822f6740

Hesser, H., and Andersson, G. (2009). The role of anxiety sensitivity and behavioral avoidance in tinnitus disability. *Int. J. Audiol.* 48, 295–299. doi: 10.1080/14992020802635325

Hesser, H., Weise, C., Westin, V. Z., and Andersson, G. (2011). A systematic review and meta-analysis of randomized controlled trials of cognitive-behavioral therapy for tinnitus distress. *Clin. Psychol. Rev.* 31, 545–553. doi: 10.1016/j.cpr.2010.12.006

Hiller, W., and Goebel, G. (2007). When tinnitus loudness and annoyance are discrepant: audiological characteristics and psychological profile. *Audiol. Neurootol.* 12, 391–400. doi: 10.1159/000106482

Hoare, D. J., Edmondson-Jones, M., Gander, P. E., and Hall, D. A. (2014). Agreement and reliability of tinnitus loudness matching and pitch likeness rating. *PLoS ONE* 9:e114553. doi: 10.1371/journal.pone.0114553

Hobson, J., Chisholm, E., and El Refaie, A. (2012). Sound therapy (masking) in the management of tinnitus in adults. *Cochrane Database Syst. Rev.* 11:CD006371. doi: 10.1002/14651858.cd006371.pub3

Hothorn, T., Bretz, F., and Westfall, P. (2008). Simultaneous inference in general parametric models. *Biom. J.* 50, 346–363. doi: 10.1002/bimj.200810425

Jasper, K., Weise, C., Conrad, I., Andersson, G., Hiller, W., and Kleinstäuber, M. (2014). Internet-based guided self-help versus group cognitive behavioral therapy for chronic tinnitus: a randomized controlled trial. *Psychother. Psychosom.* 83, 234–246. doi: 10.1159/000360705

Kleinstäuber, M., Jasper, K., Schweda, I., Hiller, W., Andersson, G., and Weise, C. (2013). The role of fear-avoidance cognitions and behaviors in patients with chronic tinnitus. *Cogn. Beh. Ther.* 42, 84–99. doi: 10.1080/16506073.2012.717301

Landgrebe, M., Azevedo, A., Baguley, D., Bauer, C., Cacace, A., Coelho, C., et al. (2012). Methodological aspects of clinical trials in tinnitus: a proposal for an international standard. *J. Psychosom. Res.* 73, 112–121. doi: 10.1016/j.jpsychores.2012.05.002

Langguth, B., and De Ridder, D. (2013). Tinnitus: therapeutic use of superficial brain stimulation. *Handb. Clin. Neurol.* 116, 441–467. doi: 10.1016/B978-0-444-53497-2.00036-X

Langguth, B., and Elgoyhen, A. B. (2012). Current pharmacological treatments for tinnitus. *Expert Opin. Pharmacother.* 13, 2495–2509. doi: 10.1517/14656566.2012.739608

Langguth, B., Kreuzer, P. M., Kleinjung, T., and De Ridder, D. (2013). Tinnitus: causes and clinical management. *Lancet Neurol.* 12, 920–930. doi: 10.1016/S1474-4422(13)70160-1

Langguth, B., Landgrebe, M., Kleinjung, T., Sand, G. P., and Hajak, G. (2011). Tinnitus and depression. *World J. Biol. Psychiatry* 12, 489–500. doi: 10.3109/15622975.2011.575178

Leaver, A. M., Renier, L., Chevillet, M. A., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2011). Dysregulation of limbic and auditory networks in tinnitus. *Neuron* 69, 33–43. doi: 10.1016/j.neuron.2010.12.002

Leaver, A. M., Seydell-Greenwald, A., Turesky, T. K., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2012). Cortico-limbic morphology separates tinnitus from tinnitus distress. *Front. Syst. Neurosci.* 6:21. doi: 10.3389/fnsys.2012.00021

Lightman, S. L., and Conway-Campbell, B. L. (2010). The crucial role of pulsatile activity of the HPA axis for continuous dynamic equilibration. *Nat. Rev. Neurosci.* 11, 710–718. doi: 10.1038/nrn2914

Lowe, A. S., and Walton, J. P. (2015). Alterations in peripheral and central components of the auditory brainstem response: a neural assay of tinnitus. *PLoS ONE* 10:e0117228. doi: 10.1371/journal.pone.0117228

Maes, I. H., Cima, R. F., Anteunis, L. J., Scheijen, D. J., Baguley, D. M., El Refaie, A., et al. (2014). Cost-effectiveness of specialized treatment based on cognitive behavioral therapy versus usual care for tinnitus. *Otol. Neurotol.* 35, 787–795. doi: 10.1097/MAO.0000000000000331

Maes, I. H., Cima, R. F., Vlaeyen, J. W., Anteunis, L. J., and Joore, M. A. (2013). Tinnitus: a cost study. *Ear. Hear.* 34, 508–514. doi: 10.1097/AUD.0b013e31827d113a

Martinez, C., Wallenhorst, C., McFerran, D., and Hall, D. A. (2015). Incidence rates of clinically significant tinnitus: 10-year trend from a cohort study in England. *Ear. Hear.* 36, e69–75. doi: 10.1097/aud.0000000000000121

McCormack, A., Edmondson-Jones, M., Somerset, S, and Hall, D. (2016). A systematic review of the reporting of tinnitus prevalence and severity. *Hear. Res.* 337, 70–79. doi: 10.1016/j.heares.2016.05.009

McKenna, L., Handscomb, L., Hoare, D. J., and Hall, D. A. (2014). A scientific cognitive-behavioral model of tinnitus: novel conceptualizations of tinnitus distress. *Front. Neurol.* 5:196. doi: 10.3389/fneur.2014.00196

Meltser, I., Cederroth, C. R., Basinou, V., Savelyev, S., Lundkvist, G. S., and Canlon, B. (2014). TrkB-mediated protection against circadian sensitivity to noise trauma in the murine cochlea. *Curr. Biol.* 24, 658–663. doi: 10.1016/j.cub.2014.01.047

Miroddi, M., Bruno, R., Galletti, F., Calapai, F., Navarra, M., Gangemi, S., et al. (2015). Clinical pharmacology of melatonin in the treatment of tinnitus: a review. *Eur. J. Clin. Pharmacol.* 71, 263–270. doi: 10.1007/s00228-015-1805-3

Newman, C. W., Sandridge, S. A., and Bolek, L. (2008). Development and psychometric adequacy of the screening version of the tinnitus handicap inventory. *Otol. Neurotol.* 29, 276–281. doi: 10.1097/MAO.0b013e31816569c4

Nondahl, D. M., Cruickshanks, K. J., Huang, G. H., Klein, B. E., Klein, R., Tweed, T. S., et al. (2012). Generational differences in the reporting of tinnitus. *Ear. Hear.* 33, 640–644. doi: 10.1097/AUD.0b013e31825069e8

Oliveira, M., Crujeiras, R. M., and Rodríguez–Casal, A. (2014). NPCirc: an R package for nonpara-metric circular methods. *J. Stat. Soft.* 61, 1–26. doi: 10.18637/jss.v061.i09

Park, J., S., Cederroth, C. R., Basinou, V., Meltser, I., Lundkvist, G., and Canlon, B. (2016). Identification of a circadian clock in the inferior colliculus and its dysregulation by noise exposure. *J. Neurosci.* 36, 5509–5519. doi: 10.1523/JNEUROSCI.3616-15.2016

Pirodda, A., Raimondi, M. C., and Ferri, G. G. (2010). Exploring the reasons why melatonin can improve tinnitus. *Med. Hypotheses* 75, 190–191. doi: 10.1016/j.mehy.2010.02.018

Probst, T., Pryss, R., Langguth, B., and Schlee, W. (2016a). Emotional states as mediators between tinnitus loudness and tinnitus distress in daily life: results from the "TrackYourTinnitus" application. *Sci. Rep.* 6:20382. doi: 10.1038/srep20382

Probst, T., Pryss, R., Langguth, B., and Schlee, W. (2016b). Emotion dynamics and tinnitus: daily life data from the "TrackYourTinnitus" application. *Sci. Rep.* 6:31166. doi: 10.1038/srep31166

Probst, T., Pryss, R. C., Langguth, B., Spiliopoulou, M., Landgrebe, M., Vesala, M., et al. (2017). Outpatient tinnitus clinic, self-help web platform, or mobile application to recruit tinnitus study samples? *Front. Aging Neurosci.* 9:113. doi: 10.3389/fnagi.2017.00113

Pryss, R., Reichert, M., Herrmann, J., Langguth, B., Schlee, W. (2015a). "Mobile crowd sensing in clinical and psychological trials - a case study," in *28th IEEE Int'l Symposium on Computer-Based Medical Systems, 22–25 June 2015* (Sao Carlos: IEEE Computer Society Press), 23–24. Available online at: http://dbis.eprints.uni-ulm.de/1144/1/PCBMS_2015.pdf (Accessed July 15, 2017)

Pryss, R., Reichert, M., Langguth, B., and Schlee, W. (2015b). "Mobile crowd sensing services for tinnitus assessment, therapy and research," in *IEEE 4th International Conference on Mobile Services (MS 2015), June 27–July 2, 2015* (New York, NY: IEEE Computer Society Press), 352–359. Available online at: http://dbis.eprints.uni-ulm.de/1152/1/ms2015rpmrblws.pdf (Accessed: July 15, 2017).

Raudenbush, S. W., and Bryk, A. S. (2002). *Hierarchical Linear Models: Applications and Data Analysis Methods*. Newbury Park, CA: Sage Publications.

Rauschecker, J. P., Leaver, A. M., and Mühlau, M. (2010). Tuning out the noise: limbic-auditory interactions in tinnitus. *Neuron* 66, 819–826. doi: 10.1016/j.neuron.2010.04.032

Rauschecker, J. P., May, E. S., Maudoux, A., and Ploner, M. (2015). Frontostriatal gating of tinnitus and chronic pain. *Trends Cogn. Sci.* 19, 567–578. doi: 10.1016/j.tics.2015.08.002

R Core Team, A. (2015). *R: A Language and Environment for Statistical Computing*. Vienna: R foundation for statistical computing. Available online at: https://www.R-project.org/ (Accessed: November 25, 2016).

Riedl, D., Rumpold, G., Schmidt, A., Zorowka, P. G., Bliem, H. R., and Moschen, R. (2015). The influence of tinnitus acceptance on the quality of life and psychological distress in patients with chronic tinnitus. *Noise Health* 17, 374–381. doi: 10.4103/1463-1741.165068

Schecklmann, M., Pregler, M., Kreuzer, P. M., Poeppl, T. B., Lehner, A., Crönlein, T., et al. (2015). Psychophysiological associations between chronic tinnitus and sleep: a cross validation of tinnitus and insomnia questionnaires. *BioMed. Res. Int.* 2015:461090. doi: 10.1155/2015/461090

Schlee, W., Pryss, R. C., Probst, T., Schobel, J., Bachmeier, A., Reichert, M., et al. (2016). Measuring the moment-to-moment variability of tinnitus: the TrackYourTinnitus smart phone app. *Front. Aging Neurosci.* 8:294. doi: 10.3389/fnagi.2016.00294

Schlee, W., Schecklmann, M., Lehner, A., Kreuzer, P. M., Vielsmeier, V., Poeppl, T. B., et al. (2014). Reduced variability of auditory alpha activity in chronic tinnitus. *Neural. Plast.* 2014:436146. doi: 10.1155/2014/436146

Singer, J. D., and Willett, J. B. (2003). *Applied Longitudinal Data Analysis: Modeling Change and Event Occurence*. New York, NY: Oxford University Press. doi: 10.1093/acprof:oso/9780195152968.001.0001

Strian, F., Lautenbacher, S., Galfe, G., and Hölzl, R. (1989). Diurnal variations in pain perception and thermal sensitivity. *Pain* 36, 125–131. doi: 10.1016/0304-3959(89)90120-6

Trull, T. J., and Ebner-Priemer, U. (2013). Ambulatory assessment. *Annu. Rev. Clin. Psychol.* 9, 151–176. doi: 10.1146/annurev-clinpsy-050212-185510

Trull, T. J., and Ebner-Priemer, U. (2014). The role of ambulatory assessment in psychological science. *Curr. Dir. Psychol. Sci.* 23, 466–470. doi: 10.1177/0963721414550706

Ueyama, T., Donishi, T., Ukai, S., Ikeda, Y., Hotomi, M., Yamanaka, N., et al. (2013). Brain regions responsible for tinnitus distress and loudness: a resting-state FMRI study. *PLoS ONE* 8:e67778. doi: 10.1371/journal.pone.0067778

Vanneste, S., Congedo, M., and De Ridder, D. (2014). Pinpointing a highly specific pathological functional connection that turns phantom sound into distress. *Cereb. Cortex* 24, 2268–2282. doi: 10.1093/cercor/bht068

Wallhäusser-Franke, E., Brade, J., Balkenhol, T., D'Amelio, R., Seegmüller, A., and Delb, W. (2012). Tinnitus: distinguishing between subjectively perceived loudness and tinnitus-related distress. *PLoS ONE* 7:e34583. doi: 10.1371/journal.pone.0034583

Weise, C., Kleinstäuber, M., Hesser, H., Westin, V. Z., and Andersson, G. (2013). Acceptance of tinnitus: validation of the tinnitus acceptance questionnaire. *Cogn. Behav. Ther.* 42, 100–115. doi: 10.1080/16506073.2013.781670

Wilson, M. B., Kallogjeri, D., Joplin, C. N., Gorman, M. D., Krings, J. G., Lenze, E. J., et al. (2015). Ecological momentary assessment of tinnitus using smartphone technology: a pilot study. *Otolaryngol. Head. Neck. Surg.* 152, 897–903. doi: 10.1177/0194599815569692

Wirz-Justice, A. (2008). Diurnal variation of depressive symptoms. *Dialogues Clin. Neurosci.* 10, 337–343.

Zeman, F., Koller, M., Figueiredo, R., Aazevedo, A., Rates, M., Coelho, C., et al. (2011). Tinnitus handicap inventory for evaluating treatment effects: which changes are clinically relevant? *Otolaryngol. Head Neck Surg.* 145, 282–287. doi: 10.1177/0194599811403882

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Outpatient Tinnitus Clinic, Self-Help Web Platform, or Mobile Application to Recruit Tinnitus Study Samples?

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For understanding the heterogeneity of tinnitus, large samples are required. However, investigations on how samples recruited by different methods differ from each other are lacking. In the present study, three large samples each recruited by different means were compared: $N = 5017$ individuals registered at a self-help web platform for tinnitus (crowdsourcing platform Tinnitus Talk), $N = 867$ users of a smart mobile application for tinnitus (crowdsensing platform TrackYourTinnitus), and $N = 3786$ patients contacting an outpatient tinnitus clinic (Tinnitus Center of the University Hospital Regensburg). The three samples were compared regarding age, gender, and duration of tinnitus (month or years perceiving tinnitus; subjective report) using chi-squared tests. The three samples significantly differed from each other in age, gender and tinnitus duration ($p < 0.05$). Users of the TrackYourTinnitus crowdsensing platform were younger, users of the Tinnitus Talk crowdsourcing platform had more often female gender, and users of both newer technologies (crowdsourcing and crowdsensing) had more frequently acute/subacute tinnitus (<3 months and 4–6 months) as well as a very long tinnitus duration (>20 years). The implications of these findings for clinical research are that newer technologies such as crowdsourcing and crowdsensing platforms offer the possibility to reach individuals hard to get in contact with at an outpatient tinnitus clinic. Depending on the aims and the inclusion/exclusion criteria of a given study, different recruiting strategies (clinic and/or newer technologies) offer different advantages and disadvantages. In general, the representativeness of study results might be increased when tinnitus study samples are recruited in the clinic as well as via crowdsourcing and crowdsensing.

Keywords: tinnitus, recruitment, crowdsourcing, crowdsensing, clinical data

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INTRODUCTION

Tinnitus is characterized by the perception of a sound without a corresponding external sound source (Baguley et al., 2013; Langguth et al., 2013). A recent review on prevalence rates of tinnitus in 16 countries found that between 5.1% and 42.7% of the population report tinnitus (McCormack et al., 2016). The prevalence rates typically vary depending on the age, the birth cohort

(Nondahl et al., 2012; Martinez et al., 2015), and the definition of tinnitus used in the epidemiological study (McCormack et al., 2016). A recent US study found that 9.6% of Americans experienced tinnitus in the last year and that only 49.4% discussed the tinnitus with a physician (Bhatt et al., 2016). In case tinnitus patients seen by physicians are not representative for the whole sample of individuals perceiving tinnitus, the representativeness of studies recruiting patients only in medical practices or hospitals might be limited. In the area of anxiety and depression, for example, researchers found differences between patients contacting an outpatient clinic, patients being treated in an Internet-based clinic, and individuals with anxiety or depressive disorders from a national epidemiological survey (Titov et al., 2010). Another study on Internet-based psychotherapy for depression found that patients recruited by online advertisements differed from patients recruited by newspaper advertisements in demographics and depression severity (Lindner et al., 2015).

In the case of tinnitus, several patient databases have been established with the aim to better understand the heterogeneity of tinnitus (Meikle, 1997; Landgrebe et al., 2010; Witsell et al., 2011). However, all these databases collect data from patients who present themselves at a tinnitus clinic. For investigating tinnitus heterogeneity, it is of high importance to know how samples recruited by different methods vary and which recruitment methods are most efficient for the recruitment of specific tinnitus subgroups.

Although the recruitment of individuals not contacting physicians is traditionally difficult, online-recruitment has been shown to be promising in clinical and health science to overcome this obstacle (e.g., Morgan et al., 2013; Bevelander et al., 2014; Gioia et al., 2016; Kayrouz et al., 2016; Thornton et al., 2016; Topolovec-Vranic and Natarajan, 2016). In a recent review on online-recruitment in health studies, Lane et al. (2015) stated, though, that "more empirical evidence is needed to make specific recommendations" (p. 1). The claim for more empirical research might be especially relevant for the relatively new participant-led research paradigm (Vayena and Tasioulas, 2013) including crowdsourcing (e.g., Swan, 2012; Ranard et al., 2014; Chandler and Shapiro, 2016) and crowdsensing (e.g., Ganti et al., 2011; Guo et al., 2014, 2015). "Crowdsourcing is a type of participative online activity in which an individual, an institution, a non-profit organization, or company proposes to a group of individuals of varying knowledge, heterogeneity, and number, via a flexible open call, the voluntary undertaking of a task. The undertaking of the task, of variable complexity and modularity, and in which the crowd should participate bringing their work, money, knowledge and/or experience, always entails mutual benefit. The user will receive the satisfaction of a given type of need, be it economic, social recognition, self-esteem, or the development of individual skills, while the crowdsourcer will obtain and utilize to their advantage what the user has brought to the venture, whose form will depend on the type of activity undertaken" (Estellés-Arolas and González-Ladrón-de-Guevara, 2012, p. 197). Guo et al. (2015) defined mobile crowdsensing and computing (MCSC) as follows: "MCSC extends the vision of participatory sensing by leveraging both participatory sensory data from mobile devices (offline) and user-contributed data from mobile social networking services (online). Further, it explores the complementary roles and presents the fusion/collaboration of machine and human intelligence in the crowdsensing and computing processes" (Guo et al., 2015, p. 1). In contrast to crowdsourcing, crowdsensing relies solely on mobile technology and integrates sensors to collect data, for example, behavioral (e.g., physical activity level, gait pattern), physiological (e.g., heart rate, electrodermal activity), and environmental (e.g., environmental sound level, GPS location) variables.

As it remains unclear whether online-recruitment by crowdsourcing and crowdsensing offers the potential to reach individuals with tinnitus that are different from the ones directly consulting a physician, the study at hand compared tinnitus patients visiting an outpatient tinnitus clinic, users of a tinnitus crowdsourcing platform (moderated self-help web platform) and users of a tinnitus crowdsensing platform (mobile application).

MATERIALS AND METHODS

Samples

The following three samples were investigated:

1. Tinnitus crowdsourcing sample "Tinnitus Talk": Tinnitus Hub[1] operates the moderated self-help platform Tinnitus Talk[2]. The platform was established in March 2011 by Markku Vesala. With 16,500 registered participants (as of December 2016) and over 210,000 unique readers every month, Tinnitus Talk is one of the most active tinnitus-dedicated platforms for information delivery, experience exchange, and self-help among individuals with tinnitus. The data of the present study relies on a survey ran from February 8th till March 13th 2016.
2. Tinnitus crowdsensing sample "TrackYourTinnitus": TrackYourTinnitus (TYT; Pryss et al., 2015a,b)[3] is an application for mobile-devices (iOS and Android) that allows the tracking of tinnitus in daily life by ecological momentary assessments. Although monitoring tinnitus repeatedly directs the attention towards the tinnitus, Schlee et al. (2016) showed that using TYT does not deteriorate the tinnitus. Other studies on TYT investigated the role of emotional states (Probst et al., 2016a) as well as emotion dynamics (Probst et al., 2016b) in tinnitus. The data presented here was collected from April 2014 to June 2016.
3. Outpatient tinnitus clinic sample "Tinnitus Center Regensburg": The University Hospital Regensburg hosts a tinnitus center[4], established 2007, and having about 300 tinnitus patients per annum. To date (December 2016), the Tinnitus database encompasses medical records for about 3000 patients.

[1] www.tinnitushub.com
[2] www.tinnitustalk.com
[3] www.trackyourtinnitus.org
[4] www.tinnituszentrum-regensburg.de

At the Tinnitus Center Regensburg, patients gave written informed consent that data were gathered and analyzed for the Tinnitus Research Initiative Database, which was approved by the Ethics Committee of the University Hospital of Regensburg. The material and the methods of TrackYourTinnitus were approved by the Ethics Committee of the University Hospital Regensburg and were carried out in accordance with the approved guidelines; written consent, however, was not possible to obtain for the users of TrackYourTinnitus. Information that the data will be used for scientific analyses is included in the mobile applications as well as on the website and, therefore, the TrackYourTinnitus users were informed that the data will be used for scientific purposes. Written informed consent was also not possible to obtain for the users of Tinnitus Hub/Tinnitus Talk, but the "Terms and Rules" of the website informed the users that the collected data will be analyzed for scientific purposes. All the data were saved anonymously.

Measures

Three variables were assessed in the three samples by self-reports: age, gender, and duration of tinnitus (month or years perceiving tinnitus; subjective report). In the Tinnitus Talk survey, the variables age (<18 years, 18–24 years, . . .; see **Table 1**) and tinnitus duration (<3 months, 4–6 months, . . .; see **Table 1**) were assessed in categories. Therefore, the metric scores of age and tinnitus duration as assessed in the Tinnitus Center Regensburg and TrackYourTinnitus were categorized accordingly to be able to perform statistical analyses.

Statistical Analysis

Chi-squared tests were performed with R and the significance value was set to $p < 0.05$.

TABLE 1 | Description of the three samples and comparisons between the samples.

	Tinnitus Talk	TrackYour Tinnitus	Tinnitus Center Regensburg	Between-group statistics
Total number	5017	867	3786	
Age in years				$X^2 = 366.7$; $p < 0.001$
% <18	1.0	1.6	0.5	
% 18–24	5.4	4.4	2.4	
% 25–34	11.4	20.7	6.6	
% 35–44	13.6	22.5	15.7	
% 45–55	20.9	26.5	29.6	
% 55–64	30.2	18.9	26.7	
% 65–74	15.1	5.2	15.5	
% >75	2.4	0.3	3.1	
Gender				$X^2 = 103.5$; $p < 0.001$
% female	42.8	27.1	35.1	
% male	57.2	72.9	64.9	
Tinnitus duration				$X^2 = 393.7$; $p < 0.001$
% <3 months	6.4	14.2	2.1	
% 4–6 months	5.7	6.8	3.4	
% 6–12 months	10.1	7.0	9.3	
% 1–2 years	14.7	7.6	16.2	
% 3–5 years	13.1	15.4	20.6	
% 5–10 years	18.3	16.0	17.1	
% 10–20 years	15.3	16.9	20.7	
% >20 years	16.4	16.1	10.6	

RESULTS

The three samples comprised $N = 9670$ individuals. Information on age was available for $n = 8766$ individuals, information on gender for $n = 9607$, and information on tinnitus duration for $n = 8409$. Full descriptions of the samples are presented in **Table 1**.

Age

A chi-squared test was calculated to test whether the distribution of age (see **Table 1**; **Figure 1**) is different between the samples. With an $X^2 = 366.7$, the hypothesis that the samples had an equal age distribution was rejected ($p < 0.001$). The most obvious differences emerged in the percentage of individuals with an age between 25 and 44 years as well as in the percentage of individuals with an age between 55 and 74 years: The 25–44 years aged individuals were more often among the TrackYourTinnitus users (25–34 years: 20.7%; 35–44 years: 22.5%) than among the Tinnitus Talk users (25–34 years: 11.4%; 35–44 years: 13.6%) and among the patients of the Tinnitus Center Regensburg (25–34 years: 6.6%; 35–44 years: 15.7%). Yet, the 55–74 years aged adults were more frequently among the Tinnitus Talk users (55–64 years: 30.2%; 65–74 years: 15.1%) and among the patients at the Tinnitus Center Regensburg (55–64 years: 26.7%; 65–74 years: 15.5%) than among the TrackYourTinnitus users (55–64 years: 18.9%; 65–74 years: 5.2%).

Gender

A chi-squared test was also calculated to investigate whether the gender distribution of the three sample groups is equal or different (see **Table 1**; **Figure 2**). Based on an $X^2 = 103.5$, the hypothesis of an equal gender distribution was rejected ($p < 0.001$). Although the participants were more frequently men than women in all three samples, TrackYourTinnitus

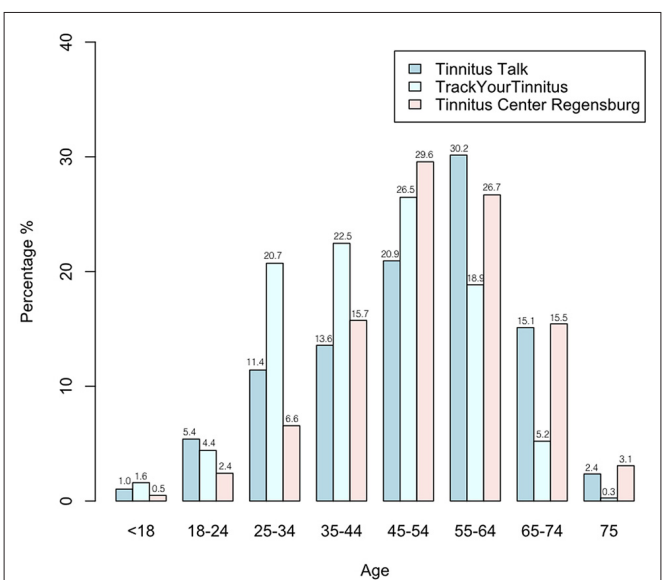

FIGURE 1 | Distribution of age in the three samples.

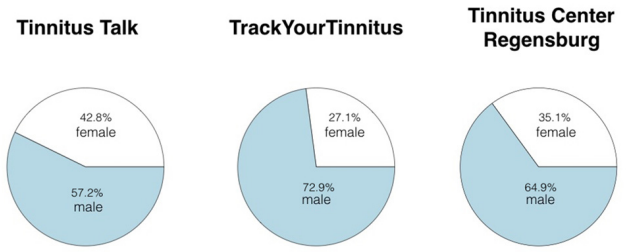

FIGURE 2 | Distribution of gender in the three samples.

had the lowest rate of females (27.1%) and Tinnitus Talk the highest (42.8%).

Tinnitus Duration

Again a chi-squared test was calculated to test whether the distribution of tinnitus duration is equal or different between the three samples (see **Table 1**; **Figure 3**). With an X^2 of 393.7, the hypothesis of an equal tinnitus duration distribution was rejected ($p < 0.001$). Differences were apparent for individuals with a tinnitus duration <3 months: 14.2% of the TrackYourTinnitus users were in this category, but only 6.4% of the Tinnitus Talk users and only 2.1% of the Tinnitus Center Regensburg patients. However, TrackYourTinnitus users reported less often 1–2 years of tinnitus duration (7.6%) than Tinnitus Talk users (14.7%) and patients at the Tinnitus Center Regensburg (16.2%). The Tinnitus Center Regensburg patients reported more often 3–5 years and 10–20 years of tinnitus duration (3–5 years: 20.6%; 10–20 years: 20.7%) than Tinnitus Talk users (3–5 years: 13.1%; 10–20 years: 15.3%) and TrackYourTinnitus users (3–5 years: 15.4%; 10–20 years: 16.9%). The individuals with tinnitus duration >20 years, however, were more frequently among the Tinnitus Talk users (16.4%) and the TrackYourTinnitus users (16.1%) than among the patients at the Tinnitus Center Regensburg (10.6%).

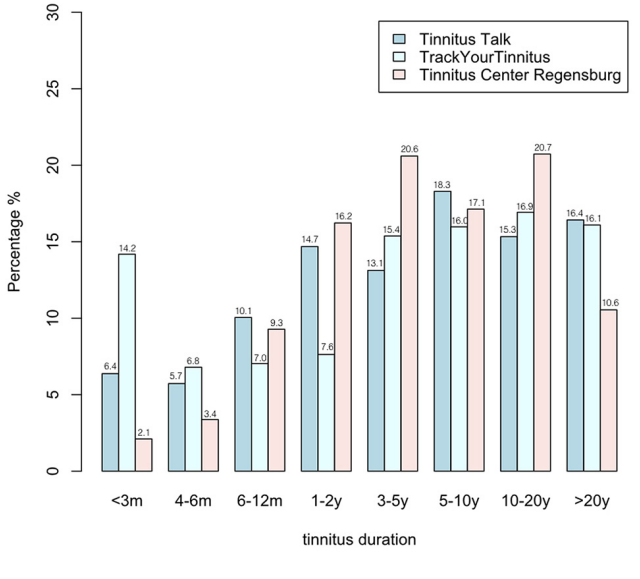

FIGURE 3 | Distribution of tinnitus duration in the three samples.

DISCUSSION

The study at hand compared individuals contacting an outpatient tinnitus clinic (Tinnitus Center Regensburg), individuals registered at a self-help web platform (crowdsourcing platform Tinnitus Talk) and users of an application for mobile devices (crowdsensing platform TrackYourTinnitus). The aim of the study was to investigate whether newer technologies (crowdsourcing and/or crowdsensing) offer possibilities for future studies to reach individuals with tinnitus that are different from the ones directly contacting an outpatient clinic. In summary, we found that the samples differed in the investigated variables. The result that patients of an outpatient clinic differed from users of newer technologies is in line with a study on anxiety and depression comparing patients of an outpatient clinic with those attending an Internet clinic (Titov et al., 2010). Moreover the results of the current study correspond with a recent review (Topolovec-Vranic and Natarajan, 2016), which reported that populations recruited by traditional methods were not comparable to samples recruited by newer technologies (the review focused on social media) in most of the studies (12 vs. 2).

One of the variables investigated in the present study was age. The results revealed that younger individuals (≤44 years) used more frequently the crowdsensing platform for tinnitus, whereas older individuals (≥55 years) were more often among the users of the crowdsourcing platform for tinnitus as well as among the patients of an outpatient tinnitus clinic. Thus, crowdsensing might be potent to recruit younger individuals but not as suited for the recruitment of older individuals. Nevertheless, crowdsensing might be appropriate to recruit older individuals in the future as the younger generation (which now uses crowdsensing) becomes older.

Another evaluated variable in the current study was gender. Gender was predominately male in all three samples and this result is in line with previous research on tinnitus: "In fact, most previous studies, but not all ... showed higher tinnitus prevalence in men than in women" (Gallus et al., 2015; p. 16). The crowdsourcing platform Tinnitus Talk had the highest percentage of female individuals, whereas the crowdsensing platform TrackYourTinnitus had the lowest. Therefore, crowdsourcing might be more appropriate than crowdsensing when aiming to recruit women by newer technologies.

The third investigated variable in the study at hand was tinnitus duration (month or years perceiving tinnitus; subjective report). Duration of tinnitus is clinically and scientifically relevant to define acute, subacute and chronic tinnitus. The results of the current study revealed that acute and subacute tinnitus are more frequent among the users of crowdsourcing and crowdsensing platforms than among the patients of an outpatient clinic. Long waiting times for appointments in specialized tinnitus clinic might contribute to this effect.

Regardless the reasons for the delay in seeing a specialist, our data indicate that individuals with tinnitus search for information or help in the Internet already early after symptom onset. As interventions are already helpful in the acute and subacute stages of tinnitus (Nyenhuis et al., 2013b) and might prevent a chronic course, offering helpful interventions for individuals with acute and subacute tinnitus is crucial. As more of these individuals can be reached by crowdsourcing or crowdsensing platforms than by an outpatient clinic, the implementation of appropriate self-help interventions in crowdsourcing or crowdsensing platforms might be promising (e.g., Heron and Smyth, 2010; Donker et al., 2013). The finding that Internet-based self-help is as effective as face-to-face interventions for tinnitus patients (e.g., Nyenhuis et al., 2013a; Jasper et al., 2014; Andersson, 2015) supports this line of argumentation. Another interesting result in the context of tinnitus duration was that individuals who perceived their tinnitus for a very long time (>20 years), were more frequent among the crowdsourcing and crowdsensing samples than among the sample of the outpatient clinic. It could be speculated that these individuals experienced past treatments as ineffective and consequently gave up seeking medical or psychological help (learned helplessness; Abramson et al., 1978) or have already established effective coping strategies. These individuals will not contact a physician or a clinic, although interventions, which these individuals have not tried yet, might be helpful. These findings at least suggest that it might be useful to provide information about interventions and current innovative treatment approaches in crowdsourcing or crowdsensing platforms.

One limitation of the study at hand is that we analyzed only one crowdsourcing platform for tinnitus, only one crowdsensing platform for tinnitus, and only one outpatient tinnitus clinic. Although our samples are relatively large, even more representative results might be obtained by multicenter studies including several clinics and several crowdsourcing (two crowdsourcing platforms were investigated, for example, by Briones and Benham, 2017) as well as several crowdsensing platforms. Another shortcoming of the crowdsourced and crowdsensed data is that the robustness and accuracy of the online-collected data is difficult to verify. Previous studies could, however, provide support for the trustworthiness of web-based research (e.g., Meyerson and Tryon, 2003; Gosling et al., 2004). Moreover comparisons with data from population-based epidemiological studies (see Titov et al., 2010) would be desirable to identify how representative the three samples of the current study are for the total tinnitus population. We abstained from such a comparison, since data from available epidemiological studies are highly variable (McCormack et al., 2016). Finally, since clinical variables were not assessed in all three samples, we could not compare the three samples regarding clinical characteristics of tinnitus (e.g., tinnitus severity). Tinnitus severity is usually important in the recruitment process of clinical studies and usually is measured by psychometrically sound instruments such as the "Tinnitus Handicap Inventory" (THI; Newman et al., 1996). Those who use an app or online site may have less bothersome tinnitus and may have no intention of seeking medical attention. At least some may be just curious but not need any treatment or intervention. Comparing the severity of these groups is an important task for future research to clarify whether recruiting individuals with tinnitus using a tinnitus app or website will aid our understanding of bothersome tinnitus. Such further studies will aid to find the best recruitment strategy for a given study.

Despite these limitations, the current study is the first one comparing tinnitus patients of an outpatient clinic with users of crowdsourcing and crowdsensing platforms for tinnitus. We showed that these newer technologies offer promising perspectives to reach individuals hard to get in contact with at an outpatient tinnitus clinic (e.g., individuals with acute/subacute tinnitus, younger individuals, as well as individuals perceiving tinnitus for a very long time).

AUTHOR CONTRIBUTIONS

TP substantially contributed to the design of the study and data preparation, drafted and revised the manuscript. RCP substantially contributed to the design of the study, data preparation, the TrackYourTinnitus platform and revised the manuscript. BL substantially contributed to the design of the study, data collection at the Tinnitus Center Regensburg, the TrackYourTinnitus platform and revised the manuscript. MS substantially contributed to the design of the study and revised the manuscript. ML substantially contributed to the data collection at the Tinnitus Center Regensburg and revised the manuscript. MV and SH substantially contributed to the Tinnitus Talk platform, and revised the manuscript. JS, MR and MS substantially contributed to the TrackYourTinnitus platform, and revised the manuscript. WS substantially contributed to the design of the study, the TrackYourTinnitus platform, drafted and revised the manuscript, and performed the statistical analyses.

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REFERENCES

Abramson, L. Y., Seligman, M. E., and Teasdale, J. D. (1978). Learned helplessness in humans: critique and reformulation. *J. Abnorm. Psychol.* 87, 49–74. doi: 10.1037/0021-843x.87.1.49

Andersson, G. (2015). Clinician-supported internet-delivered psychological treatment of tinnitus. *Am. J. Audiol.* 24, 299–301. doi: 10.1044/2015_AJA-14-0080

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Bevelander, K. E., Kaipainen, K., Swain, R., Dohle, S., Bongard, J. C., Hines, P. D., et al. (2014). Crowdsourcing novel childhood predictors of adult obesity. *PLoS One* 9:e87756. doi: 10.1371/journal.pone.0087756

Bhatt, J. M., Lin, H. W., and Bhattacharyya, N. (2016). Prevalence, severity, exposures and treatment patterns of tinnitus in the United States. *JAMA Otolaryngol. Head. Neck. Surg.* 142, 959–965. doi: 10.1001/jamaoto.2016.1700

Briones, E. M., and Benham, G. (2017). An examination of the equivalency of self-report measures obtained from crowdsourced versus undergraduate student samples. *Behav. Res. Methods* 49, 320–334. doi: 10.3758/s13428-016-0710-8

Chandler, J., and Shapiro, D. (2016). Conducting clinical research using crowdsourced convenience samples. *Annu. Rev. Clin. Psychol.* 12, 53–81. doi: 10.1146/annurev-clinpsy-021815-093623

Donker, T., Petrie, K., Proudfoot, J., Clarke, J., Birch, M. R., and Christensen, H. (2013). Smartphones for smarter delivery of mental health programs: a systematic review. *J. Med. Internet Res.* 15:e247. doi: 10.2196/jmir.2791

Estellés-Arolas, E., and González-Ladrón-de-Guevara, F. (2012). Towards an integrated crowdsourcing definition. *J. Inf. Sci.* 38, 189–200. doi: 10.1177/0165551512437638

Gallus, S., Lugo, A., Garavello, W., Bosetti, C., Santoro, E., Colombo, P., et al. (2015). Prevalence and determinants of tinnitus in the Italian adult population. *Neuroepidemiology* 45, 12–19. doi: 10.1159/000431376

Ganti, R. K., Ye, F., and Lei, H. (2011). Mobile crowdsensing: current state and future challenges. *IEEE Commun. Mag.* 49, 32–39. doi: 10.1109/mcom.2011.6069707

Gioia, C. J., Sobell, L. C., Sobell, M. B., and Agrawal, S. (2016). Craigslist versus print newspaper advertising for recruiting research participants for alcohol studies: cost and participant characteristics. *Addict. Behav.* 54, 24–32. doi: 10.1016/j.addbeh.2015.11.008

Gosling, S. D., Vazire, S., Srivastava, S., and John, O. P. (2004). Should we trust web-based studies? A comparative analysis of six preconceptions about internet questionnaires. *Am. Psychol.* 59, 93–104. doi: 10.1037/0003-066x.59.2.93

Guo, B., Wang, Z., Yu, Z., Wang, Y., Yen, N. Y., Huang, R., et al. (2015). Mobile crowd sensing and computing: the review of an emerging human-powered sensing paradigm. *ACM Comput. Surv.* 48:7. doi: 10.1145/2794400

Guo, B., Yu, Z., Zhou, X., and Zhang, D. (2014). "From participatory sensing to mobile crowd sensing," in *2014 IEEE International Conference on Pervasive Computing and Communications Workshops (PERCOM Workshops)* (Budapest), 593–598.

Heron, K. E., and Smyth, J. M. (2010). Ecological momentary interventions: incorporating mobile technology into psychosocial and health behaviour treatments. *Br. J. Health Psychol.* 15, 1–39. doi: 10.1348/135910709X466063

Jasper, K., Weise, C., Conrad, I., Hiller, W., Andersson, G., and Kleinstäuber, M. (2014). Internet-based guided self-help versus cognitive behavioral group therapy for chronic tinnitus: a randomized controlled trial. *Psychother. Psychosom.* 83, 234–246. doi: 10.1159/000360705

Kayrouz, R., Dear, B. F., Karin, E., and Titov, N. (2016). Facebook as an effective recruitment strategy for mental health research of hard to reach populations. *Internet Interv.* 4, 1–10. doi: 10.1016/j.invent.2016.01.001

Landgrebe, M., Zeman, F., Koller, M., Eberl, Y., Mohr, M., Reiter, J., et al. (2010). The Tinnitus Research Initiative (TRI) database: a new approach for delineation of tinnitus subtypes and generation of predictors for treatment outcome. *BMC Med. Inform. Decis. Mak.* 10:42. doi: 10.1186/1472-6947-10-42

Lane, T. S., Armin, J., and Gordon, J. S. (2015). Online recruitment methods for web-based and mobile health studies: a review of the literature. *J. Med. Internet Res.* 17:e183. doi: 10.2196/jmir.4359

Langguth, B., Kreuzer, P. M., Kleinjung, T., and De Ridder, D. (2013). Tinnitus: causes and clinical management. *Lancet Neurol.* 12, 920–930. doi: 10.1016/S1474-4422(13)70160-1

Lindner, P., Nyström, M. B. T., Hassmén, P., Andersson, G., and Carlbring, P. (2015). Who seeks ICBT for depression and how do they get there? Effects of recruitment source on patient demographics and clinical characteristics. *Internet Interv.* 2, 221–225. doi: 10.1016/j.invent.2015.04.002

Martinez, C., Wallenhorst, C., McFerran, D., and Hall, D. A. (2015). Incidence rates of clinically significant tinnitus: 10-year trend from a cohort study in England. *Ear Hear.* 36, e69–e75. doi: 10.1097/AUD.0000000000000121

McCormack, A., Edmondson-Jones, M., Somerset, S., and Hall, D. (2016). A systematic review of the reporting of tinnitus prevalence and severity. *Hear. Res.* 337, 70–79. doi: 10.1016/j.heares.2016.05.009

Meikle, M. B. (1997). Electronic access to tinnitus data: the Oregon Tinnitus Data Archive. *Otolaryngol. Head Neck Surg.* 117, 698–700. doi: 10.1016/s0194-5998(97)70055-x

Meyerson, P., and Tryon, W. W. (2003). Validating internet research: a test of the psychometric equivalence of internet and in-person samples. *Behav. Res. Methods Instrum. Comput.* 35, 614–620. doi: 10.3758/bf03195541

Morgan, A. J., Jorm, A. F., and Mackinnon, A. J. (2013). Internet-based recruitment to a depression prevention intervention: lessons from the Mood Memos study. *J. Med. Internet Res.* 15:e31. doi: 10.2196/jmir.2262

Newman, C. W., Jacobson, G. P., and Spitzer, J. B. (1996). Development of the tinnitus handicap inventory. *Arch. Otolaryngol. Head Neck Surg.* 122, 143–148. doi: 10.1001/archotol.1996.01890140029007

Nondahl, D. M., Cruickshanks, K. J., Huang, G. H., Klein, B. E., Klein, R., Tweed, T. S., et al. (2012). Generational differences in the reporting of tinnitus. *Ear Hear.* 33, 640–644. doi: 10.1097/AUD.0b013e31825069e8

Nyenhuis, N., Golm, D., and Kröner-Herwig, B. (2013a). A systematic review and meta-analysis on the efficacy of self-help interventions in tinnitus. *Cogn. Behav. Ther.* 42, 159–169. doi: 10.1080/16506073.2013.803496

Nyenhuis, N., Zastrutzki, S., Weise, C., Jäger, B., and Kröner-Herwig, B. (2013b). The efficacy of minimal contact interventions for acute tinnitus: a randomised controlled study. *Cogn. Behav. Ther.* 42, 127–138. doi: 10.1080/16506073.2012.655305

Probst, T., Pryss, R., Langguth, B., and Schlee, W. (2016a). Emotional states as mediators between tinnitus loudness and tinnitus distress in daily life: results from the "TrackYourTinnitus" application. *Sci. Rep.* 6:20382. doi: 10.1038/srep20382

Probst, T., Pryss, R., Langguth, B., and Schlee, W. (2016b). Emotion dynamics and tinnitus: daily life data from the "TrackYourTinnitus" application. *Sci. Rep.* 6:31166. doi: 10.1038/srep31166

Pryss, R., Reichert, M., Herrmann, J., Langguth, B., and Schlee, W. (2015a). "Mobile crowd sensing in clinical and psychological trials—a case study," in *28th IEEE Int'l Symposium on Computer-Based Medical Systems* (São Carlos), 23–24.

Pryss, R., Reichert, M., Langguth, B., and Schlee, W. (2015b). "Mobile crowd sensing services for tinnitus assessment, therapy and research," in *2015 IEEE 4th International Conference on Mobile Services* (New York, NY), 352–359.

Ranard, B. L., Ha, Y. P., Meisel, Z. F., Asch, D. A., Hill, S. S., Becker, L. B., et al. (2014). Crowdsourcing—harnessing the masses to advance health and medicine, a systematic review. *J. Gen. Intern. Med.* 29, 187–203. doi: 10.1007/s11606-013-2536-8

Schlee, W., Pryss, R., Probst, T., Schobel, J., Bachmeier, A., Reichert, M., et al. (2016). Measuring the moment-to-moment variability of tinnitus: the TrackYourTinnitus smart phone app. *Front. Aging Neurosci.* 8:294. doi: 10.3389/fnagi.2016.00294

Swan, M. (2012). Crowdsourced health research studies: an important emerging complement to clinical trials in the public health research ecosystem. *J. Med. Internet Res.* 14:e46. doi: 10.2196/jmir.1988

Thornton, L., Batterham, P. J., Fassnacht, D. B., Kay-Lambkin, F., Calear, A. L., and Hunt, S. (2016). Recruiting for health, medical or psychosocial research using Facebook: systematic review. *Internet Interv.* 4, 72–81. doi: 10.1016/j.invent.2016.02.001

Titov, N., Andrews, G., Kemp, A., and Robinson, E. (2010). Characteristics of adults with anxiety or depression treated at an internet clinic: comparison with a national survey and an outpatient clinic. *PLoS One* 5:e10885. doi: 10.1371/journal.pone.0010885

Topolovec-Vranic, J., and Natarajan, K. (2016). The use of social media in recruitment for medical research studies: a scoping review. *J. Med. Internet Res.* 18:e286. doi: 10.2196/jmir.5698

Vayena, E., and Tasioulas, J. (2013). Adapting standards: ethical oversight of participant-led health research. *PLoS Med.* 10:e1001402. doi: 10.1371/journal.pmed.1001402

Witsell, D. L., Schulz, K. A., Moore, K., Tucci, D. L., and CHEER Investigators. (2011). Implementation and testing of research infrastructure for practice-based research in hearing and communication disorders. *Otolaryngol. Head Neck Surg.* 145, 565–571. doi: 10.1177/0194599811406369

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METHODS
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Measuring the Moment-to-Moment Variability of Tinnitus: The TrackYourTinnitus Smart Phone App

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Tinnitus, the phantom perception of sound without a corresponding external sound, is a frequent disorder which causes significant morbidity. So far there is no treatment available that reliably reduces the tinnitus perception. The research is hampered by the large heterogeneity of tinnitus and the fact that the tinnitus perception fluctuates over time. It is therefore necessary to develop tools for measuring fluctuations of tinnitus perception over time and for analyzing data on single subject basis. However, this type of longitudinal measurement is difficult to perform using the traditional research methods such as paper-and-pencil questionnaires or clinical interviews. Ecological momentary assessment (EMA) represents a research concept that allows the assessment of subjective measurements under real-life conditions using portable electronic devices and thereby enables the researcher to collect longitudinal data under real-life conditions and high cost efficiency. Here we present a new method for recording the longitudinal development of tinnitus perception using a modern smartphone application available for iOS and Android devices with no costs for the users. The TrackYourTinnitus (TYT) app is available and maintained since April 2014. A number of 857 volunteers with an average age of 44.1 years participated in the data collection between April 2014 and February 2016. The mean tinnitus distress at the initial measurement was rated on average 13.9 points on the Mini-Tinnitus Questionnaire (Mini-TQ; max. 24 points). Importantly, we could demonstrate that the regular use of the TYT app has no significant negative influence on the perception of the tinnitus loudness nor on the tinnitus distress. The TYT app can therefore be proposed as a safe instrument for the longitudinal assessment of tinnitus perception in the everyday life of the patient.

Keywords: chronic tinnitus, smartphone application, ecological momentary assessment, moment-to-moment analysis, crowd sourcing, ecological validity

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INTRODUCTION

Tinnitus is the perception of a sound when no corresponding external sound is present. The severity of tinnitus varies largely between tinnitus sufferers. While a large percentage of cases is only minimally impaired during their daily routing, the severe cases of tinnitus are affected by anxiety, depression, insomnia and concentration problems all of which can impair their quality of life (Dobie, 2003; Heller, 2003; Kreuzer et al., 2013).

Epidemiological studies of tinnitus indicate a prevalence between 6%–26% with 1.2%–1.6% reporting severely annoying tinnitus (Davis and Rafaie, 2000; Hasson et al., 2010; Gallus et al., 2015). There is currently little evidence for an effective treatment of tinnitus loudness and no pharmacological treatment approved by the US Food and Drug Administration (FDA) or the European Medicines Evaluation Agency (EMEA; Langguth and Elgoyhen, 2012). Among other factors the large heterogeneity of the tinnitus patient population represents a major barrier for the development of effective tinnitus treatments (for a review see e.g., Elgoyhen et al., 2015). A recent review of tinnitus identified at least 13 different types of causal factors for tinnitus (Baguley et al., 2013) that can be described on various dimensions such as its etiology, perceptual characteristics of the sound (i.e., pitch and loudness), time since onset, continuous or intermittent, levels of conscious awareness and perceived distress and comorbidities.

As tinnitus is a purely subjective phenomenon, its assessment is not trivial (Langguth et al., 2007). For the assessment of tinnitus severity standardized questionnaires have been developed, whereas tinnitus loudness can be assessed either by visual analog scale (VAS; Adamchic et al., 2012) or by psychoacoustic measurements. All these measurements are based on the assumption that tinnitus is a rather static phenomenon. However recent data demonstrates that tinnitus loudness and annoyance fluctuate significantly from moment to moment (Henry et al., 2012; Wilson et al., 2015). Henry et al. (2012) conducted a pilot study in which tinnitus symptoms were measured in 24 participants over 2-weeks at four random time points per day using a personal digital assistant (PDA) device. They presented results, which suggest a large between-days variability of tinnitus distress for some study participants. Notably, the frequent measurement of tinnitus over the 2 weeks period had no negative impact on the perceived tinnitus distress as measured by the tinnitus handicap inventory (THI, Newman et al., 1996) indicating that directing the attention on tinnitus several times per day does not worsen the tinnitus (Henry et al., 2012). Using a similar study design (4 assessments per day for 2 weeks), Wilson et al. (2015) also investigated the intra-individual variability of tinnitus distress using smart phone notifications. Likewise, to the study by Henry et al. (2012) they showed that tinnitus fluctuations vary strongly across individuals. Some individuals report strong fluctuations of their tinnitus distress while others report relatively low fluctuations. A coefficient of variation (CV) calculated over the tinnitus self-reports of each participant ranged between 11.5% and 109.9% with a median of 48.4% (Wilson et al., 2015). So far it is not understood why tinnitus fluctuates in some cases and what are the underlying mechanisms for the fluctuations of tinnitus from one moment to the other. Better assessment of the fluctuations of tinnitus is of highest importance for many different reasons: (1) exact assessment of tinnitus fluctuations are of diagnostic importance as intra-individual fluctuations may be an important characteristic feature of an individual's tinnitus; (2) exact measurement of perceptual fluctuations is a precondition for investigating the neurobiological mechanisms of tinnitus changes over time; (3) knowledge of tinnitus fluctuations over time is important for patient management (e.g., for tinnitus counselling); and (4) information of tinnitus fluctuations is highly relevant for clinical studies, e.g., when effects of specific therapeutic interventions are assessed.

In this article, we want to present a conceptual and technical framework for an ecologically momentary assessment (EMA; Stone and Shiffman, 1994), which allows to systematically assess the moments of different tinnitus symptom severity. The method of EMA (also called Experience sampling method, e.g., Csikszentmihalyi and Larson, 2014) was originally developed for the collection of self-reports of behavior, cognition or emotions in the daily lives of the participants. In the context of tinnitus, we extend this framework to also collect self-reports about the perception of the phantom tinnitus sound and objective measurements of contextual variables (here: sound pressure of the environmental sounds to discover tinnitus masking). The EMA method offers several benefits for the assessment of tinnitus.

First, the EMA approach minimizes the retrospective bias. Several studies from multiple disciplines have demonstrated that multiple biases may jeopardize the retrospective data collection, e.g., in pain reports (Erskine et al., 1990) or for coping strategies (Todd et al., 2004). The retrospective recall is based on a process of mental reconstruction rather than a correct retrieval whereby the current circumstances and the peak of symptoms are emphasized (for a review see e.g., Fredrickson, 2000 on this peak-and-end rule). EMA approaches with momentary assessments of the tinnitus symptoms allow the measurements with exact time stamps in real life and should thereby be well suited for minimizing this retrospective bias.

Second, longitudinal assessment can reveal dynamic processes and temporal relationships. Even though it has been shown that tinnitus symptoms fluctuate from one moment to the other (Henry et al., 2012; Wilson et al., 2015), many questions about this dynamic process are still open: how much varies the extent of fluctuations from one person to the other? How strong are the fluctuations within and between days? Is there a temporal pattern (e.g., tinnitus symptoms are higher/lower in the morning compared to the evening)? Is there a temporal relationship with other factors like stress, emotion, concentration, sleep etc. which can suggest a causal relationship (e.g., emotional arousal increases lead to an increase of tinnitus or vice versa)? Are there context-specific factors for tinnitus improvement or worsening? All these questions can be systematically addressed by the EMA approach at the level of individual subjects (for a review see e.g., Wichers, 2013 about EMA assessment in mental disorders). With this manuscript, we want to introduce the technical platform of the TrackYourTinnitus (TYT) app that will be able to answer a large number of these questions. Some work using TYT has already been published investigating the relationships between tinnitus and emotional states (Probst et al., 2016a) as well as between tinnitus and emotion dynamics (Probst et al., 2016b). Further analyses are currently under preparation.

Third, assessment in real-life situations is characterized by large ecological validity. There are multiple examples in clinical research where a large discrepancy can be observed between the measurements in the clinic (or laboratory) and the measurements in everyday life. For instance, blood pressure readings made by a physician in the clinical context are often higher than the ambulatory blood pressure recordings done outside the clinic—typically described as the "white coat effect" (Hansen et al., 2006). On the other side, measurements of clinical symptoms outside the clinical or laboratory settings are hampered by low controllability, which can lead to noisy signals. An adequate item selection (items should measure the respective moment, should be short and can be answered in a view seconds) with respect to the research question is a key element for using the EMA approach. We consider both types of measurements, in the clinical/laboratory context and in real life, as important for both comprehensive diagnostic assessment and evaluation of treatment effects. While there is already a rich collection of tinnitus research tools than can be used in the laboratory or clinical context, the research tools for assessing tinnitus in real life are still limited and shall be improved by the EMA approach presented here.

Fourth, the technical integration of additional sensor data allows the objective measurement of contextual and biological variables. With the current development, we are using the microphone of the smart phone to measure the environmental sound pressure level for assessing the effect of external sounds on perceived tinnitus loudness and distress. This goes beyond earlier developments where this integration was not possible (Henry et al., 2012; Wilson et al., 2015). In this manuscript we only want to mention this option of the technical platform. Detailed analyses will be reported elsewhere and will also take into account the variance of these measures in the different types of smartphones. Further implementation in the future might also integrate biosensors for objective assessment of biological parameters (e.g., heart rate).

Fifth, the TYT is a non-commercial product and is available all over the world for no costs and without advertisements. To ensure the longitudinal assessment, the technical framework is constantly maintained and updated by the team. Furthermore, the technical implementation allows offline use to ensure that permits the use even in areas without internet connection.

MATERIALS AND METHODS

"TYT"[1] was implemented as a technical realization of the envisaged EMA approach. The TYT platform will be outlined below. A detailed description of the technical aspects is published elsewhere (Pryss et al., 2015a,b; Schickler et al., 2015).

Recruitment

TYT is an open-access platform at no costs for the users. Upon registration, the users have to agree with the terms of the smartphone app use, which includes that the anonymized data can be used for scientific purposes. The analysis of anonymized data from the smart phone app has been approved by the Ethics Committee of the University Clinic of Regensburg. Before the start of the study, all volunteers agreed with informed consent and no vulnerable populations were involved. To recruit the patients, the TYT app was advertised at the webpages and Facebook pages of the Tinnitus Research Initiative, the TINNET COST Action and the webpage of the participating research groups.

Data Collection

There are three types of data collected by the TYT platform:

1. The "registration questionnaires" consists of three questionnaires that were completed by the app-user upon registration. The registration questionnaires include the Tinnitus Sample Case History Questionnaire (TSCHQ, Langguth et al., 2007), the short Version of the Tinnitus Questionnaire (miniTQ, Hiller and Goebel, 2004) and a short questionnaire asking for the individually most disturbing tinnitus related aspect.
2. The "state questionnaire" is designed to assess tinnitus and situation-specific variables with eight short questions during everyday life. The state questionnaires constitute the main part of this EMA study. The smartphone app will notify the user at several time points during the day to fill out the state questionnaire. The state questionnaires are delivered randomly within a time frame that can be set by the user (see the "Technical Realization" Section for more details). We decided to give the user more freedom for this setting in order to enhance the usability of the app and allow adaptation to the individual needs. This, however, also enhances the variability in the number of sampling points and the time lag between them. The selection of data analysis methods need to take this into account.

   The state questionnaire consists of eight questions. With the first question we ask the patient if she/he perceives the tinnitus at this moment (answer with yes or no). The second and third question ask about the loudness of the tinnitus and how stressful the tinnitus is. The patients can give their answers on a VAS by moving a slider between the endpoints. Technically, the VAS was implemented as a slider without pre-set values to avoid anchoring affects (Tversky and Kahneman, 1974). The endpoints are "not audible" (question 2) or "not stressful" (question 3) on the left side and "maximal loudness" (question 2) or "maximal stressful" (question 3). The questions 4 and 5 ask for the emotional valence and arousal respectively, using the self-assessment manikins (SAM) developed by Bradley and Lang (1994). Question 6 asks if the person feels currently stressed on a VAS (endpoints "not stressed" and "maximal stressed"). Question 7 asks how much the user concentrated on the task that she/he was doing at the moment (VAS with the endpoints "not at all" and "fully concentrated"). Question 8 asked if the user was irritated at this moment (answer with yes/no).

[1] www.trackyourtinnitus.org

The state questionnaire was implemented to allow fast answering by the user. Typically, it takes less than a minute to complete the state questionnaire.

3. The sound pressure of environmental sounds was measured using the built-in microphone of the smartphone while the user was answering the state questionnaire. The microphone recordings were set to record with a resolution of 16 bit for both, the Android and the iOS devices. While inter-subject comparison of the sound pressure measures is limited because of the different smartphone manufacturers, an intra-subject comparison between different moments will be possible. The sound pressure measurements are not reported in this manuscript and is subject to further analyses.

Technical Realization

The Front-End (**Figure 1**) of the TYT includes two smart phone applications for iOS (implemented using Objective C) and Android devices (implemented using Java), and the www.trackyourtinnitus.org website (implemented using the Open-Source PHP-Framework LARAVEL: Code Bright, Otwell, 2016) for the registration and display of the results. Both smart phone apps are freely available for English and German speaking users in the respective app stores. The Back-End consists of a MySQL database running on a Linux Server for the storage of the collected data from all mobile devices. The data on the smart phone devices are stored in the internal SQLite database of the mobile device. If the user is online while he is answering the state questionnaire, the answers between the mobile device and the server will be synchronized immediately. If the user is offline, this synchronization process will be triggered the next time the user is online. In all cases, only anonymized data is transferred and the data transfer is encrypted using the secure sockets layer (SSL) technology. The user has the option to review his own data within the app or download the dataset using the web interface. The anonymized data of all users are stored in a central database maintained by the University of Ulm for research purposes. The user can select between two settings for the state questionnaires: in the "standard settings", the user receives the state questionnaires at random time points between 8 a.m. and 10 p.m. (can be adjusted) following a randomization algorithm described by Pryss et al. (2015b). A maximum number of 12 state questionnaires per day is allowed by system. In the "custom" settings, the user can define an own schedule with time points where she/he wants to receive the state questionnaire. The TYT platform was developed by the authors of the manuscript (RCP, AB, JS, MR) with additional support by several programmers from the University of Ulm (see "Acknowledgments" Section, Jochen Herrmann, Robin Kraft, Robin Zöller, Aliyar Aras, Marc Schickler). The TYT platform is developed for users with chronic tinnitus. No prior knowledge of EMA is required to use the app.

Data Management

High emphasis is placed on data management of the TYT platform. Given the automated procedures, the data collection is highly standardized for all participants. Data entry masks are designed carefully to only allow the entry of meaningful data and minimize the risk of wrong entries (e.g., defined value ranges). The data collection of TYT is continuously ongoing. Therefore, strict rules for data analysis were defined in order to reduce reporting biases: at fixed time points during the year, the database is frozen. Data analysis will always be done using the most recent database freeze. The data reported in this article contains the data collected between April 2014 (start of the TYT platform) and February 2016. Further analysis on the dataset have been published elsewhere (Probst et al., 2016a,b) and additional analyses will be performed in the future. This will also include time-series analyses to explore temporal relationships between the tinnitus loudness or distress and the emotional state or the perceived stress level.

Data Analysis

The influence of the duration of app-use on the perceived tinnitus loudness and distress was tested by means of regression

FIGURE 1 | Frontend of the TrackYourTinnitus (TYT) framework including the website and the smart phone applications for iOS and Android.

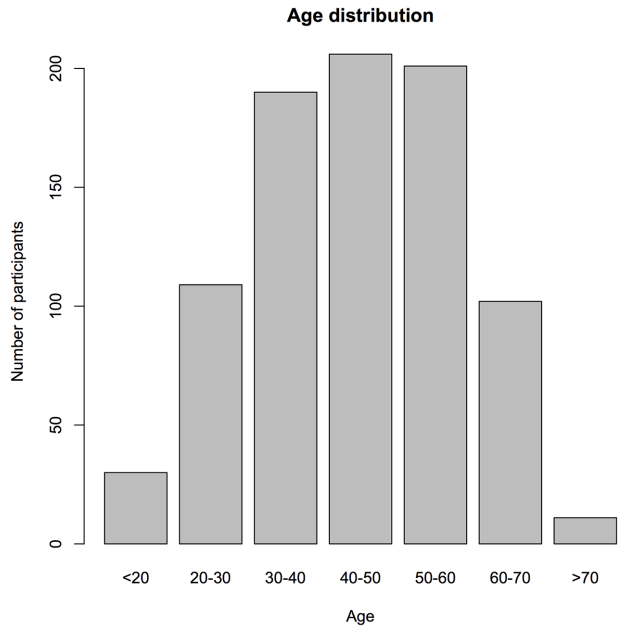

FIGURE 2 | Age distribution of the participants.

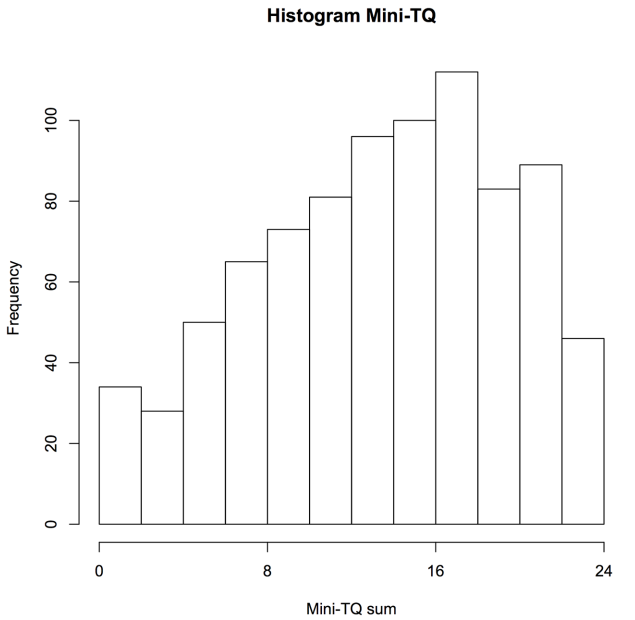

FIGURE 3 | Histogram of the Mini-Tinnitus Questionnaire (Mini-TQ) sum scores of the participants. During the registration process, the participants are required to fill out the Mini-TQ. The Mini-TQ measures the strength of the tinnitus distress on a scale from 0 to 24.

analysis. Statistical analysis was done using the statistical software package R[2].

RESULTS

Study Sample

A number of 857 volunteers (26.9% female, 73.1% male) participated in the data collection between the launch of the app in April 2014 and February 2016. To recruit the patients, the TYT app was advertised at the webpages and Facebook pages of the Tinnitus Research Initiative, the TINNET COST Action and the webpage of the participating research groups. The average age of the participants was 44.1 years (standard deviation: 14.1 years, **Figure 2**). Upon registration, the participants completed the Mini-Tinnitus Questionnaire (Mini-TQ) for the assessment of tinnitus-related distress. The average sum score over all participants was 13.9 points (SD 6.0, **Figure 3**, maximum score of the Mini-TQ is 24 points).

Influence of Study Participation on Tinnitus Symptoms

An important question is whether the continuous participation in the study, with repeated measurements of tinnitus-related symptoms, leads to a worsening or improvement of tinnitus symptoms. To analyze this question, we selected a subgroup of subjects that have used the app regularly for at least a month. Linear regression analysis was calculated for these users (n = 66) in this timeframe to test the influence of the study duration on the tinnitus loudness.

The duration of app-use measured in days was used as a regressor in this model, the tinnitus loudness as regressand (tinnitus loudness $\sim$ app-use duration). Duration of app-use did not predict tinnitus loudness, $\beta < 0.001$, $t_{(6291)} = 1.26$, $p = 0.21$. Furthermore, the duration of the app-use did not explain the variance of the tinnitus loudness significantly, R-squared <0.001, $F_{(1,6291)} = 1.58$, $p = 0.21$. Similarly, we investigated the influence of the duration of app-use on tinnitus distress (tinnitus distress $\sim$ app-use duration). The duration of app-use did not predict tinnitus distress, $\beta < 0.001$, $t_{(6264)} = 1.3$, $p = 0.19$. Also, the duration of the app-use did not explain the variance of the tinnitus distress significantly, R-squared <0.001, $F_{(1,6264)} = 1.7$, $p = 0.19$. Additionally, we calculated a paired t-test comparing the mean of the first 5 sampling state questionnaires against the mean of the last 5 state questionnaires for each user. Again, there was no significant influence of time neither on tinnitus loudness ($t_{(61)} = 1.25$, $p > 0.2$) nor on tinnitus distress ($t_{(61)} = 0.38$, $p > 0.7$), indicating, that app-use did not have a negative impact on the users' tinnitus.

The same type of paired t-test was repeated for the subjects, which used the app for less than 1 month. We selected all users with more than 5 days of app-use and less than 30 days of use. Also for this group of users, there was no significant influence of time neither on tinnitus loudness ($t_{(133)} = 0.53$, $p > 0.5$) nor on tinnitus distress ($t_{(129)} = 0.79$, $p > 0.4$).

To give an impression of the fluctuation of tinnitus loudness and tinnitus distress over time, a random selection of three participants is illustrated in **Figure 4**.

[2]www.r-project.org, version 3.3.1.

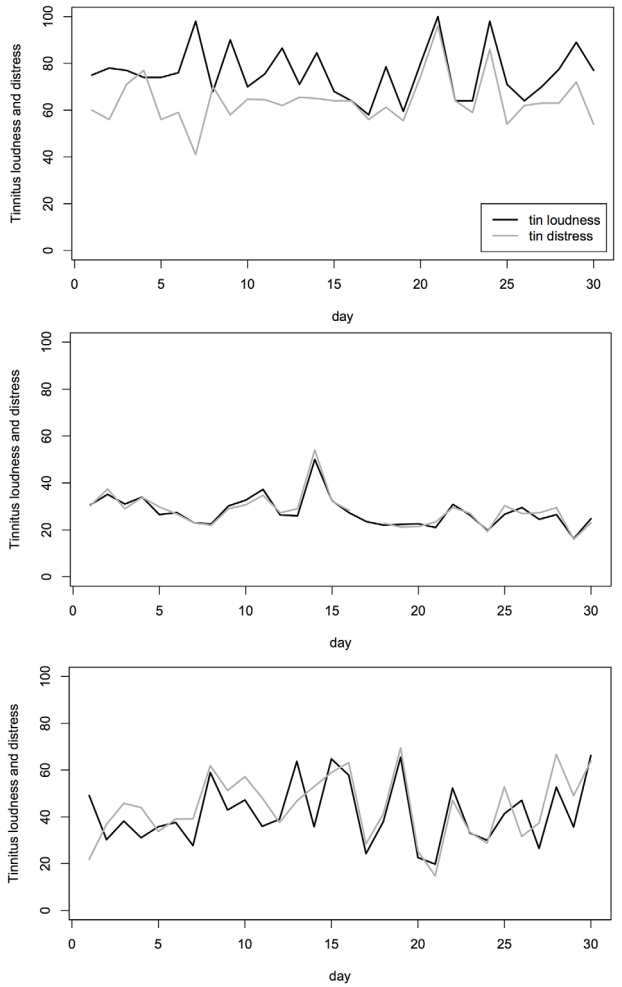

FIGURE 4 | Example of three individual tinnitus patients. Measurements of the tinnitus loudness (black) and tinnitus distress (gray) on 30 consecutive days are shown for the three individuals. The tinnitus loudness and tinnitus distress were both measured on a visual analog scale (VAS) and converted to numerical values in the range of 0 ("not audible"/"not stressful") to 100 ("maximal loudness"/"maximal stressful").

DISCUSSION

We have introduced the TYT app as a new platform that allows the assessment of tinnitus-related symptoms during the everyday life of people with tinnitus. The app is available in the two major app stores for Android and iOS devices. Between April 2014 and February 2016, the app was used by 857 people with tinnitus, with the majority of users between 30 and 60 years old. The amount of tinnitus-related distress as measured with the Mini-TQ covered the full range of the measurement spectrum showing that the app was used by people with various levels of tinnitus distress. However, further studies will need to clarify, whether the population using the app is representative to the general tinnitus population. The recruitment of patients via the internet and the app stores introduces a new way in patient recruitment compared to earlier studies. It is therefore important to mention that our results are in line with the previous studies on mobile assessment of tinnitus (Henry et al., 2012; Wilson et al., 2015). The large amount of sampled data allows the analysis of more specific research questions about tinnitus which are reported elsewhere (Probst et al., 2016a,b).

From a clinical point of view, there could be the concern that the tinnitus increases by the repeated assessment (and therefore a reminder) of the tinnitus perception. It is notable here that the tinnitus perception did not change as a result of repeated sampling, neither tinnitus loudness nor tinnitus distress. This is in line with the study by Henry et al. (2012) in which it was shown that tinnitus did not change during a 2 week assessment period with four measurements each day. Furthermore, we also analyzed the data of users with only a short duration of app-use. In principle it would be possible that users where the app-use has a negative impact on their tinnitus stop using the app, while the users without negative impact use the app for a longer time. This is clearly not the case since the paired *t*-tests on the subgroup of subjects with short app-use duration did not reveal a significant difference between the first and the last state measurements either. These findings further support the notion that assessment of tinnitus symptoms during everyday life is a safe method that is not increasing neither tinnitus loudness nor tinnitus distress.

The aim of the TYT platform is: (1) to enable real-time assessment of tinnitus variations and reduce the effect of recall bias; (2) to investigate the factors influencing the increase and decrease of tinnitus symptoms at the individual subject level; (3) to enable the longitudinal assessment of tinnitus symptoms and their dynamic processes; (4) to introduce tinnitus measurements with higher ecological validity in real-life conditions as opposed to tinnitus measurements in the lab or the clinic, and therefore establish ecological momentary assessment (EMA) methods for tinnitus research; and (5) to implement and validate tinnitus assessment with smart phones as a cost-effective assessment tool that can be applied for large populations.

Future technical implementation will also enable monitoring of clinical treatments. The effects of clinical treatments are currently typically assessed by weekly or monthly questionnaires and can therefore hardly be used to investigate dynamic changes during the treatment phase. Although EMA has been used in other domains to monitor treatment progress, e.g., for antidepressant treatment (Barge-Schaapveld and Nicolson, 2002), it has not been used for the assessment of tinnitus treatments yet. Another benefit of EMA was revealed by the study of Barge-Schaapveld and Nicolson (2002), which demonstrated an increased reporting of side-effects. The side effect of increased dizziness was reported by 35 patients using EMA while only seven patients reported increased dizziness to their general practitioner (Barge-Schaapveld and Nicolson, 2002). This suggests that the recall bias of retrospective assessment not only influences the measurement of treatment related symptom reduction but also the reporting of treatment-related side effects. Additionally, frequent assessment of treatment effects with smart phones

could help to learn more about reasons for study drop out by providing more precise data of what has happened immediately before drop out.

However, beside all the excitement about the new opportunities and advantages of EMA, we also want to mention the drawbacks of this method, which mainly arise from the low controllability of our sampling method. Since the assessment with smart phones is not completed under the supervision of clinical staff, the correct usage of the device as well as the identity of the user cannot be guaranteed. Also, there is little control over the situation and circumstances of the app usage, which on one side enhances the ecological validity of the measurement, but on the other side reduces its controllability. It is, therefore, important to mention that EMA should not be considered as a substitute for standard questionnaires used in the clinical routine, but rather be seen as a complementary method with additional value for both clinical practice and for basic as well as clinical research. We are looking forward to a new wave of studies using EMA for its various applications in tinnitus research.

AUTHOR CONTRIBUTIONS

WS: substantial contribution to the design of the study, data analysis, conception and implementation of the TrackYourTinnitus app, drafted and revised the manuscript. RCP: substantial contribution to the design of the study, data analysis, conception, implementation and maintenance of the TrackYourTinnitus app, drafted and revised the manuscript. TP and BL: substantial contribution to the design of the study and data analysis, drafted and revised the manuscript. JS, AB and MR: substantial contribution to the conception, implementation and maintenance of the TrackYourTinnitus app.

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REFERENCES

Adamchic, I., Langguth, B., Hauptmann, C., and Tass, P. A. (2012). Psychometric evaluation of visual analog scale for the assessment of chronic tinnitus. *Am. J. Audiol.* 21, 215–225. doi: 10.1044/1059-0889(2012/12-0010)

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Barge-Schaapveld, D. Q. C. M., and Nicolson, N. A. (2002). Effects of antidepressant treatment on the quality of daily life: an experience sampling study. *J. Clin. Psychiatry* 63, 477–485. doi: 10.4088/jcp.v63n0603

Bradley, M. M., and Lang, P. J. (1994). Measuring emotion: the self-assessment manikin and the semantic differential. *J. Behav. Ther. Exp. Psychiatry* 25, 49–59. doi: 10.1016/0005-7916(94)90063-9

Csikszentmihalyi, M., and Larson, R. (2014). "Validity and reliability of the experience-sampling method", in *Flow and the Foundations of Positive Psychology*, ed. M. Csikszentmihalyi (Berlin: Springer Netherlands), 35–54.

Davis, A., and Rafaie, E. A. (2000). "Epidemiology of tinnitus," in *Tinnitus Handbook*, ed. R. S. Tyler (San Diego, CA: Singular), 1–24.

Dobie, R. A. (2003). Depression and tinnitus. *Otolaryngol. Clin. North Am.* 36, 383–388. doi: 10.1016/s0030-6665(02)00168-8

Elgoyhen, A. B., Langguth, B., De Ridder, D., and Vanneste, S. (2015). Tinnitus: perspectives from human neuroimaging. *Nat. Rev. Neurosci.* 16, 632–642. doi: 10.1038/nrn4003

Erskine, A., Morley, S., and Pearce, S. (1990). Memory for pain: a review. *Pain* 41, 255–265. doi: 10.1016/0304-3959(90)90002-u

Fredrickson, B. L. (2000). Extracting meaning from past affective experiences: the importance of peaks, ends, and specific emotions. *Cogn. Emot.* 14, 577–606. doi: 10.1080/026999300402808

Gallus, S., Lugo, A., Garavello, W., Bosetti, C., Santoro, E., Colombo, P., et al. (2015). Prevalence and determinants of tinnitus in the italian adult population. *Neuroepidemiology* 45, 12–19. doi: 10.1159/000431376

Hansen, T., Jeppesen, J., Rasmussen, S., Ibsen, H., and Torp-Pederson, C. (2006). Ambulatory blood pressure monitoring and risk of cardiovascular disease: a population based study. *Am. J. Hypertens.* 19, 243–250. doi: 10.1016/j.amjhyper.2005.09.018

Hasson, D., Theorell, T., Westerlund, H., and Canlon, B. (2010). Prevalence and characteristics of hearing problems in a working and non-working Swedish population. *J. Epidemiol. Community Health* 64, 453–460. doi: 10.1136/jech.2009.095430

Heller, A. J. (2003). Classification and epidemiology of tinnitus. *Otolaryngol. Clin. North Am.* 36, 239–248. doi: 10.1016/s0030-6665(02)00160-3

Henry, J. A., Galvez, G., Turbin, M. B., Thielman, E. J., McMillan, G. P., and Istvan, J. A. (2012). Pilot study to evaluate ecological momentary assessment of tinnitus. *Ear Hear.* 33, 179–290. doi: 10.1097/AUD.0b013e31822f6740

Hiller, W., and Goebel, G. (2004). Rapid assessment of tinnitus-related psychological distress using the Mini-TQ. *Int. J. Audiol.* 43, 600–604.

Kreuzer, P. M., Vielsmeier, V., and Langguth, B. (2013). Chronic tinnitus: an interdisciplinary challenge. *Dtsch. Arztebl. Int.* 110, 278–284. doi: 10.3238/arztebl.2013.0278

Langguth, B., and Elgoyhen, A. B. (2012). Current pharmacological treatments for tinnitus. *Expert Opin. Pharmacother.* 13, 2495–2509. doi: 10.1517/14656566.2012.739608

Langguth, B., Goodey, R., Azevedo, A., Bjorne, A., Cacace, A., Crocetti, A., et al. (2007). Consensus for tinnitus patient assessment and treatment outcome measurement: tinnitus research initiative meeting, regensburg, July 2006. *Prog. Brain. Res.* 166, 525–536. doi: 10.1016/S0079-6123(07)66050-6

Newman, C. W., Jacobson, G. P., and Spitzer, J. B. (1996). Development of the tinnitus handicap inventory. *Arch. Otolaryngol. Head Neck Surg.* 122, 143–148. doi: 10.1001/archotol.1996.01890140029007

Otwell, T. (2016). Laravel - The PHP Framework for Web Artisans. Laravel.com. Last accessed 30 November 2016. Available online at: http://www.laravel.com

Probst, T., Pryss, R., Langguth, B., and Schlee, W. (2016a). Emotion dynamics and tinnitus: daily life data from the "TrackYourTinnitus" application. *Sci. Rep.* 6:31166. doi: 10.1038/srep31166

Probst, T., Pryss, R., Langguth, B., and Schlee, W. (2016b). Emotional states as mediators between tinnitus loudness and tinnitus distress in daily life: Results from the "TrackYourTinnitus" application. *Sci. Rep.* 6:20382. doi: 10.1038/srep20382

Pryss, R., Reichert, M., Herrmann, J., and Langguth, B. (2015a). "Mobile crowd sensing in clinical and psychological trials–A case study," in *28th IEEE International Symposium on Computer-Based Medical Systems* (Sao Carlos, Brazil).

Pryss, R., Reichert, M., Langguth, B., and Schlee, W. (2015b). "Mobile crowd sensing services for tinnitus assessment, therapy and research," in *IEEE 4th International Conference on Mobile Services (MS)* (New York, NY).

Schickler, M., Reichert, M., Pryss, R., Schobel, J. Schlee, W., and Langguth, B. (2015). *Entwicklung Mobiler Apps: Konzepte, Anwendungsbausteine und Werkzeuge im Business und E-Health.* Berlin: Springer Vieweg.

Stone, A. A., and Shiffman, S. (1994). Ecological momentary assessment (EMA) in behavorial medicine. *Ann. Behav. Med.* 16, 199–202.

Todd, M., Tennen, H., Carney, M. A., Armeli, S., and Affleck, G. (2004). Do we know how we cope? Relating daily coping reports to global and time-limited

retrospective assessments. *J. Pers. Soc. Psychol.* 86, 310–319. doi: 10.1037/0022-3514.86.2.310

Tversky, A., and Kahneman, D. (1974). Judgement under uncertainty: heuristics and biaises. *Sciences* 185, 1124–1131. doi: 10.126/science.185

Wichers, M. (2013). The dynamic nature of depression: a new micro-level perspective of mental disorder that meets current challenges. *Psychol. Med.* 44, 1349–1360. doi: 10.1017/S0033291713001979

Wilson, M. B., Kallogjeri, D., Joplin, C. N., Gorman, M. D., Krings, J. G., Lenze, E. J., et al. (2015). Ecological momentary assessment of tinnitus using smartphone technology: a pilot study. *Otolaryngol. Head Neck Surg.* 152, 897–903. doi: 10.1177/0194599815569692

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Call for an Evidence-Based Consensus on Outcome Reporting in Tinnitus Intervention Studies

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Tinnitus is a very common symptom affecting 5.1–42.7% of the population (1) and is frequently seen in family medicine and primary care settings. Despite its considerable socioeconomic relevance (2), real progress in developing an effective cure for tinnitus has been fruitless (3). In most of the cases, the proposed therapies remain palliative; aiming at alleviating the negative consequences of tinnitus. Although the need for effective management options for tinnitus is clear, methodological and reporting quality of clinical trials have been low (4, 5) making useful recommendations and practical guidelines for family medicine and primary health-care practitioners almost impossible to draw. Indeed, Baguley and colleagues (6) concluded that, with the exception of cognitive behavior therapy for tinnitus, evidence for the effectiveness of different treatment strategies is insufficient (pp. 1605).

The CONSORT statement[1] is perhaps the most well-known guideline for solving problems arising from inadequate reporting of randomized controlled trials, but other tinnitus-specific statements have been around since the 1990s. Unfortunately, these recommendations have not yet transformed standards in the tinnitus field so far. Indeed, recent systematic reviews of published clinical trials aiming at evaluating tinnitus therapeutic interventions have shown that reporting is still flawed by poor methodology and poor reporting (4, 5).

In this opinion, we discuss the selection and reporting of outcomes; perhaps, the most important aspect of determining whether a treatment works for patients and whether this treatment should be implemented in the medical practice either in primary or secondary settings. Selecting an appropriate outcome for determining clinical efficacy is one of those key trial design decisions. As Noble eloquently put it: "critical to any form of treatment for tinnitus is the reliance placed on measures to assess the effectiveness of the intervention" (pp. 20) (7). Just over 10 years later, Landgrebe et al. (8) made the same point stressing that "assessment of outcome is probably the single most important factor in conducting a clinical trial in tinnitus" (pp. 9). This is so because in clinical trials, therapeutic benefit is evaluated according to its effect on primary (and secondary) outcome measures that should be purposefully chosen according to the complaints (domains) of tinnitus considered to be most important from the perspective of determining therapeutic benefit (9). More specifically, the primary outcome measure is that which confirms whether or not the primary hypothesis is supported by the data. It is typically a variable relating to clinical efficacy but could also be one relating to safety, tolerability, or quality of life, if that is the primary research question. Generally speaking, the primary outcome should also be the endpoint that is clinically relevant from the patients' perspective and to health-care providers' in their everyday practice, not just significant from a statistical point of view. In support of this, the ICH E9 states that "The primary variable should be that variable capable of providing the most clinically relevant and convincing evidence directly related to the primary objective of the trial" (pp. 5) (10).

[1] www.consort-statement.org/.

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Bearing in mind that tinnitus is a subjective condition for which patients experience a diversity of complaints, there is no straightforward outcome instrument. Outcome reporting typically relies on self-report, often in the form of a multi-item tinnitus questionnaire that asks questions about a range of complaints (not all of which are the same across existing questionnaires). A number of important issues have been raised and debated over the years, but many of those concerns remain unresolved. **Table 1** summarizes conclusions/recommendations about clinical trial outcomes in tinnitus. Although this may not be exhaustive, it nevertheless serves to illustrate the status of the field spanning across three decades.

Our observations are as follows:

(i) Are instruments really validated for use in assessing tinnitus treatment-related change?

While early conclusions implied that questionnaires are the "best" primary outcomes and that adequate "validated" questionnaires exist for this purpose, by 2007 some researchers were

TABLE 1 | Concluding remarks or recommendations about clinical trial outcomes in tinnitus made in various review articles.

Reference	Conclusions and recommendations concerning outcomes
Tyler (29)	Benefit should be measured with established questionnaires and with measures of the magnitude of tinnitus. A persuasive tinnitus treatment will be one that shows a large treatment effect, can be generalized across patients and clinicians, is specific and credible, and changes the way we think about tinnitus
Tyler et al. (9)	Several scaling procedures are available, but we believe a 100-point interval scale is superior. Several validated and reliable questionnaires are available and can serve as adequate primary measures. Secondary measures that quantify the magnitude of the tinnitus should also be obtained
Langguth et al. (15)	It was generally agreed that a questionnaire is required that is specifically designed for the assessment of treatment outcomes, and which is validated in many languages and in many cultural and socioeconomic groups. The consensus agreement is that at the present time one validated questionnaire, which can be Tinnitus Handicap Inventory (THI), Tinnitus Handicap Questionnaire (THQ), TRQ, or Tinnitus Questionnaire (TQ), is an essential part of patient assessment. Therapeutic trials should use one of these questionnaires also as outcome measurement. Assessment of tinnitus severity with at least one additional questionnaire is highly recommended
Meikle et al. (11)	While the tinnitus questionnaires that are currently available provide valuable information on which to base diagnostic and screening decisions, they were not originally developed in such a way as to maximize their sensitivity to treatment-related changes in tinnitus. As a result, their construct validity for measuring treatment benefit has not received appropriate attention
Tyler et al. (9)	When the treatment is intended to reduce the tinnitus, we recommend measuring the magnitude of the tinnitus. We provide arguments and data to support the use of the THQ as a measure of the reaction to the tinnitus. We suggest that the current quality of life measures are not valid for measuring lifestyle effects of alleviating tinnitus. A clinically meaningful effect should represent a valid and reliable statistical change for an individual
Meikle et al. (13)	It is to be hoped that investigators will address the need for information about the responsiveness of all the various types of tinnitus measures. The fact that measures of sensory impairment versus functional disability and handicap each provide unique insights into treatment-related changes in tinnitus reinforces the notion that both approaches are needed for insightful assessment of tinnitus treatment outcomes
Hesser (30)	If we restrict the assessment to one particular aspect of tinnitus-related disability, definitive claims about overall treatment benefits will be difficult to make. Moreover, the measures we use need to be validated and psychometrically robust. Although several psychometrically examined measures are available to assess tinnitus impact and severity (e.g., THI, Tinnitus Reaction Questionnaire), there is no standard outcome measure that is obligatory to include in a trial. I do believe that treatment evaluations within the field would not only benefit from calculating and reporting average effects but also must rely on data on the individual level, e.g., as clinical significant change, in determining the effects of treatment
Kamalski et al. (12)	The Health-Related-Quality of Life (HR-QoL) instruments used in tinnitus trials (THI, TQ, TRQ, TSI, THQ, and TSQ) appear not to be validated to measure effectiveness of interventions. Using tests or instruments that are valid and reliable is a crucial component of research quality, and both should therefore be studied before final conclusions can be drawn from the questionnaires in upcoming clinical trials. The validity, reliability, and responsiveness of each tinnitus-specific HR-QoL should be studied before final conclusions can be drawn regarding the utility of these questionnaires in future clinical studies
Landgrebe et al. (8)	Basic requirements for clinical trials in tinnitus include: – Definition of one or more main outcome measure(s) (i.e., a validated tinnitus questionnaire). – THI should be included in every trial at least as secondary outcome to improve inter-study comparability
Newman et al. (31)	Although psychometrically robust measures of tinnitus HR-QoL do exist, there is no unanimity in, for example, what tests should be included in the tinnitus assessment, and how studies of HR-QoL should be conducted. The current authors suggest that future studies employ more rigorous designs and contain (minimally) the following characteristics: (1) utilization of randomized control groups and blinding; (2) appropriate statistical testing including "dropouts" that should be used in an "intention to treat" analysis rather than elimination from the final data set; (3) long-term follow-up assessment to evaluate responsiveness; (4) appropriate inclusion criteria to avoid "ceiling" and "floor" effects; and (5) suitable sample sizes based on the application of power analyses
Fackrell et al. (14)	– We recommend that the "gold standard" would be to carry out a systematic review of the literature before selecting any given tinnitus questionnaire for a service audit or clinical trial. – In addition to the measurement properties, selection might also give consideration to the suitability of the tinnitus questionnaire for the study population, the potential burden of completing the questionnaire (e.g., length, question difficulty, emotional impact of certain questions), and the practical aspects (e.g., copyright costs, complexity of scoring method)

(*Continued*)

TABLE 1 | **Continued**

Reference	Conclusions and recommendations concerning outcomes
Hall et al. (27)	The overall ambition of the working group is to establish an international standard for outcome measurements in clinical trials of tinnitus. The standard will be achieved by a two-step effort to produce core outcome sets of domains and instruments that harmonize viewpoints across both professional and patient stakeholder groups. A roadmap has been proposed, which sets out a provisional plan for delivery. This roadmap reflects the two-step process with Stage 1 identifying and agreeing on outcome domains and Stage 2 identifying and agreeing on outcome instruments
Plein et al. (4)	(There is) a need in the literature for high-quality tinnitus research that is adequately randomized, ensures adequate follow-up, and does not exclude a large range of common otologic conditions that can result in tinnitus, which would result in improved external validity. Analysis of external validity is essential to the development of further guidelines and should be taken into account if we hope to develop recommendations that are of most benefit to clinicians and patients
Hall et al. (5)	– Generic names and terms such as "handicap" and "severity" perpetuate the difficulty that many trialists experience in understanding what construct(s) a particular questionnaire instrument measures. – Safety, tolerability, side effects, and withdrawals might be domains that all inform the measurement of adverse events. To improve trial reporting, we draw attention to the specialized CONSORT guidelines for reporting harms-related issues in a randomized controlled trial. – We advise caution if pooling findings from the THI in a meta-analysis since it is unclear whether all translations achieve equivalence with the British original

beginning to challenge the validity of existing tinnitus questionnaires for use in assessing treatment-related change (11). In 2010, a particularly critical evaluation of the psychometric properties of six of the commonly used multi-item questionnaires assessing tinnitus burden, including the Tinnitus Handicap Inventory, TQ, and Tinnitus Handicap Questionnaire, was published by Kamalski et al. (12). For each identified tinnitus-specific Health-Related-Quality of Life questionnaire, they systematically searched for published details regarding the questionnaire's test characteristics including number of domains, construct validity, internal consistency, reproducibility, and responsiveness. Like Meikle et al. (13), they were critical that none of the six questionnaires assessed had been validated for evaluative purposes, which is necessary to be useful in clinical trials. In particular, responsiveness, which measures the ability to detect a clinically important change over time, had not been reported for any of the six instruments.

Fackrell et al. (14) raised a new issue about the dynamic nature of psychometric properties. What questionnaire properties hold for one patient population might not for another. Indeed, for this reason we prefer the term psychometric "exploration" not "validation," and we hope that questionnaire developers might be sympathetic to adaptations in order to maintain equivalence across cultures [see also Ref. (15)].

(ii) Can we reduce the diversity of outcome instruments?

Two recent systematic reviews of outcome instruments in tinnitus trials have confirmed unacceptable heterogeneity in measurement tools (4, 5) For example, we found 78 different primary outcome instruments across 228 trials (5). This makes comparisons between studies elusive.

(iii) What is a clinically meaningful effect?

Tyler et al. (9) highlighted the importance of benefit from the individual patient experience, although this psychometric property of tinnitus questionnaires has generally not been investigated or quantified [see Meikle et al. (16), for a good example].

In the race to develop and utilize tinnitus questionnaires in our research, we are losing sight of understanding "what" it is that needs to be measured at the expense of "how" it is measured. Do we really know which tinnitus-related complaints are the most relevant both from the first and second line health-care providers' and patients' perspectives? We would argue not. Primary care physicians and patients in particular have been left out of the questionnaire development process.

Involving primary care physicians and patients in developing outcome reporting standards would go a long way to resolving heterogeneity in outcome assessment and ensuring its relevance. It could then help the tinnitus community to focus efforts on conducting an appropriate psychometric exploration of whatever are the preferred instruments. As an important first step, we are therefore leading a consensus exercise to develop an agreed minimum reporting set of outcomes for trials of interventions in tinnitus. What is urgently needed are targeted discussions around "what" needs to be measured for each therapeutic approach (sound therapies, pharmacological therapies, psychological interventions, and neuromodulation) since not all interventions seek to alleviate the same tinnitus-related complaints.

Such consensus for a minimal (core) set of outcome measurements that should be used in every clinical trial clearly calls for a predefined, multidisciplinary (including patients and primary care professionals), international, and methodologically driven clinimetric approach (17). The rationale underpinning this scientific approach of outcome assessment has already been theorized. For example, the Core Outcome Measures in Effectiveness Trials (COMET) initiative brings together from all over the world researchers interested in the development and application of agreed standardized "Core Outcome Sets" (COS) (18). A COS represents the minimum that should be measured and reported in all clinical trials for a specific condition (19). COS could also be suitable for a use in clinical audit or research other than randomized trials. One should note that COS are not limitative. If necessary, other outcomes might be added to those in the relevant COS in a particular trial. But, it is recommended that all the COS items should be collected and reported, making it easier for the

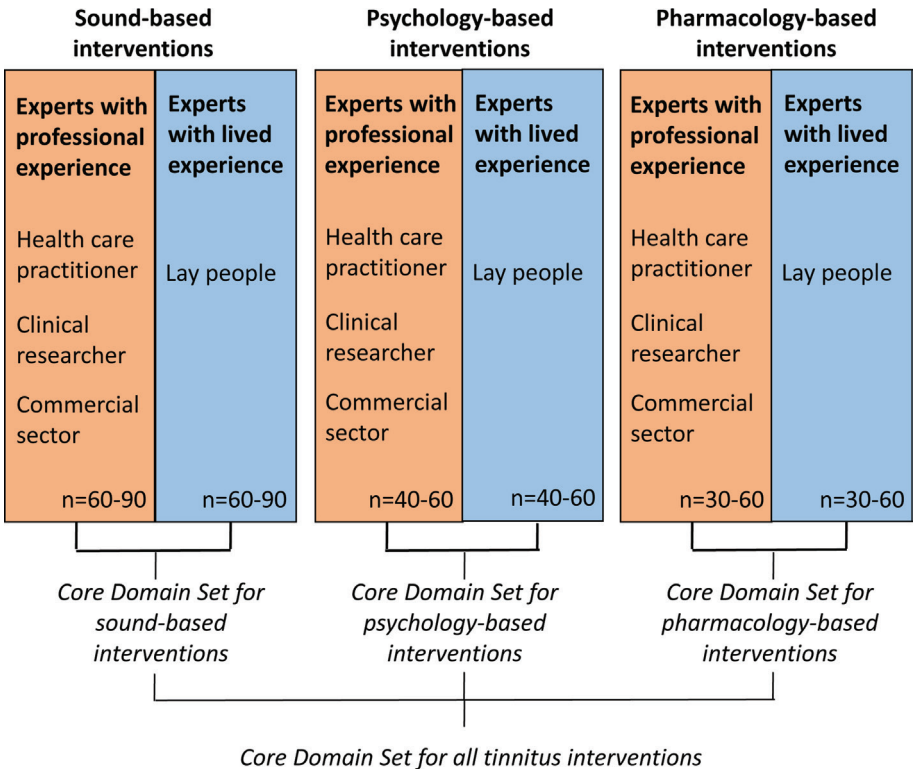

FIGURE 1 | Proposed Delphi survey strategy to identify different core outcome sets that are appropriately tailored according to different tinnitus interventions.

results to be compared, contrasted, and merged as appropriate. Indeed, similar international initiatives aiming at harmonizing outcome assessment are already existing such as for eczema (HOME for Harmonizing Outcome Measures for Eczema) (20) or, in the auditory field, for hearing loss (ICF for International Classification of Functioning, disability, and health core sets for hearing loss) (21). Then, we urge the tinnitus community to adopt and adapt these international standards of outcome definition and evaluation to the tinnitus field.

Deciding which outcome domains should be in this minimal core set requires a great deal of interactions and collaboration between stakeholders, professionals, and patients. This objective lends itself well to an international and multidisciplinary effort. We propose to follow a Delphi methodology to reach this goal. Delphi might be defined as "a method for structuring a group communication process so that the process is effective in allowing a group of individuals, as a whole, to deal with a complex problem" (22). Delphi methodology has been already proposed with success to reach a consensus in a variety of complex medical issues [e.g., see Ref. (23–25)]. Delphi methodology has also been used in the tinnitus field (26). Adapting a Delphi protocol to define a consensus core set of domains and a core set of instruments for tinnitus assessment in clinical trials is the aim of the recently launched Core Outcome Measures in Tinnitus (COMiT) initiative on behalf of the EU COST BM1306 action.[2] The activities of our COMiT initiative are registered on the website of the COMET initiative[3] according to roadmap that plans out a program of work. The first step is aimed at identifying and agreeing on core domains (27). It has started with two systematic reviews in order to establish existing knowledge and practice: the first review reflects the view of professional stakeholders by systematically looking at the current reported outcome domains in tinnitus intervention studies. The results of this review have already been published according to the PRISMA checklist of items to include when reporting a systematic review (5) establishing which outcome domains and outcome instruments have been measured in recent registered and published clinical trials. The second one represents the opinion of tinnitus people who experience the condition and/or their significant others by systematically searching in the literature and the internet the dimensions or domains that relate to tinnitus perceived intrusiveness. This second study will summarize the findings of narrative syntheses of qualitative data to establish which domains are important to patient and their significant others (28).

The data synthesis arising from both of these reviews will inform our online Delphi process, which will seek a consensus about what outcome domains are important both from healthcare professionals' and patients' perspectives. The methods for reaching consensus will use an iterative series of questionnaires, with an international multidisciplinary panel of patients, clinicians, and other professional stakeholder groups (such as

[2]http://www.cost.eu/COST_Actions/bmbs/BM1306.

[3]www.comet-initiative.org.

industry) all contributing with their views. We plan to conduct three independent Delphi processes devoted to different types of interventions (sound-, psychology-, and pharmacology-based interventions) that may require different COS. This Delphi process is illustrated in **Figure 1**. It is expected that the whole Delphi process will cover the 4-year period of the COST Action BM1306 grant. Our strong belief is that this innovative effort will improve not only trial reporting in the tinnitus domain but also the practical guidelines recommended in primary or secondary care settings for this common condition mistakenly considered to be always untreatable.

AUTHOR CONTRIBUTIONS

AL and DAH participated in conceiving, drafting, and revising the article and gave final approval of the version to be submitted.

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REFERENCES

1. McCormack A, Edmondson-Jones M, Somerset S, Hall D. A systematic review of the reporting of tinnitus prevalence and severity. *Hear Res* (2016) 337:70–9. doi:10.1016/j.heares.2016.05.009
2. Vio MM, Holme RH. Hearing loss and tinnitus: 250 million people and a US$10 billion potential market. *Drug Discov Today* (2005) 10(19):1263–5. doi:10.1016/S1359-6446(05)03594-4
3. Langguth B. Treatment of tinnitus. *Curr Opin Otolaryngol Head Neck Surg* (2015) 23(5):361–8. doi:10.1097/MOO.0000000000000185
4. Plein CT, Harounian J, Floyd E, Irizarry R, Ferzli G, Kidwai S, et al. A systematic review of eligibility and outcomes in tinnitus trials: reassessment of tinnitus guideline. *Otolaryngol Head Neck Surg* (2016) 154(1):24–32. doi:10.1177/0194599815608160
5. Hall DA, Haider H, Szczepek AJ, Lau P, Rabau S, Jones-Diette J, et al. Systematic review of outcome domains and instruments used in clinical trials of tinnitus treatments in adults. *Trials* (2016) 17(1):270. doi:10.1186/s13063-016-1399-9
6. Baguley D, McFerran D, Hall D. Tinnitus. *Lancet* (2013) 382(9904):1600–7. doi:10.1016/S0140-6736(13)60142-7
7. Noble W. Tinnitus self-assessment scales: domains of coverage and psychometric properties. *Hear J* (2001) 54(11):20–6. doi:10.1097/01.HJ.0000293150.63349.c7
8. Landgrebe M, Azevedo A, Baguley D, Bauer C, Cacace A, Coelho C, et al. Methodological aspects of clinical trials in tinnitus: a proposal for an international standard. *J Psychosom Res* (2012) 73(2):112–21. doi:10.1016/j.jpsychores.2012.05.002
9. Tyler RS, Oleson J, Noble W, Coelho C, Ji H. Clinical trials for tinnitus: study populations, designs, measurement variables, and data analysis. *Prog Brain Res* (2007) 166:499–509. doi:10.1016/S0079-6123(07)66048-8
10. *Guideline for Good Clinical Practice – E9_Guideline*. (1998). Available from: http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E9/Step4/E9_Guideline.pdf
11. Meikle MB, Stewart BJ, Griest SE, Martin WH, Henry JA, Abrams HB, et al. Assessment of tinnitus: measurement of treatment outcomes. *Prog Brain Res* (2007) 166:511–21. doi:10.1016/S0079-6123(07)66049-X
12. Kamalski DM, Hoekstra CE, van Zanten BG, Grolman W, Rovers MM. Measuring disease-specific health-related quality of life to evaluate treatment outcomes in tinnitus patients: a systematic review. *Otolaryngol Head Neck Surg* (2010) 143(2):181–5. doi:10.1016/j.otohns.2010.03.026
13. Meikle MB, Stewart BJ, Griest SE, Henry JA. Tinnitus outcomes assessment. *Trends Amplif* (2008) 12(3):223–35. doi:10.1177/1084713808319943
14. Fackrell K, Hall DA, Barry JG, Hoare DJ. Psychometric properties of the Tinnitus functional index (TFI): assessment in a UK research volunteer population. *Hear Res* (2016) 335:220–35. doi:10.1016/j.heares.2015.09.009
15. Langguth B, Goodey R, Azevedo A, Bjorne A, Cacace A, Crocetti A, et al. Consensus for tinnitus patient assessment and treatment outcome measurement: Tinnitus research initiative meeting, Regensburg, July 2006. *Prog Brain Res* (2007) 166:525–36. doi:10.1016/S0079-6123(07)66050-6
16. Meikle MB, Henry JA, Griest SE, Stewart BJ, Abrams HB, McArdle R, et al. The tinnitus functional index: development of a new clinical measure for chronic, intrusive tinnitus. *Ear Hear* (2012) 33(2):153–76. doi:10.1097/AUD.0b013e31822f67c0
17. de Vet HCW, Terwee CB, Mokkink LB, Knol DL. *Measurement in Medicine: a Practical Guide*. Cambridge, New York, NY: Cambridge University Press (2011). 338 p.
18. Prinsen CAC, Vohra S, Rose MR, King-Jones S, Ishaque S, Bhaloo Z, et al. Core outcome measures in effectiveness trials (COMET) initiative: protocol for an international Delphi study to achieve consensus on how to select outcome measurement instruments for outcomes included in a "core outcome set". *Trials* (2014) 15:247. doi:10.1186/1745-6215-15-247
19. Williamson P, Altman D, Blazeby J, Clarke M, Gargon E. Driving up the quality and relevance of research through the use of agreed core outcomes. *J Health Serv Res Policy* (2012) 17(1):1–2. doi:10.1258/jhsrp.2011.011131
20. Chalmers JR, Simpson E, Apfelbacher CJ, Thomas KS, von Kobyletzki L, Schmitt J, et al. Report from the fourth international consensus meeting to harmonize core outcome measures for atopic eczema/dermatitis clinical trials (HOME initiative). *Br J Dermatol* (2016) 175(1):69–79. doi:10.1111/bjd.14773
21. Granberg S, Swanepoel DW, Englund U, Möller C, Danermark B. The ICF core sets for hearing loss project: international expert survey on functioning and disability of adults with hearing loss using the international classification of functioning, disability, and health (ICF). *Int J Audiol* (2014) 53(8):497–506. doi:10.3109/14992027.2014.900196
22. Linstone HA, Turoff M, editors. *The Delphi Method: Techniques and Applications*. Reading, MA: Addison-Wesley Pub. Co., Advanced Book Program (1975). 620 p.
23. Potter S, Brookes ST, Holcombe C, Ward JA, Blazeby JM. Exploring methods for the selection and integration of stakeholder views in the development of core outcome sets: a case study in reconstructive breast surgery. *Trials* (2016) 17(1):463. doi:10.1186/s13063-016-1591-y
24. Hughes A-M, Bouças SB, Burridge JH, Alt Murphy M, Buurke J, Feys P, et al. Evaluation of upper extremity neurorehabilitation using technology: a European Delphi consensus study within the EU cost action network on robotics for neurorehabilitation. *J Neuroeng Rehabil* (2016) 13(1):86. doi:10.1186/s12984-016-0192-z
25. Casini A, de Moerloose P; The Congenital Fibrinogen Disorders Group. Management of congenital quantitative fibrinogen disorders: a Delphi consensus. *Haemophilia* (2016) 22(6):898–905. doi:10.1111/hae.13061
26. Sereda M, Hoare DJ, Nicholson R, Smith S, Hall DA. Consensus on hearing aid candidature and fitting for mild hearing loss, with and without Tinnitus: Delphi review. *Ear Hear* (2015) 36(4):417–29. doi:10.1097/AUD.0000000000000140
27. Hall DA, Haider H, Kikidis D, Mielczarek M, Mazurek B, Szczepek AJ, et al. Toward a global consensus on outcome measures for clinical trials in Tinnitus: report from the first International Meeting of the COMiT initiative, November 14, 2014, Amsterdam, The Netherlands. *Trends Hear* (2015) 4:19. doi:10.1177/2331216515580272
28. Haider H, Fackrell K, Kennedy V, Hall DA. Dimensions of tinnitus-related complaints reported by patients and their significant others: protocol for a systematic review. *BMJ Open* (2016) 6(10):e009171. doi:10.1136/bmjopen-2015-009171
29. Tyler RS. The use of science to find successful tinnitus treatments. *Proceedings of the Sixth International Tinnitus Seminar*; Cambridge, UK; 1999 September 5–9. London: The Tinnitus and Hyperacusis Centre. (1999). 3 p.
30. Hesser H. Methodological considerations in treatment evaluations of tinnitus distress: a call for guidelines. *J Psychosom Res* (2010) 69(3):305–7.

31. Newman CW, Sandridge SA, Jacobson GP. Assessing outcomes of tinnitus intervention. *J Am Acad Audiol* (2014) 25(1):76–105.

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Different Teams, Same Conclusions? A Systematic Review of Existing Clinical Guidelines for the Assessment and Treatment of Tinnitus in Adults

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Background: Though clinical guidelines for assessment and treatment of chronic subjective tinnitus do exist, a comprehensive review of those guidelines has not been performed. The objective of this review was to identify current clinical guidelines, and compare their recommendations for the assessment and treatment of subjective tinnitus in adults.

Method: We systematically searched a range of sources for clinical guidelines (as defined by the Institute of Medicine, United States) for the assessment and/or treatment of subjective tinnitus in adults. No restrictions on language or year of publication were applied to guidelines.

Results: Clinical guidelines from Denmark, Germany, Sweden, The Netherlands, and the United States were included in the review. There was a high level of consistency across the guidelines with regard to recommendations for audiometric assessment, physical examination, use of a validated questionnaire(s) to assess tinnitus related distress, and referral to a psychologist when required. Cognitive behavioral treatment for tinnitus related distress, use of hearing aids in instances of hearing loss and recommendations against the use of medicines were consistent across the included guidelines. Differences between the guidelines centered on the use of imaging in assessment procedures and sound therapy as a form of treatment for tinnitus distress respectively.

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Conclusion: Given the level of commonality across tinnitus guidelines from different countries the development of a European guideline for the assessment and treatment of subjective tinnitus in adults seems feasible. This guideline would have the potential to benefit the large number of clinicians in countries where clinical guidelines do not yet exist, and would support standardization of treatment for patients across Europe.

Keywords: tinnitus, clinical guidelines, assessment, treatment, systematic review

INTRODUCTION

Tinnitus is essentially made up of two components, the phantom perception of a sound in the ears or head, and the degree of emotional reaction to that percept. Tinnitus can co-occur with several medical-otological disorders such as presbycusis, though etiology is unknown for the majority of tinnitus patients (Baguley et al., 2013b). In rare cases tinnitus indicates a serious underlying pathology such as vascular troubles, vestibular schwannoma (VS), or otosclerosis (Baguley et al., 2013a). In most cases however subjective tinnitus is a benign symptom. In many patients co-morbidities exist such as anxiety, depression, insomnia, and concentration problems, all of which severely impair quality of life (Langguth et al., 2011). In 1–3% of cases tinnitus causes severe health problems, with a wide range of effects on daily life functioning (Davis and Refaie, 2000; Fujii et al., 2011; Kim et al., 2015). Evidence corroborates that the aversive psychological reactions, such as cognitive problems, negative emotions, and dysfunctional attentional processes are of main importance in leading to a severe tinnitus condition (Erlandsson and Hallberg, 2000; Andersson et al., 2006; Cima et al., 2011; Kleinstauber et al., 2013; McKenna et al., 2014; Handscomb et al., 2017).

During the last decades, efforts have been made to better understand tinnitus pathophysiology and provide specialized treatments to patients (Kamalski et al., 2010; Cima et al., 2012; Langguth et al., 2013; Hoekstra et al., 2014). A large number of management strategies including various assessment and treatment procedures exist and have evolved but lack empirical support. For example, there is no evidenced treatment or licensed pharmacological therapy to eliminate the tinnitus percept (Langguth and Elgoyhen, 2012). The Cochrane Library lists 10 completed systematic reviews on different tinnitus treatments, all of which reported small numbers of studies of variable quality (e.g., Martinez-Devesa et al., 2010). These facts combined makes it difficult for healthcare professionals to decide what is best for which tinnitus patient. This is evidenced by the discrepancy between scientific and clinical perspectives on the management of tinnitus and the *actual* day-to-day practice in European healthcare settings (Hoare et al., 2012); tinnitus patient care is fragmented and *ad hoc* (Hoare and Hall, 2011; Hoare et al., 2012). To date there has been no overview of the number of existing clinical practice guidelines for tinnitus, the details included, their comparability, or their purpose. Clinical practice guidelines are defined as systematically developed statements to assist practitioner and patient decisions about appropriate health care for specific clinical circumstances (Field and Lohr, 1990). They have the benefit of simplifying and standardizing assessment and treatment options for clinicians and patients. A European Union guideline would extend this benefit to 28 countries. This systematic review aims to identify, review, and examine the clinical guidelines which do exist for tinnitus. The tinnitus assessments (diagnostics and measures), processes, and treatment options recommended by the respective guidelines will be compared and summarized.

METHODS

The aims, the work plan, and the protocol for this systematic review were developed by TINNET Working Group 1, a COST Action BM1306 (2014–2018) to create a pan-European tinnitus research network (http://tinnet.tinnitusresearch.net/). This review was registered with PROSPERO, the international register of systematic reviews (protocol number: CRD42016038588) prior to commencing the literature search. The review was exempt from human ethics procedures as there were no human participants and only secondary sources of data (the clinical guidelines) were used.

Eligibility Criteria

Records were considered eligible for inclusion if they fit the definition of a guideline by describing and making recommendations on the assessment, diagnosis, and or treatment of subjective tinnitus for adults (i.e., people aged 16 years or older). Those records were required to identify or describe themselves as guidelines, and be the most recent guideline form the country of origin. No publication date or language restrictions were imposed on the eligibility of the guidelines.

Guidelines were excluded if they were for objective tinnitus, pediatrics, referred only to the triage or referral pathways for assessing and treating tinnitus, or if they were a guide for only one specific type of assessment or treatment procedure for tinnitus.

Literature Search

The literature search for clinical guidelines included the Medline, PubMed, and the Cumulative Index to Nursing and Allied Health Literature (CINAHL), and EMBASE databases. In addition to these the National Guideline Clearinghouse (www.guideline.gov), National Institute for Health and Clinical Excellence (NICE; https://www.nice.org.uk/), Guideline International Network (GIN; http://www.g-i-n.net/), Google, and hand-search of reference lists of any included guidelines was undertaken. International experts were also contacted to ask if they were aware of any guidelines that had not already been identified from

the search results. The date that the search for guidelines was first conducted was 2 May 2016 and was undertaken by TEF and HH using "tinnitus" and "guideline" as the two key terms. The final search was conducted on 24 June 2016.

Study Selection

Two reviewers independently screened search results by title and abstract, and then by full text if required. The first 20 pages of search results from Google, and all search results from GIN, NICE, and the National Guideline Clearinghouse were screened. In the event of disagreements, a third reviewer (BM) acted as an arbiter. As an additional check and in line with other systemic review searches using internet search engines, a *post-hoc* rule of stopping searching after three consecutive pages without new search results was applied. In this case, no new search results were identified after the first eight pages.

Data Extraction

Data extraction was undertaken using a tailored form that had been pilot tested and was emailed to reviewers in the form of an Excel spreadsheet. A document with guidance on the extraction of information for each of the items was provided to each of the reviewers to improve consistency of data extraction. Data extraction from each guideline was undertaken by at least two reviewers who were native speakers of or fluent in the language in which the guideline was published. Reviewers extracted information from the guidelines regarding items about the: country and year of publication, availability, author details, sponsor/funder involved, scope, target audience, developers and process related to the guideline, recommendations for assessment and treatment procedures, the level of evidence and type of rating system used (e.g., Oxford) related to the recommendations, and items related to the implementation and revision of the guideline.

Data Management

HH and TEF were responsible for data management and maintained editorial rights. All identified records were saved into a Microsoft word master file and then saved in pdf-copy.

Quality Assessment and Risk of Bias

All reviewers of the guidelines also completed the AGREE II tool (Brouwers et al., 2010) to assess the quality of the guidelines. AGREE II is an international tool to assess the quality and reporting of practice guidelines (www.agreetrust.org). It contains 23 items grouped under six guideline domains. Each item is scored on a 1–7 scale where 1 = "Strongly disagree" and 7 = "Strongly agree." Scores are standardized to provide an overall percentage score. Previous reviews have used a 60% marker to distinguish high and low quality guidelines (Sanclemente et al., 2014; Ruszczynski et al., 2016).

Details relating to the sources of funding, professional affiliations, and editorial independence of the guideline developers were extracted as indicative of risk of bias.

Data Synthesis

Data extracted by the reviewers were collated and integrated into summary tables and a narrative synthesis describing the similarities and differences between the clinical practice guidelines was completed.

RESULTS

Five clinical guidelines for tinnitus were ultimately included in this review (see **Figure 1** for details of the search and selection process). They were guidelines from Denmark (Jørgensen et al., 2007), Germany (The Association of the Scientific Medical Societies, 2015), The Netherlands (Dutch Association for Ear Nose Throat and Head surgery [Nederlandse Vereniging voor Keel – Neus – Oor heel kunde en Heelkunde van het Hoofd – Halsgebied], in press), Sweden (Idrizbegovic et al., 2011), and United States (Tunkel et al., 2014). Several documents were excluded as by definition not providing a guideline. For example, the Australian audiology clinical practice standards (Audiology Australia, 2013) underwent full-text screening but was not included as it only related to audiological management and had a brief section on tinnitus assessment. The UK Good Practice Guide (Department of Health, 2009) also was excluded as it explicitly states: "This Good Practice Guide to the delivery of services is not, and does not aim to be, an evidence-based guideline for clinical practice with individual patients" (p. 5). The Tinnitus Research Initiative (TRI) algorithm (Biesinger et al., 2010), after some debate within the review team, was also excluded because it was judged not to be a "clinical guideline." A list of full text documents considered but excluded is in Appendix 1.

Although there was not a restricting time period for the guidelines, no guidelines older than 10 years were identified. With exception of the Danish guideline (published in 2007) all were developed during the last 5 years.

Details about Development of the Clinical Guidelines

Table 1 provides detailed information about the stakeholder involvement, rigor of development, and the editorial independence associated with the respective clinical guidelines.

All the guidelines included information on the professional backgrounds of the participants in the respective development groups and in three out of the five cases (American, Dutch, and German), provided information on how views of funding bodies and competing interests were addressed. Although patient groups and the public were consulted in the development of three guidelines (American, Dutch, and German), the actual expected users of the guidelines were health professionals.

Details were provided in all guidelines (with the exception of those from Sweden which did not provide methodological information) about how literature was located and used to inform the respective recommendations. That is, details of search strategies using MeSH and other search terms and databases such as Medline and PsychInfo were included. Tools and criteria used to assess the evidence included the: Oxford Centre for Evidence Based Medicine (U.S. and German guidelines) and American Academy of Paediatrics' (American guideline) evidence criteria respectively, the AMSTAR checklist (Dutch guideline), and

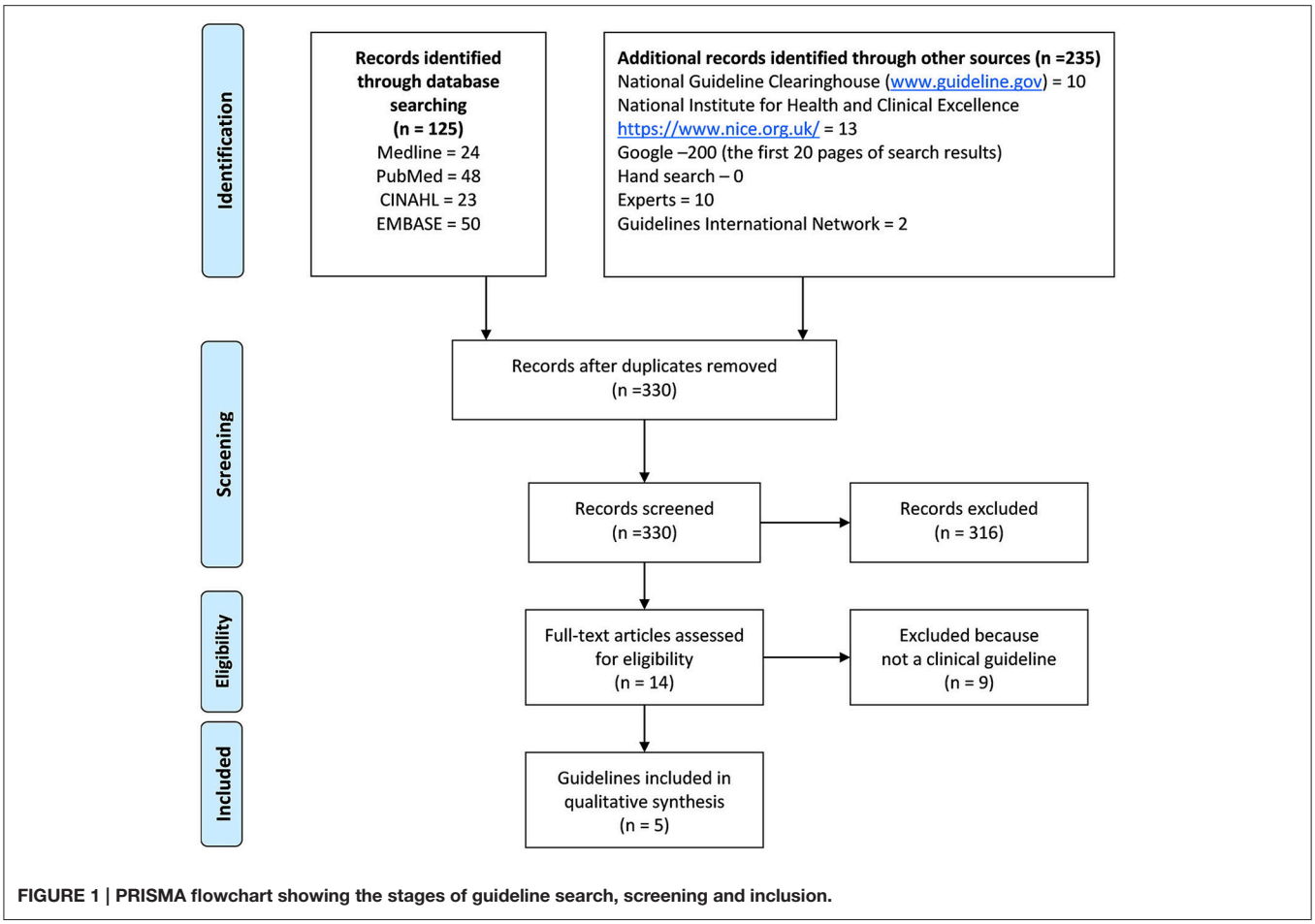

FIGURE 1 | PRISMA flowchart showing the stages of guideline search, screening and inclusion.

the GRADE ranking system of trust in conclusions of the literature (Dutch guideline). American, Danish, Dutch and German guidelines all provided information and referred to the research literature associated with each recommendation as well as describing their methods for reaching consensus on each recommendation. The Dutch, German and U.S. guidelines consider the strengths and limitations of the research literature and were reviewed externally prior to publication. Similarly, those three guidelines also state a year by and/or describe conditions under which they would be revised.

Assessment Recommendations in the Clinical Guidelines

Table 2 compares assessment recommendations between the respective national tinnitus guidelines. All guidelines, except the Danish, recommend a clinical history (anamnesis/targeted history/special tinnitus anamnesis) be taken.

All guidelines describe the need for physical examination by an ENT doctor, although physical examination is not explicitly referred to in the Swedish guideline. The American guideline recommends examination to exclude objective tinnitus, cardiovascular disease and vascular lesions, neurologic diseases, middle or outer ear infection/disease, vertigo, head-neck masses, or other treatable conditions. The German guideline additionally mentions cervical, dental, and temporomandibular joint functional exploration in a silent environment to evaluate tinnitus modulation.

Audiological assessment was recommended in all the included guidelines. The majority refers to audiometry as a general category, but the German guideline provides most detail. For example, it specifies details relating to the assessment of oto-acoustic measurements, brainstem auditory evoked responses, caloric tests, determination of tinnitus loudness and frequency using narrow-band noise and pure tones, residual inhibition, Feldmann masking curves (Feldmann, 1984), and loudness discomfort level. None of the other guidelines included in this review recommend psychoacoustic measurements of tinnitus frequency or intensity.

The German guideline does not refer to specific psychological assessments though the other guidelines do in varying terms. For example, when tinnitus is severe or accompanied by psychological factors, the Swedish guideline recommends psychological assessment while the Danish guideline recommends a structured interview. The American guideline on the other hand recommends that clinicians distinguish between patients with or without bothersome tinnitus for subsequent referral (when necessary) to a psychologist or psychiatrist.

TABLE 1 | Summary of guideline development by country.

Country	Professionals involved	Views of patients considered	Target users	Views of funding body	Competing interests
Germany	Audiologists, psychiatrist, psychologists, otolaryngologists, dentists, pediatricians, neurologists, and patient representative groups	Patient representative groups were included in the guideline development group; contributed to external review on draft documents, and patient related information was also considered from the results of a literature review	Physicians (especially ENT), phoniatry and pediatric audiology, psychiatry, psychosomatic, neurology, mouth, jaw, and facial surgeons and dentists, psychologists, general practitioners	A statement concerning financial and other interests and editorial independence is included.	Competing interests are declared and when relevant, stakeholders with competing interests were excluded
Denmark	Speech Pathologist and hearing therapists	NS	Hearing therapists	NS	NS
Netherlands	Details provided. ENT-doctors, psychologist, clinical physicist-audiologist	Dutch Association of the Hearing Impaired consulted. A literature review regarding patient preferences was also conducted	ENT doctors, audiology centers, GP's, psychologists, psychiatrists	A statement of independence was signed by professionals involved	Competing interests are declared
USA	Paediatric and adult otolaryngologists, otologists/neurotologists, geriatrician, behavioral neuroscientist, neurologist, audiologist, family physician, radiologist, psychiatrist, psycho-acoustician, nurse, physician, and consumer advocates	Yes: also included a draft of the guideline being made available for public comment	Any clinician, health care provider, specialty physicians, and non-physician providers such as audiologists and mental health professionals	Funded by American Academy of Otolaryngology—Head and Neck Surgery Foundation but no statement of independence from the process	Competing interests are declared
Sweden	Partial details provided—included medical doctors, and professional representatives from the tinnitus teams for diagnostics and rehabilitation	NS	Staff at the audiology and balance clinic at Karolinska University Hospital and professionals that might refer to the clinic (GPs, ENTs or audiologist)	NS	NS

(Continued)

TABLE 1 | Continued

Country	Methods used	Evidence criteria	Category of evidence*	Strengths and limitations	Methods for reaching consensus	Consequences of the recommendations	Link between evidence and recommendations	Peer review?	Update of the guidelines?
Germany	Systematic methods used, details provided in the guideline	Classified according to Oxford Centre of Evidence-based Medicine criteria	1a	Strengths and limitations of the body of evidence are clearly described	Formal consensus technique	The guideline includes health benefits, side effects and risks formulating the recommendations	There is an clear link between the recommendations and the supporting evidence	External review	Due in 2020.
Denmark	Systematic methods used, details provided in the guideline	NS	The guidelines are based on literature, and articles based on the consensus of leading professionals in the field of audiology (evidence level IV)	NS	Informal consensus. All recommendations are based on the ICF model	NS	Each recommendation is provided with an argument based on relevant literature	Not peer reviewed	NS
Netherlands	Systematic methods used, details provided in the guideline	Based on AMSTAR checklist	1a, 1b, IV	The strength of the evidence is specified according to GRADE. Evidence tables describe limitations and strengths of the included studies	Recommendations were evidence based and the importance the workgroup gave to them conforms to GRADE	Recommendations were made considering the scientific value, preferences of the patient, costs, and availability of the organization	There is a clear link between the recommendations and the supporting evidence	External review	Update due in 2020 or sooner if new compelling evidence warrants earlier consideration
USA	Systematic methods used, details provided in the guideline	Based on criteria from the Oxford Centre for Evidence-Based Medicine	American Academy of Pediatrics Categories of evidence (A, B, C, D, and X) updated to be in accordance with Oxford Centre for Evidence-Based Medicine	Strengths and limitations of the body of evidence are clearly described	This guideline was developed using an explicit and transparent a priori protocol for creating actionable statements based on supporting evidence and the associated balance of benefit and harm	The benefits and harms of the recommendations have been considered for each recommendation.	There is an clear link between the recommendations and the supporting evidence	External review	Update due in 2018/9 or sooner if new compelling evidence warrants earlier consideration
Sweden	No method reported	No evidence criteria	No evidence provided	None provided.	NS	NS	NS	NS	NS

**Unless stated, the level of evidence refers to/uses the Oxford Centre for Evidence Based Medicine criteria (GRADE system consists of 4 grades of degree of trust in conclusions of the literature: high, moderate, low, and very low) NS, not specified; ENT, Ear Nose Throat; GP, General Practitioner.*

TABLE 2 | Clinical guideline recommendations regarding assessment of patients with tinnitus.

Guideline	Physical examination	Hearing and audiology tests	Psychological assessment	Assessment tools/questionnaires recommended	Other assessment procedures	Procedures not recommended
Germany	Orientating neurological assessment of cervical spine, vestibular is with examination of denture (including TMJ) in silence to screen modulation of tinnitus • Orientating examination of functioning of N. facialis • ENT examination including tympanic membrane microscopy, asopharyngoscopy and eustachian respectively • stethoscopic examination of the ear and of the carotid artery, particularly in pulsatile tinnitus	• Pure tone audiometry • discomfort, possibly with categorical loudness scaling • determining of tinnitus loudness and frequency using narrow-band noise and pure tones • residual inhibition • determining the minimum masking level by white noise and pure tones; masking curves according to Feldmann • tympanometry and acoustic reflex including recording possible changes due to breathing or heart rate • TEOAE and/or DPOAE • brainstem auditory evoked response (BAER) • preliminary vestibular examination possibly including caloric testing • Brainstem audiometry (BERA) when medically justified, economically viable and likely to be useful in informing counseling might be of potential benefit	NS	• Goebel-Hiller Tinnitus Questionnaire, • VAS or other validated scales	• Special tinnitus anamnesis (see Structured Tinnitus Interview (Goebel and Hiller, 2001) • X-rays of the cervical spine, if further indicated also functional images	• Acoustic examination with more than 84 dB 1 week after acute tinnitus or tinnitus exacerbation
Denmark	NS	• Audiometry (performed by ENTs) • LDL/UCL • If necessary also: ABR	• Structured interview	• THI-DK • VAS-scale for hyperacusis • Tværfaglig Tinnitus Screening (Danish tool assessing signs of anxiety)	If necessary also: • ABR, • CT/MRI, • blood samples, • other neurological tests	NS
Netherlands	• Anamnesis, • ENT-assessment inclusive otoscopy and tuning fork tests, • Blood pressure measurement, • Flexible nasofaryngoscopy, • Palpation of neck and area around ear	• Audiometry (Air and bone conduction) • Speech audiometry	• Detailed assessment regarding the nature how tinnitus impacts on daily life and functioning, comorbid symptoms	• TQ, mini-TQ • THI • TFI • THQ • HADS	• MRI/MRA, • CT, • DSA (angiography)	• Not to use MRI with every patient with non-pulsatile, unilateral tinnitus.

(Continued)

TABLE 2 | Continued

Guideline	Physical examination	Hearing and audiology tests	Psychological assessment	Assessment tools/questionnaires recommended	Other assessment procedures	Procedures not recommended
USA	• Targeted history and physical examination of the head and neck including otoscopy and neurologic examination. • When pulsatile tinnitus is reported, the examination should focus on identification of cardiovascular disease and vascular lesions	• Prompt, comprehensive audiological examination (Tonal and Speech audiometry and Immittance) in patients with tinnitus that is unilateral, persistent (≥6 months), or associated with hearing difficulties (Strong recommendation); • Initial comprehensive audiological examination (including ear specific masked air and bone conduction) in patients who present with tinnitus regardless of laterality, duration, or perceived hearing status (Option)	• Distinction between patients with bothersome tinnitus from patients with non-bothersome tinnitus. • Assess degree of tinnitus related disability (including baseline measurement for the purpose to establish effects of treatment). • Assess if further psychological treatment required	• TQ, • TEQ, • THQ, • TRQ, • THI, • TFI	NS	• Imaging studies unless patients have one or more of the following: tinnitus that localises to one ear, pulsatile tinnitus, focal neurological abnormalities, or asymmetric hearing loss
Sweden	NS	• Audiometry (including LDL when necessary) • Speech and speech in noise test and impedance audiometry • ABR and MRI when necessary	• In case of severe tinnitus: the first encounter with the psychologist/ psychiatrist is investigative and informative. (1) symptoms tinnitus, (2) individual's mental status, (3) the overall life situation	• BAS (basic own questionnaire), • THI • HADS (when necessary)	• Anamnesis focused on tinnitus onset, laterality, character and patients' problems. • Consideration of psychological factors and somatosensory factors	NS

ABR, Auditory brainstem response; BAER, Brainstem auditory evoked response; CT, Computer tomography; DSA, Digital subtraction angiography; DPOAE, Distortion product optoacoustic emission; ENT, Ear nose throat; GP, General Practitioner; HADS, Hospital Anxiety and Depression Scale; LDL, Loudness discomfort level; MRA, Magnetic resonance angiography; MRI, Magnetic resonance imaging; TEOAE, Transient evoked optoacoustic emission; TEQ, Tinnitus evaluation questionnaire; TFI, Tinnitus functional index; THI, Tinnitus handicap inventory; THQ, Tinnitus handicap questionnaire; TMJ, Temporomandibular joint; TQ, Tinnitus questionnaire; TRQ, Tinnitus reaction questionnaire; UCL, Uncomfortable listening level; VAS, Visual analog scale.

The Tinnitus Handicap Inventory (THI; Newman et al., 1996, 1998) is the most frequently referred to assessment questionnaire followed by Tinnitus Questionnaire (TQ; Goebel and Hiller, 1994). Visual Analog Scales (VAS; e.g., Germany, Denmark) and the Hospital Anxiety and Depression Scale (HADS; Zigmond and Snaith, 1983, e.g., The Netherlands, Sweden) were referred to by at least two guidelines. The American guideline referred to a large number of questionnaires including the: TQ (Goebel and Hiller, 1994), THI (Newman et al., 1996, 1998), Tinnitus Effects Questionnaire (TEQ; Hallam et al., 1988), Tinnitus Handicap Questionnaire (THQ; Kuk et al., 1990), Tinnitus Reaction Questionnaire (TRQ; Wilson et al., 1991), and Tinnitus Functional Index (TFI; Meikle et al., 2012).

Several guidelines make recommendations for or against the use of other assessment related procedures. For example, the German guideline refers to X-rays of the cervical spine. Although three guidelines recommend magnetic resonance imaging (MRI) as an assessment of tinnitus, The American and the Dutch guideline recommend against it, unless patients have one or more of: tinnitus that localizes to one ear, pulsatile tinnitus, focal neurological abnormalities, or asymmetric hearing loss. The German guideline also recommends against acoustic examination using sound pressure levels more than 84 dB 1 week after acute tinnitus or tinnitus exacerbation.

Summary of Recommendations Regarding the Assessment of Subjective Tinnitus

- Conduct a thorough physical examination to exclude possible (physical) causes of tinnitus (three of five guidelines; not stated in Danish and Swedish).
- Complete a thorough audiological assessment (all guidelines).
- Establish the degree to which a patient experiences subjective tinnitus as bothersome or distressing using a validated and reliable multi-item questionnaire such as the TQ, THI, TFI, or HADS (all guidelines).
- In situations where patients appear to be experiencing a degree of distress or difficulties related to living with tinnitus, consider making a referral for an assessment by a psychologist or psychiatrist (four of five guidelines; not stated in German guideline).
- Variation exist in recommendations regarding the use of imaging studies (e.g., MRI).

Treatment Recommendations across the Guidelines

Table 3 compares therapeutic recommendations for the treatment of subjective tinnitus between the respective national tinnitus guidelines; note the Danish guideline is not included in this table as it provides only recommendations regarding assessment procedures. Across the guidelines there is generally a high degree of consistency in the recommendations for or against: the use of medicines (prescribed drugs and herbal supplements); audiological and psychological interventions; and, transcranial magnetic stimulation. Greatest variation occurs in the recommendations concerning the use of therapies involving sound such as Tinnitus Retraining Therapy (TRT).

There is a consensus that medicines should not be prescribed for the treatment of subjective tinnitus, though some variation in the level of specificity that each guideline has. For example, the German guideline lists specific medicines that should not be prescribed for the treatment of tinnitus. The German and Swedish do however note that medicines such as antidepressants might be prescribed to treat comorbid conditions. Herbal supplements such as Gingko biloba are also specifically recommended against being used in all guidelines except for Sweden which does not make recommendations for or against their use.

The use of hearing aids is recommended by all guidelines but only when clinically meaningful hearing loss is also present in people suffering from tinnitus. The use of a cochlear implant is mentioned in the Dutch and German guidelines and only recommended when there is profound hearing loss or deafness in addition to tinnitus. The Dutch guideline is the only one to provide scores on tinnitus questionnaires (e.g., TQ, THI) for when such interventions should be considered (e.g., it recommends referral to specialized stepped-care CBT for tinnitus in cases where TQ score is greater than 30, in combination with a clinically relevant request for healthcare by the patient, as is judged by the referring party).

Psychological interventions for tinnitus can potentially include a wide range of components but there is general consensus on the use of two of them. In particular, the provision of information and education about tinnitus and treatment options is consistently recommended across the guidelines although there is some variation in the specificity of the content that each provides. Second, specialized CBT for tinnitus is specifically recommended by all the guidelines except for Sweden which mentions it only in relation to the presence of stress, anxiety, or depression.

Least consistency exists across the guidelines in relation to TRT. Specifically, the Dutch guideline recommends that TRT can only be contemplated if tinnitus is very mild (TQ < 30) and the patients specifically asks for TRT, the American guideline indicates that sound therapies "may" be recommended to patients with tinnitus, while the Swedish guideline recommends that sound stimulation be used as part of TRT for people without hearing loss. The German guideline recommends the use of notched music therapy, but recommends against the use of TRT.

In relation to other less commonly used treatments (such as acupuncture or hyperbaric oxygen), the guidelines mostly indicate that there is an insufficient body of evidence to be able to make recommendations for or against their use.

Lastly, three guidelines (Germany, The Netherlands, and U.S.) either caution that there is insufficient evidence, or make additional recommendations against the use of transcranial magnetic stimulation (rTMS), transcranial direct current stimulation (tDCS), dietary supplements, neuromodulation treatments, and hearing aids for tinnitus patients without hearing loss.

Summary of Therapeutic Recommendations regarding the Treatment of Subjective Tinnitus

- Provide information about tinnitus and treatment options (all guidelines).
- Use hearing aids only when patients also experience hearing loss (all guidelines).

TABLE 3 | Tinnitus guideline recommendations regarding treatments for tinnitus.

Country	Medicine	Audiological	Psychological	Sound therapies	Other	Treatments recommended against using
Germany	• None for tinnitus but refers to, for example, some for co-morbid depression, e.g., glutamate-antagonists.	• Hearing aids for patients with hearing loss; Cochlear Implants for patients with deafness.	• General counseling (including information provision). • Tinnitus specific CBT (aimed at reducing attention focusing toward the ear noise, reappraisal of the tinnitus and its consequences) individual or group-settings, also treatment for comorbidities. • Hospital treatment for decompensated tinnitus and/or with severe psychiatric comorbidity. • An absence of conclusive evidence of effectiveness for self-help groups.	• Audio therapy including "notched music," "coordinated reset" or music therapy.	• Absence of evidence of effectiveness for: acupuncture, cervical vertebral spine therapy/ physiotherapy, hyperbaric oxygen; and, electric stimulation (e.g., transcutaneous electric stimulation, ear and cervical spine; vagus stimulation); Acoustic Coordinated-Reset Neuromodulation. • Uncertain recommendation for rTMS.	• Sound therapy including Noiser and TRT. • Hearing aids for patients with only tinnitus. • Medicines (including: steroids, melatonin, antidepressants, Sulpirid, Apraxolam, Sertraline, Botox A, Pramiprexol, Nortriptyline, Piribedil, Vardenafil, Trazodone, Atorvastatin, Gabapentin, anticonvulsants, Paroxetine, Lamotrigine, Cyclandelat, Baclofen, Nicotinamide, Tocainid, Misoprostol, Egb 761, Amitriptyline, Misoprostol, Pramipexole Dopamine. • Herbal medicines and vitamins (including Ginkgo biloba zinc).
Netherlands	None	• Consider a trial of hearing aids. • In patients with high TQ (>60) or THI (>78) scores, and have severe hearing loss or deafness and have not responded to CBT, consider Cochlear Implant.	• Educational material about tinnitus and treatment options considered essential. • Specialised CBT for patients with TQ > 30 or THI >36.	• TRT can only be contemplated in case tinnitus is very mild (TQ<30) and the patients specifically asks for TRT.	None	• rTMS. • TDCS. • Gingko biloba. • Acupuncture. • Auditive perceptual training. • Hyperbaric oxygen.
Sweden	• None for tinnitus specifically but does state that if necessary, sleeping pills or antidepressants, can be used to treat sleep disorders or depression (no drug types, names, or dosage provided).	• For people with tinnitus and hearing loss hearing aids are fitted.	• Individual or group tinnitus information meetings. For patients without hearing loss, this is based on a modified version of TRT protocol. • There is reference to CBT in case of stress/ anxiety/ depression, but no clear recommendation.	• Sound stimulation as part of TRT for people without hearing loss.	• For middle ear dysfunctions such as otosclerosis, surgery is possible – no clear recommendation is provided. • For tensions or pain in the jaw, neck, shoulders or back, referral to "bite" therapist, or physiotherapist.	None

(Continued)

TABLE 3 | Continued

Country	Medicine	Audiological	Psychological	Sound therapies	Other	Treatments recommended against using
USA	None	• Clinicians *should* recommend a hearing aid evaluation for patients with hearing loss and persistent, bothersome tinnitus.	• Clinicians *should* educate patients with persistent, bothersome tinnitus about management strategies. • Clinicians *should* recommend cognitive behavior therapy to patients with persistent, bothersome tinnitus.	• Clinicians *may* recommend sound therapy (e.g., TMT, TRT) to patients with persistent, bothersome tinnitus but patients must be informed of potential outcomes as well as costs associated with sound therapy.	• *No recommendation* can be made regarding the effect of acupuncture in patients with persistent bothersome tinnitus based on the poor quality of trials, no benefit, and minimal harm.	Clinicians *should not* routinely recommend: • Medicine (including antidepressants, anticonvulsants, anxiolytics, or intratympanic medications for a primary indication of treating persistent, bothersome tinnitus. • Dietary supplements and herbal medicines (e.g., Ginkgo biloba, melatonin, zinc). • TMS.

CBT, Cognitive behavioral therapy; NS, not specified; rTMS, repetitive Transcranial magnetic stimulation; TCDS, Transcranial direct stimulation; THI, Tinnitus handicap inventory; TMS, Transcranial magnetic stimulation; TMT, Tinnitus management therapy; TQ, Tinnitus questionnaire; TRT, Tinnitus retraining therapy.

- Specialised CBT for tinnitus should be offered to patients (three of four guidelines; Sweden refers to use of CBT in context of co-morbid anxiety or depression).
- There is a lack of consensus on the use of TRT for tinnitus.
- Prescribed medicines and herbal supplements should not be used for the treatment of tinnitus (all guidelines).
- Treatment with TMS is recommended against by Dutch and U.S. guidelines, and German guidelines give an "uncertain" recommendation.

Quality Assessment of the Guidelines

The AGREE II tool was used by the authors who undertook data extraction of the respective guidelines and the summarized results are shown in **Table 4**. In general the domains of "stakeholder involvement" and "clarity of presentation" respectively by guideline developers were rated high (good quality). Conversely, ratings on the domain of "applicability" which refers to how the guidelines might be disseminated, implemented and evaluated were low. For the domains addressing the scope and purpose of the guidelines, rigor of development and editorial independence, a pattern emerged whereby the American, Dutch and German guidelines were rated considerably higher (AGREE II scores >60% on all domains) than the Danish and Swedish guidelines (AGREE II scores <60% on all domains).

DISCUSSION

This systematic review aims to compare existing clinical guidelines for the assessment and treatment of subjective tinnitus in adults. Five guidelines, developed in the last 10 years within Europe, Scandinavia, and North America were included in the review. Although there are differences in some specific recommendations for assessment and treatment procedures across the guidelines, in general, commonalities across guidelines were high. The fact that there are differences in some of the recommendations is not surprising and appears to reflect the relatively young state of the field and the evolving nature of assessment and treatments for subjective tinnitus—a symptom with a high level of heterogeneity. On the other hand, the level of agreement, for example, in the recommendation of specialized cognitive behavioral therapy reflects the growing evidence base for the effectiveness of this treatment to alleviate patients' distress and impairment, even though significant changes in the tinnitus percept itself as a result of CBT have been proposed, though not yet assessed across studies.

When the methods of the development of guidelines were reported, it was clear that the respective groups were making efforts to be transparent, systematic, and using the best available evidence base, and frequently linking recommendations to specific research literature. For example, systematic reviews and meta-analyses were referred to whenever available to inform recommendations. It should be noted though that there is a lack of high quality studies or powered randomized trials of some treatments either for practical or methodological reasons. Regardless, the strengths and limitations of the evidence for particular recommendations were included for the majority

TABLE 4 | Summary of AGREE II domain scores (%) by country.

	Scope & Purpose	Stakeholder involvement	Rigour of development	Clarity of presentation	Applicability	Editorial independence
Germany	61	94	83	89	71	67
Denmark	52	44	24	59	2	17
Netherlands	81	100	97	100	9	100
USA	86	97	93	100	71	88
Sweden	42	42	1	33	2	13
Median	61	94	83	89	9	67
Average	64	75	60	76	31	57

of the guidelines and thus enable the user/reader to make informed decisions about following the recommended actions. Furthermore, target users were generally clearly defined and the development groups were comprised of a range of the health professionals often involved in the assessment and treatment of tinnitus. These two factors are important not only for providing expert input into the guideline, but also for garnering "buy-in" from potential users of the guidelines and focussing the content.

Differences between the Guidelines

Differences in recommended assessment procedures tend to relate to specific techniques (questionnaires, diagnostic tests, types of scanning techniques) rather than general principles [e.g., trying to establish tinnitus severity, hearing loss, psycho-social problem(s)], or the presence or absence of severe physical pathology that might be causing the tinnitus. Differences related to, for example, the recommended questionnaires for assessing tinnitus related interference and distress. While all the guidelines referred to the THI (the German guideline indirectly refers to this), only the American, Dutch and German guidelines referred to the TQ. Recommendations for specific questionnaires to measure psychological distress (especially symptoms of anxiety and depression) also varied with some guidelines not mentioning any (e.g., United States) and others such as the Dutch and Swedish guidelines which referred to the HADS. Differences also existed between the recommendations to assess loudness discomfort levels with the American and Dutch guidelines not recommending the use of such tests while the other guidelines did.

With regard to treatments, differences are found primarily regarding recommendations for the use of sound therapies. TRT specifically is not recommended by the German guideline, conditionally by the Dutch guideline and the American guideline indicates that clinicians "may" recommend it; TRT is currently being tested in a large multicenter trial in the U.S. (clinical trials ID: NCT01177137). A lack of evidences about other treatments such as acupuncture, hyperbaric oxygen and some herbal supplements leads most groups to recommend against them. The American guideline though is more cautious and simply states that because there is a lack of evidence they can neither recommend for or against the use of such treatments.

Differences in the recommendations of assessment and treatment procedures could be explained by a combination of factors including the time of the development of the guideline and availability of translated versions of the questionnaires (e.g., the TFI was published in 2012 which was after that of the Danish and Swedish guidelines), the known psychometric properties of the questionnaires themselves [e.g., concerns have been raised about the cross-cultural use of the HADS (Maters et al., 2013)], and the different methods used to reach consensus by the different guideline groups.

Consistencies across the Guidelines

Across the guidelines consensus appears to exist on a number of important general features of assessment relating to subjective tinnitus. Specifically, there is consensus about the initial need for excluding a physical cause of the tinnitus, conducting an audiometric assessment of the patient, using standardized questionnaires to measure degrees of tinnitus related distress, and when relevant, making referrals for further psychological assessment.

Regarding the therapeutic recommendations for the treatment of subjective tinnitus, all guidelines recommend against the use of medicines for the treatment of the tinnitus specifically but note that medicines are appropriate for treating co-morbid conditions. There is also agreement in the recommendations to use hearing aids for patients experiencing hearing loss and CBT to facilitate adjustment to the symptom, alleviate distress and tinnitus-related interference in daily life.

As a group of tinnitus researchers and clinicians, we endorse the specific principles and practices of assessment and treatment that are consistently found across the guidelines. Further, while a treatment for removing the tinnitus percept does not exist, we reiterate the importance of providing patients with bothersome tinnitus, evidence based cost-effective treatment(s) in a way (such as stepped care) that is minimally burdensome to the patient. That is patients who are assessed as having relatively little tinnitus related distress and interference should receive less intensive treatment in the first instance, than someone who is assessed as having severe levels of distress and interference in activities of daily living.

Strengths and Limitations of the Review

There are two critical factors that affect the conclusions that can be drawn from the included guidelines. Firstly, and as with all systematic reviews, the search strategy and inclusion criteria used determine what is located and subsequently included. In this review, we used the search terms "tinnitus" and "guideline" to conduct the search in a wide range of databases, repositories of clinical guidelines, and search engines, with the intention of being focussed enough to identify the most relevant documents within a manageable number of

search results. Only including the term "guideline" though might have resulted in relevant documents, albeit not called "guidelines," being omitted from search results. Similarly, our use of inclusion/exclusion criteria that led to the decision to exclude documents such as the TRI flowchart could be problematic as it (the TRI flowchart) is a comprehensive document potentially used in many situations to inform assessment and treatment decisions.

To minimize the risk of omitting relevant search results we contacted a range of international experts and members of guideline development groups. In addition to this, we conducted hand searches of the references lists of included guidelines for relevant sources. We also recruited native speakers to extract data from the respective guidelines in an effort to ensure that data collection was as accurate as possible. It is possible, that different search and inclusion criteria might have led to different documents being included. However, given the large range of assessment and treatment options and the limited evidence base around many treatments in particular, it is unlikely that our conclusions would differ significantly if further guidelines had been identified at this time. Future systematic reviews though will be able to use this as a reference point.

IMPLICATIONS AND CONCLUSIONS

As researchers from around the world are collecting and making efforts to better understand the heterogeneity of subjective tinnitus in adults and systematically evaluate assessment and treatment options, we have, for the first time, described the major similarities and differences between existing clinical guidelines for subjective tinnitus in adults. The results reveal true guidelines from only five countries and thus highlight a need to develop guidelines that are endorsed by the range of professionals involved in assessing and treating tinnitus. Although we do not place a great deal of weight on the quality assessment ratings of the guidelines, they do suggest that there is room for improvement particularly with regard to implementation and evaluation. The absence of guidelines contributes to the variations that exist in assessment and treatment of tinnitus internationally.

While it would be tempting to do so, it is beyond the scope of this paper to formulate a new or composite guideline based on the results that we have obtained. Instead, the results of this review in conjunction with those from a survey of European tinnitus healthcare providers and researchers (Cima et al., 2016) will form the basis of further work on the development of a set of European clinical guidelines for the assessment and treatment of tinnitus being undertaken by the COST-action TINNET: Working Group I "*Clinical*." As with existing clinical guidelines, attention will need to be given to how the future European guideline is disseminated, subsequently evaluated, and the implications for resource management considered. We expect it to be challenging task but one that will hopefully result in a more reliable and equitable assessment and treatment of tinnitus patients across Europe.

AUTHOR CONTRIBUTIONS

TF, HH, DK, AL, BM, AN, SR and RC, conceived and designed the study. TF and HH wrote and revised the protocol and manuscripts, conducted the literature search, data extraction, and data synthesis. SR, RL, CC, NE, PB, SNR, AK, DL, and BM extracted data. DH, AN, DK, and RC reviewed and edited manuscripts and contributed intellectually to the content of the manuscript. All authors approved the manuscript for submission.

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REFERENCES

American Academy of Audiology (2000). *Audiologic Guidelines for the Diagnosis and Management of Tinnitus Patients*. Available online at: http://www.audiology.org/publications-resources/document-library/audiologic-guidelines-diagnosis-management-tinnitus-patients [Accessed 22 November 2016].

American Speach Language Hearing Association (2016). *Tinnitus Triage Guidelines.* Available online at: http://www.asha.org/aud/Articles/Tinnitus-Triage-Guidelines/ [Accessed 22 November 2016].

Andersson, G., Juris, L., Classon, E., Fredrikson, M., and Furmark, T. (2006). Consequences of suppressing thoughts about tinnitus and the effects of cognitive distraction on brain activity in tinnitus patients. *Audiol. Neurotol.* 11, 301–309. doi: 10.1159/000094460

Audiology Australia (2013). *Audiology Australia Professional Practice Standards - Part B Clinical Standards*. Melbourne, VCA: Audiology Australia.

Baguley, D., Andersson, G., McFerran, D., and McKenna, L. (eds.). (2013a). *Tinnitus: A Multidisciplinary Approach*. West Sussex: John Wiley & Sons, Ltd.

Baguley, D., McFerran, D., and Hall, D. (2013b). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Biesinger, E., Del Bo, L., De Ridder, D., Goodey, R., Herraiz, C., Kleinjung, T., et al. (2010). *Algorithm for the Diagnostic and Therapeutic Management of Tinnitus.* Available online at: http://www.tinnitusresearch.org/en/documents/downloads/TRI_Tinnitus_Flowchart.pdf [Accessed 30 Jan 2015].

Brouwers, M. C., Kho, M. E., Browman, G. P., Burgers, J. S., Cluzeau, F., Feder, G., et al. (2010). AGREE II: advancing guideline development, reporting and evaluation in health care. *CMAJ* 182, E839–E842. doi: 10.1503/cmaj.090449

Cima, R. F., Crombez, G., and Vlaeyen, J. W. (2011). Catastrophizing and fear of tinnitus predict quality of life in patients with chronic tinnitus. *Ear Hear.* 32, 634–641. doi: 10.1097/AUD.0b013e31821106dd

Cima, R. F., Haider, H., Mazurek, B., Cederroth, C. R., Lapira, A., Kikidis, D., et al. (2016). "TINNET COST Action BM1306 - clinical WG1: establishment of a standard for Tinnitus; patient assessment, characterization, and treatment options," in *10th International Tinnitus Research Initiative Conference* (Nottingham: Tinnitus Research Initiative).

Cima, R. F., Maes, I. H., Joore, M. A., Scheyen, D. J., El Refaie, A., Baguley, D. M., et al. (2012). Specialised treatment based on cognitive behaviour therapy versus usual care for tinnitus: a randomised controlled trial. *Lancet* 379, 1951–1959. doi: 10.1016/S0140-6736(12)60469-3

Davis, A., and Refaie, A. E. (2000). "Epidemiology of tinnitus," in *Handbook of Tinnitus*, ed R. S. Tyler (San Diego: Singular thompson Learning), 1–24.

Department of Health (2009). *Provision of Services for Adults with Tinnitus: A Good Practice Guide*. London: Department of Health.

Dutch Association for Ear Nose Throat and Head surgery [Nederlandse Vereniging voor Keel – Neus – Oor heel kunde en Heelkunde van het Hoofd – Halsgebied] (in press). *Guideline Tinnitus [Richtlijn Tinnitus]*. Utrecht.

Erlandsson, S. I., and Hallberg, L. R. (2000). Prediction of quality of life in patients with tinnitus. *Br. J. Audiol.* 34, 11–20. doi: 10.3109/03005364000000114

Feldmann, H. (1984). Tinnitus masking curves (updates and review). *J. Laryngol. Otol.* 98, 157–160. doi: 10.1017/S1755146300090375

Field, M. J., and Lohr, K. N. (eds.). (1990). *Clinical Practice Guidelines: Directions for a New Program*. Washington, DC: National Academy of Sciences.

Fujii, K., Nagata, C., Nakamura, K., Kawachi, T., Takatsuka, N., Oba, S., et al. (2011). Prevalence of tinnitus in community-dwelling Japanese adults. *J. Epidemiol.* 21, 299–304. doi: 10.2188/jea.JE20100124

Goebel, G., and Hiller, W. (1994). The tinnitus questionnaire. A standard instrument for grading the degree of tinnitus. Results of a multicenter study with the tinnitus questionnaire. *HNO* 42, 166–172.

Goebel, G., and Hiller, W. (2001). *Verhaltensmedizinische Tinnitus-Diagnostik. Eine Praktische Anleitung zur Erfassung Medizinischer und Psychologischer Merkmale Mittels des Strukturierten Tinnitus-Interviews (STI)*. Göttingen: Hogrefe.

Hallam, R. S., Jakes, S. C., and Hinchcliffe, R. (1988). Cognitive variables in tinnitus annoyance. *Br. J. Clin. Psychol.* 27(Pt 3), 213–222. doi: 10.1111/j.2044-8260.1988.tb00778.x

Handscomb, L. E., Hall, D. A., Shorter, G. W., and Hoare, D. J. (2017). Positive and negative thinking in tinnitus: factor structure of the tinnitus cognitions questionnaire. *Ear Hear.* 38, 126–132. doi: 10.1097/AUD.0000000000000365

Henry, J. A., Zaugg, T. L., Myers, P. J., Schmidt, C. J., Ribbe, C., Edmonds, K., et al. (2015). *Adult Tinnitus Management Clinical Practice Recommendation*. Portland, OR: Department of Veterans Affairs.

Henry, J. A., Zaugg, T. L., and Schechter, M. A. (2005a). Clinical guide for audiologic tinnitus management I: assessment. *Am. J. Audiol.* 14, 21–48. doi: 10.1044/1059-0889(2005/004)

Henry, J. A., Zaugg, T. L., and Schechter, M. A. (2005b). Clinical guide for audiologic tinnitus management II: treatment. *Am. J. Audiol.* 14, 49–70. doi: 10.1044/1059-0889(2005/005)

Hoare, D. J., Gander, P. E., Collins, L., Smith, S., and Hall, D. A. (2012). Management of tinnitus in English NHS audiology departments: an evaluation of current practice. *J. Eval. Clin. Pract.* 18, 326–334. doi: 10.1111/j.1365-2753.2010.01566.x

Hoare, D. J., and Hall, D. (2011). Clinical guidelines and practice: a commentary on the complexity of tinnitus management. *Eval. Health Prof.* 34, 413–420. doi: 10.1177/0163278710390355

Hoekstra, C. E., Wesdorp, F. M., and van Zanten, G. A. (2014). Socio-demographic, health, and tinnitus related variables affecting tinnitus severity. *Ear Hear.* 35, 544–554. doi: 10.1097/AUD.0000000000000045

Idrizbegovic, E., Kjerulf, E., and Team for Diagnostics Hearing Habilitation Children and Youth and Hearing Rehabilitation for Adults (2011). *Tinnitus Care Program [Tinnitus Vårdprogram]*. Stockholm: Karolinska Institute.

Jørgensen, H. S., Amt, F., Nemholt, S. S., Kristensen, R., and Ellesøe, H. (2007). "Guidance for Diagnosing Tinnitus and Hyperacusis [Vejledning for udredning af tinnitus og hyperakusis]," in *Vejledninger i Udredning* (Denmark: S.l. Danske Tale-Høre-Synsinstitutioner: eksp. Center for Hjælpemidler og Kommunikation).

Kamalski, D. M., Hoekstra, C. E., van Zanten, B. G., Grolman, W., and Rovers, M. M. (2010). Measuring disease-specific health-related quality of life to evaluate treatment outcomes in tinnitus patients: a systematic review. *Otolaryngol. Head Neck Surg.* 143, 181–185. doi: 10.1016/j.otohns.2010.03.026

Kim, H. J., Lee, H. J., An, S. Y., Sim, S., Park, B., Kim, S. W., et al. (2015). Analysis of the prevalence and associated risk factors of tinnitus in adults. *PLoS ONE* 10:e0127578. doi: 10.1371/journal.pone.0127578

Kleinstauber, M., Jasper, K., Schweda, I., Hiller, W., Andersson, G., and Weise, C. (2013). The role of fear-avoidance cognitions and behaviors in patients with chronic tinnitus. *Cogn. Behav. Ther.* 42, 84–99. doi: 10.1080/16506073.2012.717301

Kuk, F. K., Tyler, R. S., Russell, D., and Jordan, H. (1990). The psychometric properties of a tinnitus handicap questionnaire. *Ear Hear.* 11, 434–445. doi: 10.1097/00003446-199012000-00005

Langguth, B., and Elgoyhen, A. B. (2012). Current pharmacological treatments for tinnitus. *Expert Opin. Pharmacother.* 13, 2495–2509. doi: 10.1517/14656566.2012.739608

Langguth, B., Kleinjung, T., and Landgrebe, M. (2011). Severe tinnitus and depressive symptoms: a complex interaction. *Otolaryngol. Head Neck Surg.* 145, 519; author reply 520. doi: 10.1177/0194599811411851

Langguth, B., Kreuzer, P. M., Kleinjung, T., and De Ridder, D. (2013). Tinnitus: causes and clinical management. *Lancet Neurol.* 12, 920–930. doi: 10.1016/S1474-4422(13)70160-1

Martinez-Devesa, P., Perera, R., Theodoulou, M., and Waddell, A. (2010). Cognitive behavioural therapy for tinnitus. *Cochrane Database Syst. Rev.* CD005233. doi: 10.1002/14651858.CD005233.pub3

Maters, G. A., Sanderman, R., Kim, A. Y., and Coyne, J. C. (2013). Problems in cross-cultural use of the hospital anxiety and depression scale: "no butterflies in the desert". *PLoS ONE* 8:e70975. doi: 10.1371/journal.pone.0070975

McKenna, L., Handscomb, L., Hoare, D. J., and Hall, D. A. (2014). A scientific cognitive-behavioral model of tinnitus: novel conceptualizations of tinnitus distress. *Front. Neurol.* 5:196. doi: 10.3389/fneur.2014.00196

Meikle, M. B., Henry, J. A., Griest, S. E., Stewart, B. J., Abrams, H. B., McArdle, R., et al. (2012). The tinnitus functional index: development of a new clinical measure for chronic, intrusive tinnitus. *Ear Hear.* 33, 153–176. doi: 10.1097/AUD.0b013e31822f67c0

Newman, C. W., Jacobson, G. P., and Spitzer, J. B. (1996). Development of the tinnitus handicap inventory. *Arch. Otolaryngol. Head Neck Surg.* 122, 143–148. doi: 10.1001/archotol.1996.01890140029007

Newman, C. W., Sandridge, S. A., and Jacobson, G. P. (1998). Psychometric adequacy of the Tinnitus Handicap Inventory (THI) for evaluating treatment outcome. *J. Am. Acad. Audiol.* 9, 153–160.

NHS Scotland (2006). *Best Practice Statement - Ear Care*. Healthcare Improvement Scotland.

Ruszczynski, M., Horvath, A., Dziechciarz, P., and Szajewska, H. (2016). Cow's milk allergy guidelines: a quality appraisal with the AGREE II instrument. *Clin. Exp. Allergy* 46, 1236–1241. doi: 10.1111/cea.12784

Sanclemente, G., Acosta, J. L., Tamayo, M. E., Bonfill, X., and Alonso-Coello, P. (2014). Clinical practice guidelines for treatment of acne vulgaris: a critical appraisal using the AGREE II instrument. *Arch. Dermatol. Res.* 306, 269–277. doi: 10.1007/s00403-013-1394-x

The Association of the Scientific Medical Societies (2015). *German S3 Guideline 017/064: Chronic Tinnitus [AWMF-Register Nr. 017/064 Klasse: S3 Chronischer Tinnitus]*. AWMF online.

Tunkel, D. E., Bauer, C. A., Sun, G. H., Rosenfeld, R. M., Chandrasekhar, S. S., Cunningham, E. R., et al. (2014). Clinical practice guideline: tinnitus. *Otolaryngol. Head Neck Surg.* 151, S1–S40. doi: 10.1177/0194599814547475

Wilson, P. H., Henry, J., Bowen, M., and Haralambous, G. (1991). Tinnitus reaction questionnaire psychometric properties of a measure of distress associated with tinnitus. *J. Speech Hear. Res.* 34, 197–201. doi: 10.1044/jshr.3401.197

Zigmond, A. S., and Snaith, R. P. (1983). The hospital anxiety and depression scale. *Acta Psychiatr. Scand.* 67, 361–370. doi: 10.1111/j.1600-0447.1983.tb09716.x

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

APPENDIX

Excluded full text documents

1. UK Good practice guide for adults with tinnitus (Department of Health, 2009).
2. Audiology Australia Professional Practice Standards - Part B Clinical Standards (Audiology Australia, 2013).
3. American Speech language and hearing association Tinnitus triage guidelines (American Speach Language Hearing Association, 2016).
4. Ear care, NHS Scotland General practice guide for ear care. (NHS Scotland, 2006).
5. TRI flowchart (Biesinger et al., 2010).
6. Clinical guide for audiologic tinnitus management: Assessment and Clinical guide for audiologic tinnitus management: Assessment (Henry et al., 2005a) and Treatment (Henry et al., 2005b).
7. Adult Tinnitus Management Clinical Practice Recommendation (Henry et al., 2015).
8. American Academy of Audiology Audiologic Guidelines for the Diagnosis and Management of Tinnitus Patients (American Academy of Audiology, 2000).

frontiers in Psychology

REVIEW
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A State-of-the-Art Review: Personalization of Tinnitus Sound Therapy

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Background: There are several established, and an increasing number of putative, therapies using sound to treat tinnitus. There appear to be few guidelines for sound therapy selection and application.

Aim: To review current approaches to personalizing sound therapy for tinnitus.

Methods: A "state-of-the-art" review (Grant and Booth, 2009) was undertaken to answer the question: how do current sound-based therapies for tinnitus adjust for tinnitus heterogeneity? Scopus, Google Scholar, Embase and PubMed were searched for the 10-year period 2006–2016. The search strategy used the following key words: "tinnitus" AND "sound" AND "therapy" AND "guidelines" OR "personalized" OR "customized" OR "individual" OR "questionnaire" OR "selection." The results of the review were cataloged and organized into themes.

Results: In total 165 articles were reviewed in full, 83 contained sufficient details to contribute to answering the study question. The key themes identified were hearing compensation, pitched-match therapy, maskability, reaction to sound and psychosocial factors. Although many therapies mentioned customization, few could be classified as being personalized. Several psychoacoustic and questionnaire-based methods for assisting treatment selection were identified.

Conclusions: Assessment methods are available to assist clinicians to personalize sound-therapy and empower patients to be active in therapy decision-making. Most current therapies are modified using only one characteristic of the individual and/or their tinnitus.

Keywords: tinnitus, treatment, therapy, review, person-centered

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INTRODUCTION

State-of-the-art reviews are a specific form of review that focus on current issues and new perspectives, often in areas with a need of further research (Grant and Booth, 2009). The last 10 years has seen the emergence of many new tinnitus therapies using sound (Hoare et al., 2013a, 2014b). Although the heterogeneity of tinnitus is widely acknowledged by clinicians, many common sound-based tinnitus treatments are applied with limited assessment of individual differences (Hoare et al., 2014b). The ambiguity underlying tinnitus mechanisms and the rapid development of commercial interests in digital sound technology for tinnitus treatment have resulted in an increase in treatment options but few selection guidelines (Searchfield, 2016). There

appears to be little information as to how to choose between treatments and how to apply them based on individual differences. In this state-of-the-art review we focus on how sound-based therapies are modified for individual characteristics and needs.

Research into the benefits of sound as a tinnitus therapy medium has not been systematic (Mckenna and Irwin, 2008; Hobson et al., 2012). There have been concerns as to whether the claims made regarding the effectiveness of sound therapy are correct (Mckenna and Irwin, 2008). We believe that some of the ambiguity surrounding sound therapy arises by applying the title "sound therapy" as a blanket term to the use of (any) sound that may have a positive effect on tinnitus. There are numerous mechanisms by which sounds could interfere with tinnitus (Norena, 2015). Tinnitus may be masked by sound interfering with encoding (e.g., energetic masking, Vernon, 1977) or pattern recognition (e.g., informational masking, Kidd et al., 1998, 2002). Sounds may desynchronize neural ensembles suspected in tinnitus generation (Eggermont and Tass, 2015). Sounds with positive emotional associations can affect mood and arousal (Handscomb, 2006; Tang et al., 2009). Hearing aids may mask tinnitus and have psychosocial benefits by improving communication (Shekhawat et al., 2013b; Searchfield, 2015). Long-term alleviation of tinnitus may occur through habituation (Jastreboff, 2000), inhibition (Teismann et al., 2011), gain reduction (Norena, 2015) or possible elevations in individual signal detection thresholds (Welch and Dawes, 2008). Attention, cognition and context of perception also appear to be important factors that manipulate long-term adaptation to tinnitus (Searchfield et al., 2012; Searchfield, 2014; Andersson et al., 2016).

Across the health sciences the personalized medicine movement has created a shift in focus from a "one size fits all" model to one that tailors diagnosis and treatment to the individual (Tutton, 2012; Schleidgen et al., 2013). At the same time health services are beginning to shift from evidence-based medicine to evidence-informed individualized care (Miles and Loughlin, 2011). Personalized medicine tends to incorporate traditional assessment methods, genotyping and genomic evaluation to explain and predict risk and treatment outcomes (Ginsburg and Willard, 2009). There is a search for biomarkers and endophenotypes in tinnitus (Sand et al., 2007). The current absence of clear genetic and blood-based biomarkers for tinnitus does not preclude personalization of treatments. The view of personalized medicine should be broadened away from just genetic markers (Anon, 2012). Tinnitus has both psychoacoustic (sound) (Tyler, 2000) and psychological (emotion, reaction) markers (Meikle et al., 2012). The context of tinnitus experience is also likely to affect its perception (Andersson et al., 2016) so that tinnitus is the result of psychosocial, psychoacoustic and individual psychological factors (Searchfield, 2014). In addition to modifying treatments based on assessment outcomes there are good arguments for applying principles of person-centered care to tinnitus therapy. In person-centered care patients are encouraged to be active participants in their treatment through the creation of a power-balanced, therapeutic relationship with their health professionals (Michie et al., 2003). Patients who participate in their own care report greater satisfaction, better adherence and health outcomes (Grenness et al., 2014). Research in the treatment of various health conditions such as chronic pain, balance disorders, and diabetes shows that self-efficacy beliefs also play an important role in treatment outcomes and management of the condition (Smith and Fagelson, 2011). Tinnitus self-efficacy is the confidence that individuals have in their capabilities to perform the treatment courses of action needed to manage their tinnitus successfully (Smith and Fagelson, 2011).

Person-centered needs-based care in rehabilitative audiology is not a new concept (Grenness et al., 2014). Most audiologists will be familiar with needs-based assessment for hearing aid selection (Dillon et al., 1987); similar principles could be applied in tinnitus therapy. Such an approach requires clinicians to take into account the needs of individual tinnitus patients, and provide custom-tailored therapeutic approaches. Our purpose in reviewing the literature was to identify the current state-of-the-art in personalizing sound-based therapies.

METHODS

A state-of-art review (Grant and Booth, 2009) was undertaken in December 2016 with cataloging of results in January and February 2017. All studies irrespective of quality were included as long as they addressed the topic and the research occurred in the last 10 years. There were no other inclusion/exclusion criteria. The research question for the current scoping review was: how do current sound-based therapies for tinnitus adjust for tinnitus heterogeneity? Sound-based therapies were defined as those that included the use of sound (either with or without counseling), but not psychological therapies used without sound or based on the scope of practice of psychologists (e.g., Cognitive Behavioral Therapy). From the included studies, all data were charted; themes and key issues were identified.

To identify relevant studies, the search was carried out using the databases Scopus, Google Scholar, Embase and PubMed for the 10-year period 2006–2016. The search strategy used the following key words: "tinnitus" AND "sound" AND "therapy" AND "guidelines" OR "personalized" OR "customized" OR "individual" OR "questionnaire" OR "selection." The search on Google scholar was terminated when two full pages of consecutive results contained no entries of relevance to the study question. After initial consideration of title relevance to the study by one author (GDS), 199 articles were shortlisted. After reading the abstracts, 150 articles were read in full independently by two authors (GDS and MD). The reference lists of these 150 articles were searched for additional pertinent articles. This returned a further 15 articles for which the text was read in full, of those 165 articles for which full text was reviewed 83 studies described personalization (customization, individual adjustment) methods sufficiently to extract meaningful data. Two authors (GDS and MD) achieved a consensus on classification of content with the third author (TL) verifying categorization.

Studies were charted according to the method used to personalize therapy. The application of psychoacoustical and/or psychosocial assessment tools to treatment selection and

management was cataloged along with the method used to customize the treatment for individual characteristics. A therapy might employ multiple types of customization and tools, in which case cross-referencing was used. In addition, evidence for person-centered care was recorded (including: getting to know patient or client as a person, sharing of power and responsibility, informed of treatment choice, accessibility and flexibility of service provider, coordination and integration, environment that is conducive to person-centered care).

RESULTS

Five themes were identified from the literature surveyed as categorical answers to the question: "how do current sound-based therapies for tinnitus adjust for tinnitus heterogeneity?" The themes were "hearing compensation" (adjustment to audiometry) "pitch matched" (adjustment using the predominant tinnitus pitch) "maskability" (adjustment to a desired level of masking) "reaction to sound" (selection based on the emotional or relaxing characteristics of sound) and "psychosocial factors" (use of psychological and/or environmental factors to select therapy). The therapy themes, the treatments using the approach, and the assessment used, are cataloged in **Table 1**.

Hearing Compensation

Treatments that modified their response on the basis of hearing sensitivity were hearing aids and sound stimulation compensating for reduced audibility (**Table 1**). Hearing aids were used to correct for loss of audibility of sounds that accompanied hearing loss. When the primary focus of hearing aid fitting was to improve hearing for speech an emphasis was placed on amplifying sound in a frequency specific manner to improve intelligibility (McNeill et al., 2012; Shekhawat et al., 2013b). When tinnitus was the primary focus a secondary goal for amplification was raising the audibility of environmental sounds (Shekhawat et al., 2013a). The basis for modification was the individuals hearing thresholds obtained using pure-tone audiometry. The amount of amplification was determined using a prescription based on the audiogram (Shekhawat et al., 2013a). Hearing aids were considered to reduce hearing handicap, reduce the levels of attention paid to tinnitus, and compensate for deafferentation, and possibly improve cognition (Searchfield, 2006, 2015; Shekhawat et al., 2013b; Sereda et al., 2015; Zarenoe et al., 2017). McNeill et al. (2012) identified those most likely to achieve benefit from hearing aids as having good low frequency hearing and tinnitus pitch within the amplification range of the hearing aids (McNeill et al., 2012). Jalilvand et al. (2015) suggested that hearing aids might be more successful in management of tinnitus from blast injuries than sound generators (Jalilvand et al., 2015). Frequency lowering processing was suggested as an alternative strategy to conventional amplification (Peltier et al., 2012). Several studies suggested sound therapy device selection based on the audiogram (from hearing aids, combination instruments and cochlear implants) (Folmer and Carroll, 2006; Mazurek et al., 2006; Tyler et al., 2015; Searchfield, 2016). Searchfield (2016) recommended normal hearing would be fitted with sound generators; high frequency hearing loss with hearing aids; hearing loss encompassing low frequencies with combination instruments; severe-profound hearing loss with cochlear implants.

Hearing loss will affect the perception of therapeutic sounds in addition to reducing the audibility of speech and environmental sounds. To address this several sound therapies adjusted the spectrum of music (Davis et al., 2007; Wazen et al., 2011; Peltier et al., 2012; Vanneste et al., 2013; Henin et al., 2016; Li et al., 2016), fractal tones (Herzfeld et al., 2014) or noise (Uriz et al., 2013) for the presence of hearing loss. The intent from threshold-adjusted sounds was to make the sound audible across a broad frequency range rather than stimulating the frequencies with best thresholds (Davis, 2006; Távora-Vieira et al., 2011). In some cases too severe a hearing loss was an exclusion factor for therapy (Davis et al., 2007). One study suggested that adjusting music levels for hearing threshold was of no benefit to tinnitus suppression (Vanneste et al., 2013).

Pitch-Based Therapy

Several therapies used the pitch or spectrum of tinnitus as the basis for stimulation. Sound stimuli were individualized to span a frequency range centered on the dominant tinnitus pitch (**Table 1**). The sounds used and intended mechanisms of effect varied greatly: some therapies attempt to change the synchronized firing of neural assemblies near tinnitus pitch using tonal stimulation (Hanley and Davis, 2008; Reavis et al., 2010, 2012; Eggermont and Tass, 2015; Hauptmann et al., 2015, 2016; Hoare et al., 2015; Williams et al., 2015); others changed the phase of sounds presented at tinnitus pitch (Herraiz et al., 2007; Vermeire et al., 2007; Choy et al., 2010; Meeus et al., 2010; Fioretti et al., 2011; Heijneman et al., 2012) and another paired tonal stimulation with vagus nerve stimulation (De Ridder et al., 2015). Tinnitus pitch was used as the basis for selecting band-pass noise (Serquera et al., 2015) notched music (Courtenay et al., 2010; Teismann et al., 2011; Györi, 2016) or noise (Lugli et al., 2009) for lateral inhibition (Courtenay et al., 2010) and one method provided extra stimulation at tinnitus pitch (Mahboubi et al., 2012) while another used tinnitus pitch-matched sound embedded in nature sounds as a therapy (Bessman et al., 2009). Another form of pitch-based therapy employed participants undertaking active training tasks in discrimination (Herraiz et al., 2007; Roberts and Bosnyak, 2011; Hoare et al., 2013b, 2014b; Wise et al., 2016) or categorization tasks (Jepsen et al., 2010). The intended mechanism of effect for these training tasks is reorganization of tonotopic maps, but their main effect may be in modifying attention to tinnitus (Hoare et al., 2010). Several studies used more complex replicas (avatars) of tinnitus for passive stimulation (Viirre, 2010) and stimulation only while asleep (Pedemonte et al., 2010). Auditory training in a game format used the individual's tinnitus avatar as a distractor (Wise et al., 2016).

The variability in tinnitus pitch matching is a critical concern issue for pitch-based treatments (Hoare et al., 2014a; Serquera et al., 2015). Variability in pitch match that is more than one octave between consecutive sessions may preclude some therapies (Hoare et al., 2014a). Pitch matching is

TABLE 1 | Therapy themes and references.

Therapy theme	Treatments	Assessment
Hearing compensation	**Hearing aids** Folmer and Carroll, 2006; Searchfield et al., 2010; McNeill et al., 2012; Peltier et al., 2012; Oz et al., 2013; Shekhawat et al., 2013b; Jalilvand et al., 2015; Searchfield, 2015; Sereda et al., 2016 **Threshold adjusted sound** Davis, 2006; Davis et al., 2007; Wazen et al., 2011; Peltier et al., 2012; Vanneste et al., 2013; Parra, 2015; Henin et al., 2016; Li et al., 2016 **Cochlear implant** Tyler et al., 2015	**Pure Tone Audiogram** Davis, 2006; Folmer and Carroll, 2006; Davis et al., 2007; Searchfield et al., 2010; Wazen et al., 2011; McNeill et al., 2012; Peltier et al., 2012; Oz et al., 2013; Shekhawat et al., 2013b; Vanneste et al., 2013; Jalilvand et al., 2015; Parra, 2015; Searchfield, 2015; Tyler et al., 2015; Henin et al., 2016; Li et al., 2016; Sereda et al., 2016
Pitched-matched	**Notched sound** Lugli et al., 2009; Courtenay et al., 2010; Teismann et al., 2011; Györi, 2016 **Band-pass noise** Serquera et al., 2015 **Tonal stimulation** Hanley and Davis, 2008; Reavis et al., 2010, 2012; De Ridder et al., 2015; Eggermont and Tass, 2015; Hauptmann et al., 2015, 2016; Hoare et al., 2015; Williams et al., 2015 **Auditory training** Herraiz et al., 2007; Hoare et al., 2010, 2014b; Jepsen et al., 2010; Roberts and Bosnyak, 2011; Spiegel et al., 2015; Wise et al., 2015, 2016 **Phase shifting** Herraiz et al., 2007; Vermeire et al., 2007; Choy et al., 2010; Meeus et al., 2010; Fioretti et al., 2011; Heijneman et al., 2012 **Hearing aids** Schaette et al., 2010; McNeill et al., 2012; Searchfield, 2016 **Replica tinnitus** Viirre, 2010; Drexler et al., 2016 **Emphasis tinnitus pitch** Bessman et al., 2009; Mahboubi et al., 2012	**Pitch matching** Herraiz et al., 2007; Vermeire et al., 2007; Hanley and Davis, 2008; Bessman et al., 2009; Lugli et al., 2009; Choy et al., 2010; Courtenay et al., 2010; Meeus et al., 2010; Reavis et al., 2010, 2012; Schaette et al., 2010; Fioretti et al., 2011; Roberts and Bosnyak, 2011; Teismann et al., 2011; Heijneman et al., 2012; Mahboubi et al., 2012; McNeill et al., 2012; Hoare et al., 2014a, 2015; Hutter et al., 2014; Eggermont and Tass, 2015; Hauptmann et al., 2015, 2016; Serquera et al., 2015; Spiegel et al., 2015; Williams et al., 2015; Györi, 2016 **Tinnitus Avatar** Viirre, 2010; Spiegel et al., 2015; Wise et al., 2015, 2016; Drexler et al., 2016
Maskability	**Partial masking** Tyler et al., 2007, 2012; Suzuki et al., 2016 **Mixing point masking** Henry et al., 2006; Huang et al., 2006; Jastreboff and Jastreboff, 2006; Jastreboff, 2007, 2011, 2015; Kim et al., 2014; Ostermann et al., 2016 **Hearing aids** McNeill et al., 2012; Searchfield, 2016 **Spatial** Oishi et al., 2013; Searchfield et al., 2016	**Patient report** Jastreboff and Jastreboff, 2006; Jastreboff, 2007, 2011, 2015; Tyler et al., 2007, 2012; McNeill et al., 2012; Oishi et al., 2013; Kim et al., 2014; Ostermann et al., 2016; Searchfield, 2016; Suzuki et al., 2016 **Calculation** Huang et al., 2006 **Localization** Searchfield et al., 2016
Reaction to sound	**Nature sounds / sound type** Handscomb, 2006; Ito et al., 2009; Piskosz, 2012; Herzfeld et al., 2014; Durai et al., 2015; Henry et al., 2015; Barozzi et al., 2016 **Music** Hann et al., 2008 Hann et al., 2008 **Notched music** Lugli et al., 2009; Courtenay et al., 2010; Teismann et al., 2011; Györi, 2016 **Filtered music** Davis, 2006; Davis et al., 2007; Hutter et al., 2014; Li et al., 2016 **Discomfort to sound** Bartnik and Skarzynski, 2006; Jastreboff and Jastreboff, 2006; Jastreboff, 2007, 2011, 2015; Durai et al., 2015	**Patient report** Davis, 2006; Handscomb, 2006; Davis et al., 2007; Hann et al., 2008; Ito et al., 2009; Lugli et al., 2009; Courtenay et al., 2010; Teismann et al., 2011; Piskosz, 2012; Herzfeld et al., 2014; Hutter et al., 2014; Durai et al., 2015; Henry et al., 2015; Barozzi et al., 2016; Györi, 2016; Li et al., 2016 **Categorization of report** Bartnik and Skarzynski, 2006; Jastreboff and Jastreboff, 2006; Jastreboff, 2007, 2011, 2015
Psychosocial factors	**Hearing aids** Searchfield, 2006; Hoare et al., 2012; Sereda et al., 2015	**COSIT** Searchfield, 2006, 2015

(Continued)

TABLE 1 | Continued

Therapy theme	Treatments	Assessment
	Partial masking Tyler et al., 2007; Anwar, 2013; Sereda et al., 2016 **Adaptation** Durai et al., 2015 **Mixing point masking** Mazurek et al., 2006; Herraiz et al., 2007; Ariizumi et al., 2010 **Selection** Tyler et al., 2006; Newman, 2008 #19; Newman et al., 2008; Tyler, 2012	**STOP** Newman et al., 2008 **TAQ** Tyler et al., 2006, 2007 **Demographics and clinical history** Mazurek et al., 2006; Herraiz et al., 2007; Hoare et al., 2012; Tyler, 2012; Sereda et al., 2015 **Motivation** Ariizumi et al., 2010 Ariizumi et al., 2010 **Personality** Tyler et al., 2006; Durai et al., 2015

The references were categorized to the major theme of the research or review. There were occasions where references were cross-referenced to different themes or treatment categories. Assessment was broadly classified into categories; there was variation between studies in how specific features were measured (e.g., different forms of pitch matching). TAQ, Tinnitus Activities Questionnaire; STOP, Sound Therapy Option Profile; COSIT, Client Oriented Scale of Improvement in Tinnitus.

not considered very useful in methods based on counseling and broad noise therapy (Baguley et al., 2013). However, tinnitus pitch within the effective range of sound therapy device may be a prognostic factor for treatment success (Schaette et al., 2010; McNeill et al., 2012; Searchfield, 2016). Momentary analysis may have a role in guiding treatments in which the feature measured (e.g., pitch) guides treatment sound selection. Incorporating such assessments into daily routine in a non-threatening manner or even game (Wise et al., 2016) may mitigate the potential negative effects of momentary analysis in priming individuals to focus on their tinnitus.

Maskability

We used a psychoacoustic definition of masking: when the perception of tinnitus is affected by the presence of another sound. The level of sound used in theory has been one of the more contentious issues in audiology-based tinnitus therapy (Jastreboff, 2007; Tyler et al., 2012). Masking can be used to totally or partially reduce the audibility of tinnitus by covering it with another sound. Tinnitus Retraining Therapy (TRT) advocates a masking level in which the sound mixes with, but does not cover, the tinnitus (Henry et al., 2006; Huang et al., 2006; Jastreboff and Jastreboff, 2006; Jastreboff, 2007, 2011, 2015; Kim et al., 2014; Ostermann et al., 2016) while others suggest use of the minimum level resulting in relief (Tyler et al., 2007, 2012; Suzuki et al., 2016). Most therapies set their target level based on patients' reports of tinnitus audibility and sound being comfortable, although Huang et al. (2006) reported that the mixing level could be predicted on the basis of the MML (Huang et al., 2006). Kim et al. (2014) reported TRT with broadband noise to have a higher success rate than mixed or narrowband noise (Kim et al., 2014). TRT and masking is typically practiced using sound presentation to both ears; Oishi et al. suggested that monaural presentation can be successful (Oishi et al., 2013) and Searchfield et al. (2016) showed the potential for binaural sound presentation using interaural cues and Head Related Transfer Function to achieve spatial as well as spectral masking.

Reaction to Sound

In order for sound therapy to be effective it must be comfortable to the user. Under this theme we cataloged therapies that considered individual's sensitivity to, or, reaction to sounds. A key factor in allocating participants to the treatment categories used in TRT was known discomfort to sound (Jastreboff and Jastreboff, 2006; Jastreboff, 2007, 2011, 2015). Personality may be a predictive factor in determining if a participant is sound responsive or sound sensitive (Durai et al., 2015). A positive individual response to therapeutic sounds will increase the individual's ability to achieve treatment goals as well as compliance to treatment. The strong emotional response to music has seen its use as a therapeutic tool (Hann et al., 2008). Music has been adopted as the sound manipulated in the Neuromonics Tinnitus Treatment (Davis et al., 2007; Li et al., 2016) and notched music therapies (Lugli et al., 2009; Courtenay et al., 2010; Teismann et al., 2011; Györi, 2016). Fractal tones also have music-like relaxation properties (Herzfeld et al., 2014). Advances in hearing aid technology allow relaxing music or nature sounds to be directly streamed from a patient's smart phone to their hearing aids (Piskosz, 2012). Studies to date suggest that modulated sounds (Henry et al., 2015) or nature sounds (Barozzi et al., 2016) achieve tinnitus benefits similar to broadband noise stimulation.

Psychosocial

We defined psychosocial factors in sound therapy as social moderators and individual thoughts and behaviors that determine the treatment approach. In an evaluation of tinnitus management in NHS audiology departments in the UK by Hoare et al. (2012) identified a wide range of factors that influenced clinicians management strategies (in order of high-low reporting): level of hearing loss, evidence of stress or anxiety, state of mind, severity, willingness to try treatment, sleep disturbance, health, understanding, lifestyle preferences, coping ability, hyperacusis, age, and depression. Although these results were not described in terms of sound-therapy specific decision-making, three of the top four treatments reported were sound-based or could use sound (hearing aids, sound generator,

TABLE 2 | Examples of tinnitus assessments that can be chosen to help guide sound therapy selection.

Assessment tool	Suggested use
TFI (Meikle et al., 2012) TPFQ (Tyler et al., 2014)	Intake and outcome questionnaires developed to be sensitive to treatment effects. Determine effect of tinnitus on individual and areas of life most affected by tinnitus. Assist in priority setting
Audiometry (Sereda et al., 2011)	Identify degree of hearing loss accompanying tinnitus, as basis for modifying audibility of sound therapy
HHI (A or E) (Zarenoe et al., 2017)	Assists in determining if hearing aids should be trialed based on effect of hearing
Psychoacoustic matching (Henry et al., 2013; Hoare et al., 2014a)	Identify tinnitus characteristics on which to base sound stimulation. Essential for pitch-based therapies, helpful in predicting hearing aid success
HADS (Zigmond and Snaith, 1983)	Measure of anxiety and depression, assists in identifying need for referral and focus of therapy (e.g., CBT vs. sound therapies)
MPQ (Tellegen, 1982; Durai and Searchfield, 2016)	Personality typing helps identify sound sensitive patients and tendency for chronic tinnitus
NIH Cognitive Toolbox (Heaton et al., 2014)	Assess cognition (attention, memory) have influence therapy selection and settings (e.g., sow processing for hearing aids)
TAQ (Tyler et al., 2006, 2007)	Identify activities requiring therapy focus
STOP (Newman et al., 2008)	Assists selection of sound therapy types
SETMQ (Smith and Fagelson, 2011)	Identify areas where patients are struggling to manage tinnitus
COSIT (Searchfield, 2006)	Identify and prioritize individuals needs and goals

TFI, Tinnitus Functional index; TPFQ, Tinnitus Primary Function Questionnaire; HHI (A or E), Hearing Handicap Inventory (Adults or Elderly); HADS, Hearing Anxiety and Depression Scale; MPQ, Multidimensional Personality Questionnaire; TAQ, Tinnitus Activities Questionnaire; STOP, Sound Therapy Option Profile; SETMQ, Self-Efficacy for Tinnitus Management Questionnaire; COSIT, Client Oriented Scale of Improvement in Tinnitus.

habituation). In a similar population psychosocial factors were used in selecting hearing aids for tinnitus and mild hearing loss (Sereda et al., 2015). Skepticism, length of treatment and attitude can influence treatment success (Herraiz et al., 2007). Willingness to pay may affect client decision-making (Tyler, 2012). Older patients and tinnitus of longer duration may benefit less from sound therapies (Mazurek et al., 2006; Anwar, 2013). Questionnaires have been used in several person-centered tinnitus therapies to guide treatment (**Table 2**). In Tinnitus Activities Treatment (TAT), the Iowa Tinnitus Activities Questionnaire is recommended to identify patient's needs and treatment priorities (Tyler et al., 2007). Searchfield (2006) advocated the use of a tinnitus version of the Client Orientated Scale of Improvement (COSIT) to identify and set goals for treatment. Newman et al. (2008) used the Sound Therapy Option Profile (STOP) to assist in therapy selection and understanding patient attitudes to different treatments and the Self-Efficacy for Tinnitus Management Questionnaire (SETMQ) can be used to assess patient confidence in using different treatments (Smith and Fagelson, 2011; Fagelson and Smith, 2016).

DISCUSSION

Tinnitus is a heterogeneous disorder: tinnitus sound can differ between individuals, it can result from many different types of injury and its effect can vary from a minor annoyance to catastrophic impact on daily life (Stouffer and Tyler, 1990).

The review of the literature identified five sound therapy themes. Some treatments were included across themes. The majority of studies considered tailored or customized therapy to be selection of treatment sound on the basis of either audiometric threshold or tinnitus pitch. There were approaches that used dimensions of tinnitus severity, sound sensitivity and hearing to categorize or subtype groups of sufferers (Jastreboff, 2011). Another approach was a hierarchy or stepped care model in which individually tailored treatments were used if less resource intensive methods were unsuccessful (Myers et al., 2014). Stepped care has been implemented in environments where universal individually focused therapy would be economically unsustainable (Department of Health, 2009; Myers et al., 2014). Unlike single characteristic therapies whole-person approaches involved patients in decision making as to which of several approaches are best suited to them (Tyler et al., 2007; Newman et al., 2008). We believe that a contributing factor to the inconsistent benefit reported with sound therapies (Mckenna and Irwin, 2008; Hobson et al., 2012) is application of a single title "sound therapy" to a very heterogeneous collection of different sound-based approaches. In addition individual needs and reaction to therapy sounds differ (Durai and Searchfield, 2017) we ascribe to the philosophy that individuals are most likely to manage their tinnitus better when the treatment plan is tailored to their needs (Fisher and Boswell, 2016).

Planning Individual Tinnitus Care

The review did not identify any comprehensive guidelines for optimal sound therapy selection. We believe that many of the sound therapies identified could be effective when selected for the right patients at the right time and appropriate context. To do this we suggest careful assessment and then use of an individual care plan. In this review we deliberately focus on sound therapy, but we strongly believe any treatment plan should consider counseling (Tyler et al., 2007; Searchfield et al., 2011), and referral for psychological therapies such as Cognitive Behavioral Therapy (Martinez-Devesa et al., 2006) when appropriate. By determining individual needs and priorities, alongside assessment measures such as pure-tone audiometry and pitch matching, a plan can

be developed that we believe reduces the risk for ineffective treatment. An effective individual care plan may also reduce the time required for treatment, reducing stress, anxiety and loss of hope for the sufferer. We believe those factors of the individual's complaint that are likely to be driving other symptoms should be addressed by therapy first (Fisher and Boswell, 2016).

Based on the review, along with our clinical experience, we suggest that the individual care plan use various clinical assessment methods. When patients first attend the clinic their clinical history, a thorough hearing assessment and tinnitus matching should be undertaken (Langguth et al., 2007). A questionnaire assessing aspects of tinnitus effects on quality of life should be used to provide an overview of tinnitus impact, and serve as a baseline for future assessments of outcomes. The Tinnitus Functional Index (Meikle et al., 2012) and Tinnitus Primary Function Questionnaire (Tyler et al., 2014) are two recent questionnaires developed for this purpose. Based on a clinical history an evaluation of anxiety and depression (e.g., the Hospital Anxiety and Depression Scale, Andersson et al., 2003) or cognition [e.g., National Institutes for Health (NIH) Toolbox Cognition Battery, Heaton et al., 2014] may be important. The comorbidity of anxiety and depression with tinnitus is well known (Andersson et al., 2003). The Hospital Anxiety and Depression Scale (HADS) (Zigmond and Snaith, 1983) or similar questionnaire scores can assist decision-making as to the necessity and priorities for psychological therapies. Tinnitus negatively impacts on cognition (Zarenoe et al., 2017). We do not know yet if differences in cognition should influence selection of sound therapy type, however research suggests that slow-acting hearing aid processing strategies may lead to better hearing when users memory is impaired (Lunner et al., 2009). We also recommend the assessment of personality. The use of personality questionnaires such as the Multidimensional Personality Questionnaire (MPQ) subscales (Tellegen, 1982) in clinics may be useful in identifying at-risk individuals for distressing tinnitus. Four key "maladaptive" personality traits are suspected in playing a role in diverting attention and processing resources toward tinnitus and which may subsequently act to prevent adaptation. These include higher levels of stress reaction, lower social closeness, lower self-control and higher alienation (Durai and Searchfield, 2016). If, for example, an individual has high stress reactions and low self-control and reacts negatively to sound psychological-based interventions may be needed before sound therapy.

Hoare et al. (2014b) recommended that clinicians be guided by the patient's point of care, patient motivation and expectations of sound therapy. The acceptability of the intervention both in terms of the sound stimuli to be used and whether patients are willing to use sound extensively or intermittently is important (Hoare et al., 2014b). A step in this direction is counseling patients about the therapies that are available. Information should be provided about the basis of the treatment, evidence for effectiveness, speed of effects, and costs. Aazh et al. (2009) suggest a poster format for this pre-consultation information; clinics websites and marketing material also can provide useful appointment scene setting. A tinnitus needs assessment can assist in the shared decision making process. The Hearing and Tinnitus clinic at the University of Auckland has used the COSIT as a decision making and goal setting tool for over a decade (Searchfield, 2006). The COSIT is a modification of the COSI, a tool frequently used in needs assessment for hearing problems (Dillon et al., 1987). Other questionnaires that may assist needs assessment include the TAQ (Tyler et al., 2007), STOP (Newman et al., 2008) and SETMQ (Smith and Fagelson, 2011). The TAQ determines the areas in which tinnitus creates problems (emotion, sleep, communication and/or concentration). The TAQ can highlight areas of life in which tinnitus is having the most debilitating effect, which can then be used to focus or tailor treatment (Tyler et al., 2007). The STOP is an 11-item tool that takes into account motivation, acceptance, expectations and willingness to use sound therapy devices (Newman et al., 2008). The SETMQ is a 40-item measure that quantifies the patient's confidence in managing tinnitus in five areas: (1) routine management, (2) emotional response, (3) internal thoughts and interaction with others, (4) tinnitus concepts, and (5) use of assistive devices such as hearing aids and maskers (Smith and Fagelson, 2011).

The relative effectiveness of these questionnaires in informing successful treatment has to be determined. However the usefulness and time savings achieved by use of these questionnaires should not be underestimated, as they can be completed by the patient prior to their appointment and can be assessed by the clinician prior to meeting the patient; saving time during the appointment. There is overlap in the questions asked by the questionnaires so not all need be used. It is important that clinicians choose those questionnaires best suited to the treatments they offer and their health care setting and patient population. We suggest that many audiologists will find the open-ended format of the COSIT familiar, and may wish to combine with one or more closed question formats (TAQ, STOP, SETMQ). The outcome of appropriately selected assessments can result in an efficient tinnitus clinic. Unnecessary or inappropriate treatments can be avoided, reducing the risk that patients become disillusioned with the clinician's methods. With patient "buy-in," motivation and compliance to treatments should be high. Understanding and choosing the treatments to use immediately, and as the impact of tinnitus changes, may be empowering to the patient.

Advancing Sound Therapy and Recognizing Its Limitations

The literature review highlighted the diverse basis and application of sound therapy. Researchers and publications need to be clear on what aspect or type of sound therapy is being used. Mckenna and Irwin (2008) wrote a useful critique of sound therapy with the provocative title "Sound therapy: sacred cow or idol worship?" Mckenna and Irwin's (2008) main arguments were that the mechanisms of sound therapy were not necessarily those claimed, effects may be cognitive or psychological rather than purely auditory, and benefits were modest, if any, above counseling alone. Sound therapy is potentially confusing, given the numerous approaches and

various potential mechanisms of effect. While more evidence for sound therapies is becoming available there is still a need to prove benefits. The individualized sound therapy approach also needs to be validated relative to single therapy protocols. In order to provide this evidence we may a need to move away from the dominant nomothetic research approach to an idiographic method that embraces individual variance (Fisher and Boswell, 2016). Group comparisons are limited in their ability to identify effective sound therapies when there is heterogeneity. Sound therapy is, in general, a slow-acting therapy that requires long-term use of some form of sound delivery device; so longitudinal data is needed. Research also needs to continue to investigate whether auditory-based therapies can be enhanced, or sped up, by combining with non-invasive brain (Shekhawat et al., 2014; De Ridder et al., 2015) or multisensory (Spiegel et al., 2015) stimulation.

CONCLUSIONS

The basis of sound therapy is the belief that increasing extrinsic sound driven activity of the auditory system reduces tinnitus. This does not mean sound therapy is uniform in its application; instead it covers many dimensions and presumed mechanisms of effect. Current commentary on sound therapy fails to fully recognize this heterogeneity in application. At the same time few sound therapies can truly be considered personalized to make the most of their purported mechanisms. Much of the literature surveyed used the terms "customized" or "tailored" in terms of a single dimension rather than viewing tinnitus as a complex combination of dimensions. Tools exist for personalizing and planning treatments, they should be integrated into patient care, and their usefulness tested.

AUTHOR CONTRIBUTIONS

GS undertook the initial database search, cataloging, and prepared the manuscript and revisions. MD was involved in manuscript preparation and cataloging of results. TL reviewed the cataloging and contributed to the manuscript with a focus on clinical application.

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REFERENCES

Aazh, H., Moore, B. C., and Roberts, P. (2009). Patient-centered tinnitus management tool: a clinical audit. *Am. J. Audiol.* 18, 7–13. doi: 10.1044/1059-0889(2009/08-0037)

Andersson, G., Heesser, H., and McKenna, L. (2016). "Psychological mechanisms and tinnitus," in *Tinnitus Clinical and Research Perspectives*, eds D. Baguley and M. Fagelson (San Diego, CA: Plural Publishing), 63–74.

Andersson, G., Kaldo-Sandstrom, V., Strom, L., and Stromgren, T. (2003). Internet administration of the hospital anxiety and depression scale in a sample of tinnitus patients. *J. Psychosom. Res.* 55, 259–262. doi: 10.1016/S0022-3999(02)00575-5

Anon (2012). What happened to personalized medicine? *Nature Biotechnology* 30:1. doi: 10.1038/nbt.2096

Anwar, M. N. (2013). Mining and analysis of audiology data to find significant factors associated with tinnitus masker. *Springerplus* 2:1. doi: 10.1186/2193-1801-2-595

Ariizumi, Y., Hatanaka, A., and Kitamura, K. (2010). Clinical prognostic factors for tinnitus retraining therapy with a sound generator in tinnitus patients. *J. Med. Dent. Sci.* 57, 45–53. doi: 10.11480/jmds.570106

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Barozzi, S., Del Bo, L., Crocetti, A., Dyrlund, O., Passoni, S., Zolin, A., et al. (2016). A comparison of nature and technical sounds for tinnitus therapy. *Acta. Acust. United Acust.* 102, 540–546. doi: 10.3813/AAA.918971

Bartnik, G. M., and Skarzynski, H. (2006). "Tinnitus retraining therapy," in *Tinnitus Treatment: Clinical Protocols*, ed R. Tyler (New York, NY: Thieme Medical Publishers, Inc.), 133–145.

Bessman, P., Heider, T., Watten, V. P., and Watten, R. G. (2009). The tinnitus intensive therapy habituation program: a 2-year follow-up pilot study on subjective tinnitus. *Rehabil. Psychol.* 54, 133–137. doi: 10.1037/a0015660

Choy, D., Lipman, R., and Tassi, G. (2010). Worldwide experience with sequential phase-shift sound cancellation treatment of predominant tone tinnitus. *L. Laryngol Otol.* 124, 366–369. doi: 10.1017/S0022215109992167

Courtenay, E. W., Schlaug, G., and Pantev, C. (2010). Listening to filtered music as a treatment option for tinnitus: a review. *Music Percept.* 27, 327–330. doi: 10.1525/mp.2010.27.4.327

Davis, P. (2006). "Music and the acoustic desensitization protocol for tinnitus," in *Tinnitus Treatment*, ed R. S. Tyler (New York, NY: Thieme), 146–160.

Davis, P. B., Paki, B., and Hanley, P. J. (2007). Neuromonics tinnitus treatment: third clinical trial. *Ear. Hear.* 28, 242–259. doi: 10.1097/AUD.0b013e3180312619

Department of Health (2009). Provision of *Services* for *Adults* with *Tinnitus*: *A Good Practice Guide*. London: Central office of information.

De Ridder, D., Kilgard, M., Engineer, N., and Vanneste, S. (2015). Placebo-controlled vagus nerve stimulation paired with tones in a patient with refractory tinnitus: a case report. *Otol. Neurotol.* 36, 575–580. doi: 10.1097/MAO.0000000000000704

Dillon, H., James, A., and Ginis, J. (1987). Client oriented scale of improvement (COSI) and its relationship to several other measures of benefit and satisfaction provided by hearing aids. *J. Am. Acad. Audiol.* 8, 27–43.

Drexler, D., Lopez-Paullier, M., Rodio, S., Gonzalez, M., Geisinger, D., and Pedemonte, M. (2016). Impact of reduction of tinnitus intensity on patients' quality of life. *Int. J. Audiol.* 55, 11–19. doi: 10.3109/14992027.2015.1072772

Durai, M., Kobayashi, K., and Searchfield, G. (2015). A preliminary examination of the roles of contextual stimuli and personality traits under the adaptation level theory model of tinnitus. *Acta. Acust United Acust.* 101, 543–551. doi: 10.3813/AAA.918851

Durai, M., and Searchfield, G. (2016). Anxiety and depression, personality traits relevant to tinnitus: a scoping review. *Int. J. Audiol.* 55, 605–615. doi: 10.1080/14992027.2016.1198966

Durai, M., and Searchfield, G. D. (2017). A mixed-methods trial of broad band noise and nature sounds for tinnitus therapy: group and individual responses modeled under the adaptation level theory of tinnitus. *Front. Aging. Neurosci.* 9:44. doi: 10.3389/fnagi.2017.00044

Eggermont, J. J., and Tass, P. A. (2015). Maladaptive neural synchrony in tinnitus: origin and restoration. *Front. Neurol.* 6:29. doi: 10.3389/fneur.2015.00029

Fagelson, M. A., and Smith, S. L. (2016). Tinnitus self-efficacy and other tinnitus self-report variables in patients with and without post-traumatic stress disorder. *Ear Hear.* 37, 541–546. doi: 10.1097/AUD.0000000000000290

Fioretti, A., Eibenstein, A., and Fusetti, M. (2011). New trends in tinnitus management. *Open Neurol. J.* 5:12. doi: 10.2174/1874205X01105010012

Fisher, A. J., and Boswell, J. F. (2016). Enhancing the personalization of psychotherapy with dynamic assessment and modeling. *Assessment* 23, 496–506. doi: 10.1177/1073191116638735

Folmer, R. L., and Carroll, J. R. (2006). Long-term effectiveness of ear-level devices for tinnitus. *Otol. Head Neck Surg.* 134, 132–137. doi: 10.1016/j.otohns.2005.09.030

Ginsburg, G. S., and Willard, H. F. (2009). Genomic and personalized medicine: foundations and applications. *Transl. Res.* 154, 277–287. doi: 10.1016/j.trsl.2009.09.005

Grant, M. J., and Booth, A. (2009). A typology of reviews: an analysis of 14 review types and associated methodologies. *Health Info. Libr. J.* 26, 91–108. doi: 10.1111/j.1471-1842.2009.00848.x

Grenness, C., Hickson, L., Laplante-Lévesque, A., and Davidson, B. (2014). Patient-centred care: a review for rehabilitative audiologists. *J. Audiol.* 53, S60–S67. doi: 10.3109/14992027.2013.847286

Györi, A. (2016). "Tailor-made notched music training-a therapy of chronic tinnitus," in *Computer Music Seminar Aachen*. Available online at: https://www.researchgate.net/publication/310046636_Tailor-Made_Notched_Music_Training_-_a_therapy_of_chronic_tinnitus

Handscomb, L. (2006). Use of bedside sound generators by patients with tinnitus-related sleeping difficulty: which sounds are preferred and why? *Acta Otolaryngol.* 126, 59–63. doi: 10.1080/03655230600895275

Hanley, P. J., and Davis, P. B. (2008). Treatment of tinnitus with a customized, dynamic acoustic neural stimulus: underlying principles and clinical efficacy. *Trends Amp.* 12, 210–222. doi: 10.1177/1084713808319942

Hann, D., Searchfield, G. D., Sanders, M., and Wise, K. (2008). Strategies for the selection of music in the short-term management of mild tinnitus. *ANZ. J. Audiol.* 30:129. doi: 10.1375/audi.30.2.129

Hauptmann, C., Ströbel, A., Williams, M., Patel, N., Wurzer, H., von Stackelberg, T., et al. (2015). Acoustic coordinated reset neuromodulation in a real life patient population with chronic tonal tinnitus. *BioMed. Res. Int*. 2015:569052. doi: 10.1155/2015/569052

Hauptmann, C., Wegener, A., Poppe, H., Williams, M., Popelka, G., and Tass, P. A. (2016). Validation of a mobile device for acoustic coordinated reset neuromodulation tinnitus therapy. *J. Am. Acad. Audiol.* 27, 720–731. doi: 10.3766/jaaa.15082

Heaton, R. K., Akshoomoff, N., Tulsky, D., Mungas, D., Weintraub, S., Dikmen, S., et al. (2014). Reliability and validity of composite scores from the NIH toolbox cognition battery in adults. *J. Int. Neuropsychol. Soc.* 20, 588–598. doi: 10.1017/S1355617714000241

Heijneman, K. M., De Kleine, E., and Van Dijk, P. (2012). A randomized double-blind crossover study of phase-shift sound therapy for tinnitus. *Otolaryngol. Head Neck Surg.* 147, 308–315. doi: 10.1177/0194599812442615

Henin, S., Fein, D., Smouha, E., and Parra, L. C. (2016). The effects of compensatory auditory stimulation and high-definition transcranial direct current stimulation (hd-tdcs) on tinnitus perception - a randomized pilot study. *PLoS ONE* 11:e0166208. doi: 10.1371/journal.pone.0166208

Henry, J. A., Frederick, M., Sell, S., Griest, S., and Abrams, H. (2015). Validation of a novel combination hearing aid and tinnitus therapy device. *Ear Hear.* 36, 42–52. doi: 10.1097/AUD.0000000000000093

Henry, J. A., Roberts, L. E., Ellingson, R. M., and Thielman, E. J. (2013). Computer-automated tinnitus assessment: noise-band matching, maskability, and residual inhibition. *J. Am. Acad. Audiol.* 24, 486–504. doi: 10.3766/jaaa.24.6.5

Henry, J. A., Schechter, M. A., Zaugg, T. L., Griest, S., Jastreboff, P. J., Vernon, J. A., et al. (2006). Outcomes of clinical trial: tinnitus masking versus tinnitus retraining therapy. *J. Am. Acad. Audiol.* 17, 104–132. doi: 10.3766/jaaa.17.2.4

Herraiz, C., Hernandez, F. J., Toledano, A., and Aparicio, J. M. (2007). Tinnitus retraining therapy: prognosis factors. *Am. J. Otol.* 28, 225–229. doi: 10.1016/j.amjoto.2006.09.004

Herzfeld, M., Enza, C., and Sweetow, R. (2014). Clinical trial on the effectiveness of widex zen therapy for tinnitus. *Hear. Rev.* 21, 24–29.

Hoare, D. J., Adjamian, P., Sereda, M., and Hall, D. A. (2013a). Recent technological advances in sound-based approaches to tinnitus treatment: a review of efficacy considered against putative physiological mechanisms. *Noise Health* 15, 107. doi: 10.4103/1463-1741.110292

Hoare, D. J., Edmondson-Jones, M., Gander, P. E., and Hall, D. A. (2014a). Agreement and reliability of tinnitus loudness matching and pitch likeness rating. *PLoS ONE* 9:e114553. doi: 10.1371/journal.pone.0114553

Hoare, D. J., Gander, P. E., Collins, L., Smith, S., and Hall, D. A. (2012). Management of tinnitus in english nhs audiology departments: an evaluation of current practice. *J. Eval. Clin. Prac.* 18, 326–334. doi: 10.1111/j.1365-2753.2010.01566.x

Hoare, D. J., Pierzycki, R. H., Thomas, H., McAlpine, D., and Hall, D. A. (2013b). Evaluation of the acoustic coordinated reset (CR ®) neuromodulation therapy for tinnitus: study protocol for a double-blind randomized placebo-controlled trial. *Trials* 14:207. doi: 10.1186/1745-6215-14-207

Hoare, D. J., Searchfield, G. D., El Refaie, A., and Henry, J. A. (2014b). Sound therapy for tinnitus management: practicable options. *J. Am. Acad. Audiol.* 25, 62–75. doi: 10.3766/jaaa.25.1.5

Hoare, D. J., Stacey, P. C., and Hall, D. A. (2010). The efficacy of auditory perceptual training for tinnitus: a systematic review. *Annals. Behav. Med.* 40, 313–324. doi: 10.1007/s12160-010-9213-5

Hoare, D. J., Whitham, D., Henry, J. A., and Shorter, G. W. (2015). Neuromodulation (desynchronisation) for tinnitus in adults. *Cochrane Database Syst. Rev.* 6:CD011760. doi: 10.1002/14651858.CD011760.

Hobson, J., Chisholm, E., and El Refaie, A. (2012). Sound therapy (masking) in the management of tinnitus in adults. *Cochrane Database Syst. Rev.* 11:CD006371. doi: 10.1002/14651858.CD006371.pub3

Huang, C.-Y., Wu, J.-L., Cheng, C.-C., Sher, Y.-J., and Chung, K.-C. (2006). Evaluation of the mixing point in tinnitus sound therapy by a psychoacoustic matching protocol with a digital tinnitus evaluation system. *Orl* 68, 110–114. doi: 10.1159/000091213

Hutter, E., Grapp, M., Argstatter, H., and Bolay, H. V. (2014). Music therapy for chronic tinnitus: variability of tinnitus pitch in the course of therapy. *J. Am. Acad. Audiol.* 25, 335–342. doi: 10.3766/jaaa.25.4.5

Ito, M., Soma, K., and Ando, R. (2009). Association between tinnitus retraining therapy and a tinnitus control instrument. *Auris Nasus Larynx* 36, 536–540. doi: 10.1016/j.anl.2009.01.003

Jalilvand, H., Pourbakht, A., and Haghani, H. (2015). Hearing aid or tinnitus masker: which one is the best treatment for blast-induced tinnitus? The results of a long-term study on 974 patients. *Audiol. Neurotol.* 20, 195–201. doi: 10.1159/000377617

Jastreboff, M. M. (2007). Sound therapies for tinnitus management. *Prog. Brain Res.* 166, 435–440. doi: 10.1016/S0079-6123(07)66042-7

Jastreboff, P. (2015). 25 Years of tinnitus retraining therapy. *HNO* 63, 307–311. doi: 10.1007/s00106-014-2979-1

Jastreboff, P. J. (2000). "Tinnitus habituation therapy (THT) and tinnitus retraining therapy (TRT)," in *Tinnitus Handbook*, ed R. Tyler (San Diego, CA: Singular Publishing Group), 357–376.

Jastreboff, P. J. (2011). "Tinnitus retraining therapy," in *Textbook of Tinnitus*, eds A. R. Møller, B. Langguth, D. DeRidder, and T. Kleinjung (New York, NY: Springer-Verlag), 575–596.

Jastreboff, P., and Jastreboff, M. M. (2006). Tinnitus retraining therapy: a different view on tinnitus. *Orl* 68, 23–30. doi: 10.1159/000090487

Jepsen, K., Sanders, M., Searchfield, G., and Kobayashi, K. (2010). Perceptual training of tinnitus. proceedings of 'tinnitus discovery': Asia-Pacific tinnitus symposium, 11-12 Sept 2009. *NZ. Med. J.* 123, 141–153.

Kidd, G. Jr., Arbogast, T. L., Mason, C. R., and Walsh, M. (2002). Informational masking in listeners with sensorineural hearing loss. *JARO* 3, 107–119. doi: 10.1007/s101620010095

Kidd, G. J., Mason, C. R., Rothla, T. L., and Deliwala, P. S. (1998). Release from masking due to spatial separation of sources in the identification of nonspeech auditory patterns. *J. Acous. Soc. Am.* 104, 422–431. doi: 10.1121/1.423246

Kim, B. J., Chung, S. W., Jung, J. Y., and Suh, M. W. (2014). Effect of different sounds on the treatment outcome of tinnitus retraining therapy. *Clin. Exp. Otorhinolaryn* 7, 87–93. doi: 10.3342/ceo.2014.7.2.87

Langguth, B., Goodey, R., Azevedo, A., Bjorne, A., Cacace, A., Crocetti, A., et al. (2007). Consensus for tinnitus patient assessment and treatment outcome measurement: tinnitus research initiative meeting, regensburg, july 2006. *Prog. Brain Res.* 166, 525–536. doi: 10.1016/S0079-6123(07)66050-6

Li, S.-A., Bao, L., and Chrostowski, M. (2016). Investigating the effects of a personalized, spectrally altered music-based sound therapy on treating tinnitus: a blinded, randomized controlled trial. *Audiol. Neurotol.* 21, 296–304. doi: 10.1159/000450745

Lugli, M., Romani, R., Ponzi, S., Bacciu, S., and Parmigiani, S. (2009). The windowed sound therapy: a new empirical approach for an effective personalized treatment of tinnitus. *Int. Tin. J.* 15, 51–61.

Lunner, T., Rudner, M., and Ronnberg, J. (2009). Cognition and hearing aids. *Scand. J. Psychol.* 50, 395–403. doi: 10.1111/j.1467-9450.2009.00742.x

Mahboubi, H., Ziai, K., and Djalilian, H. R. (2012). Customized web-based sound therapy for tinnitus. *Int. Tin. J.* 17, 26–30.

Martinez-Devesa, P., Waddell, A., and Theodoulou, M. (2006). Cognitive behavioural therapy for tinnitus. *Cochrane Database Syst. Rev.* 1:1. doi: 10.1002/14651858.CD005233.pub3.

Mazurek, B., Fischer, F., Haupt, H., Georgiewa, P., Reisshauer, A., and Klapp, B. F. (2006). A modified version of tinnitus retraining therapy: observing long-term outcome and predictors. *Audiol. Neurotol.* 11, 276–286. doi: 10.1159/000093526

Mckenna, L., and Irwin, R. (2008). Sound therapy for tinnitus–sacred cow or idol worship? an investigation of the evidence. *Audiol. Med.* 6, 16–24. doi: 10.1080/16513860801899389

McNeill, C., Távora-Vieira, D., Alnafjan, F., Searchfield, G. D., and Welch, D. (2012). Tinnitus pitch, masking, and the effectiveness of hearing aids for tinnitus therapy. *Int. J. Audiol.* 51, 914–919. doi: 10.3109/14992027.2012.721934

Meeus, O., Heyndrickx, K., Lambrechts, P., De Ridder, D., and Van de Heyning, P. (2010). Phase-shift treatment for tinnitus of cochlear origin. *Eur. Arch. Otorhinolaryngol.* 267, 881–888. doi: 10.1007/s00405-009-1145-y

Meikle, M. B., Henry, J. A., Griest, S. E., Stewart, B. J., Abrams, H. B., McArdle, R., et al. (2012). The tinnitus functional index: development of a new clinical measure for chronic, intrusive tinnitus. *Ear Hear.* 32, 153–176. doi: 10.1097/AUD.0b013e31822f67c0

Michie, S., Miles, J., and Weinman, J. (2003). Patient-centredness in chronic illness: what is it and does it matter? *Patient Ed. Couns.* 51, 197–206. doi: 10.1016/S0738-3991(02)00194-5

Miles, A., and Loughlin, M. (2011). Models in the balance: evidence-based medicine versus evidence-informed individualized care. *J. Eval. Clin. Prac.* 17, 531–536. doi: 10.1111/j.1365-2753.2011.01713.x

Myers, P. J., Griest, S., Kaelin, C., Legro, M. W., Schmidt, C. J., Zaugg, T., et al. (2014). Development of a progressive audiologic tinnitus management program for veterans with tinnitus. *J. Rehab. Res. Dev.* 51:609. doi: 10.1682/JRRD.2013.08.0189

Newman, C. W., Sandridge, S. A., Meit, S. S., and Cherian, N. (2008). Strategies for managing patients with tinnitus: a clinical pathway model. *Sem. Hear.* 29, 300–309. doi: 10.1055/s-0028-1082035

Norena, A. J. (2015). Revisiting the cochlear and central mechanisms of tinnitus and therapeutic approaches. *Audiol. Neurootol.* 20(Suppl. 1), 53–59. doi: 10.1159/000380749

Oishi, N., Shinden, S., Kanzaki, S., Saito, H., Inoue, Y., and Ogawa, K. (2013). Effects of tinnitus retraining therapy involving monaural noise generators. *Eur. Arch. Oto. Rhino. Laryngol.* 270, 443–448. doi: 10.1007/s00405-012-1951-5

Ostermann, K., Lurquin, P., Horoi, M., Cotton, P., Herve, V., and Thill, M. P. (2016). Somatic tinnitus prevalence and treatment with tinnitus retraining therapy. *B-ENT* 12, 59–65.

Oz, I., Arslan, F., Hizal, E., Erbek, S. H., Eryaman, E., Senkal, O. A., et al. (2013). Effectiveness of the combined hearing and masking devices on the severity and perception of tinnitus: a randomized, controlled, double-blind study. *ORL J. Otorhinolaryngol. Relat. Spec.* 75, 211–220. doi: 10.1159/000349979

Parra, P. C. (2015). Tinnitus: mechanisms, measures and sound treatments. *Loquens* 2:24. doi: 10.3989/loquens.2015.024

Pedemonte, M., Drexler, D., Rodio, S., Geisinger, D., Bianco, A., Pol-Fernandes, D., et al. (2010). Tinnitus treatment with sound stimulation during sleep. *Inter. Tin. J.* 16, 37–43.

Peltier, E., Peltier, C., Tahar, S., Alliot-Lugaz, E., and Cazals, Y. (2012). Long-term tinnitus suppression with linear octave frequency transposition hearing AIDS. *PLoS ONE* 7:e51915. doi: 10.1371/journal.pone.0051915

Piskosz, M. (2012). The Role of wireless streaming in tinnitus management. *Hear. Rev.* 12–15.

Reavis, K. M., Chang, J. E., and Zeng, F.-G. (2010). Patterned sound therapy for the treatment of tinnitus. *Hear. J.* 63, 21–22. doi: 10.1097/01.HJ.0000390817.79500.ed

Reavis, K. M., Rothholtz, V. S., Tang, Q., Carroll, J. A., Djalilian, H., and Zeng, F.-G. (2012). Temporary suppression of tinnitus by modulated sounds. *JARO* 13, 561–571. doi: 10.1007/s10162-012-0331-6

Roberts, L. E., and Bosnyak, D. J. (2011). "Auditory training in tinnitus," in *Textbook of Tinnitus*, eds A. R. Møller, B. Langguth, D. DeRidder, and T. Kleinjung (New York, NY: Springer-Verlag), 563–573.

Sand, P., Langguth, B., Kleinjung, T., and Eichhammer, P. (2007). Genetics of chronic tinnitus. *Prog. Brain Res.* 166, 159–168. doi: 10.1016/S0079-6123(07)66014-2

Schaette, R., Konig, O., Hornig, D., Gross, M., and Kempter, R. (2010). Acoustic stimulation treatments against tinnitus could be most effective when tinnitus pitch is within the stimulated frequency range. *Hear. Res.* 269, 95–101. doi: 10.1016/j.heares.2010.06.022

Schleidgen, S., Klingler, C., Bertram, T., Rogowski, W. H., and Marckmann, G. (2013). What is personalized medicine: sharpening a vague term based on a systematic literature review. *BMC Med. Ethics* 14:55. doi: 10.1186/1472-6939-14-55

Searchfield, G. D. (2006). "Hearing aids and tinnitus," in *Tinnitus Protocols*, ed R. Tyler (New York, NY: Thieme), 161–175.

Searchfield, G. D. (2014). Tinnitus what and where: an ecological framework. *Front. Neurol.* 5:271. doi: 10.3389/fneur.2014.00271

Searchfield, G. D. (2015). "Hearing aids for tinnitus," in *Tinnitus: Clinical and Research Perspectives*, eds D. Baguley and M. Fagelson (San Diego, CA: Plural Publishing), 197–212.

Searchfield, G. D. (2016). "Tinnitus sound therapy options," in *The Consumer Handbook on Tinnitus, 2nd Edn.* ed R. Tyler (Sedona, AZ: Auricle Ink Publishers), 179–195.

Searchfield, G. D., Kaur, M., and Martin, W. H. (2010). Hearing aids as an adjunct to counseling: tinnitus patients who choose amplification do better than those that don't. *Int. J. Audiol.* 49, 574–579. doi: 10.3109/14992021003777267

Searchfield, G. D., Kobayashi, K., Hodgson, S.-A., Hodgson, C., Tevoitdale, H., and Irving, S. (2016). Spatial masking: development and testing of a new tinnitus assistive technology. *Assist Tech* 28, 115–125. doi: 10.1080/10400435.2015.1110214

Searchfield, G. D., Kobayashi, K., and Sanders, M. (2012). An adaptation level theory of tinnitus audibility. *Front. Syst. Neurosci.* 6:46. doi: 10.3389/fnsys.2012.00046

Searchfield, G. D., Magnusson, J., Shakes, G., Biesinger, E., and Kong, O. (2011). "Counseling and psycho-education for tinnitus management," in *Textbook of Tinnitus*, eds A. R. Møller, B. Langguth, D. DeRidder, and T. Kleinjung (New York, NY: Springer-Verlag), 535–556.

Sereda, M., Davies, J., and Hall, D. A. (2016). Pre-market version of a commercially available hearing instrument with a tinnitus sound generator: feasibility of evaluation in a clinical trial. *Int. J. Audiol.* 56, 286–294. doi: 10.1080/14992027.2016.1254822

Sereda, M., Hall, D. A., Bosnyak, D. J., Edmondson-Jones, M., Roberts, L. E., Adjamian, P., et al. (2011). Re-examining the relationship between audiometric profile and tinnitus pitch. *Int. J. Audiol.* 50, 303–312. doi: 10.3109/14992027.2010.551221

Sereda, M., Hoare, D. J., Nicholson, R., Smith, S., and Hall, D. A. (2015). Consensus on hearing aid candidature and fitting for mild hearing loss, with and without tinnitus: delphi review. *Ear Hear.* 36:417. doi: 10.1097/AUD.0000000000000140

Serquera, J., Schlee, W., Pryss, R., Neff, P., and Langguth, B. (2015). "Music technology for tinnitus treatment within tinnet," in *Audio Engineering Society Conference: 58th International Conference: Music Induced Hearing Disorders: Audio Engineering Society* (Aalborg).

Shekhawat, G. S., Searchfield, G. D., Kobayashi, K., and Stinear, C. M. (2013a). Prescription of hearing-aid output for tinnitus relief. *Int. J. Audiol.* 52, 617–625. doi: 10.3109/14992027.2013.799787

Shekhawat, G. S., Searchfield, G. D., and Stinear, C. M. (2013b). Role of hearing aids in tinnitus intervention: a scoping review. *J. Am. Acad. Audiol.* 24, 747–762. doi: 10.3766/jaaa.24.8.11

Shekhawat, G. S., Searchfield, G. D., and Stinear, C. M. (2014). Randomized trial of transcranial direct current stimulation and hearing aids for tinnitus management. *Neurorehabil. Neural Repair* 28, 410–419. doi: 10.1177/1545968313508655

Smith, S. L., and Fagelson, M. (2011). Development of the self-efficacy for tinnitus management questionnaire. *J. Am. Acad. Audiol.* 22, 424–440. doi: 10.3766/jaaa.22.7.4

Spiegel, D. P., Linford, T., Thompson, B., Petoe, M. A., Kobayashi, K., Stinear, C. M., et al. (2015). Multisensory attention training for treatment of tinnitus. *Sci. Rep.* 5:10802. doi: 10.1038/srep10802

Stouffer, J. L., and Tyler, R. S. (1990). Characterization of tinnitus by tinnitus patients. *J. Speech Hear. Dis.* 55, 439–453. doi: 10.1044/jshd.5503.439

Suzuki, B., Suzuki, F. A., Yonamine, F. A., Onishi, F. K., Penido, E. T., and Oliveira, N. (2016). Effectiveness of sound therapy in patients with tinnitus resistant to previous treatments: importance of adjustments. *Brazil J. Otorhinolaryngol.* 82, 297–303. doi: 10.1016/j.bjorl.2015.05.009

Tang, H. Y., Harms, V., Speck, S. M., Vezeau, T., and Jesurum, J. T. (2009). Effects of audio relaxation programs for blood pressure reduction in older adults. *Eur. J. Cardiovasc. Nurs.* 8, 329–336. doi: 10.1016/j.ejcnurse.2009.06.001

Távora-Vieira, D., Eikelboom, R. H., and Miller, S. (2011). Neuromonics tinnitus treatment for patients with significant level of hearing loss: an adaptation of the protocol. *In. J. Audiol.* 50, 881–886. doi: 10.3109/14992027.2011.606286

Teismann, H., Okamoto, H., and Pantev, C. (2011). Short and intense tailor-made notched music training against tinnitus: the tinnitus frequency matters. *PLoS ONE* 6:e24685. doi: 10.1371/journal.pone.0024685

Tellegen, A. (1982). *Brief Manual for the Multidimensional Personality Questionnaire*. Minneapolis: University of Minnesota Minneapolis, 1031–1010.

Tutton, R. (2012). Personalizing medicine: futures present and past. *Soc. Sci. Med.* 75, 1721–1728. doi: 10.1016/j.socscimed.2012.07.031

Tyler, R. (2000). "Psychoacoustical measurement," in *Handbook of Tinnitus*, ed R. Tyler (San Diego, CA: Singular Publications), 149–180.

Tyler, R., Ji, H., Perreau, A., Witt, S., Noble, W., and Coelho, C. (2014). Development and validation of the tinnitus primary function questionnaire. *Am. J. Audiol.* 23, 260–272. doi: 10.1044/2014_AJA-13-0014

Tyler, R. S. (2012). Patient preferences and willingness to pay for tinnitus treatments. *J. Am. Acad. Audiol.* 23, 115–125. doi: 10.3766/jaaa.23.2.6

Tyler, R. S., Coelho, C., and Noble, W. (2006). Tinnitus: standard of care, personality differences, genetic factors. *ORL* 68, 14–22. doi: 10.1159/000090486

Tyler, R. S., Gogel, S. A., and Gehringer, A. K. (2007). Tinnitus activities treatment. *Prog. Brain Res.* 166, 425–434. doi: 10.1016/S0079-6123(07)66041-5

Tyler, R. S., Keiner, A. J., Walker, K., Deshpande, A. K., Witt, S., Killian, M., et al. (2015). A series of case studies of tinnitus suppression with mixed background stimuli in a cochlear implant. *Am. J. Audiol.* 24, 398–410. doi: 10.1044/2015_AJA-15-0005

Tyler, R. S., Noble, W., Coelho, C. B., and Ji, H. (2012). Tinnitus retraining therapy: mixing point and total masking are equally effective. *Ear. Hear.* 33, 588–594. doi: 10.1097/AUD.0b013e31824f2a6e

Uriz, A. J., Agüero, P. D., Tulli, J. C., Moreira, J. C., González, E. L., Moscardi, G., et al. (2013). "A development and implementation of a tinnitus treatment method," in *Journal of Physics: Conference Series,* Vol. 477 (Tucumán: IOP Publishing), 012026.

Vanneste, S., van Dongen, M., De Vree, B., Hiseni, S., van der Velden, E., Strydis, C., et al. (2013). Does enriched acoustic environment in humans abolish chronic tinnitus clinically and electrophysiologically? A double blind placebo controlled study. *Hear. Res.* 296, 141–148. doi: 10.1016/j.heares.2012.10.003

Vermeire, K., Heyndrickx, K., De Ridder, D., and Van de Heyning, P. (2007). Phase-shift tinnitus treatment: an open prospective clinical trial. *B-ENT* 3(Suppl. 7), 65–69.

Vernon, J. (1977). Attempts to relieve tinnitus. *J. Am. Acad. Audiol.* 2, 124–131.

Viirre, E. (2010). Customized Sound Therapy (CST): a therapy for low-level tinnitus. *Hear. J.* 63, 30–34. doi: 10.1097/01.HJ.0000390819.17619.2c

Wazen, J. J., Daugherty, J., Pinsky, K., Newman, C. W., Sandridge, S., Battista, R., et al. (2011). Evaluation of a customized acoustical stimulus system in the treatment of chronic tinnitus. *Otol. Neurotol.* 32, 710–716. doi: 10.1097/MAO.0b013e318217d459

Welch, D., and Dawes, P. J. (2008). Personality and perception of tinnitus. *Ear Hear.* 29, 684–692. doi: 10.1097/AUD.0b013e318177d9ac

Williams, M., Hauptmann, C., and Patel, N. (2015). Acoustic CR neuromodulation therapy for subjective tonal tinnitus: a review of clinical outcomes in an independent audiology practice setting. *Front. Neurol.* 6:54. doi: 10.3389/fneur.2015.00054

Wise, K., Kobayashi, K., and Searchfield, G. (2015). Feasibility study of a game integrating assessment and therapy of tinnitus. *J. Neurosci. Methods* 249, 1–7. doi: 10.1016/j.jneumeth.2015.04.002

Wise, K., Kobayashi, K., Magnusson, J., Welch, D., and Searchfield, G. D. (2016). Randomized controlled trial of a perceptual training game for tinnitus therapy. *Games Heal. J.* 5, 141–149. doi: 10.1089/g4h.2015.0068

Zarenoe, R., Hallgren, M., Andersson, G., and Ledin, T. (2017). Working memory, sleep, and hearing problems in patients with tinnitus and hearing loss fitted with hearing aids. *J. Am. Acad. Audiol.* 28, 141–151. doi: 10.3766/jaaa.16023

Zigmond, A. S., and Snaith, R. P. (1983). The hospital anxiety and depression scale. *Acta. Psychiatr. Scand.* 67, 361–370. doi: 10.1111/j.1600-0447.1983.tb09716.x

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A Mixed-Methods Trial of Broad Band Noise and Nature Sounds for Tinnitus Therapy: Group and Individual Responses Modeled under the Adaptation Level Theory of Tinnitus

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Objectives: A randomized cross-over trial in 18 participants tested the hypothesis that nature sounds, with unpredictable temporal characteristics and high valence would yield greater improvement in tinnitus than constant, emotionally neutral broadband noise.

Study Design: The primary outcome measure was the Tinnitus Functional Index (TFI). Secondary measures were: loudness and annoyance ratings, loudness level matches, minimum masking levels, positive and negative emotionality, attention reaction and discrimination time, anxiety, depression and stress. Each sound was administered using MP3 players with earbuds for 8 continuous weeks, with a 3 week wash-out period before crossing over to the other treatment sound. Measurements were undertaken for each arm at sound fitting, 4 and 8 weeks after administration. Qualitative interviews were conducted at each of these appointments.

Results: From a baseline TFI score of 41.3, sound therapy resulted in TFI scores at 8 weeks of 35.6; broadband noise resulted in significantly greater reduction (8.2 points) after 8 weeks of sound therapy use than nature sounds (3.2 points). The positive effect of sound on tinnitus was supported by secondary outcome measures of tinnitus, emotion, attention, and psychological state, but not interviews. Tinnitus loudness level match was higher for BBN at 8 weeks; while there was little change in loudness level matches for nature sounds. There was no change in minimum masking levels following sound therapy administration. Self-reported preference for one sound over another did not correlate with changes in tinnitus.

Conclusions: Modeled under an adaptation level theory framework of tinnitus perception, the results indicate that the introduction of broadband noise shifts internal adaptation level weighting away from the tinnitus signal, reducing tinnitus magnitude. Nature sounds may modify the affective components of tinnitus via a secondary, residual pathway, but this appears to be less important for sound effectiveness. The different rates of adaptation to broadband noise and nature sound by the auditory system may explain the different tinnitus loudness level matches. In addition to group effects there

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also appears to be a great deal of individual variation. A sound therapy framework based on adaptation level theory is proposed that accounts for individual variation in preference and response to sound.

Clinical Trial Registration: www.anzctr.org.au, identifier #12616000742471.

Keywords: clinical trial, tinnitus, auditory perception, adaptation, psychoacoustics, ecology model, sound therapy

INTRODUCTION

Subjective tinnitus is the involuntary perception of one or more sounds by an individual, in the absence of an external physical source (Henry et al., 2005; Moller, 2006; Kaltenbach, 2011; De Ridder et al., 2014). It is now broadly understood to arise as a result of peripheral lesions in the auditory system resulting in altered cortical input. This triggers compensatory neuroplasticity changes across several overlapping brain networks (Schecklmann et al., 2013a,b; Vanneste et al., 2011, 2013; Husain and Schmidt, 2014). Final tinnitus magnitude is thought to result from differences in personality and activity within auditory, emotion, attention, and memory networks (Searchfield et al., 2012; Searchfield, 2014; Durai et al., 2015). Fifteen to twenty percentage of the tinnitus population experience significant disruption to quality of life (Heller, 2003; Hoffmann and Reed, 2004), manifesting as impaired concentration, problems with hearing, irritation, frustration and annoyance, anxiety, depression, disruption of everyday activities, and disturbed sleep (Davis and El Refaie, 2000; Heller, 2003; Bartels et al., 2010; Malouff et al., 2011). Reports of tinnitus affect vary a great deal from individual to individual, leading to models of tinnitus that include individual psychology and personality as strong contributors (Searchfield et al., 2012; Searchfield, 2014). A failure to account for the heterogeneous nature of tinnitus has likely contributed to the difficulties in identifying useful therapies.

Sound therapy is currently used in several tinnitus treatment paradigms. Sound therapy uses external sounds to modify tinnitus perception and/or reactions to it (Scott et al., 1990; Jastreboff, 1999; Henry et al., 2006; Tyler, 2006; Hoare et al., 2011; Searchfield et al., 2012). Immediate effects are provided by masking (Scott et al., 1990; Tyler, 2006), and long-term changes in tinnitus functional networks have also been observed (Noreña and Eggermont, 2006; Távora-Vieira et al., 2011; Tyler et al., 2012). The potential for tinnitus and external sound to interact exists as both undergo similar auditory processing within the system, including feature extraction, schema formation, and semantic objective formation (Searchfield et al., 2012; Searchfield, 2014). Although categorization of patient characteristics has been used to guide focus of treatments [e.g., hearing aids, counseling, use of sound therapy (Jastreboff, 1998)] and some sound therapies alter the therapeutic sound based on pitch (Stein et al., 2016) and give participants choices about the stimuli, how sound is selected based on individual needs does not appear to be widespread or documented. Sounds used in therapy include broadband noise (BBN), narrow-band noise (either pitch-matched or unmatched to tinnitus), nature sounds or music (Sandlin and Olsson, 1999; Vernon and Meikle, 2000; Folmer and Carroll, 2006). Despite its popularity, there is no consensus as to the most appropriate sound parameters for tinnitus therapy, or if the treatment provides independent benefit over psychological effects (Tyler, 2006; McKenna and Irwin, 2008; Hobson et al., 2010) or hearing aids (Henry et al., 2015). Several recent studies using different types of sound have shown small (Kim et al., 2014) or no significant differences in effect (Barozzi et al., 2016) between different therapy sounds on tinnitus. There is some evidence that dynamic sounds that temporally vary may provide greater benefit for reducing tinnitus symptoms compared to fixed intensity sounds (Vernon and Meikle, 2000; Henry et al., 2004; Davis, 2006; Hann et al., 2008). Customized music and counseling applied via the Neuromonics Tinnitus Treatment for 6 months resulted in greater alleviation of tinnitus symptoms and greater user acceptability than when participants were provided with counseling and BBN, or counseling only (Davis et al., 2008). Schreitmüller et al. (2013) observed that nature sounds, even though they presented with higher dynamics and higher masking thresholds, were accepted more by the listener than white noise. Ocean or wave sounds have recently been introduced by several hearing aid manufacturers in their tinnitus therapy devices (Callaway, 2014; Dos Santos and Powers, 2015).

The reasons why temporally varying sound may be more effective in treating tinnitus in some individuals are unclear. The added therapeutic success of dynamic sounds, particularly sounds relevant to an individual's everyday environment, may be due to the provision of greater informational (central) auditory masking, whereby both therapeutic sound and tinnitus compete for cognitive resources (Kidd et al., 2002). Informational or "central" masking is possible with tinnitus, as the phenomenon is due to central processing itself. Another way in which music or nature sounds can promote relief is by engaging the emotional regions of the brain; as relaxation aids (Davis et al., 2008; Hanley and Davis, 2008). Unpleasant sounds mimicking tinnitus have been found to activate the tinnitus network more strongly than neutral tones (Schlee et al., 2008). Simulation of tinnitus (using an aversive tinnitus-like auditory stimuli) in patients without tinnitus have been shown to activate neural networks comparable to that of tinnitus, including recruitment of the limbic system (Mirz et al., 2000a,b). Differences in processing of pleasant sounds have also been observed between tinnitus

Abbreviations: ALT, Adaptation Level Theory; ANOVA, Analysis of Variance; ANZCTR, Australian New Zealand Clinical Trial Registry; BBN, Broadband Noise; CAB, Comprehensive Attention Battery; DASS, Depression, Anxiety and Stress Scale; EAP, Equal Annoyance Point; ELP, Equal Loudness Point; Hz, Hertz; LLM, Loudness Level Match; MML, Minimum Masking Level; MPQ, Multidimensional Personality Questionnaire; NZ, New Zealand; PANAS, Positive and Negative Affect Scale; TCHQ, Tinnitus Case History Questionnaire; TFI, Tinnitus Functional Index; THQ, Tinnitus Handicap Questionnaire; COSIT, Client Oriented Scale of Improvement for Tinnitus.

patients compared to those without hearing loss or tinnitus (Carpenter-Thompson et al., 2014), as greater activation of the bilateral hippocampus and right insula. It is possible that tinnitus and emotionally negative auditory perceptions from known sources may share similar neural processing networks, which are counteracted by the presence of pleasant stimuli. Short term exposure to emotional stimuli in the auditory modality (but not visual modality) influences ratings of tinnitus: with presentation of more unpleasant sounds resulting in increased tinnitus magnitude (Durai et al., 2017b).

An ecological model (Searchfield, 2014) of tinnitus that incorporates Adaptation Level Theory (ALT; Helson, 1964; Searchfield et al., 2012) has been proposed to account for individual differences in responses to sound therapy, in which a multitude of inherent, and environmental factors interact to determine final tinnitus magnitude. The ALT is based on Helson's (Helson, 1948, 1964) theory, whereby an adaptation level (AL) acts as an internal anchor/reference point used to make sensory magnitude estimations, and this is susceptible to change over time and context (Helson, 1948, 1964; Coren and Ward, 1989). For loud and/or annoying tinnitus, a high internal AL is established—thus the tinnitus is perceived as being of high magnitude. The final AL magnitude estimates of tinnitus, as well as distress judgements, are derived by interactions between the focal component (tinnitus), contextual component (any background noise or applied sounds), and various residual components (individual cognitive and behavioral characteristics such as personality traits, memory, and past experiences, emotion, etc.) (Searchfield et al., 2012; Searchfield, 2014; Durai et al., 2015). The ALT model of tinnitus predicts that BBN and sounds that fluctuate or are emotive (such as nature sounds in our soundscape) should both affect tinnitus positively but through different mechanisms. Variables affecting the success of different sounds might include individual-specific top-down processing related to personality, memory, prediction, attention, and emotion as well as bottom up processes related to primitive auditory analysis such as contrast (Searchfield et al., 2012). Up until this study there have been no controlled trials to test sound therapy based on the ALT model. The presence of several influencing factors on tinnitus-external sound interactions might account for individual success (or lack of success) with sound therapy. A successful sound therapy is not one that affects tinnitus alone; it must be comfortable as well. Testing different parameters and individual preferences of sound therapy are therefore significant in strengthening support for, and improving, sound therapy effectiveness (Barros Suzuki et al., 2016).

We hypothesized that nature sounds would affect top-down processing, and this, along with positive effects on emotion would result in greater reduction in tinnitus magnitude than BBN, that would primarily affect bottom-up processing. Barozzi et al. (2016) found that nature and BBN resulted in similar reductions of the Tinnitus Handicap Questionnaire (THQ) following 6 months of administration, but they did not explore individual characteristics and mechanism of benefit relative to study outcomes. An experimental study piloting some of the methods employed here (Durai et al., unpublished manuscript) found that 30 min administration of unpredictable surf-like sound resulted in significantly lower tinnitus loudness than a predictable surf sound. A 2 week feasibility trial found greater number of participants preferred the unpredictable surf sound (Durai et al., unpublished manuscript). The effects of other contributory factors (e.g., greater relaxation to one sound over the other, different emotions evoked by the two sounds, anticipation) were not controlled for in that short-term trial. A longer-term clinical trial comparing BBN and nature sounds measuring various individual residuals (e.g., emotion, attention) was deemed critical to understand sound therapy effects.

METHODS

This study was approved by the University of Auckland Human Participants Ethics Committee. All participants gave written informed consent in accordance with the Declaration of Helsinki. This trial was retrospectively registered on Australian New Zealand Clinical Trials Registry (ANZCTR; Trial #12616000742471).

Trial Design

A randomized controlled, cross-over study design using mixed (qualitative and quantitative) methods was employed. Repeated outcome measures were obtained at three time points: baseline when the sound was first fitted, 4 weeks after administration, and 8 weeks after administration for both BBN and nature sound therapies. There was a 3 week wash-out period in between the two conditions. The outcome measures taken at each appointment and time-frame protocol for data collection are presented in **Table 1** and **Figure 1**, respectively.

Participants

The inclusion criteria were: adults aged between 18 and 69 years residing in the Auckland region (NZ), constant tinnitus and a minimum weighted score of 21 on the Tinnitus Functional Index (TFI; this cut-off score is calculated based on convergent validity results between TFI mean scores and response levels of a tinnitus global severity item; a score of 21 delineates individuals who consider their tinnitus as problematic from those who do not view tinnitus as a problem; Meikle et al., 2012), normal middle ear function, and a maximum of a moderate degree of hearing loss (<70 dB loss on average across frequencies). A participant information sheet was provided to participants that outlined the background and aims of the trial and details of measurements to be taken at various appointments.

Initial Assessments

Following a comprehensive case history [Tinnitus Case History Questionnaire; TCHQ; (Langguth et al., 2007)], a hearing assessment was conducted in a sound treated room (ISO 8253–1:2010). Pure tone audiometry (0.25–16 kHz, Carhart and Jerger, 1959) was undertaken using a GSI-61 two-channel audiometer and TDH-50P headphones or E.A.RTONE 3A insert earphones and Sennheiser HDA-200 high-frequency headphones. Tympanometry was undertaken

TABLE 1 | Outcome measurements taken at the different time points of the trial.

	1st baseline + 1st sound fitting	4 week follow-up	8 week follow-up	2nd baseline + 2nd sound fitting	4 week follow-up	8 week follow-up
Quantitative questionnaires	TFI	TFI	TFI	TFI	TFI	TFI
	Tinnitus loudness rating (1–10)	Tinnitus loudness rating (1–10)	Tinnitus loudness rating (1–10)	Tinnitus loudness rating (1–10)	Tinnitus loudness rating (1–10)	Tinnitus loudness rating (1–10)
	Tinnitus annoyance rating (1–10)	Tinnitus annoyance rating (1–10)	Tinnitus annoyance rating (1–10)	Tinnitus annoyance rating (1–10)	Tinnitus annoyance rating (1–10)	Tinnitus annoyance rating (1–10)
	PANAS	PANAS	PANAS	PANAS	PANAS	PANAS
	DASS	DASS	DASS	DASS	DASS	DASS
Psychoacoustic measurements	LLM	LLM	LLM	LLM	LLM	LLM
	MML	MML	MML	MML	MML	MML
Attention measurements	CAB		CAB	CAB		CAB
Qualitative		Qualitative interview schedule	Qualitative interview schedule		Qualitative interview schedule	Qualitative interview schedule

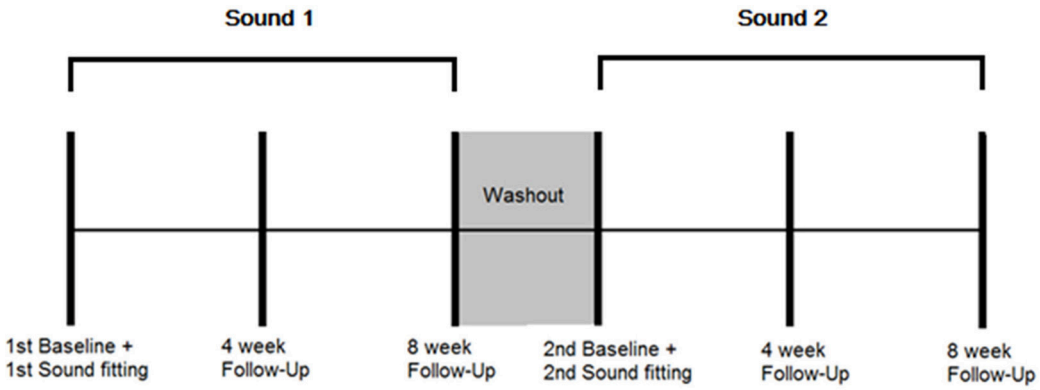

FIGURE 1 | Protocol for data collection. Multiple outcome measurements were taken at the following time points: 1st sound fitting (Baseline), and 4 and 8 weeks after first fitting while the sound was being used. A washout period of 3 weeks followed in which no sound was administered. Multiple outcome measurements were then taken at the following time points: 2st sound fitting (Baseline), and 4 and 8 weeks after second fitting while the sound was being used.

using a GSI Immittance audiometer to check middle-ear function. Tinnitus pitch match was carried out using tinnitus testing software (The University of Auckland) using high frequency circumaural headphones (Sennheiser HDA-200). Tinnitus pitch match was assessed throughout the test frequency range of 0.25–16 kHz using a two-alternative forced-choice (2AFC) method. Each tone was presented at a sensation level of 15 dB SL. Pitch match was then compared to tones one octave above and below to rule out octave confusion. The measurement was repeated until two repeatable responses were obtained. The Multidimensional Personality Questionnaire (MPQ; Tellegen, 1982) was also administered at the initial appointment to measure levels of individual personality traits.

Interventions

Sound Therapy Stimuli

Broadband noise (BBN) was generated using Audacity 2.1.2. (A.2.1.2., 2016). The natural sounds were Surf, Cicadas/Farm Sounds and Rain sounds directly recorded from the natural setting by the researchers using a Roland R-05 WAV/MP3 Recorder with CS-10 EM binaural ear level microphones and edited to 30 min duration using Audacity 2.1.2. software (A.2.1.2., 2016). All stimuli were adjusted for sound level such that the long-term average loudness (dB SPL) was equivalent. The detailed acoustic parameters and spectrum of each sound stimulus are provided in Appendix A in Supplementary Material. Each sound therapy was administered for 8 weeks each via a Philips ViBE SA4VBE08KF/97 4GB MP3 Player and Panasonic

RP-HJE290GUK Premium Black Earphones with a Budloks Earphone Sports Grip earpiece attached for secure retention within the ear. Participants were instructed to listen to the sound therapy for a minimum of 1 h per day.

Tinnitus Loudness and Annoyance Functions and Selection of Nature Sound

BBN and the three nature sounds were played for 2 min each (in randomized order) at the participants desired comfort level. At the end of each sound, participants were asked to rate the sound on a scale of 1–10, with 1 corresponding to a highly negative and/or unpleasant sound and 10 corresponding to highly positive and/or pleasant sound.

BBN and the three nature sounds again were played (in randomized order) to participants at increasing sound levels: from the threshold at which the sound was first heard to the minimum masking level (MML) where the sound first masked the individual's tinnitus. Tinnitus annoyance, tinnitus loudness ratings and noise annoyance ratings (on a scale of low 1–10 high) were undertaken at fixed sound level intervals from 0 dB SL to MML. Participants were also asked to judge the relative loudness of tinnitus and noise on a scale of 1–10 as each sound was increased in sound level, with 1 corresponding with the nature noise being not audible (tinnitus is only audible) and 10 being tinnitus is not audible (fully masked by the sound).

At the end of the task, participants selected which nature sound stimuli they preferred to use in the trial, and this was administered as the Nature sound intervention. Participants were also asked the following questions:

1. Why did you select this particular sound?
2. What kind of feelings (if any) does this sound elicit?

The average valence rating, Equal Loudness Level (the sound level where the combined tinnitus and noise loudness rating given to a noise level was 5, indicating that both tinnitus and noise were of equal perceived loudness) and Equal Annoyance Level (the sound level tinnitus at which annoyance rating functions and noise annoyance rating functions intersect, indicating that both tinnitus and noise are of equal perceived annoyance) of all participants were calculated and recorded for BBN and each environmental sound.

The MP3 volume was initially set for BBN and the nature sound to be one step (10%) below Equal Loudness Level, and if participants preferred it to be slightly higher or lower due to comfort reasons, the sounds were further adjusted accordingly. The final sound set was therefore at an audibility where sound interfered with tinnitus perception but that was also comfortable for the user.

Outcomes

Assessments: Questionnaires for Clinical Evaluation

The Tinnitus Functional Index (TFI; Meikle et al., 2012) was the primary outcome measure, in addition, the following outcome questionnaires were used: Tinnitus Loudness Rating (scale of 1–10), Tinnitus Annoyance Rating (scale of 1–10), Positive and Negative Affect Schedule (PANAS; Tellegen, 1982; Watson et al., 1988), and the Depression, Anxiety and Stress Scale (DASS Scale; Lovibond and Lovibond, 1995). The TFI (Meikle et al., 2012) is a recently developed questionnaire and assesses both severity of tinnitus and its impact on life over eight diverse subscales of intrusiveness, sense of control, cognitive, sleep, auditory, relaxation, quality of life, and emotional. TFI shows high responsiveness to treatment-related change and has been validated as an intake questionnaire with good test-retest reliability in the NZ population (Chandra et al., 2014). Tinnitus loudness ratings were made on a 10-point rating scale where 1 corresponded to a very quiet and 10 with extremely loud. Annoyance ratings were made on a similar scale with one being very low in distress and/or annoyance and 10 being extremely high in distress and/or annoyance. PANAS measures the extent to which positive and negative emotional states are experienced by an individual over the period of the past week. The DASS scale measures levels of affective symptoms.

Assessments: Psychoacoustic Tinnitus Characteristics

Tinnitus psychoacoustic outcomes were measured using tinnitus testing software (The University of Auckland) using high frequency circumaural headphones (Sennheiser HDA-200). Loudness level matching (LLM) was obtained using the pitch-matched stimulus sound at 30 dB above the threshold level and decreasing it slowly in 2 dB steps until the participant stated it was same loudness as their tinnitus. This was repeated three times, and the average of the last two runs was taken. This was subtracted from the threshold level to obtain a level match in dB SL. Minimum masking level (in dB SL) was obtained using a narrow-band noise (NBN) stimulus of 1/3 octave width, raising it from the threshold level until the participant reported that the tinnitus was no longer audible. This procedure was repeated three times, and the average level was calculated. This was subtracted from the threshold level to give the MML match in dB SL.

Assessments: Attention

The Comprehensive Attention Battery (CAB®; Rodenbough, 2003) was used to behaviorally measure individual attention and concentration ability. The CAB is a reliable computer supervised test battery and can be repeated before and after intervention administration to assess for any resulting change. The Discrimination Reaction Time Task (measuring focused attention) and Reaction Time Task (measuring alertness needed for general cognitive task performance; Zomeren and Brouwer, 1994) were utilized in this trial from the CAB series of tests, as in previous studies these domains showed the greatest interaction with tinnitus (Wise, 2012). Focused attention requires attention to be directed toward one aspect of sensory information while excluding others, and is analogous to selective attention (Eysenck and Keane, 2015). Alertness consists of three components: (1) Expectancy, (2) Orientation to various stimuli, and (3) Readiness to produce a motor output. Decreased Reaction Time Task or Discrimination Reaction Time Task scores over time can therefore indicate loss of concentration or increased cognitive load, or inability to focus attention selectively, which can result if tinnitus is increased in magnitude.

For the Reaction Time Task, a gray square was presented in the middle of an otherwise dark/black computer screen. The visual assessment required the participant to respond as soon as possible (touching the square) when it quickly changed to a green color. The presentation lasted 200 ms and occurred after a time delay randomly varying from 1 to 4 s (1000–4000 ms).

For the Discrimination Reaction Time Task, the visual task involved watching a gray square presented in the center of a dark/black computer screen. Random visual presentations of three different colored squares occurred: red, blue or green. Participants were required to touch the square as soon as possible, registering their response, if the square changed to the target color (red) while ignoring non-target colors (blue or green). The target presentations lasted 200 ms, interspersed with 1800 ms time delays. In the auditory task condition, random auditory presentations (spoken) occurred of three different color words: Red, blue, or green. Participants responded whenever they heard the target color word (green) and ignored verbalizations of non-target color words (red or blue). Word presentation lasted ∼300 ms. In the mixed visual and auditory condition, participants heard verbal instructions "The Target Is" followed by either: (1) The gray square changing in color to indicate a visual target, or (2) An auditory presentation (spoken) denoting an auditory target, color word. Whenever the target was seen or heard (depending on whether the target given was visual or auditory in nature), the participant was required to press the square as quickly as possible. While anticipating the indicated target, participants experienced randomized presentation of visual and auditory non-targets; spoken color words or visually presented color changes for the gray square. Targets were altered seven times during the assessment. The tasks resulted in assessment of pure visual reaction time (50 stimuli), pure auditory reaction time (50 stimuli), and visual and auditory reaction time (100 stimuli).

Assessments: Qualitative Interviews

At each follow-up appointment and at the end of the trial all participants were interviewed, and the interviews were digitally recorded, transcribed, and responses coded into themes (Gale et al., 2013). The interview schedule for each follow-up appointment was as follows:

1. How often did you use sounds stimuli?
2. In which particular environments did you find yourself using the sounds?
3. How is the quality of the intervention sound?
4. How you feel the sound is interacting with your tinnitus?
5. Has the quality (characteristics of your tinnitus such as the pitch, duration, fluctuation, etc.) of your tinnitus changed over the last month? If yes, how?

Additional questions asked during the final end-of-trial interview were:

6. Which of the two stimuli (BBN or nature) did you prefer the most? Why?
7. Will you be willing to wear this device as a form of tinnitus management for the next 6 months? Why or why not?
8. How can each of the sound stimuli be improved and why would this be an improvement?
9. Any other comments?

Sample Size

A power analysis indicated that 21 participants would need to enter this two-treatment crossover study. The probability was 80% that the study will detect a treatment difference at a two-sided 0.05 significance level, if the true difference between treatments was 13.0 units on the TFI. This is based on the assumption that the standard deviation of the difference in the TFI is 20.

Randomization

The order of sound presentation for participants (Order 1 = BBN then Nature OR Order 2 = Nature then BBN) was decided using an online, free True Random Number Generator (https://www.random.org/). There were no significant differences in personality trait scores between participants placed in Order 1 compared to Order 2. Throughout the trial the same researcher tested all participants. The only blinding applied was participants were not shown the results of their tinnitus outcome measures at the different time points until the end of the trial. Blinding to intervention type could not be provided due to the distinct perceptual sound characteristics of the two sound stimuli. No tinnitus counseling was provided; participants had their hearing tests and tinnitus results explained, and instructions were provided on use of the MP3 player and how to set volume relative to their tinnitus. The nature sound trialed was that chosen by the user.

Statistical Methods

A 2 × 3 repeated-measures Analysis of Variance (ANOVA) was used to examine changes in outcome measures between the two sound types (BBN, natures sounds) at the three time points (baseline, 4 weeks of intervention and 8 weeks of intervention). All assumptions were tested for all outcomes for each independent variable to see if they were met before running ANOVA. In cases where a significant main effect was observed, Bonferroni *post-hoc* tests were administered.

For outcome measures where there was no group effect for intervention observed at 8 weeks, further bivariate correlation and ANOVA analyses of changes in outcome measures (8 weeks-baseline) was conducted in order to explore whether age, gender, and degree of hearing loss [categorized as slight, mild, moderate, moderately severe, severe, or profound (Clark, 1981) based on average of 3000, 4000, and 6000 Hz hearing thresholds bilaterally] effects were present.

In order to extract potential converging information of the different outcome measures and identify key factors influencing the effect of sound therapy administration on tinnitus over time, a Principal Component Analysis (PCA) was conducted. Changes in all outcome measures (regardless of BBN or Nature sounds) between 8 weeks and baseline as well as baseline measures of personality were included. All components with Eigenvalue >1

were extracted. Following inspection of data and the scree plot, a decision was made regarding the final number of components to be included in rotational analysis with Direct Oblimin rotation. Correlations about 0.5 were criterion used to define and load key variables to respective components and construct dimensions.

The framework method (Gale et al., 2013) was used to analyse the qualitative interviews, consisting of five steps: familiarization, identification of a thematic framework, indexing, charting, and mapping and interpretation. Familiarization involved careful listening to the digital recordings and transcribing, and re-reading the transcription. Common themes were identified in the transcripts, and in the charting phase, the data was rearranged according to theme. In the mapping and interpretative stages, the charted data was compared, and contrasted to identify patterns within the data. Quotations from participants and their thematic analysis were included in the results following standard practice in qualitative methodology (Rossman and Wilson, 1985; Onwuegbuzie and Leech, 2005).

RESULTS

Participants Flow and Baseline Data

Thirty-one participants from the University of Auckland Tinnitus Research Volunteer Database expressed interest in the trial; seven participants did not meet the criteria or were excluded for other reasons (one participant was administered the intervention sounds but the data was excluded from final analysis due to a too low baseline TFI score; removed due to basement effects). The data from 24 participants (8 females, 16 males, mean age = 56.31, range 37–65) was taken for the final trial (**Figure 2**). Eighteen participants (7 female, 11 male, mean age = 60.63, range 38–65) completed the trial, retention was 76%. Three participants were lost to follow-up (did not respond to emails, attend follow-up appointments and/or did not finish trialing both sounds) and three participants had early termination. Early termination of trial refers to cases where participants voluntarily expressed they wanted to stop the trial between the 4 and 8 week appointment period for one or both of the Intervention sounds, but still attended follow-up appointments.

The average baseline outcome measures of participants at the start of the trial is provided in **Table 2** (individual baseline outcome measures are provided in Appendix B in Supplementary Material). The mean Tinnitus Functional Index (TFI) score of participants was 41.5 (SD = 15.5). All participants had experienced chronic bothersome tinnitus for a minimum of 4 years with an average length of time since tinnitus onset of 17.5 years (SD = 12.2, ranging from 4 to 45 years). Thirty-nine percent of participants described tinnitus quality as cricket sounds, 39% as tonal and 22% as noise. Measured tinnitus pitch ranged from 800 to 15,750 Hz, and there was no clustering observed around any particular pitch match. Fifty percent of participants had not used any form of tinnitus treatment in the past, 25% had tried one treatment and 25% had tried more than one treatment. Three out of the 18 participants (17%) wore pairs of hearing aids. When asked whether loud sounds tended to make their tinnitus worse, 42% responded that it did exacerbate it, 32% responded no and

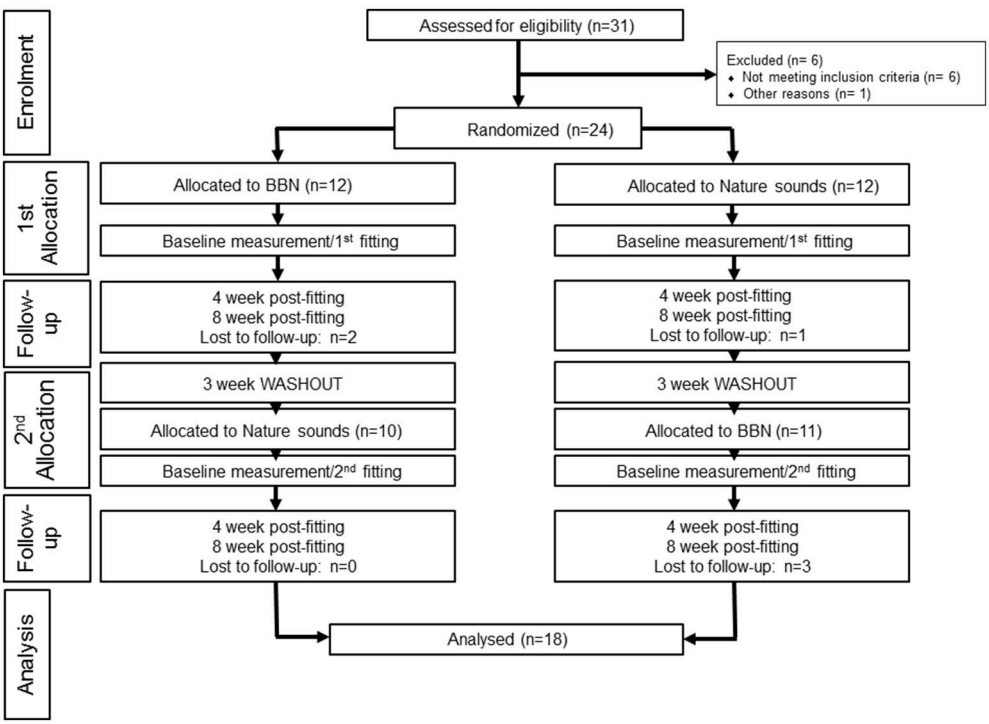

FIGURE 2 | CONSORT flow diagram of participants.

TABLE 2 | Average baseline characteristics of participants as measured at the start of the trial.

		Order 1	Order 2
Demographics	Gender	Five female, four male	Two female, six male
	Age	59.3 (9.6)	56.6 (7.7)
Tinnitus characteristics	Duration (Years)	18.2 (15.5)	14 (8.5)
	Loudness rating (1–10)	6.2 (1.5)	7 (0.8)
	Annoyance rating (1–10)	5.7 (2.2)	5.6 (1.2)
	Total TFI score (weighted)	38.6 (12.5)	47.4 (17)
	LLM	3 (4)	4.8 (4.5)
	MML	6 (6)	7.4 (6.4)
Emotional/ psychological	Positive emotionality	34.2 (6)	34.1 (6.7)
	Negative emotionality	16.2 (5.4)	16.5 (6.7)
	Anxiety	5 (6.4)	4.6 (5.7)
	Depression	3.1 (4)	2.9 (3.6)
	Stress	6.8 (5)	7.1 (7.7)
Personality traits	Stress reaction	7.6 (2.1)	6.3 (4.9)
	Social closeness	5.1 (2.5)	6.1 (2.8)
	Self-control	14.4 (3.1)	13.4 (3.1)
	Alienation	1.1 (1.2)	1.5 (1.5)

Values inside brackets in columns represent one standard deviation. Order 1, BBN then Nature as order of intervention presentation for participants. Order 2, Nature then BBN as order of intervention presentation for participants.

26% did not know. Forty-two percent of participants felt that their tinnitus was reduced by music or by certain types of nature sounds (such as the noise of a waterfall, running shower water, etc.) and the remaining 58% did not know.

Loudness and Annoyance Functions for Sound Therapy Stimuli and Tinnitus

All the sounds resulted in decreased tinnitus loudness and annoyance, and increases in sound loudness and annoyance occurred as noise level was raised (**Figures 3**, **4**). The average ratings for sound therapy stimuli at MML are provided in **Table 3**. When the sounds were ranked based on average rating changes with noise level increases, Rain was ranked #1 (equal) for tinnitus loudness decline, #4 (equal) for sound loudness growth, #1 (equal) for tinnitus annoyance decline, and #2 (equal) for sound annoyance growth. Cicadas was ranked as #1 (equal) in tinnitus loudness decline, #4 (equal) for sound loudness growth, #1 (equal) for tinnitus annoyance decline, and #1 for sound annoyance growth. Surf was ranked #4 for tinnitus loudness decline, #1 for sound loudness growth, #4 for tinnitus annoyance decline and #2 (equal) for sound annoyance growth. BBN was ranked #1 (equal) for tinnitus loudness decline, #1 for sound loudness growth, #1 (equal) for tinnitus annoyance decline, and #4 for sound annoyance growth. The Equal Loudness Level ranking was: Rain/BBN > Cicadas > Surf. The Equal Annoyance Level ranking was: Surf > Rain > Cicadas > BBN.

Selection of Nature Sound

Eleven out of the 18 participants (61%) selected the Rain sound to be used in the trial, and key reasons were that it was soothing and interacted more with tinnitus. Five participants selected the Surf sound (28%) while two selected Cicadas (11%).ain had the highest valence rating (most pleasant) by participants, followed by the Surf and Cicadas respectively. BBN was the least pleasant of all the sounds (**Figure 5**). All participants except one (who expressed neutral feelings) reported the nature sound was pleasant, soothing, relaxing and elicited happy feelings.

Intervention Outcomes: Tinnitus Measures

There was a significant main effect of sound therapy time on TFI scores, with a 5.7 point decrease in TFI scores at 8 weeks compared to baseline [$F_{(2, 28)} = 4.144$, $p < 0.05$; **Figure 6**]. There was a significant effect of sound types at 8 weeks [$F_{(1, 28)} = 6.875$, $p < 0.05$], with BBN sound administration resulting in a mean 8.2 point decrease in scores, while nature sounds resulted in a 3.2 point decrease. The small change in TFI in response to the nature sounds at 4 weeks (4.2 point decrease) was not statistically significant. There were no significant difference in tinnitus measures between before the washout (8 week follow-up appointment for the first sound) and immediately after the washout (sound fitting appointment for the second sound). There was also no effect of order: the degree of change in tinnitus outcome measures was not significantly different between the first and second sound administered.

There was no difference in loudness or annoyance ratings following sound therapy at 4 weeks compared to baseline. At 8 weeks the loudness ratings were 13% lower than at baseline irrespective of the BBN or nature sound condition [$F_{(2, 28)} = 1.551$, $p < 0.05$; **Figure 7**]. At 8 weeks, annoyance ratings were 25% lower than at baseline irrespective of BBN or nature sound condition [$F_{(2, 28)} = 2.815$, $p < 0.05$]. There was no significant difference in tinnitus loudness ratings and annoyance ratings between BBN or nature sound conditions at either 4 or 8 weeks.

There was a significant effect of sound type on psychoacoustic loudness level matches at 8 weeks [$F_{(1, 28)} = 3.134$, $p < 0.05$], with BBN sound administration resulting in a greater mean increase in loudness level match (2.6 dB increase in LLM) while nature sounds had slight increase (0.47 dB increase in LLM; **Figure 8**). There was no significant difference between BBN or nature sound conditions at 4 weeks. There was no significant main effect of sound therapy time on tinnitus minimum masking levels between baseline and 4 weeks or baseline and 8 weeks. There was no significant change in minimum masking levels between 4 weeks and baseline. There was no significant difference between minimum masking levels with sound types at either 4 or 8 weeks.

Intervention Outcomes: Psychological Measures

There was a significant effect of sound therapy time on PANAS positive emotionality scores [$F_{(2, 28)} = 2.210$, $p < 0.05$] with lower levels reported 8 weeks compared to baseline (1.4 points; **Figure 9**). There was no significant difference between sound types on positive emotionality scores at either 4 or 8 weeks.

There was a very small but significant effect of sound therapy time on negative emotionality scores [$F_{(2, 28)} = 1.247$, $p < 0.05$], with an increase in scores at 8 weeks compared to baseline (0.2 points). There was no change in negative emotionality scores

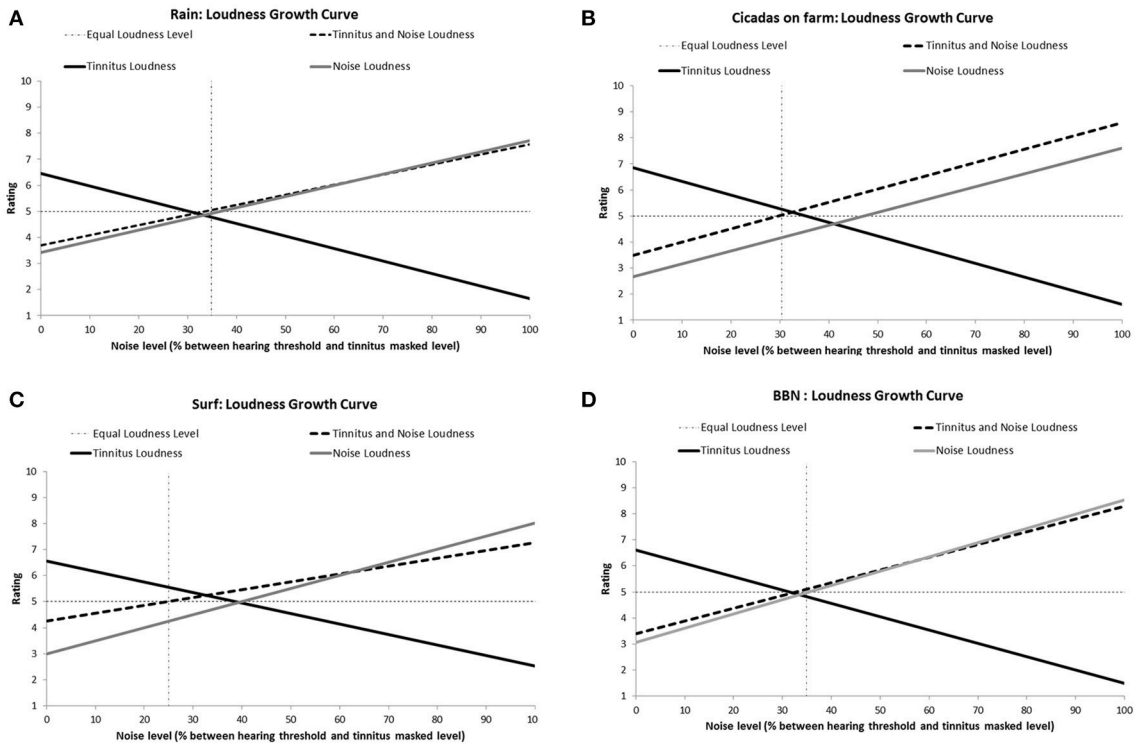

FIGURE 3 | Loudness ratings growth curves for each therapy sound [Rain (A), Cicadas on farm **(B)**, Surf **(C)**, BBN **(D)**] as a function of noise level (% between hearing threshold and minimum masking level for tinnitus). Loudness functions show decreases in tinnitus loudness (solid black line), increases in sound loudness (solid gray line), and increases in combined tinnitus and sound loudness as a function of sound level (dashed line). The Equal Loudness Level (vertical dashed line) defines the sound level at which both tinnitus and sound were of equal perceived loudness (tinnitus and sound loudness rating = 5).

between 4 weeks and baseline. There was no significant difference between sound types on negative emotionality scores at either 4 or 8 weeks.

There was a significant effect of sound therapy time on all outcomes measures of anxiety, depression and stress (**Figure 10**). Reduced anxiety scores were observed between 4 weeks and baseline (0.3 points), and 8 weeks and baseline (1.1 points) [$F_{(2, 28)} = 3.721$, $p < 0.05$]; reduced depression scores were observed between 4 weeks and baseline (0.6 points), and 8 weeks and baseline (1.4 points) [$F_{(2, 28)} = 2.44$, $p < 0.05$]; stress scores were increased between 4 weeks and baseline (2.3 points), and decreased between 8 weeks and baseline (1.1 points) [$F_{(2, 28)} = 3.01$, $p < 0.05$].

There were no significant effects of sound therapy time or sound type on either attention reaction response times or attention discrimination response times.

Principal Component Analysis

The Kaiser-Meyer-Olkin measure (KMO) verified the sampling adequacy for the analysis (KMO = 0.62). Bartlett's test of sphericity indicated that inter-measure correlations were sufficiently large for PCA ($p < 0.001$). The majority (87.5%) of variation in outcome variables following sound therapy administration over time were accounted for by changes in tinnitus impact on life (27%), tinnitus perceptual characteristics (9%), stress reduction/relaxation (21%), changes in positive mood (16%), and changes in negative mood (14%). This was a satisfactory amount of variation. The individual correlations/strength of loadings of each intervention outcome measure on to each principle component is provided in **Table 4**.

Correlations and Differences of Age, Gender, and Hearing Loss on Outcome Measures

The effects of age, gender, or hearing loss on outcome measure changes were investigated. Participants with mild hearing loss had a decrease in LLM (5.7 dB SL), while those with moderately severe hearing loss showed a slight increase in LLM (0.83 dB SL) between baseline and 8 weeks [$F_{(3, 15)} = 2.32$, $p < 0.05$]. During administration of BBN and natures sounds, significant differences by gender were present for negative emotionality [$F_{(1, 15)} = 6.393$, $p < 0.05$]; females displayed an increase in scores between baseline and 4 weeks (2.4 point increase), while males had a decrease in scores between baseline and 4 weeks (4.4 point decrease). Significant differences by gender were present for depression [$F_{(1, 15)} = 3.096$, $p < 0.05$], anxiety [$F_{(1, 15)} = 5.532$, $p < 0.05$], and stress [$F_{(1, 15)} = 6.37$, $p < 0.05$]. Females had a slight increase in depression scores (0.52 points) and anxiety scores (0.82 points) between baseline and 4 weeks; males had a decrease in depression scores (3.35 points) and anxiety scores (2.09 points)

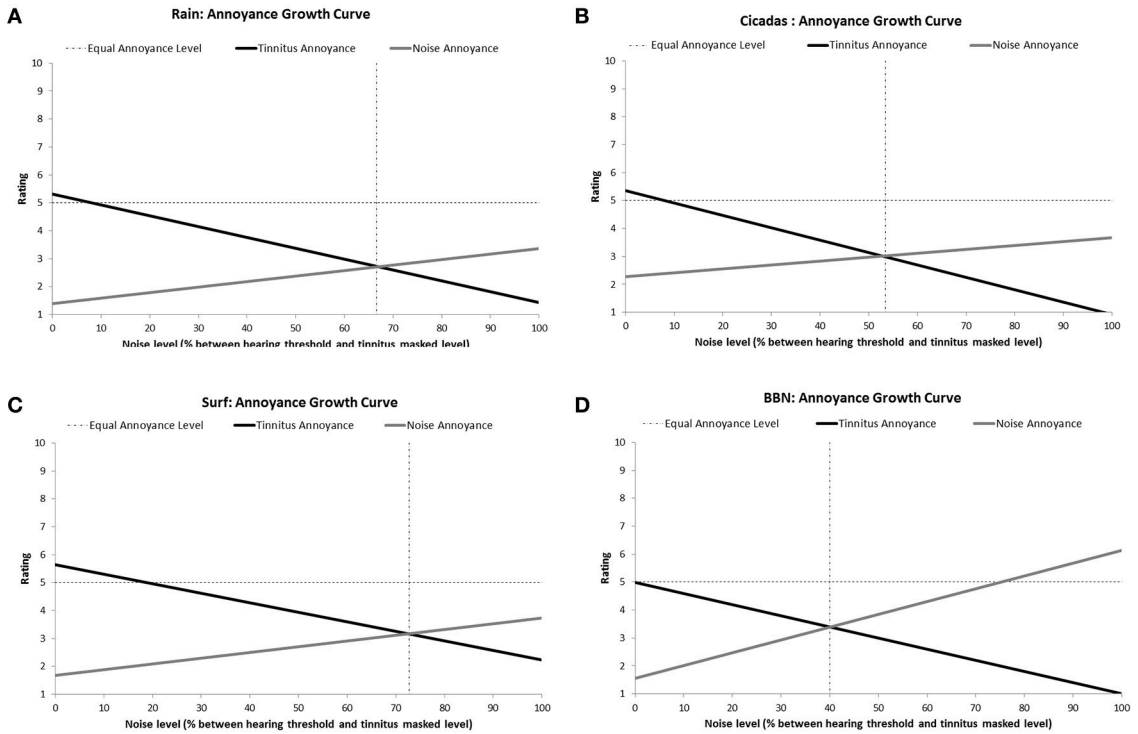

FIGURE 4 | Annoyance ratings growth curves of each therapy sound [Rain (A), Cicadas on Farm **(B)**, Surf **(C)**, BBN **(D)**] as a function of noise level (% between hearing threshold and minimum masking level for tinnitus]. Annoyance functions show decreases in tinnitus annoyance (solid black line) and increases in sound annoyance (solid gray line) as a function of sound level. The equal annoyance point (vertical dashed line) defines the sound level at which both tinnitus and sounds were of equal perceived annoyance (point of intersection between tinnitus annoyance and sound annoyance functions).

TABLE 3 | Ratings on a scale of 1–10 of tinnitus and sound loudness and annoyance at the MML of tinnitus.

Rating at MML (on scale of 1–10)	Rain	Cicadas	Surf	BBN
Tinnitus loudness	2 (2=)	2 (2=)	3 (4)	1 (1)
Noise loudness	8 (1=)	8 (1=)	8 (1=)	9 (4)
Tinnitus annoyance	1 (1=)	1 (1=)	2 (4)	1 (1=)
Sound annoyance	3 (1)	4 (2=)	4 (2=)	6 (4)

For example, BBN at MML was rated as being louder and more annoying than other sounds; however, the administration of BBN at this level also resulted in one of the lowest tinnitus loudness and annoyance ratings. The number in brackets represents ranking from 1 (best on measure for sound therapy) to 4 (worst on measure for sound therapy).

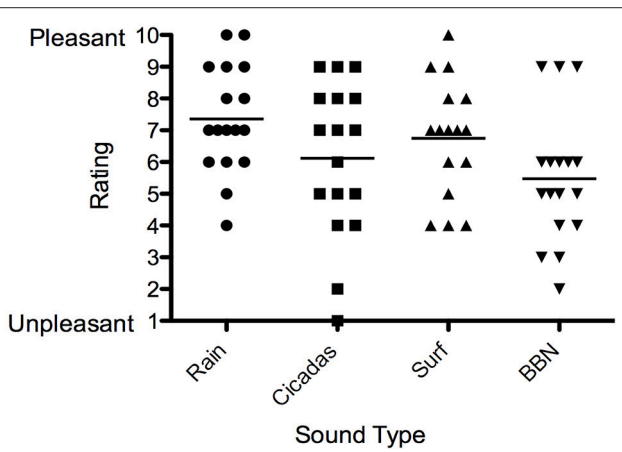

FIGURE 5 | Valence ratings of sound therapy stimuli [Rain (circles), Cicadas on Farm (squares), Surf (triangles), BBN (inverted triangles)] by participants. Horizontal lines represent average valence ratings for each sound stimulus.

for the same time period. Females had an increase in stress scores (2.96 points) between baseline and 8 weeks; males had a decrease in stress scores (2.42 points) for the same time period.

Individual Differences

For all outcome measures where there were no effects of sound types after 8 weeks of trial, individual results were explored in a descriptive manner for any patterns (**Figure 11**). Overall, there was a considerably large amount of individual variability present in responses to sound therapy.

A greater number of participants seemed to experience a decrease in tinnitus loudness ratings at 8 weeks compared to baseline (than an increase or no change); this was slightly more likely to occur during administration of nature sound than BBN. For those who experienced an increase in tinnitus loudness rating with the presence of sound, this was most likely to occur regardless of whether BBN or nature sound was administered. Likewise more participants seemed to experience a decrease in

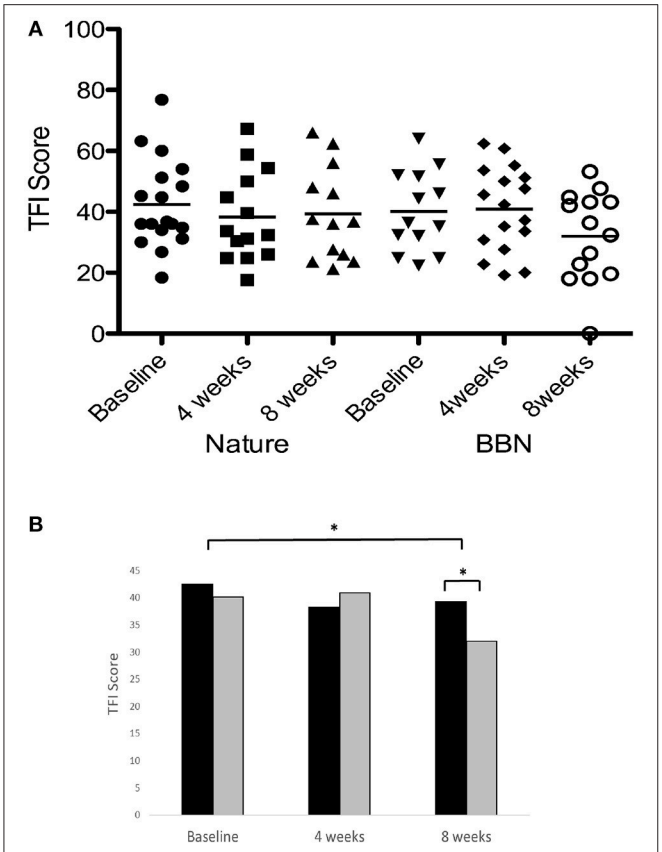

FIGURE 6 | (A) Individual TFI scores of participants at baseline, at 4 weeks follow-up and at 8 weeks follow-up following administration of BBN and nature sound stimuli. Horizontal lines represent average TFI scores. **(B)** Average TFI scores of participants at baseline (filled circles for nature sounds; inverted triangles for BBN), at 4 weeks follow-up (squares for nature sounds; diamonds for BBN) and at 8 weeks follow-up (triangles for nature sounds; open circles for BBN) following administration of BBN (black) and nature (gray) sound stimuli. The significant difference is indicated by $^*p < 0.05$. Error bars represent ± one standard error.

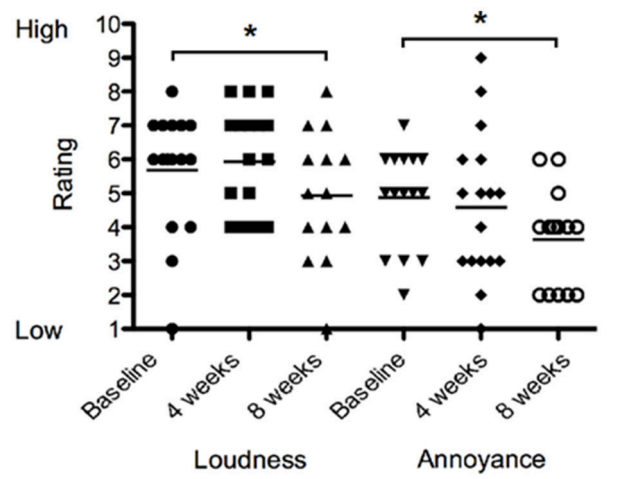

FIGURE 7 | Tinnitus loudness ratings (on a scale of 1–10, where 1 corresponded with very quiet tinnitus and 10 with extremely loud tinnitus) and annoyance ratings (on a scale of 1–10, where 1 corresponded low in distress and/or annoyance and 10 with extremely high distress) of participants at baseline (filled circles for loudness ratings; inverted triangles for annoyance ratings), at 4 weeks follow-up (squares for loudness ratings; diamonds for annoyance ratings) and at 8 weeks follow-up (triangles for loudness ratings; open circles for annoyance ratings). Significant differences are indicated by $^*p < 0.05$. Horizontal lines represent average rating scores.

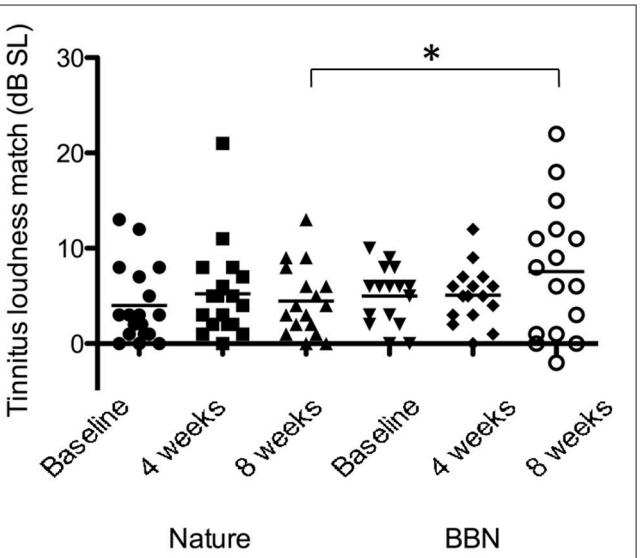

FIGURE 8 | Average LLMs (in dB SL) at baseline (filled circles for nature sounds; inverted triangles for BBN), at 4 weeks follow-up (squares for nature sounds; diamonds for BBN) and at 8 weeks follow-up (triangles for nature sounds; open circles for BBN) following administration of BBN (black) and nature (gray) sound stimuli. Horizontal lines represent average tinnitus loudness matches (in dB SL). Significant differences are indicated by $^*p < 0.05$. Error bars represent ± one standard error.

tinnitus annoyance ratings at 8 weeks compared to baseline (than an increase or no change); however this was more likely to occur during administration of BBN sound. For those who experienced an increase in tinnitus loudness rating with the presence of sound, this was most likely to occur for a specific sound type (either BBN or nature sound, but not both).

More participants seemed to experience a decrease in MML and anxiety scores at 8 weeks compared to baseline; for both these outcome measures, a decrease was more likely to occur during administration of BBN than nature sound. For negative emotionality, positive emotionality, depression, stress, attention reaction, and discrimination response time scores, individuals were roughly equally distributed by whether there was an increase, a decrease, or no change between 8 weeks and baseline. One participant had a significant decrease in depression scores under the BBN condition; the decrease in depression scores seemed to be considerably less for nature sound administered to the same individual. Another participant had a considerable decrease in depression scores with nature sound; BBN however led to an increase in depression scores in the same person.

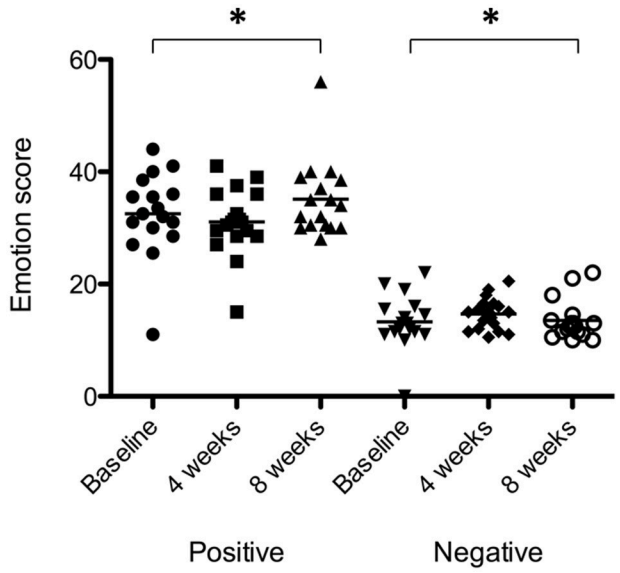

FIGURE 9 | Positive emotionality and negative emotionality scores at baseline (filled circles for positive emotionality; inverted triangles for negative emotionality), at 4 weeks follow-up (squares for positive emotionality; diamonds for negative emotionality) and at 8 weeks follow-up (triangles for positive emotionality; open circles for negative emotionality). Horizontal lines represent average scores. Significant differences are indicated by $^*p < 0.05$.

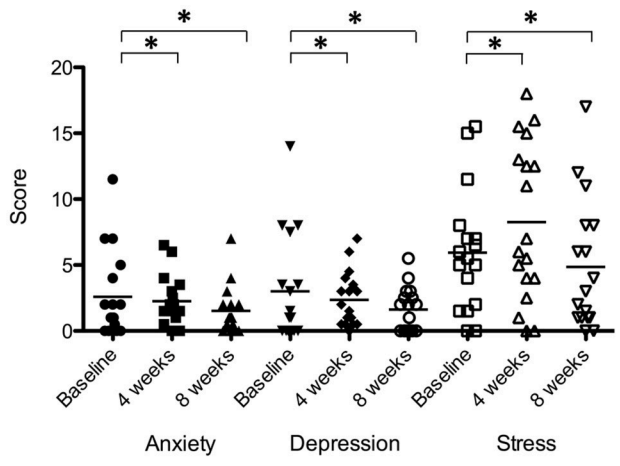

FIGURE 10 | Anxiety, depression, and stress scores of participants at baseline (filled circles for anxiety; inverted triangles for depression; open squares for stress), at 4 weeks follow-up (squares for anxiety; diamonds for depression; open triangles for stress) and at 8 weeks follow-up (triangles for anxiety; unfilled circles for depression; open inverted triangles for stress). Horizontal lines represent average scores. Significant differences are indicated by $^*p < 0.05$.

Intervention Outcomes: Qualitative Reports

Following qualitative analysis using the framework method, certain key areas emerged with regards to the sound trial. Common threads identified during the qualitative interviews are outlined below (relevant excerpts are included in Appendix C in Supplementary Material).

Hours and Environments of Use

Most participants used both sounds for the minimum amount required each day and reported usage ranged from 0.5 to 1.5 h for BBN. The nature sounds were listened to for longer periods of time: nine participants reported consistently using the nature sound for 2 h or more. One participant used the sounds at work (7 h/day). If participants were involved in engaging activities, they often let the sounds run on. The vast majority of participants used the sounds in more than one environment: 48% in quiet, usually in the evenings or in bed reading, 26% working on quiet tasks around the house, garden or in the car, and 26% at the office or doing computer work. A few participants reported experimenting with the sounds in some situations with extra sound such as TV, radio, while having conversations, or in traffic noise. The use of sounds in the presence of noise did not make the tinnitus worse.

Early Termination of Trial

For 17% of participants (three participants), the trial had to be terminated early due to significant exacerbation of tinnitus. In two out of the three cases, termination occurred during administration of BBN sound. The reason for variation was a specific life event (death of brother-in-law, disruption to sleep activity) and an incident (workplace incident exposure to loud noise). The third case terminated during nature sound administration, as they had disruption to sleep activity and did not observe any benefit.

Effectiveness of Intervention Sounds at 4 Weeks

At 4 weeks 42% of participants reported experiencing a worsening of tinnitus with both sounds. Among those who reported no change in tinnitus (39%), a slightly higher proportion reported this during administration of BBN than nature sound. In contrast, slightly more participants (19%) obtained tinnitus relief with nature sound compared to BBN. Six participants felt tinnitus was exacerbated with BBN sound and nine participants with nature sound. For some participants the increase in tinnitus was not observed while the sound was playing, but immediately, or shortly, after the sound was stopped. There was no noticeable change in tinnitus for six participants listening to BBN sound and five participants listening to nature sound.

Effectiveness of Intervention Sounds at 8 weeks

At 8 weeks 52% of participants did not perceive any change. A lack of perceived change was more prevalent following administration of the nature sound than the BBN. The group that had benefit (13% of participants) was almost three times more likely to benefit from BBN than nature sounds. However, BBN was also reported more likely to make tinnitus became worse (34%). There were no statistically significant underlying differences in baseline outcome measures (e.g., baseline TFI score, LLM, etc.) or demographic factors (e.g., age, gender, etc.) between participant groups who reported benefit, no change or worse tinnitus at 8 weeks follow-up.

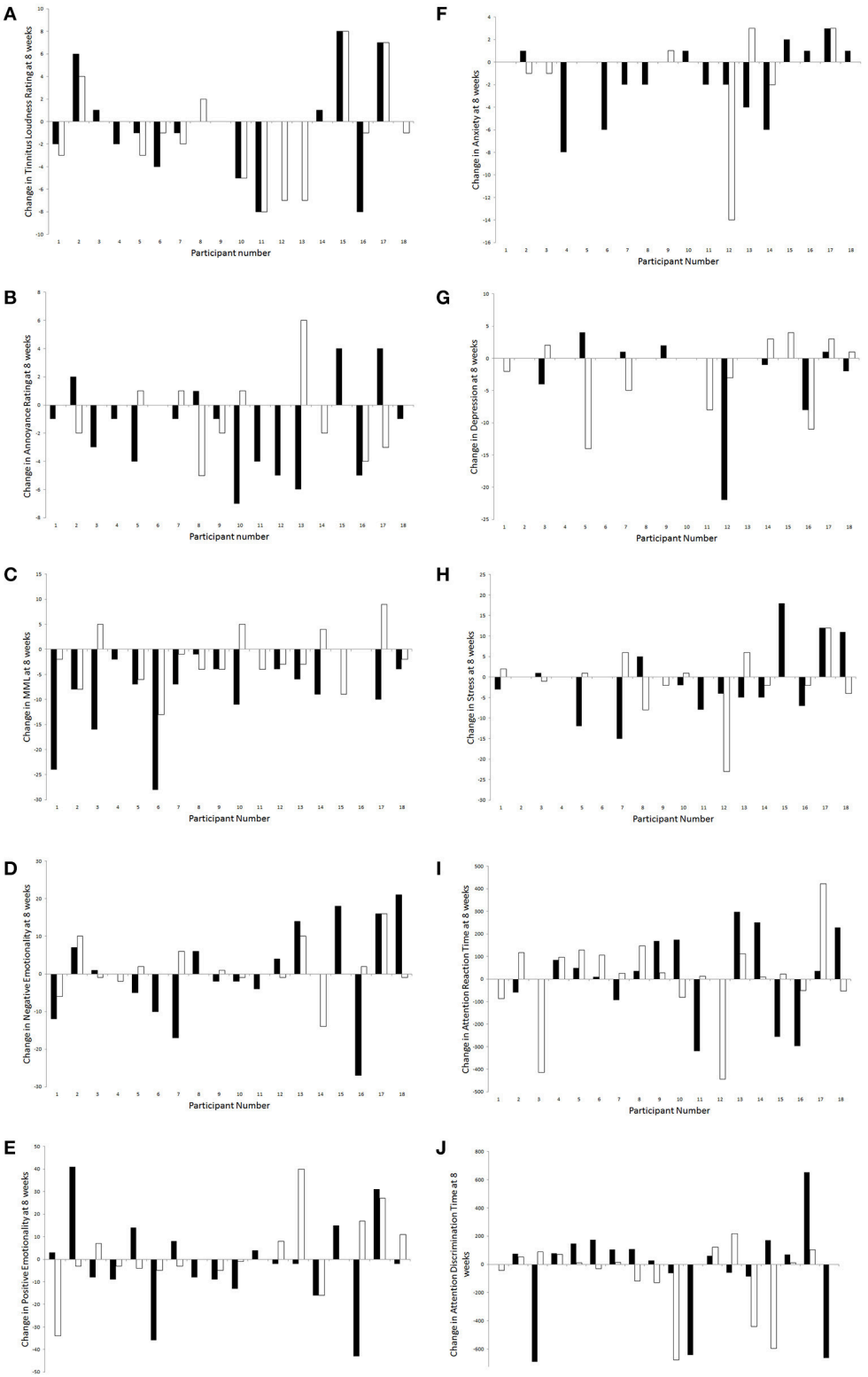

FIGURE 11 | Changes in outcome measures [(A), tinnitus loudness ratings; **(B)**, tinnitus annoyance ratings; **(C)**, MML; **(D)**, negative emotionality; **(E)**, positive emotionality; **(F)**, anxiety; **(G)**, depression; **(H)**, stress; **(I)**, attention reaction time; **(J)**, attention discrimination time] for each participant following administration of BBN (black) and nature (white) sound stimuli.

TABLE 4 | Principal components analysis (with direct oblimin rotation) loadings of intervention study outcomes.

	Tinnitus		Psychological			h^2
	Component 1	Component 2	Component 3	Component 4	Component 5	
	Tinnitus influence—impact on life	Tinnitus—perceptual characteristics	Stress reduction/relaxation	Positive mood	Negative mood	
TFI total score	**0.787**	**0.608**	0.076	0.027	0.003	0.995
Tinnitus loudness ratings	−0.493	**0.860**	0.075	0.005	−0.050	0.990
Tinnitus annoyance ratings	**0.984**	0.010	−0.052	−0.053	−0.085	0.982
Tinnitus loudness level match	**0.984**	0.010	−0.052	−0.053	−0.085	0.982
Tinnitus minimum masking level	0.134	**0.967**	0.047	−0.031	−0.115	0.969
Positive emotionality	−0.032	0.083	−0.310	**0.898**	0.039	0.912
Negative emotionality	0.118	0.112	−0.032	−0.318	**0.788**	0.750
Depression	−0.050	0.109	**0.601**	**−0.730**	−0.017	0.905
Anxiety	−0.171	0.053	**0.887**	−0.102	0.142	0.850
Stress	0.091	−0.037	**0.856**	−0.270	0.282	0.896
Attention reaction response times	**−0.547**	0.061	**0.584**	−0.278	−0.397	0.879
Attention discriminatory response times	−0.074	−0.447	0.258	**0.685**	−0.246	0.803
Personality: stress reaction	0.073	−0.027	**−0.730**	−0.041	**0.558**	0.853
Personality: self-control	−0.044	0.326	−0.252	**0.636**	0.481	0.808
Personality: social closeness	−0.083	−0.258	0.211	0.390	**0.773**	0.869
Personality: alienation	0.168	0.080	−0.072	−0.066	**−0.713**	0.552
% of variance explained by each factor	0.27	0.09	0.21	0.16	0.14	0.875

Eigen-value > 1 criteria applied. Correlations above 0.5 and total variance explained by principal components are presented in bold.

Preference of Intervention Sound at 8 weeks

Preference for one sound was asked, regardless of its interaction pattern with tinnitus. Of the 18 participants, only three (26%) did not have any preference. Thirty-two percent of participants preferred the BBN and a slightly higher 42% preferred the nature sound. Chi-squared tests showed that participants were not significantly more likely to choose any one of BBN, nature sound, or no preference as a response than the other. Nature sounds were reported as relaxing and had a distracting element to them that had a psychological benefit. BBN sound was described as interacting better with the tinnitus, and led to a noticeable difference in tinnitus. Among those who preferred BBN, the nature sound was commonly described as the more pleasant sound, but BBN was more efficient for treatment. In contrast, others did not like the distracting effect of the nature sound and found attention was directed toward the tinnitus instead. Participants also mentioned that they initially conceptualized BBN to be less pleasant to listen to, but discovered that it was more tolerable than they had imagined. There were no significant differences in hearing observable by sound preference; those with poorer hearing on average were less likely to have a preference although this was not statistically significant.

Long-Term Use of Sound Device for Tinnitus Management

Nine (half of total) participants were interested in continuing using the device for long-term tinnitus management and believed their tinnitus would change as a result. There was roughly equal split as to whether participants wanted to listen to BBN or nature sound over time. Two participants were interested in continuing sound therapy but did not believe their tinnitus would change. Eight participants were not interested in continuing, predominantly because there was either (1) no benefit, (2) tinnitus became louder in volume as a result of sound therapy, and/or (3) sounds made them more aware of their tinnitus as discussed previously.

Quality of Intervention Sounds

There were no concerns regarding the sound quality of both s from the majority of participants; however one participant felt

their volume control increased dramatically from one step to another for the BBN.

Relationship between Intervention Outcomes and Qualitative Reports

No trends were observed when grouping participant's tinnitus quantitative intervention outcome measures (loudness rating, annoyance rating, and LLM of tinnitus) by whether participants reported benefit or not from sound. There were also no observable trends when grouping by participant preference for an intervention sound.

DISCUSSION

The administration of sound therapy led to reduction in tinnitus over 8 weeks. This effect was largely due to BBN sound therapy which resulted in a 8.2 point reduction of TFI scores (Meikle et al., 2012); this was significantly different to the 3.2 point reduction following 8 weeks of Nature sound administration. The TFI reflects impact of tinnitus on quality of life (Meikle et al., 2012). Both the TFI changes were not large enough to meet one suggested clinical criterion for meaningful reduction in TFI outcome scores [a 13-point reduction (Meikle et al., 2012)] but BBN did if a different criteria of 7–8 point change (Folmer et al., 2015) was applied. For most participants sound resulted in small but significant changes in secondary outcome measures of tinnitus (reduced loudness rating scale and reduced annoyance rating scale) and psychologically related measures (increased positive emotionality, reduced anxiety, reduced depression, and reduced stress). Unlike response to rating scales, the loudness level matches increased for BBN, while there was minimal increase for loudness level matches for Nature sounds between baseline and 8 weeks follow-up. There was no significant change in MML matches following sound therapy administration. For BBN, while there was a slight decrease in loudness level matches for nature sound between baseline and 8 weeks follow-up. The results showed large individual preferences.

In this study participants played the sounds for 1–1.5 h/day, which is less than many tinnitus treatment paradigms suggest (e.g., Neuromonics Tinnitus Treatment and Tinnitus Retraining Therapy recommend 6–8 h use; Davis et al., 2008; Hanley and Davis, 2008). The time frame (8 weeks) of administration was also less than the 6 months or greater suggested by these treatments. The degree of change observed with sound may be different if used for longer periods of time per day or administered over a longer time frame (e.g., individual responses might converge or diverge over a greater amount of time).

Individual Effects (Age, Gender, Hearing Loss)

There were some interesting differences observed in gender and hearing loss with regards to some of the changes in outcome measures (Hunter and Gillen, 2009). For the psychological outcomes of negative emotionality, depression, anxiety and stress, females had an initial worsening of symptoms between baseline and 4 weeks, while males had a decrease. LLMs significantly decreased over 8 weeks among individuals with mild hearing loss (by 6 points) while those with moderately severe hearing loss actually had an increase in LLM (by slightly <1 point). The introduction of sound therapy was most beneficial in cases of mild deafferentation and/or auditory pathway damage. This may be interpreted in two ways: the tinnitus characteristics of those with lower levels of deafferentation may be more driven by attentional and psychological variables, such that new sound provides attention diversion and relief translating into lower tinnitus loudness measurements, or in instances of severe damage/deafferentation to the hearing system, sound therapy is not able to reach the appropriate cortical regions to elicit any changes, even when set a comfortable and audible listening level (Schaette et al., 2010). This has implications clinically when setting levels for sound therapy, especially when user-set. The counter to increasing levels to create greater tinnitus interaction is that if the level is set too high, there is a risk of triggering negative emotion and discomfort to the sound itself (Scott et al., 1990; Searchfield, 2014); thereby also preventing any AL shifts (Searchfield et al., 2012).

Individual Effects (Personality Traits)

The four personality traits examined in this study have been associated with tinnitus perception and distress. Tinnitus sufferers displaying higher levels of stress reaction, lower social closeness, lower self-control, and higher alienation than individuals with hearing loss (but not tinnitus; Rizzardo et al., 1998; Scott and Lindberg, 2000; Sirois et al., 2006; Welch and Dawes, 2008; Bartels et al., 2010; Durai et al., 2015; Durai and Searchfield, 2016). In this study, personality traits of self-control and social closeness were significantly negatively correlated, and social closeness and alienation were positively correlated. This is similar to previous findings applying the MPQ to tinnitus groups (Durai et al., 2017a). Females in this study had greater levels of social closeness than males. Males in this study had higher alienation than females. Welch and Dawes (2008) observed an elevation in alienation scores among men in their general population sample of 32-year-olds. Males also displayed higher emotional suppression scores than females in the study by Durai and Searchfield (Durai et al., 2017b). Thus, underlying personality differences appear to exist between males and females who experience tinnitus.

Participants with moderate hearing loss also had significantly higher self-control scores than those with severe hearing loss. Both genetic and environmental factors interact to create an individual's personality (Bouchard and Loehlin, 2001; Specht et al., 2011). Some of the personality traits identified in this study, such as stress reaction and social closeness, are difficult to change (Welch and Dawes, 2008). If any change is possible, it will be gradual and dependent on the age of the individual—absolute level changes have been reported to be more pronounced during adolescence and the elderly years of life, due to biological maturation, social expectations and conditioning processes (Costa and McRae, 1992; Costa and McCrae, 2006; Roberts et al., 2006; Corr and Matthews, 2009).

Personality differences can add to the heterogeneity presented in tinnitus, although this has been given little attention. It may

be valuable to attempt to understand this contributory factor further by incorporating personality into assessment and for sub grouping to see how it shapes tinnitus perception, distress, and emotional response.

Attention Effects

Attention (focused attention and general alertness; Zomeren and Brouwer, 1994) was the only measurement dimension that did not change over the 8 weeks. However, there were significant correlations present between changes in reaction time task attention scores and changes in MML and stress scores at 8 weeks. At 8 weeks, changes in discrimination time task attention scores significantly correlated with changes in TFI, depression, and anxiety scores. This suggests a complex interaction between attention, cognitive, and psychological affect, tinnitus perceptual characteristics and tinnitus impact on life, as suggested in the ecological model of tinnitus (Searchfield, 2014).

Interpretation under the Adaptation Level Theory

Under the ALT model, the "presence of sound effect" (decrease in tinnitus outcomes after administration of either sound therapy stimuli) suggests a shifting of internal AL steadily away from the tinnitus and towards background noise stimulus (**Figure 12**). This may occur due to component weighting shifts and attention diversion. Increased positive psychological benefits may also create a facilitating residual effect, which also shifts AL. It is possible that characteristics of specific sound stimuli may work by placing greater emphasis on altering one pathway than another (e.g., BBN has been reported to aid in attention diversion; nature sounds were reported as eliciting high valence emotions). Durai et al. (2015) explored the possibility that tinnitus distress and loudness may be underlined by different perceptual and decision making processes that can be represented by two distinct adaptation levels. An AL can exist for any sensory modality, but also within each modality (Helson, 1948, 1964; Coren and Ward, 1989; Masuyama, 1994). The AL for distress might be more prone to contextual and indirect psychological influences, given the complexity of non-auditory region involvement such as the emotional, arousal, attention, and memory networks (Zenner et al., 2006; Jastreboff, 2007; De Ridder et al., 2011; Kaltenbach, 2011). De Ridder et al. (2014) have outlined a "tinnitus core" sub-network within the brain. It has been suggested that the minimal set of brain areas that needs to be simultaneously active in order for tinnitus to be consciously perceived. Affective components of tinnitus are represented by additional and overlapping networks. There is a possibility that tinnitus signal AL weighting decreases via the direct pathway toward external sound (involvement of core networks) while the affective component decreases occur via a residual pathway.

It is postulated that high levels of stress reaction, low social closeness, low self-control and high alienation may act as "maladaptive" personality residuals under this framework, diverting attention and auditory processing resources toward the tinnitus, thus increasing its AL weighting. The subsequent co-activation of various sub networks encoding tinnitus characteristics in the cortex with increased awareness/salience might then potentially explain the relationship between personality trait and psychoacoustic tinnitus characteristics (De Ridder et al., 2014, 2015). The relationship of attention within ALT is complex and it is difficult to determine weighting changes directly, due to the overlapping nature of networks possibly involved.

ALT stresses the active interaction between an individual experiencing tinnitus, cognition and their environment (both the immediate surroundings and broader factors, including their culture, beliefs, work, and social environment). The influence of

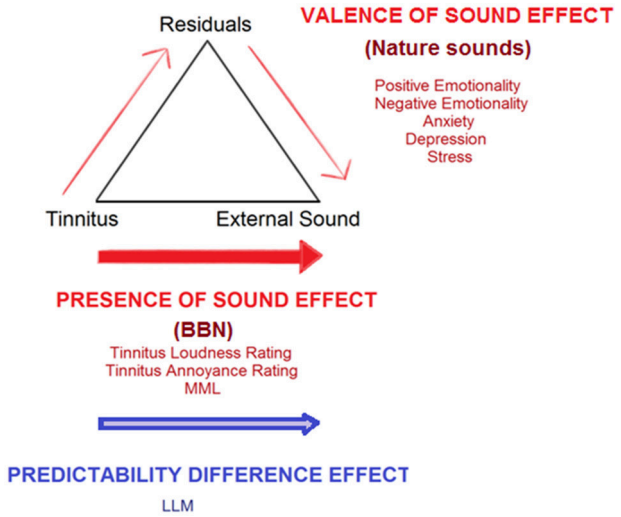

FIGURE 12 | Conceptualization of current study findings under an adaptation level theory (ALT) framework for tinnitus perception (Schecklmann et al., 2013b). Tinnitus is envisaged as a sensory stimulus with an existing internal adaptation level (AL) which acts as a reference point for all tinnitus-related judgments and is able to be manipulated by context and time. A high tinnitus AL results in tinnitus that is judged by the sufferer as being of high magnitude and/or eliciting high distress. Three key components set the final AL: (1) the focal component/stimuli being attended to (tinnitus), (2) background stimuli, as well as (3) residuals (various psychological and cognitive individual influences, including emotion, personality, past experiences, arousal, and level of prediction elicited by sound stimuli). The "presence of sound effect" (red arrows) illustrates steady shifts in AL away from the tinnitus and toward sound therapy stimuli, which can occur by directly increasing the weighting placed on external sound, via attention, and auditory streaming shifts. A valence of sound effect increases weighting placed on external sound via the residual pathway. The latter occurs as external sounds provide psychological relief from tinnitus and can counteract tinnitus-related negative emotions, anxiety, stress, and depression, thereby creating a facilitating residual effect which reduces tinnitus severity. The "predictability difference effect" (blue arrow) illustrates a potential difference between BBN and nature sounds in terms of the amount of prediction errors elicited; this may also influence the degree of adaptation each sound undergoes over time. Shifts in AL away from tinnitus toward external sound would discontinue upon adaptation of the auditory system to the external sound itself. Auditory system adaptation to BBN and natural sound therapy may occur at different rates. Adaptation to BBN occurs sometime between 4 and 8 weeks after the first introduction of the sound, leading to the need to increase the sound level required to match tinnitus in Loudness Level Matching. There may be small but immediate valence effects, but nature sounds due to their intermittent nature may take longer to reach peak adaptation, such that at 8 weeks no change in Loudness Level Matching may be observed.

the environment and health factors was evident in qualitative reports by participants, e.g., "Still feeling sick from flu, not feeling well at all, so not sure how accurate tinnitus perception might be," "Stressed at work, because in my view I feel tinnitus is stress or noise related, so hear it more." The physical location as well as time of day can alter the magnitude of tinnitus. Overall the success of sound appears to be partially related to individual influences, which interact to determine final tinnitus magnitude and its impact. It is not yet possible clinically to prescribe sounds that are tailored to an individual's tinnitus with confidence that they are the best sounds. However, the use of the rating functions described in this study may assist selection of sound type for therapy.

Factors Influencing Sound Therapy Effect on Tinnitus

Two components relating to sound therapy effects on tinnitus were interpreted, influencing impact of life such as presence, distress, and reactions to tinnitus (encompassing TFI scores, annoyance ratings, and LLMs) in addition to altering perceptual tinnitus characteristics such as subjective loudness and maskability (TFI scores, loudness ratings, and MML). However, we also cannot rule out placebo effects on either the qualitative or quantitative results, in scenarios where placebo effects are relevant, choice over treatment can increase these effects (Geers and Rose, 2011) which may account for qualitative preferences for the nature sounds, but does not account for the greater effects on the TFI with the BBN sound. TFI scores were the only variable which loaded onto both components of impact of life and perceptual characteristics. This is in line with one of the aims of the TFI, which is to comprehensively cover the broad range of symptoms associated with tinnitus severity (Meikle et al., 2012).

Based on the pattern of results it was also reasoned that three residuals of sound therapy effects on tinnitus were stress reduction/relaxation, and positive mood and negative mood. Under broad classification, the components map well onto the ALT model explanation of tinnitus-related and residual psychology-related effects of sound therapy discussed. The discrepancy in mood change (both positive and negative) in relation to sound therapy administration is interesting. Negative Emotionality and the personality trait of Social Closeness loaded strongly positively on the negative mood dimension, while Alienation as a personality trait loaded strongly negatively. In contrast, strong negative loadings of Stress Reaction on Stress reduction/relaxation and Self Control moderately positively loaded on positive mood. One possible interpretation is that stress and self-control are indices for discerning subgroups of individuals with exacerbated tinnitus following sound therapy. The sequence of events resulting in increased tinnitus may follow the indirect residual pathway (driven by an increase in negative affect) and the presence of certain underlying personality trait levels (e.g., social closeness, stress reaction) may determine the extent to which this pathway occurs and the magnitude of shifts in weighting toward tinnitus. However, this is only speculation and further research is needed in this regard.

Attention reaction response times loaded moderately negatively on tinnitus impact of life and moderately positively on stress reduction/relaxation. Attention discriminatory response times loaded moderately positively on positive mood. One possible explanation for this observation is that decreased tinnitus impact and increased psychological well-being in general may be related to increased attentional response times. Various studies suggest that reaction time is shorter under conditions of physiological stress (Desiderato, 1964; Ohmura et al., 2009).

Sound Adaptation as a Confound

The loudness level match is commonly used to psychoacoustically measure changes in tinnitus; however its interpretation in cases where external sound is administered for long periods of time can be difficult. Discrepancies between subjective loudness rating scores and loudness level match measures have been observed in the past, and termed the tinnitus loudness paradox (Penner, 1986; Henry and Meikle, 1996; Searchfield et al., 2012). Interpreted under ALT, the loudness paradox arises because subjective loudness judgments estimate the current tinnitus AL: it is made in a sound proof booth with no contextual noise stimuli (Penner, 1986; Henry and Meikle, 1996). In contrast, the objective match is made when an external test stimulus is introduced and the individual has to match it with the existing tinnitus AL. If the AL is initially set high, the matching sound level does not have to be increased as much before it is perceived as being of equal loudness as the tinnitus. However, if the matching sound undergoes adaptation to sound over time it would appear quieter, and would therefore have to be raised in order to match the intensity of tinnitus loudness (which undergoes slower adaptation; Searchfield et al., 2012; Durai et al., 2015). The auditory system may adjust to sound therapy stimuli over time; this would eventually stop further AL shifts and/or result in shifts back toward tinnitus.

It is highly likely that adaptation to the intervention sound may confound the interpretation of loudness level matches in this study. Underlying neural changes can occur through gain control, or adjustment of input-output sound functions of auditory neurons (Marks and Arieh, 2006; Robinson and McAlpine, 2009). Studies have observed stimulus-specific adaptation effects at all levels of the auditory system from early auditory encoding (Marks and Arieh, 2006) to the auditory cortex (Robinson and McAlpine, 2009; Rabinowitz et al., 2011). Adaptation of the auditory system to BBN and nature sounds may occur at different rates. Because BBN is a predictable sound, it may be adapted to at a faster rate, and lead to an increase when an external sound is used to match tinnitus in loudness level matching. Unpredictable natural sounds are adapted to more slowly; therefore no change in loudness level match is obtained.

It is possible that intermittent tinnitus masking may not appear to alter tinnitus due to auditory continuity effects (Näätänen et al., 2001) whereby the brain "fills in the gap' where masking sound is applied and tinnitus appears as a continuous percept. This learning effect involves several networks in the brain that overlap with that of tinnitus, including the limbic structures, basal ganglia, and prefrontal cortex (Hassler, 1978).

Davis et al. (2007) observed more consistent benefit over 12 months if Neuromonics Tinnitus Treatment involved masking of tinnitus for the first 2 months followed by intermittent perception of tinnitus compared to where there is intermittent perception of tinnitus throughout treatment. It may be useful to run future trials in which the temporal structure of sounds are changed often to maintain novelty and prevent sound adaptation, continuity illusion, and facilitate AL shifts toward external sound (Schreitmüller et al., 2013).

Clinical Implications and Sub Grouping of Tinnitus Characteristics

Participants often demonstrated a clear preference for one nature sound over the others in the initial selection task; mostly Rain (highest valence ratings by participants and reported as having greater interaction with tinnitus, possibly due to its broad frequency spectrum, and more consistent nature) over Surf or Cicadas. Similar loudness and annoyance growth curves for tinnitus and interactions between all sounds (nature and BBN) were present with increasing volume levels. However, BBN had higher sound annoyance rating at tinnitus masking level than all three nature sounds. BBN sound therapy has been recommended (Jastreboff and Hazell, 2004) as it is proposed to be more easily tolerated, neutral in nature, and better for facilitating habituation than tones or NBN (Jastreboff, 1999; Jastreboff and Hazell, 2004). The results of this study would agree with these suggestions, although we believe the process of tinnitus adaptation is more complex than habituation (Searchfield et al., 2012). Also, considering the BBN had a stronger effect on adaptation level while nature sounds influence residual emotion pathways, and nature sounds are more accepted (Schreitmüller et al., 2013), another clinical application would be the combing the two sounds or staging their use.

Sixteen percent of the participants experienced an exacerbation of tinnitus sufficient enough to terminate the trial early; however, this was mainly due to external situational factors or incidents. It was not possible to identify any characteristics (e.g., personality trait, age, gender, duration of tinnitus, other tinnitus variables) which isolated these individuals from others in the study. From the qualitative reports at 4 weeks after administration, there were subgroups in self-reported response to sound therapy: 42% had worsening of tinnitus, 39% no change, and 19% obtained relief. Moreover, at 8 weeks after administration, there was variation in responses: 52% had worsening, 34% had no change, and 13% had relief from sounds. The number of participants reporting benefit was lower than anticipated based on hearing aid (Folmer and Carroll, 2006; Hobson et al., 2010; Searchfield et al., 2010) and tinnitus aid (Bauer and Brozoski, 2011; Barozzi et al., 2016) studies. This may, at least in part, be due to mode of sound delivery. MP3 players and earbuds were used as a lower cost intervention than tinnitus aids. Improvements in implementation of MP3 players from a previous study were made based on participant reports (Durai et al., unpublished manuscript); an easier user interface was implemented by switching from Apple iPod shuffles to the Philips ViBE MP3 Player (with more accessible manual controls) and use of retaining hooks with the earbuds. However, even these improvements resulted in less use than hearing aids. Patient reports suggest the MP3 players were used 1–1.5 h per day, significantly less than that usually recommended for sound therapy using ear-level devices (6–8 h per day; Jastreboff, 1999; Jastreboff and Jastreboff, 2000). The sounds also did not compensate for hearing loss. Threshold adjusted noise (Searchfield et al., 2002) is implemented in several tinnitus aids (e.g., Siemens, Phonak, Oticon). The flat frequency response we used may have led to less interaction with tinnitus in the region of hearing loss, but would be similar for both the intervention sounds. Tinnitus aids can use sound in a number of ways through inbuilt sounds or by streaming sounds (Searchfield, 2016). A future trial should build on the findings in sound selection described here using tinnitus aids streaming sounds that are downloaded to tablet computers or smartphones, e.g., from hearing aid manufacturers Apps (e.g., Tinnitus Balance App, https://www.phonak.com/us/en/support/apps.html) or independent online sources (e.g., TinnitusTunes, http://www.tinnitustunes.com). Another factor to consider is the long duration of tinnitus (mean time since onset was 17 years) among participants in this study. Tinnitus neural networks tend to change as tinnitus duration increases (Schlee et al., 2009; Vanneste et al., 2011) which may impose limitations on brain plasticity and the extent of change triggered by the presence of sound.

Interestingly, there was no correlation between the reported effects of sound or preference of sound and the intervention outcomes measured in this clinical trial. These findings highlight that qualitative and quantitative measures do not always clinically equate. Melin et al. (1987) observed similar discrepancies in their study in which hearing aids were fit to individuals with tinnitus for 6 weeks. The results were explained in terms of demand characteristics: when asked during interviews, subjects may tend to exaggerate (or underestimate) the ability of the intervention to change tinnitus, while the scaling may not be as sensitive to these effects. The extent of this reporting bias might differ between numerical rating scales and qualitative interviews. The inclusion of systematic qualitative methods in sound therapy treatment paradigms may be more advantageous than quantitative measures in identifying shifts in environmental factors or significant change in factors outside of the individual which can influence tinnitus (Malterud, 2001), such as individual health or stress determinants (e.g., changes in tinnitus characteristics present as a result of illness, as reported in this trial) or to identify concerns which arise (e.g., situational factors which may result in discontinuation of sound, as reported in this trial).

Subgroups have been identified among tinnitus sufferers, which vary based on pathophysiology, perceptual features, co-morbid conditions, and how they respond to specific treatments (Stouffer and Tyler, 1990; Lockwood et al., 2002; Heller, 2003; Tyler et al., 2008). The results support the existence of different classes of tinnitus, and the ALT may eventually be most supported as a framework for only a particular tinnitus subgroup. Both auditory and non-auditory residual factors may shift the AL more readily among such individuals. Moreover, age, gender, hearing loss, personality traits, and duration of

sound therapy may all (hypothetically) be factors which can help delineate individuals.

The measures used in this study were selected based on validity and usefulness from past pilot studies. It is acknowledged that there are other standardized measures available such as the Clinical Global Impression Scale (Busner and Targum, 2007) and the COSIT (Dillon et al., 1997; Searchfield, 2016) for measuring perceived improvement of tinnitus. In this trial tinnitus counseling was deliberately not provided. Current tinnitus treatment paradigms such as Tinnitus Activities Treatment (Tyler et al., 2007) and Tinnitus Retraining Therapy (Jastreboff and Jastreboff, 2000; Jastreboff and Hazell, 2004; Jastreboff, 2007) use sound therapy alongside counseling of some form. Some trials of these therapies have been criticized (Sandlin and Olsson, 1999; McKenna and Irwin, 2008) as the benefits of sound therapy, over and above counseling, have not been determined. The results reported here are independent of counseling but it is strongly advocated that the use of sound clinically should be in addition to (not instead of) counseling. Sound therapy involves more than passive exposure to sound (Bauer and Brozoski, 2008; Davis et al., 2008), and participants need to be informed of this, and counseled also about how to use the sound. For some of the participants sound therapy ended up diverting attention toward the tinnitus instead. Individually tailoring sound therapy and counseling to target different components of the ALT would be expected to demonstrate an additive effect in shifting AL weighting away from tinnitus if administered together, than if each was administered in isolation. Attention training (Searchfield et al., 2007; Wise, 2012; Wise et al., 2016) might enhance adaptation to tinnitus through the presence of sound effect, while psychoeducation (or mindfulness or Cognitive Behavioral Therapy; Sweetow, 1984, 1986; Martinez-Devesa et al., 2010) might increase tinnitus adaptation through the valence sound effect.

CONCLUSIONS

Overall, the presence of sound had a positive effect on the TFI; after 8 weeks of administration, sound therapy with BBN resulted in a greater reduction of TFI than nature sound. The positive effect of sound on tinnitus was supported by secondary tinnitus and psychological-related outcome measures, but not interviews. BBN and nature sounds did not differ significantly on secondary outcome measures of tinnitus, emotion, attention, and psychological state after 8 weeks of administration. Interpreted under an ALT framework, internal AL weighting shifts away from the tinnitus signal and toward the sound therapy stimuli may occur, via a direct pathway toward external sound (involvement of core networks) while the affective component of tinnitus decreases via the residual pathway. The auditory system may adjust to sound therapy stimuli over time; this may eventually stop further AL shifts and result in shifts back toward tinnitus. It such cases predictable BBN might undergo loudness adaptation at a faster rate than unpredictable nature sounds.

This study provides further evidence for the heterogeneous nature of tinnitus. ALT appears to provide a framework for sound selection that could be applied to improve future sound-therapies. In this study, the selection of sound therapy stimuli by individuals was found to be, at least in part, governed by certain characteristics of the stimuli itself. Within a clinical setting, it is important to understand individual variation and that each person presents with different needs. Individual preferences were shown within this study that might be applied to improve outcomes if known apriori. It may be beneficial to have a wide range of sounds available in the clinic. The results of the Principal Component Analysis and ALT model interpretation are both compatible with an ecological framework of tinnitus, a multitude of factors (e.g., attention and personality, characteristics of and preference for sound stimuli) appeared to determine the magnitude and experience of tinnitus at any one time. Regular qualitative assessments will allow for a more comprehensive picture to be obtained regarding various factors influencing sound success. Selecting sounds based on the ALT model would involve weighing treatment sound stimuli and sound levels based on sound valence ratings, tinnitus and sound loudness and annoyance (dependent on the individual's profile at a particular point in time) as well as alternating presentation of sounds that evoke positive feelings (through the valence sound effect) and sounds with high interaction with tinnitus (for a presence of sound effect) over time, dependent on the individual's profile at a particular point in time. Trials of sound therapy selection based on Adaptation Level Therapy are needed.

ETHICS STATEMENT

Fifty individuals were invited by email invitations sent out to randomly selected members on the University of Auckland Tinnitus Research Volunteer Database (372 people from throughout New Zealand, majority from within Auckland). These are individuals with debilitating tinnitus who are interested in volunteering for tinnitus studies and clinical trials related to tinnitus relief. The inclusion criteria were: adults aged between 18 and 69 years residing in the Auckland region (NZ), constant tinnitus, and a minimum score of 38 on Tinnitus Functional Index (TFI), normal middle ear function, and a maximum of a moderate degree of hearing loss (<70 dB loss on average across frequencies). All subjects gave written informed consent in accordance with the Declaration of Helsinki. Participants read the Participant Information Sheet which listed any risks, benefits and layout of the trial as well as contact details. If participants were interested in continuing, a consent form was signed at the start of the trial.

AUTHOR CONTRIBUTIONS

MD was involved in manuscript preparation, collection of environmental sound recordings, development of sound therapy stimuli, sound therapy administration, data collection, and analysis. GS was involved in manuscript preparation, collection of environmental sound recordings, advise in development of sound therapy, and study design.

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REFERENCES

A.2.1.2. (2016). Audacity: Free Audio Editor and Recorder.

Barozzi, S., Del Bo, L., Crocetti, A., Dyrlund, O., Passoni, S., Zolin, A., et al. (2016). A comparison of nature and technical sounds for tinnitus therapy. *Acta Acustica United Acustica* 102, 540–546. doi: 10.3813/AAA.918971

Barros Suzuki, F. A., Suzuki, F. A., Yonamine, F. K., Onishi, E. T., and Penido, N. O. (2016). Effectiveness of sound therapy in patients with tinnitus resistant to previous treatments: importance of adjustments. *Braz. J. Otorhinolaryngol.* 82, 297–303. doi: 10.1016/j.bjorl.2015.05.009

Bartels, H., Middel, B., Pedersen, S. S., Staal, M. J., and Albers, F. W. (2010). The distressed (Type D) personality is independently associated with tinnitus: a case–control study. *Psychosomatics* 51, 29–38. doi: 10.1176/appi.psy.51.1.29

Bauer, C. A., and Brozoski, T. J. (2008). "Tinnitus: theories mechanisms and treatments," in *Auditory Trauma, Protection, and Repair*, *Springer Handbook of Auditory Research*, eds J. Schacht, A. N. Popper, and R. R. Fay (New York, NY: Springer), 101–129.

Bauer, C. A., and Brozoski, T. J. (2011). Effect of tinnitus retraining therapy on the loudness and annoyance of tinnitus: a controlled trial. *Ear Hear.* 32, 145–155. doi: 10.1097/AUD.0b013e3181f5374f

Bouchard, T. Jr., and Loehlin, J. (2001). Genes, evolution, and personality. *Behav. Genet.* 31, 243–273. doi: 10.1023/A:1012294324713

Busner, J., and Targum, S. D. (2007). The clinical global impressions scale: applying a research tool in clinical practice. *Psychiatry (Edgmont)* 4, 28–37.

Callaway, S. L. (2014). *The Oticon Approach to Care of the Tinnitus Patient [Pamphlet]*. Oticon.

Carhart, R., and Jerger, J. (1959). Preferred methods for clinical determination of pure-tone thresholds. *J. Speech Hear. Res.* 24, 330–345. doi: 10.1044/jshd.2404.330

Carpenter-Thompson, J. R., Kwaku, A., Schmidt, S. A., Dolcosa, F., and Husain, F. T. (2014). Alterations of the emotional processing system may underlie preserved rapid reaction time in tinnitus. *Brain Res.* 1567, 28–41. doi: 10.1016/j.brainres.2014.04.024

Chandra, N., Lee, A., and Searchfield, G. D. (2014). "Validation of the Tinnitus Functional Index in New Zealand," in *8th International TRI Tinnitus Conference* (Auckland), 31.

Clark, J. G. (1981). Uses and abuses of hearing loss classification. *ASHA* 23, 493–500.

Coren, S., and Ward, L. M. (1989). *Sensation and Perception.* New York, NY: Harcourt Brace Jovanovich.

Corr, P. J., and Matthews, G. (2009). *The Cambridge Handbook of Personality Psychology*. New York, NY: Cambridge University Press.

Costa, P. T., and McRae, R. R. (1992). *Revised NEO Personality Inventory (NEO-PI-R) and NEO Five-Factor Inventory (NEO-FFI) Professional Manual.* Odessa, FL: Psychological Assessment Resources, Inc.

Costa, P. T., and McCrae, R. R. (2006). Age changes in personality and their origins: comment on Roberts, Walton, and Viechtbauer (2006). *Psychol. Bull.* 132, 26–28. doi: 10.1037/0033-2909.132.1.26

Davis, A., and El Refaie, A. (2000). "Epidemiology of tinnitus," in *Tinnitus Handbook*, ed R. S. Tyler (San Diego, CA: Singular Publishing), 1–23.

Davis, P. B. (2006). "Music and the acoustic desensitization protocol for tinnitus," in *Tinnitus Treatment: Clinical Protocols*, ed R. S. Tyler (New York, NY: Thieme), 146–160.

Davis, P. B., Paki, B., and Hanley, P. J. (2007). The neuromonics tinnitus treatment: third clinical trial. *Ear Hear.* 28, 242–259. doi: 10.1097/AUD.0b013e3180312619

Davis, P. B., Wilde, R. A., Steed, L. G., and Hanley, P. J. (2008). Treatment of tinnitus with a customized acoustic neural stimulus: a controlled clinical study. *ENT J.* 87, 330–339.

De Ridder, D., Congedo, M., and Vanneste, S. (2015). The neural correlates of subjectively perceived and passively matched loudness perception in auditory phantom perception. *Brain Behav.* 5:e00331. doi: 10.1002/brb3.331

De Ridder, D., Elgoyhen, A. B., Romo, R., and Langguth, B. (2011). Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8075–8080. doi: 10.1073/pnas.1018466108

De Ridder, D., Vanneste, S., Weisz, N., Londero, A., and Schlee, W. (2014). An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci. Biobehav. Rev.* 44, 16–32. doi: 10.1016/j.neubiorev.2013.03.021

Desiderato, O. (1964). Generalization of conditioned suppression. *J. Comp. Physiol. Psychol.* 57, 434–437. doi: 10.1037/h0047275

Dillon, H., James, A., and Ginis, J. (1997). The Client Oriented Scale of Improvement (COSI) and its relationship to several other measures of benefit and satisfaction provided by hearing aids. *J. Am. Acad. Audiol.* 8, 27–43.

Dos Santos, G. M., and Powers, L. (2015). *Tinnitus: The Siemens Package [Pamphlet]*. Erlangen: Sivantos.

Durai, M., and Searchfield, G. D. (2016). Anxiety and depression, personality traits relevant to tinnitus: a scoping review. *Int. J. Audiol.* 55, 605–615. doi: 10.1080/14992027.2016.1198966

Durai, M., O'Keeffe, M. G., and Searchfield, G. D. (2017a). The personality profile of tinnitus sufferers and a non-tinnitus control group. *J. Am. Acad. Audiol.* doi: 10.3766/jaaa.15103. [Epub ahead of print].

Durai, M., O'Keeffe, M. G., and Searchfield, G. D. (2017b). Examining the short term effects of emotion under an adaptation level theory model of tinnitus perception. *Hear. Res.* 345, 23–29. doi: 10.1016/j.heares.2016.12.013

Durai, M., Searchfield, G. D., and Kobayashi, K. (2015). A preliminary examination of the roles of contextual stimuli and personality traits under the adaptation level theory model of tinnitus. *Acta Acustica United Acustica* 101, 543–555. doi: 10.3813/AAA.918851

Eysenck, M. W., and Keane, M. T. (2015). *Cognitive Psychology: A Student's Handbook.* New York, NY: Psychology Press.

Folmer, R. L., and Carroll, J. R. (2006). Long-term effectiveness of ear-level devices for tinnitus. *Otolaryngol. Head Neck Surg.* 134, 132–137. doi: 10.1016/j.otohns.2005.09.030

Folmer, R. L., Theodoroff, S. M., Casiana, L., Shi, Y., Griest, S., and Vachhani, J. (2015). Repetitive transcranial magnetic stimulation treatment for chronic tinnitus: a randomized clinical trial. *JAMA Otolaryngol. Head Neck Surg.* 141, 716–722. doi: 10.1001/jamaoto.2015.1219

Gale, N. K., Heath, G., Cameron, E., Rashid, S., and Redwood, S. (2013). Using the framework method for the analysis of qualitative data in multi-disciplinary health research. *BMC Med. Res. Methodol.* 13:117. doi: 10.1186/1471-2288-13-117

Geers, A., and Rose, J. (2011). Treatment choice and placebo expectation effects. *Soc. Personal. Psychol. Compass* 5, 734–750. doi: 10.1111/j.1751-9004.2011.00385.x

Hanley, P. J., and Davis, P. B. (2008). Treatment of tinnitus with a customized, dynamic acoustic neural stimulus: underlying principles and clinical efficacy. *Trends Amplif.* 12, 210–222. doi: 10.1177/1084713808319942

Hann, D., Searchfield, G. D., Sanders, M., and Wise, K. (2008). Strategies for the selection of music in the short-term management of mild tinnitus. *Aust. N.Z. J. Audiol.* 30, 129–140. doi: 10.1375/audi.30.2.129

Hassler, R. (1978). Striatal control of locomotion, intentional actions and of integrating and perceptive activity. *J. Neurol. Sci.* 36, 187–224. doi: 10.1016/0022-510X(78)90082-5

Heller, A. J. (2003). Classification and epidemiology of tinnitus. *Otolaryngol. Clin. North Am.* 36, 239–248. doi: 10.1016/S0030-6665(02)00160-3

Helson, H. (1948). Adaptation level as a basis for a quantitative theory of frames of reference. *Psychol. Rev.* 55, 297–313. doi: 10.1037/h0056721

Helson, H. (1964). *Adaptation Level Theory*. New York, NY: Harper and Row.

Henry, J. A., and Meikle, M. A. (1996). "Loudness recruitment only partially explains the small size of tinnitus loudness matches," in *Proceedings of the Fifth International Tinnitus Seminar*, eds G. E. Reichand and J. A. Vernon (Portland: American Tinnitus Association), 148–157.

Henry, J. A., Dennis, K. C., and Schechter, M. A. (2005). General review of tinnitus: prevalence, mechanisms, effects, and management. *J. Speech Lang. Hear. Res.* 48, 1204–1235. doi: 10.1044/1092-4388(2005/084)

Henry, J. A., Frederick, M., Sell, S., Griest, S., and Abrams, H. B. (2015). Validation of a novel combination hearing aid and tinnitus therapy device. *Ear Hear.* 36, 42–52. doi: 10.1097/AUD.0000000000000093

Henry, J. A., Rheinsburg, B., and Zaugg, T. (2004). Comparison of custom sounds for achieving tinnitus relief. *J. Am. Acad. Audiol.* 15, 585–598. doi: 10.3766/jaaa.15.8.6

Henry, J. A., Schechter, M. A., Zaugg, T. L., Griest, S., Jastreboff, P. J., Vernon, J. A., et al. (2006). Outcomes of clinical trial: tinnitus masking versus tinnitus retraining therapy. *J. Am. Acad. Audiol.* 17, 104–132. doi: 10.3766/jaaa.17.2.4

Hoare, D. J., Kowalkowski, V. L., Kang, S., and Hall, D. A. (2011). Systematic review and meta-analyses of randomized controlled trials examining tinnitus management. *Laryngoscope* 121, 1555–1564. doi: 10.1002/lary.21825

Hobson, J., Chisholm, E., and El Refaie, A. (2010). Sound therapy (masking) in the management of tinnitus in adults. *Cochrane Database Syst. Rev.* CD006371. doi: 10.1002/14651858.CD006371.pub2

Hoffmann, H. J., and Reed, G. W. (2004). "Epidemiology of tinnitus," in *Tinnitus: Theory and Management*, ed J. B. Snow (Hamilton: B.C. Decker), 16–41.

Hunter, I. R., and Gillen, M. C. (2009). Stress coping mechanisms in elderly adults: an initial study of recreational and other coping behaviors in nursing home patients. *Adultspan J.* 8, 43–53. doi: 10.1002/j.2161-0029.2009.tb00056.x

Husain, F. T., and Schmidt, S. A. (2014). Using resting state functional connectivity to unravel networks of tinnitus. *Hear. Res.* 307, 153–162. doi: 10.1016/j.heares.2013.07.010

Jastreboff, P. J. (1998). "Tinnitus; the method of," in *Current Therapy in Otolaryngology Head and Neck Surgery*, ed G. A. Gates (St. Louis, MO: Mosby), 90–95.

Jastreboff, P. J. (1999). "Optimal sound use in TRT - Theory and practice," in *Proceedings of the Sixth International Tinnitus Seminar*, ed J. W. P. Hazell (London: Ed Hazell Publishing THC), 491–494.

Jastreboff, P. J. (2007). Tinnitus retraining therapy. *Prog. Brain Res.* 166, 415–423. doi: 10.1016/S0079-6123(07)66040-3

Jastreboff, P. J., and Hazell, J. W. P. (2004). *Tinnitus Retraining Therapy: Implementing the Neurophysiological Model*. Cambridge: Cambridge University Press.

Jastreboff, P. J., and Jastreboff, M. M. (2000). Tinnitus Retraining Therapy (TRT) as a method for treatment of tinnitus and hyperacusis patients. *J. Am. Acad. Audiol.* 11, 162–177.

Kaltenbach, J. A. (2011). Tinnitus: models and mechanisms. *Hear. Res.* 276, 52–60. doi: 10.1016/j.heares.2010.12.003

Kidd, G., Mason, C. R., and Arbogast, T. L. (2002). Similarity, uncertainty, and masking in the identification of nonspeech auditory patterns. *J. Acoust. Soc. Am.* 111, 1367–1376. doi: 10.1121/1.1448342

Kim, B. J., Chung, S. W., Jung, J. Y., and Suh, M. W. (2014). Effect of different sounds on the treatment outcome of tinnitus retraining therapy. *Clin. Exp. Otorhinolaryngol.* 7, 87–93. doi: 10.3342/ceo.2014.7.2.87

Langguth, B., Goodey, R., Azevedo, A., Bjorne, A., Cacace, A., Crocetti, A., et al. (2007). Consensus for tinnitus patient assessment and treatment outcome measurement: tinnitus research initiative meeting, Regensburg, July 2006. *Prog. Brain Res.* 166, 525–536. doi: 10.1016/S0079-6123(07)66050-6

Lockwood, A. H., Salvi, R. J., and Burkard, R. F. (2002). Tinnitus. *N.Engl. J. Med.* 347, 904–910. doi: 10.1056/NEJMra013395

Lovibond, S. H., and Lovibond, P. F. (1995). *Manual for the Depression Anxiety Stress Scales*. Sydney: Psychology Foundation.

Malouff, J. M., Schutte, N. S., and Zucker, L. A. (2011). Tinnitus-related distress: a review of recent findings. *Curr. Psychiatry Rep.* 13, 31–36. doi: 10.1007/s11920-010-0163-1

Malterud, K. (2001). The art and science of clinical knowledge: evidence beyond measures and numbers. *Lancet* 358, 397–400. doi: 10.1016/S0140-6736(01)05548-9

Marks, L. E., and Arieh, Y. (2006). Differential effects of stimulus context in sensory processing. *Eur. Rev. Appl. Psychol. Rev.* 56, 213–221. doi: 10.1016/j.erap.2005.09.009

Martinez-Devesa, P., Perera, R., Theodoulou, M., and Waddell, A. (2010). Cognitive behavioural therapy for tinnitus. *Cochrane Database Syst. Rev.* CD005233. doi: 10.1002/14651858.cd005233.pub3

Masuyama, E. (1994). Application of adaptation level theory to human experiment on temperature sensation. *Jpn. J. Erg.* 30, 201–208. doi: 10.5100/jje.30.201

McKenna, L., and Irwin, R. (2008). Sound therapy for tinnitus sacred cow or idol worship?: an investigation of the evidence. *Audiol. Med.* 6, 16–24. doi: 10.1080/16513860801899389

Meikle, M. B., Henry, J. A., Griest, S. E., Stewart, B. J., Abrams, H. B., McArdle, R., et al. (2012). The tinnitus functional index: development of a new clinical measure for chronic, intrusive tinnitus. *Ear Hear.* 33, 153. doi: 10.1097/AUD.0b013e31822f67c0

Melin, L., Scott, B., Lindberg, P., and Lyttkens, L. (1987). Hearing aids and tinnitus -an experimental group study. *Br. J. Audiol.* 21, 91–97. doi: 10.3109/03005368709077782

Mirz, F., Gjedde, A., Ishizu, K., and Pedersen, C. B. (2000a). Cortical networks subserving the perception of tinnitus – a PET study. *Acta Otolaryngol. Suppl.* 543, 241–243. doi: 10.1080/000164800454503

Mirz, F., Gjedde, A., Sodkilde-Jrgensen, H., and Pedersen, C. B. (2000b). Functional brain imaging of tinnitus-like perception induced by aversive auditory stimuli. *Neuroreport* 11, 633–637. doi: 10.1097/00001756-200002280-00039

Moller, A. R. (2006). *Hearing: Anatomy, Physiology, and Disorders of the Auditory System*. Boston, MA: Academic Press.

Näätänen, R., Tervaniemi, M., Sussman, E., Paavilainen, P., and Winkler, I. (2001). 'Primitive intelligence' in the auditory cortex. *Trends Neurosci.* 24, 283–288. doi: 10.1016/S0166-2236(00)01790-2

Noreña, A. J., and Eggermont, J. J. (2006). Enriched acoustic environment after noise trauma abolishes neural signs of tinnitus. *Neuroreport* 17, 559–563. doi: 10.1097/00001756-200604240-00001

Ohmura, Y., Yamaguchi, T., Futami, Y., Togashi, H., Izumi, T., Matsumoto, M., et al. (2009). Corticotropin releasing factor enhances attentional function as assessed by the five-choice serial reaction time task in rats. *Behav. Brain Res.* 198, 429–433. doi: 10.1016/j.bbr.2008.11.025

Onwuegbuzie, A. J., and Leech, N. L. (2005). On Becoming a pragmatic researcher: the importance of combining quantitative and qualitative research methodologies. *Int. J. Soc. Res. Methodol.* 8, 375–387. doi: 10.1080/13645570500402447

Penner, M. J. (1986). Magnitude estimation and the "paradoxical" loudness of tinnitus. *J. Speech Hear. Res.* 29, 407–412. doi: 10.1044/jshr.2903.407

Rabinowitz, N. C., Willmore, B. D., Schnupp, J. W., and King, A. J. (2011). Contrast gain control in auditory cortex. *Neuron* 70, 1178–1191. doi: 10.1016/j.neuron.2011.04.030

Rizzardo, R., Savastano, M., Maron, M. B., Mangialaio, M., and Salvadori, L. (1998). Psychological distress in patients with tinnitus. *J. Otolaryngol.* 27, 21–25.

Roberts, B. W., Walton, K. E., and Viechtbauer, W. (2006). Patterns of mean-level change in personality traits across the life course: a meta-analysis of longitudinal studies. *Psychol. Bull.* 132, 1–25. doi: 10.1037/0033-2909.132.1.1

Robinson, B. L., and McAlpine, D. (2009). Gain control mechanisms in the auditory pathway. *Curr. Opin. Neurobiol.* 19, 402–407. doi: 10.1016/j.conb.2009.07.006

Rodenbough, J. R. (2003). *Comprehensive Attention Battery® (Version 5.0)*. Greensboro, NC: Neuropsych Works, Inc.

Rossman, G. B., and Wilson, B. L. (1985). Numbers and words: combining quantitative and qualitative methods in a single large-scale evaluation study. *Eval. Rev.* 9, 627–643. doi: 10.1177/0193841X8500900505

Sandlin, R. E., and Olsson, R. J. (1999). Evaluation and selection of maskers and other devices used in the treatment of tinnitus and hyperacusis. *Trends Amplif.* 4, 6–26. doi: 10.1177/108471389900400102

Schaette, R., Konig, O., Hornig, D., Gross, M, and Kempter, R. (2010). Acoustic stimulation treatments against tinnitus could be most effective when tinnitus pitch is within the stimulated frequency range. *Hear. Res.* 269, 95–101. doi: 10.1016/j.heares.2010.06.022

Schecklmann, M., Landgrebe, M., Poeppl, T. B., Kreuzer, P., Männer, P., Marienhagen, J., et al. (2013a). Neural correlates of tinnitus duration and distress: a positron emission tomography study. *Hum. Brain Mapp.* 34, 233–240. doi: 10.1002/hbm.21426

Schecklmann, M., Lehner, A., Poeppl, T. B., Kreuzer, P. M., Rupprecht, R., Rackl, J., et al. (2013b). Auditory cortex is implicated in tinnitus distress: a voxel-based morphometry study. *Brain Struct. Funct.* 218, 1061–1070. doi: 10.1007/s00429-013-0520-z

Schlee, W., Hartmann, T., Langguth, B., and Weisz, N. (2009). Abnormal resting-state cortical coupling in chronic tinnitus. *BNC Neurosci.* 10:11. doi: 10.1186/1471-2202-10-11

Schlee, W., Weisz, N., Bertrand, O., Hartmann, T., and Elbert, T. (2008). Using auditory steady state responses to outline the functional connectivity in the tinnitus brain. *PLoS ONE* 8:e3720. doi: 10.1371/annotation/c71826c3-da07-42e8-a563-bde0257a0845

Schreitmüller, S., von Wedel, H., Walger, M., and Meister, H. (2013). Untersuchungen von Rauschsignalen für die akustische Tinnitustherapie [Effect of dynamics on acceptance and concealment effect]. *HNO* 61, 38–45. doi: 10.1007/s00106-012-2642-7

Scott, B., and Lindberg, P. (2000). Psychological profile and somatic complaints between help-seeking and non-help-seeking tinnitus subjects. *Psychosomatics* 41, 347–352. doi: 10.1176/appi.psy.41.4.347

Scott, B., Lindberg, P., Lyttkens, L., and Melin, L. (1990). Predictors of tinnitus discomfort, adaptation and subjective loudness. *Br. J. Audiol.* 24, 51–62. doi: 10.3109/03005369009077842

Searchfield, G. D. (2014). Tinnitus What and Where: An Ecological Framework. *Front. Neurol.* 5:271. doi: 10.3389/fneur.2014.00271

Searchfield, G. D. (2016). "Hearing aids for tinnitus," in *Tinnitus: Clinical and Research Perspectives*, eds D. M. Baguley and M. Fagelson (San Diego, CA: Plural Publishing, Inc.).

Searchfield, G. D., Kaur, M., and Martin, W. H. (2010). Hearing aids as an adjunct to counseling: tinnitus patients who choose amplification do better than those who don't. *Int. J. Audiol.* 49, 574–579. doi: 10.3109/14992021003777267

Searchfield, G. D., Kobayashi, K., and Sanders, M. (2012). An adaptation level theory of tinnitus audibility. *Front. Syst. Neurosci.* 6:46. doi: 10.3389/fnsys.2012.00046

Searchfield, G. D., Morrison-Low, J., and Wise, K. (2007). Object identification and attention training for treating tinnitus. *Prog. Brain Res.* 166, 441–460. doi: 10.1016/S0079-6123(07)66043-9

Searchfield, G. D., Warr, A. A., Kuklinski, J. V., and Purdy, S. C. (2002). "Digital instruments for tinnitus:mixing point identification and threshold-adjusted noise," in *Proceedings of the Seventh International Tinnitus Seminar*, ed R. Patuzzi (Perth, WA: The University of Western Australia), 191–195.

Sirois, F. M., Davis, C. G., and Morgan, M. S. (2006). "Learning to live with what you can't rise above": control beliefs, symptom control, and adjustment to tinnitus. *Health Psychol.* 25, 119–123. doi: 10.1037/0278-6133.25.1.119

Specht, J., Egloff, B., and Schmukle, S. C. (2011). Stability and change of personality across the life course: the impact of age and major life events on mean-level and rank-order stability of the Big Five. *J. Pers. Soc. Psychol.* 101, 862–882. doi: 10.1037/a0024950

Stein, A., Wunderlich, R., Lau, P., Engell, A., Wollbrink, A., Shaykevich, A., et al. (2016). Clinical trial on tonal tinnitus with tailor-made notched music training. *BMC Neurol.* 16:38. doi: 10.1186/s12883-016-0558-7

Stouffer, J. L., and Tyler, R. S. (1990). Characterization of tinnitus by tinnitus patients. *J. Speech Hear. Disord.* 55, 439–453. doi: 10.1044/jshd.5503.439

Sweetow, R. W. (1984). Cognitive behavioral modification in tinnitus management. *Hear. Instrum.* 35, 14–18.

Sweetow, R. W. (1986). Cognitive aspects of tinnitus-patient management. *Ear Hear.* 7, 390–396. doi: 10.1097/00003446-198612000-00008

Távora-Vieira, D., Eikelboom, R. H., and Miller, S. (2011). Neuromonics tinnitus treatment for patients with significant level of hearing loss: an adaptation of the protocol. *Int. J. Audiology* 50, 881–886. doi: 10.3109/14992027.2011.606286

Tellegen, A. (1982). *Brief manual for the Multidimensional Personality Questionnaire.* Minneapolis, MN: University of Minnesota.

Tyler, R. S. (2006). *Tinnitus Treatment: Clinical Protocols*. New York, NY: Thieme.

Tyler, R. S., Gogel, S. A., and Gehringer, A. K. (2007). Tinnitus activities treatment. *Prog. Brain Res.* 166, 425–434. doi: 10.1016/S0079-6123(07)66041-5

Tyler, R. S., Noble, W., Coelho, C. B., and Ji, H. (2012). Tinnitus retraining therapy: mixing point and total masking are equally effective. *Ear Hear.* 33, 588–594. doi: 10.1097/AUD.0b013e31824f2a6e

Tyler, R., Coelho, C., Tao, P., Ji, H., Noble, W., and Gehringer, A. (2008). Identifying tinnitus subgroups with cluster analysis. *Am. J. Audiol.* 17, 176–184. doi: 10.1044/1059-0889(2008/07-0044)

Vanneste, S., Song, J. J., and De Ridder, D. (2013). Tinnitus and musical hallucinosis: the same but more. *Neuroimage* 82, 373–383. doi: 10.1016/j.neuroimage.2013.05.107

Vanneste, S., Van de Heyning, P., and De Ridder, D. (2011). The neural network of phantom sound changes over time: a comparison between recent-onset and chronic tinnitus patients. *Eur. J. Neurosci.* 34, 718–731. doi: 10.1111/j.1460-9568.2011.07793.x

Vernon, J. A., and Meikle, M. B. (2000). "Tinnitus masking," in *Tinnitus Handbook*, ed R. S. Tyler (San Diego, CA: Singular Thompson Learning), 313–356.

Watson, D., Clark, L. A., and Tellegan, A. (1988). Development and validation of brief measures of positive and negative affect: the PANAS scales. *J. Pers. Soc. Psychol.* 54, 1063–1070. doi: 10.1037/0022-3514.54.6.1063

Welch, D., and Dawes, P. J. (2008). Personality and perception of tinnitus. *Ear Hear.* 29, 684–692. doi: 10.1097/AUD.0b013e318177d9ac

Wise, K. J. (2012). *Tinnitus and Attention Training, Audiology.* Auckland: University of Auckland.

Wise, K., Kobayashi, K., Magnusson, J., Welch, D., and Searchfield, G. D. (2016). Randomized controlled trial of a perceptual training game for tinnitus therapy. *Games Health J.* 5, 141–149. doi: 10.1089/g4h.2015.0068

Zenner, H. P., Pfister, M., and Birbaumer, N. (2006). Tinnitus sensitization: sensory and psychophysiological aspects of a new pathway of acquired centralization of chronic tinnitus. *Otol. Neurotol.* 27, 1054–1063. doi: 10.1097/01.mao.0000231604.64079.77

Zomeren, A. H., and Brouwer, W. H. (1994). *Clinical Neuropsychology of Attention.* Oxford: Oxford University Press.

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CORRECTION
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Corrigendum: A Mixed-Methods Trial of Broad Band Noise and Nature Sounds for Tinnitus Therapy: Group and Individual Responses Modeled under the Adaptation Level Theory of Tinnitus

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Keywords: clinical trial, tinnitus, auditory perception, adaptation, psychoacoustics, ecology model, sound therapy

A corrigendum on

A Mixed-Methods Trial of Broad Band Noise and Nature Sounds for Tinnitus Therapy: Group and Individual Responses Modeled under the Adaptation Level Theory of Tinnitus
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Figure/Table Legend

In the original article, there was a mistake in the legend **Figure 12** as published. The reference for the figure should be Searchfield et al. (2012) instead of Schecklmann et al. (2013b). The correct legend appears below.

FIGURE 12 | Conceptualization of current study findings under an adaptation level theory (ALT) framework for tinnitus perception (Searchfield et al., 2012). Tinnitus is envisaged as a sensory stimulus with an existing internal adaptation level (AL) which acts as a reference point for all tinnitus-related judgments and is able to be manipulated by context and time. A high tinnitus AL results in tinnitus that is judged by the sufferer as being of high magnitude and/or eliciting high distress. Three key components set the final AL: (1) the focal component/stimuli being attended to (tinnitus), (2) background stimuli, as well as (3) residuals (various psychological and cognitive individual influences, including emotion, personality, past experiences, arousal, and level of prediction elicited by sound stimuli). The "presence of sound effect" (red arrows) illustrates steady shifts in AL away from the tinnitus and toward sound therapy stimuli, which can occur by directly increasing the weighting placed on external sound, via attention, and auditory streaming shifts. A valence of sound effect increases weighting placed on external sound via the residual pathway. The latter occurs as external sounds provide psychological relief from tinnitus and can counteract tinnitus-related negative emotions, anxiety, stress, and depression, thereby creating a facilitating residual effect which reduces tinnitus severity. The "predictability difference effect" (blue arrow) illustrates a potential difference between BBN and nature sounds in terms of the amount of prediction errors elicited; this may also influence the degree of adaptation each sound undergoes over time. Shifts in AL away from tinnitus toward external sound would discontinue upon adaptation of the auditory system to the external sound itself. Auditory system adaptation to BBN and natural sound therapy may occur at different rates. Adaptation to BBN occurs sometime

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between 4 and 8 weeks after the first introduction of the sound, leading to the need to increase the sound level required to match tinnitus in Loudness Level Matching. There may be small but immediate valence effects, but nature sounds due to their intermittent nature may take longer to reach peak adaptation, such that at 8 weeks no change in Loudness Level Matching may be observed.

Incorrect Citation

In the original article, the citation for the text in the final paragraph under Discussion, Sound Adaptation as a Confound, "Changed often to maintain novelty and prevent sound adaptation, continuity illusion, and facilitate AL shifts toward external sound" was incorrectly cited as (Schreitmüller et al., 2013). It should be (Searchfield et al., 2012).

Text Correction

In the original article there was an error. The paragraph was "There were no concerns regarding the sound quality of both s from the majority of participants; however one participant felt their volume control increased dramatically from one step to another for the BBN." A correction has been made to Results, Intervention Outcomes: Qualitative Reports, Quality of intervention sounds: "There were no concerns regarding the sound quality of both sounds from the majority of participants; however one participant felt their volume control increased dramatically from one step to another for the BBN."

The authors apologize for these errors and state that this does not change the scientific conclusions of the article in any way.

REFERENCES

Schreitmüller, S., von Wedel, H., Walger, M., and Meister, H. (2013). Untersuchungen von Rauschsignalen für die akustische Tinnitustherapie [Effect of dynamics on acceptance and concealment effect]. *HNO* 61, 38–45. doi: 10.1007/s00106-012-2642-7

Searchfield, G. D., Kobayashi, K., and Sanders, M. (2012). An adaptationlevel theory of tinnitus audibility. *Front. Syst. Neurosci.* 6:46. doi: 10.3389/fnsys.2012.00046

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10 Hz Amplitude Modulated Sounds Induce Short-Term Tinnitus Suppression

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Objectives: Acoustic stimulation or sound therapy is proposed as a main treatment option for chronic subjective tinnitus. To further probe the field of acoustic stimulations for tinnitus therapy, this exploratory study compared 10 Hz amplitude modulated (AM) sounds (two pure tones, noise, music, and frequency modulated (FM) sounds) and unmodulated sounds (pure tone, noise) regarding their temporary suppression of tinnitus loudness. First, it was hypothesized that modulated sounds elicit larger temporary loudness suppression (residual inhibition) than unmodulated sounds. Second, with manipulation of stimulus loudness and duration of the modulated sounds weaker or stronger effects of loudness suppression were expected, respectively.

Methods: We recruited 29 participants with chronic tonal tinnitus from the multidisciplinary Tinnitus Clinic of the University of Regensburg. Participants underwent audiometric, psychometric and tinnitus pitch matching assessments followed by an acoustic stimulation experiment with a tinnitus loudness growth paradigm. In a first block participants were stimulated with all of the sounds for 3 min each and rated their subjective tinnitus loudness to the pre-stimulus loudness every 30 s after stimulus offset. The same procedure was deployed in the second block with the pure tone AM stimuli matched to the tinnitus frequency, manipulated in length (6 min), and loudness (reduced by 30 dB and linear fade out). Repeated measures mixed model analyses of variance (ANOVA) were calculated to assess differences in loudness growth between the stimuli for each block separately.

Results: First, we found that all sounds elicit a short-term suppression of tinnitus loudness (seconds to minutes) with strongest suppression right after stimulus offset [$F_{(6,\ 1331)} = 3.74$, $p < 0.01$]. Second, similar to previous findings we found that AM sounds near the tinnitus frequency produce significantly stronger tinnitus loudness suppression than noise [vs. Pink noise: $t_{(27)} = -4.22$, $p < 0.0001$]. Finally, variants of the AM sound matched to the tinnitus frequency reduced in sound level resulted in less suppression while there was no significant difference observed for a longer stimulation duration. Moreover, feasibility of the overall procedure could be confirmed as scores of both tinnitus loudness and questionnaires were lower after

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the experiment [tinnitus loudness: $t_{(27)} = 2.77$, $p < 0.01$; Tinnitus Questionnaire: $t_{(27)} = 2.06$, $p < 0.05$; Tinnitus Handicap Inventory: $t_{(27)} = 1.92$, $p = 0.065$].

Conclusion: Taken together, these results imply that AM sounds, especially in or around the tinnitus frequency, may induce larger suppression than unmodulated sounds. Future studies should thus evaluate this approach in longitudinal studies and real life settings. Furthermore, the putative neural relation of these sound stimuli with a modulation rate in the EEG α band to the observed tinnitus suppression should be probed with respective neurophysiological methods.

Keywords: tinnitus, acoustic stimulation, sound therapy, amplitude modulation, frequency modulation, residual inhibition, entrainment, alpha

1. INTRODUCTION

Subjective tinnitus is defined as "the perception of sound(s) in the absence of an external sound source" (Eggermont and Roberts, 2004; Erlandsson and Dauman, 2013) and is deemed chronic after 12 months since first occurrence (Mazurek et al., 2010). No less than 35% of the general (US) population are haunted by this phantom auditory perception at some point during their lifetime (Jastreboff, 1990). 10–15% report their tinnitus percept as being frequent or continuous and ∼1–2% suffer heavily under the condition (Langguth et al., 2013). With a steadily aging demographic, tinnitus is becoming increasingly prevalent and relevant (Hoffman and Reed, 2004; Nondahl et al., 2012). Besides the tantalizing phantom sound or comorbidities like depression, stress and anxiety (Langguth et al., 2013), tinnitus also impacts daily life functions in healthy aging as impaired hearing, sound localization and speech perception can lower the quality of life in tinnitus sufferers (Moon et al., 2015; Gilles et al., 2016; Hyvärinen et al., 2016).

In the majority of cases tinnitus manifests as a single tone, ringing or noise with a definable pitch and loudness, which is perceived bilaterally or with a slight preference to one side, or alternatively lateralized to one ear (Lockwood et al., 2002). Tinnitus pitch, laterality and loudness can be therefore considered as the main (subjective) perceptual parameters of interest in addition to maskability and residual inhibition by external sounds (Henry and Meikle, 2000). Usually, tinnitus is considered to be caused by either objective (Eggermont and Roberts, 2004; Schaette and Kempter, 2006; Mazurek et al., 2010) or hidden hearing loss (Weisz et al., 2006; Schaette and McAlpine, 2011; Adjamian et al., 2012; Xiong et al., 2013), where loss of cochlear hair cells in objective hearing loss has been shown to lead to maladaptive plasticity throughout the auditory pathway and brain. Tinnitus pitch seems to average near the frequency of maximal hearing loss, especially in sufferers with pure-tone tinnitus (Schecklmann et al., 2012). Related to this maladaptive plasticity, a similarity of tinnitus to phantom limb or general phantom (pain) perception following sensory deafferentation has also been proposed (De Ridder et al., 2011). Although models of pathogenesis and physiology are still being debated and are limited by an underlying inherent heterogeneity of the disorder, it can be stated with confidence that both the inner ear and the brain are involved (Jastreboff, 1990; Eggermont and Roberts, 2004; Adjamian et al., 2009; De Ridder et al., 2011, 2014; Vanneste and De Ridder, 2012; Elgoyhen et al., 2015).

Acoustic stimulations have been used in various forms to counteract or alleviate the malicious phantom percept (Jastreboff, 2007). From a clinical routine perspective, acoustic stimulation or sound therapies are proposed as symptom-oriented treatment options besides cognitive behavioral therapy and neuromodulation or -stimulation if chronic subjective tinnitus persists after standard clinical assessment and intervention (Langguth et al., 2013). Traditionally, masking approaches using broadband or narrow-band noise, or pure tones, were established first (Feldmann, 1971; Vernon, 1977; Watanabe et al., 1997; Henry et al., 2004; Hazell and Wood, 2009). These maskers have also been administered in hearing aids (Vernon and Meikle, 2003) with slightly better effects than hearing aids without maskers as shown in a study by Henry et al. (2015). In recent times, two major acoustic stimulation techniques for long-term, daily intervention have been developed building on the model of lateral inhibition (Pantev et al., 2012; Adamchic et al., 2014). Following peripheral hearing loss, central tonotopic map reorganization and hyperactivity in regions of the reorganization responsible for the tinnitus sensation (Eggermont and Komiya, 2000; Eggermont and Roberts, 2004), lateral inhibition is theorized to counteract or reverse this maladaptive hyperactivity. Pantev and colleagues therefore proposed to apply a notch filter in a single octave band around the tinnitus frequency to music. The energy of the sound signal at the edges of the notch filter is theorized to inhibit the frequencies around the tinnitus pitch therefore reversing the maladaptive plasticity, which has been shown to be effective in long-term intervention (Okamoto et al., 2010). The width of the notch filter did not significantly influence treatment effects in a further study (Wunderlich et al., 2015b) while the spectral contrast (i.e., increased sound pressure at frequencies neighboring the filter edges) seems to improve the treatment effects as shown in a further follow up study (Stein et al., 2015). Building on similar reasoning about frequencies neighboring the tinnitus pitch and lateral inhibition, Tass and colleagues (Tass et al., 2012) established a method where sine tones are presented in a randomized fashion around the tinnitus frequency for several hours a day with similar longitudinal therapeutic effects.

While the established approaches focus on the retraining of auditory and related cortical structures in longitudinal

therapeutic interventions (Pantev et al., 2012; Adamchic et al., 2014), only few studies looked at the effect of sounds on the temporary suppression of tinnitus (Roberts et al., 2006, 2008; Reavis et al., 2012) to identify possible candidates for future tinnitus sound therapies. Acoustic stimulation with amplitude modulation (AM) and frequency modulation (FM) (Reavis et al., 2012; Tyler et al., 2014) has just recently entered this line of research building on results of electrical stimulation of the cochlea (Zeng et al., 2011). The results of these studies indicate that especially AM sounds in the higher, tinnitus-relevant frequencies of 3,000–9,000 Hz produce a more pronounced tinnitus suppression during and after the stimulation compared to their unmodulated pendants or white noise. In any case, longitudinal data on efficacy and long-term as well as momentary neuroplastic alterations of continuous modulated or patterned, sounds is missing. Therefore, approaches showing efficacy and feasibility in single session experiments with short stimulation duration measuring tinnitus suppression (i.e., residual inhibition) should be tested in longitudinal, prospective placebo-controlled studies to assess long-term efficacy. While recent studies with AM and/or FM sounds, used 40 Hz for the modulation rate (Reavis et al., 2012; Tyler et al., 2014), which is known to produce the largest neural responses in auditory cortex through entrainment as shown in auditory steady-state response (ASSR) paradigms (Picton et al., 2003), no former study tested the influence of lower modulation rates in different carrier sounds, including the tinnitus pitch, for tinnitus suppression. Of special interest here, several reviewed studies in Picton et al. (2003) could also show entrainment effects for different bands including the alpha frequency band. Cortical auditory α activity has been shown to be decreased in tinnitus patients in MEG (Weisz et al., 2005; Schlee et al., 2014), EEG (Moazami-Goudarzi et al., 2010) and possibly also reduced in variability (Schlee et al., 2014). Looking at modulation depth of the stimuli and strength of (entrainment) effect as measured by EEG or MEG, several studies have reliably shown entrainment effects of monaural AM stimuli (100% modulation depth) superior to binaural AM stimuli (Picton et al., 2003; Schwarz and Taylor, 2005; Draganova et al., 2008; Becher et al., 2015). A modulation rate in the α frequency band as well as monaural stimuli with a maximized entrainment effect may therefore enable a normalization of reduced auditory α and thereby concomitantly reduce the tinnitus percept. Based on this preliminary reasoning we here investigated the effects of AM sounds in the α band for tinnitus sound therapy. Yet, the focus of this study was set on the behavioral level to proof the concept and feasibility in the absence of neurophysiological methods.

In the exploratory study at hand, we therefore tested the influence of 10 Hz AM sounds (two pure tones, noise, music and FM sounds) and unmodulated sounds (pure tone, noise) on the temporary suppression of subjective tinnitus loudness in participants with tonal tinnitus in block 1 of the experiment. We hypothesize that all sounds may elicit a short-term suppression of tinnitus loudness (seconds to minutes) with strongest suppression right after stimulus offset (Roberts et al., 2006, 2008; Reavis et al., 2012; Tyler et al., 2014). Given the different types of modulated and unmodulated sounds with frequencies in or around the actual tinnitus pitch, we expect to find differential suppression patterns between the stimuli with AM sounds possibly eliciting enhanced suppression (Reavis et al., 2012). Additionally, with the manipulation of stimulation length and loudness in block 2 of the experiment, we anticipate more pronounced or weaker effects of tinnitus loudness suppression, respectively.

2. METHODS

2.1. Participants

Patients with chronic tonal tinnitus (>12 months tinnitus duration), who had consulted the multidisciplinary Tinnitus Clinic of the University of Regensburg, were included in the study if their age was between 18 and 75 years. Patients with history or presence of severe and relevant somatic, neurological, or mental disorders were excluded. Intake of psychotropic medication or ongoing participation in tinnitus therapies were further exclusion criteria. The study was approved by the Ethics Committee of the University of Regensburg (16-101-0061). All participants gave written informed consent after a comprehensive explanation of the procedures.

After signing the consent, form all participants completed the tinnitus questionnaire (TQ) (Hallam et al., 1988; Goebel and Hiller, 1994), the Tinnitus Handicap Inventory (THI) (Newman et al., 1996), and a visual analog scale (VAS) (Adamchic et al., 2012) with respect to tinnitus loudness (spanning from inaudibility to maximal imaginable loudness). The Tinnitus Sample Case History Questionnaire (TSCHQ) was used to gather clinical and demographic data of all patients (Langguth et al., 2007). Furthermore, hearing level was measured with a standard audiogram using frequencies ranging from 125 Hz to 8 kHz in octave steps with semi-octave steps between 2 and 4 (i.e., 3 kHz), and 4 and 8 kHz (i.e., 6 kHz), respectively (Madsen Midimate 622D; GN Otometrics, Denmark). Headphones used for audiometry, tinnitus matching, as well as for the stimulation procedure were quasi-linear in their frequency response over the whole audible spectrum (Sennheiser HDA 2000; Sennheiser, Germany).

Questionnaire scores and participants characteristics are listed in **Table 1**. The distribution of sexes in the sample was slightly skewed with 11 female and 18 male participants. 3 participants reported a purely left-sided, 2 participants a purely right-sided tinnitus. The majority of participants indicated some form of bilateral or diffuse tinnitus location, with 8 participants indicating tinnitus in both ears, 4 inside the head, 7 both ears with a tendency to the left side, and 4 with a tendency to the right side. A specific tinnitus laterality was not considered as an inclusion criterion due to the diotic presentation of the stimuli. Hearing thresholds slightly differed between ears [right side: mean = 40.63, $SD = 13.24$; left side: mean = 39.46, $SD = 12.17$; $t_{(28)} = 2.10$, $p = 0.044$].

2.2. Tinnitus Matching

After filling in the questionnaires and audiometry, participants were seated in front of a screen with a computer mouse and

TABLE 1 | Participants characteristics ($n = 29$).

	Mean	SD[a]	Median	Minimum	Maximum
Age (years)	52.34	12.78	54	24	75
Tinnitus duration (months)	123.66	117.74	71	12	431
Hearing Loss (both ears, dB)	38.29	11.78	37.27	15.91	62.73
TQ[b] total score (0–84)	39.41	14.06	40	10	69
THI[c] total score (0–100)	43.97	18.48	44	10	92
Tinnitus loudness (%)	67.59	14.74	70	30	100
VAS[d] loudness (0–100)	54.93	17.26	55	22	86
Tinnitus awareness (%)	66.55	26.73	60	0	100
Tinnitus frequency (matching, Hz)	5,334.77	2,904.96	6,000	911	10,500
Tinnitus loudness (matching, dBA)	45.46	14.92	43.90	23.50	81.60

[a]*SD, Standard Deviation;* [b]*TQ, Tinnitus Questionnaire (Goebel and Hiller, 1994);* [c]*THI, Tinnitus Handicap Inventory (Newman et al., 1996);* [d]*VAS, visual analog scale.*

instructed for the tinnitus matching via software. The matching procedure was designed around a sine tone generator (Meyer et al., 2014) where pitch (in single Hz resolution), amplitude and laterality (panning) could be defined and controlled using MAX software (MAX 7; Cycling'74, USA). First, the loudness and lateralization of the tinnitus was roughly defined followed by the actual pitch by the study personnel (Penner and Bilger, 1992; Henry and Meikle, 2000). Participants were then made familiar with the handling of the pitch dial on the graphical user interface and informed about the possibility to adjust the tinnitus pitch in 1 Hz steps while holding down the shift key on the keyboard. Following that, participants proceeded with the actual pitch matching self-reliantly. To ensure reliability and validity of the procedure, the final pitch indicated by the participant was shifted an octave down and up and checked with the participant, respectively, to control for possible octave confusion. Finally, the matched tone was evaluated in a short discussion with the study personnel and rated on a 5 point likert scale (1 = not at all matching the tinnitus percept, 5 = perfect fit). Frequency and loudness results of the matching procedure are listed in **Table 1**.

2.3. Sound Stimuli

A set of 3 amplitude modulated, 2 notch filter amplitude modulated as well as 2 unmodulated sounds were prepared in MATLAB (Matlab R2015a; Mathworks, USA). Besides sine tones in 4 and noise in 2 conditions, a variety of popular music songs was provided to the participants out of which they could choose their favorite song for notch filter modulated presentation in one condition. A sum total of 7 acoustic stimuli or conditions with 3 min of duration was therefore produced for each participant for block 1. In the remainder of this manuscript, including tables and figures, we termed the different stimuli as follows: "AMTinnitus" for AM sounds centered at the tinnitus frequency (**Figure 1A**), "Tinnitus pure tone" for unmodulated sounds centered at the tinnitus frequency (**Figure 1B**), "AMFM" for the AM FM sound (**Figure 1C**), "AMLow" for AM of the 108 Hz sound (**Figure 1D**), "AMMusic" for the AM of musical songs (**Figure 1E**), "AMPinknotch" for the filter AM of pink noise (**Figure 1F**), and "Pink noise" for the pink noise sound (**Figure 1G**). For block 2, participants could choose their favorite stimulus, besides AM in the tinnitus frequency (AMTinnitus), after completing block 1. The AMTinnitus and the chosen stimulus were then manipulated in length, or loudness, or faded (linear fade out in the last minute of the stimulus) resulting in 3 conditions for two stimuli in block 2.

For AMTinnitus, a carrier sine tone was generated and amplitude modulated (100% modulation depth) with a sinusoidal function according to the following principle, where the first part of the equation represents the carrier sound and the second part the modulator. Note that the information in brackets in the legend of the equation is indicative of study-specific settings:

$$s = ca * \sin(2 * \pi * cf * t) * mia * \cos(2 * \pi * mf * t + \phi) \quad (1)$$

where:

s	sinusoidally amplitude modulated sound
ca	carrier amplitude
cf	carrier frequency (=tinnitus frequency)
t	time
mia	modulator index/amplitude (=1)
mf	modulator frequency (=10 Hz)
ϕ	phase

For the AMPinknotch and AMMusic sounds the target of the 10 Hz modulator was the notch filter amplitude. The notch filter used (Butterworth, filter order = 4) was centered around the matched tinnitus frequency with a filter bandwidth of 1 octave (Okamoto et al., 2010; Wunderlich et al., 2015a). With the filter amplitude modulation applied, the resulting sounds where rhythmically suppressed in the octave around the tinnitus frequency, giving the acoustic impression of a slight flutter in the stimulus.

For the AMFM sound, a FM sweep from 0 Hz up to the tinnitus frequency with a modulation rate of 10 Hz served as the carrier sound, which was then amplitude modulated like AMTinnitus (i.e., 100% modulation depth). AMLow with a low frequency carrier sound (108 Hz instead of tinnitus frequency) was generated analogously to AMTinnitus. Finally, the unmodulated stimuli, namely Tinnitus pure tone and Pink noise, were generated. Possible transient artifacts were avoided in the beginning and the end of the stimuli through ramping (linear

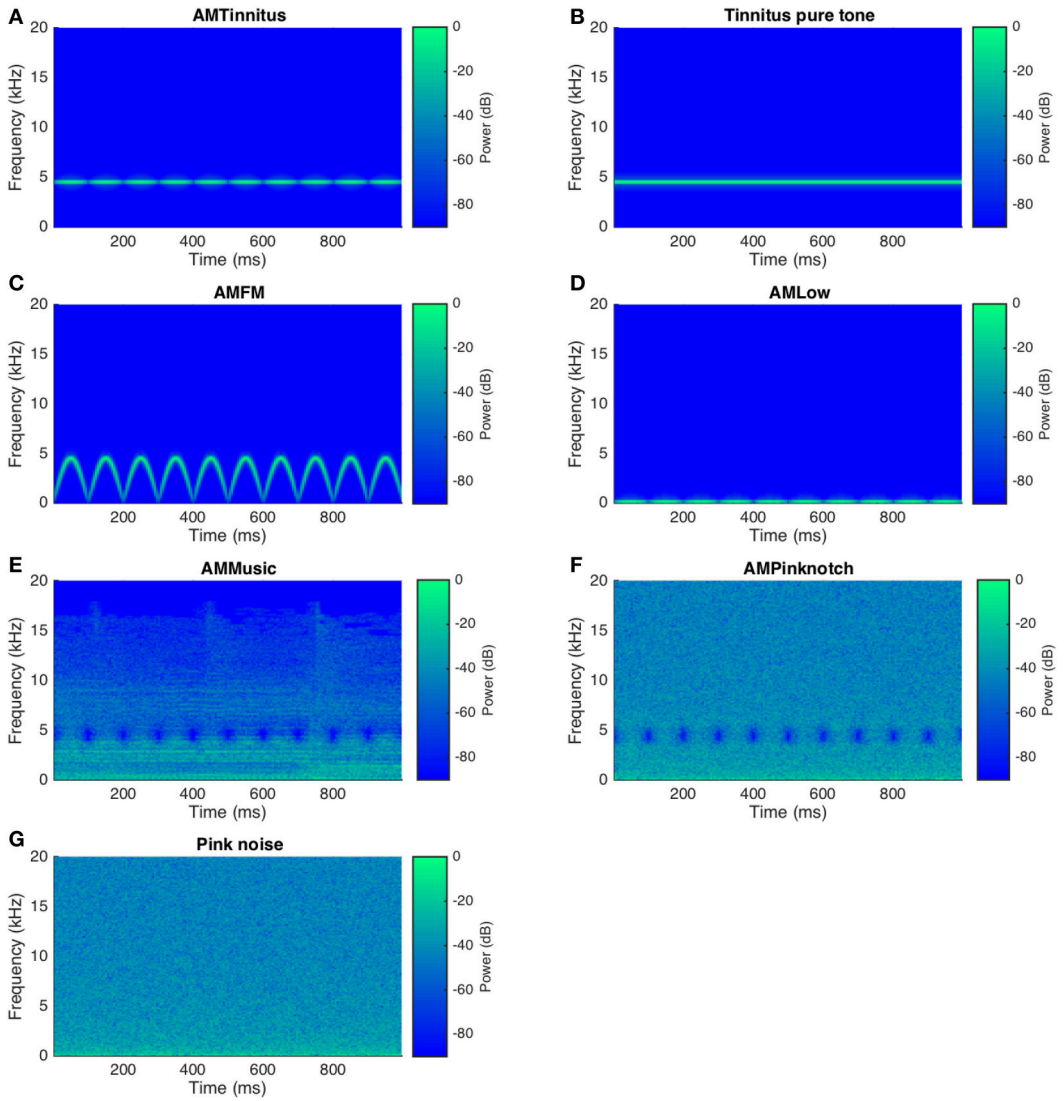

FIGURE 1 | Spectrograms of all sound stimuli (1 s snippets). For all of the plotted representative stimuli an arbitrary tinnitus frequency of 4,500 Hz was chosen and stimuli normalized to full digital displacement. The modulation rate was constant at 10 Hz in modulated sounds **(A,C–F)** whereas **(B,G)** represent the unmodulated stimuli. Stimulus presentation was set to 3 min for all stimuli and block 1. In block 2 AMTinnitus **(A)** underwent loudness (loudness reduction by 30 dB and linear fade out) and temporal (duration of 6 min) manipulations resulting in 4 stimuli including the standard AMTinnitus stimulus from block 1.

fade with 100 ms window). Stimuli were then normalized in sound level and finally exported for the experimental procedure.

2.4. Acoustic Stimulation Procedure

All stimuli were presented at sound levels of 60 dB SL in block 1 (i.e., in broadband stimuli noise and music to the average hearing threshold, whereas in frequency specific stimuli the nearest frequency of the audiogram was chosen as reference for the level adjustment). For block 2, the AMTinnitus and the stimulus of choice were (1) presented for 6 min, (2) reduced in sound level (30 instead of 60 dB SL) and (3) processed with a linear sound level fade out in the last minute of the stimulus. By varying these core parameters of stimulation length and sound level in block 2, we tested differential tinnitus suppression patterns within single stimuli classes with a focus on AMTinnitus. To ensure comfort and safety of the participants, 80 dBA was the upper limit for the sound level of all stimuli. Sound level was carefully checked with an SPL meter (NTi Audio XL2; NTi Audio, Lichtenstein) before actual stimulation. Participants were reminded of the option to interrupt the procedure whenever a sound was deemed uncomfortable at any point of the experiment.

For the acoustic stimulation procedure participants were seated comfortably facing a window with a view on trees to avoid distraction and ensure calmness. No particular instruction was given to focus their attention on either the sound or tinnitus. The presentation sequence of the stimuli was randomized in the two

blocks for each participant. Participants were instructed to relax during the acoustic stimulation and to rate the loudness of their tinnitus in percent, compared to the pre-stimulation loudness, after each stimulation at time points 0, 30, 60, 90, 120, 150, and 180 s. A similar approach of tinnitus loudness growth was used in the study by Reavis and colleagues (Reavis et al., 2012). However, we diverged from the former study by not measuring suppression during acoustic stimulation, having no reference tones in and after the stimulation and deploying a loudness regime tied to hearing loss with 60 dB SL (Reavis et al. (2012) presented stimuli slightly below matched tinnitus loudness). There was a short break between the blocks to maintain vigilance and comfort of the participants. At the end of the study after block 2, the VAS for tinnitus loudness and tinnitus questionnaires were again filled in by the participants. Participants were then thanked for their participation and finally dismissed.

2.5. Data Analysis

A repeated measures mixed model analysis of variance (ANOVA) was calculated with the factors time and condition as well as a random intercept per participant to assess the effect of temporary tinnitus suppression in the loudness growth paradigm. *Post hoc* tests of the ANOVA controlled for multiple comparisons contrasting the suppression profiles between the stimuli were performed using the Tukey method. Finally, paired two-tailed *t*-tests were used to compare tinnitus questionnaire scores and tinnitus loudness VAS before and after acoustic stimulation procedure. As the 3 variables subjected to the paired comparisons were considered within an independent analysis and not part of any primary outcome statistical model or search space, we refrained from a correction for multiple comparisons (e.g., bonferroni) for this secondary analysis. R statistic toolbox with the supplementary libraries "nlme" and "lsmeans" was used for all statistical calculations (R version 3.3.2; R Foundation for Statistical Computing, Austria).

3. RESULTS

3.1. Tinnitus Loudness Growth after Acoustic Stimulation

The results of the ANOVA for the tinnitus loudness growth curves of all stimuli in block 1 are shown in **Table 2** and respective corrected *post-hoc* contrasts in **Table 3**. Notably, there was a significant effect of condition, time, and interaction condition*time on the tinnitus loudness. Mean tinnitus loudness suppression curves are plotted in **Figure 2**.

Post hoc contrasts between each of the 7 stimuli elicited significant differences ($p < 0.05$) for AMMusic vs. AMTinnitus [$t_{(27)} = 4.42$, $p < 0.0001$], Pink noise vs. AMTinnitus [$t_{(27)} = 4.22$, $p = 0.001$], AMLow vs. AMTinnitus [$t_{(27)} = 3.70$, $p = 0.004$], AMFM vs. AMMusic [$t_{(27)} = -3.31$, $p = 0.016$], and AMFM vs. Pink Noise [$t_{(27)} = -3.12$, $p = 0.031$], respectively. These results are indicative of a pattern of enhanced tinnitus suppression of AMTinnitus and AMFM compared to Pink Noise, AMMusic, and AMLow [except AMFM vs. AMLow with $t_{(27)} = -2.60$, $p = 0.127$].

TABLE 2 | Results of ANOVA block 1 ($n = 28$).

	numDF[a]	denDF[b]	*F*-value	*p*-value
(Intercept)	1	1,331	2,845.28	<0.0001
Condition	6	1,331	5.40	<0.0001
Time	1	1,331	185.81	<0.0001
Condition:Time	6	1,331	3.74	0.0011

[a]*numDF, degrees of freedom of numerator;* [b]*denDF, degrees of freedom of denominator.*

TABLE 3 | *Post-hoc* contrasts block 1 ($n = 28$, Tukey-adjusted).

Contrast	Estimate	*t*-value	*p*-value
AMFM - AMMusic	**−5.204**	**−3.31**	**0.016**
AMFM - AMPinknotch	−2.245	−1.43	0.786
AMFM - AMLow	−4.082	−2.60	0.127
AMFM - Pink noise	**−4.898**	**−3.12**	**0.031**
AMFM - AMTinnitus	1.735	1.11	0.927
AMFM - Tinnitus pure tone	−2.041	−1.30	0.852
AMMusic - AMPinknotch	2.959	1.88	0.491
AMMusic - AMLow	1.122	0.72	0.992
AMMusic - Pink noise	0.306	0.20	>0.999
AMMusic - AMTinnitus	**6.939**	**4.42**	**<0.0001**
AMMusic - Tinnitus pure tone	3.163	2.01	0.406
AMPinknotch - AMLow	−1.837	−1.17	0.906
AMPinknotch - Pink noise	−2.653	−1.69	0.623
AMPinknotch - AMTinnitus	3.98	2.53	0.148
AMPinknotch - Tinnitus pure tone	0.204	0.13	>0.999
AMLow - PinkNoise	−0.816	−0.52	0.999
AMLow - AMTinnitus	**5.816**	**3.70**	**0.004**
AMLow - Tinnitus pure tone	2.041	1.30	0.852
Pink noise - AMTinnitus	**6.633**	**4.22**	**0.001**
Pink noise - Tinnitus pure tone	2.857	1.82	0.535
AMTinnitus - Tinnitus pure tone	−3.776	−2.40	0.198

Degrees of freedom = 1,331; Standard error = 1.517; Significant differences are highlighted in bold.

To counteract possible effects of the stimulation sequence in block 1, we furthermore tested the data for order effects with no significant results for position [$F_{(1, 1317)} = 0.05$, $p = 0.832$], condition*position [$F_{(6, 1317)} = 0.94$, $p = 0.468$], time*position [$F_{(1, 1317)} = 3.05$, $p = 0.081$], and interaction condition*time*position [$F_{(6, 1317)} = 0.70$, $p = 0.646$].

For block 2, we report the results for tinnitus loudness growth of the manipulated variations of AMTinnitus (long (6 min of duration), fade, and reduced sound level) with the addition of the data of AMTinnitus of block 1 (standard) in **Table 4**. *Post-hoc* contrasts are indicated in **Table 5** and mean tinnitus loudness suppression curves are plotted in **Figure 3**. Of special interest and according to our expectations, longer stimulation (long, 6 min) resulted in a larger suppression compared to stimulations reduced in sound level [fade vs. long: $t_{(27)} = 3.88$, $p = 0.00065$; reduced sound level vs. long: $t_{(27)} = 4.00$, $p = 0.00041$] but no significant differences with the AMTinnitus stimulation for

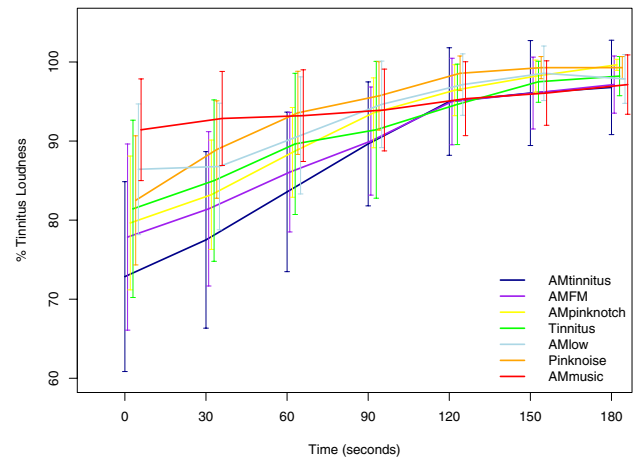

FIGURE 2 | Mean tinnitus loudness suppression after stimulus offset of all sound stimuli in block 1. Confidence intervals at 95% are plotted for each condition and time point. Notably, after 90–120 s tinnitus loudness suppression generally diminishes and curves of the different stimuli converge. Significant differences between stimuli (conditions) are listed in **Table 3**.

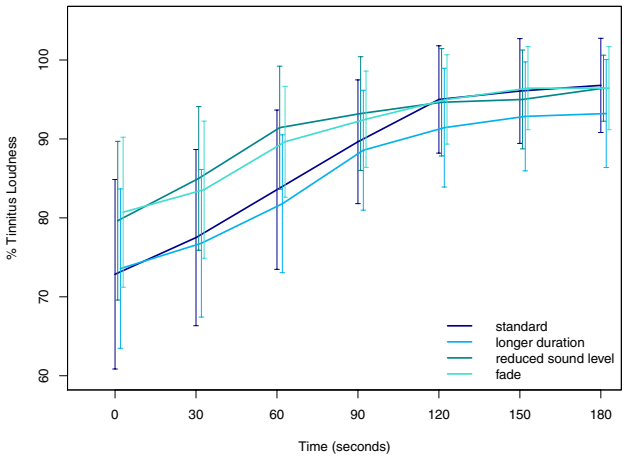

FIGURE 3 | Mean tinnitus loudness suppression after stimulus offset of AMTinnitus and its variations in block 2. Confidence intervals at 95% are plotted for each condition and time point. Standard and longer duration of the stimulus are colored in blue whereas stimuli with reduced sound level or fade out are colored in green. Significant differences between stimuli (conditions) are listed in **Table 5**.

TABLE 4 | Results of ANOVA for AMTinnitus in block 2 ($n = 28$).

	numDF[a]	denDF[b]	*F*-value	*p*-value
(Intercept)	1	749	746.20	<0.0001
Condition	3	749	7.62	0.0001
Time	1	749	201.14	<0.0001
Condition:Time	3	749	2.70	0.0443

[a]*numDF, degrees of freedom of numerator;* [b]*denDF, degrees of freedom of denominator.*

TABLE 5 | *Post-hoc* contrasts block 2 ($n = 28$, Tukey-adjusted).

Contrast	Estimate	*t*-value	*p*-value
Fade - Reduced sound level	−0.153	−0.12	0.999
Fade - Long	**5.153**	**3.88**	**0.00065**
Fade - Standard	3.265	2.46	0.067
Reduced sound level - Long	**5.306**	**4.00**	**0.00041**
Reduced sound level - Standard	**3.418**	**2.57**	**0.050**
Long - Standard	−1.887	−1.42	0.486

Degrees of freedom = 749; Standard error = 1.328; Significant differences are highlighted in bold.

3 min from block 1 [long vs. standard: $t_{(27)} = -1.42$, $p = 0.486$]. Furthermore, AMTinnitus elicited marginally increased suppression compared to the faded stimulus [fade vs. standard: $t_{(27)} = 2.46$, $p = 0.067$, trend] and the stimulus with reduced sound level [reduced sound level vs. standard: $t_{(27)} = 2.57$, $p = 0.050$]. The comparison of the two stimuli with manipulated sound level resulted in no significant difference [fade vs. reduced sound level: $t_{(27)} = -0.12$, $p = 0.999$].

3.2. Responder Patterns and Overall Feasibility

The evaluation of the matched tinnitus pitch resulted in a mean of 4.0 ($SD = 0.55$, with 5 indicating perfect fit) highlighting the reasonable quality of the matching procedure. The response criterion for temporary tinnitus suppression was set to any suppression per stimuli (here at t0, right after the offset of the auditory stimulation) as similarly done before (Reavis et al., 2012). Applying this criterion, the following descriptive responder pattern emerges: In the AMTinnitus condition 19 out of 28 participants indicated a suppression at t0, in AMPinknotch 19/28, in AMFM 16/28, in Pink noise 16/28, in AMLow 13/28, in Tinnitus pure tone 13/28, and in AMMusic 8/20.

Differences in tinnitus loudness (VAS) and total scores of standardized questionnaires (TQ and THI) comparing assessments before and after experimental procedures are listed in **Table 6** and summarized in the following: Tinnitus loudness (VAS) was significantly reduced after experimental procedures compared to the baseline assessment [$t_{(27)} = 2.774$, $p = 0.01$]. Furthermore, TQ and THI scores measuring tinnitus-related distress were also both lower after the experiment. While TQ scores are below the p-value threshold of $p = 0.05$, we can only report a trend for the THI [TQ: $t_{(27)} = 2.062$, $p = 0.049$; THI: $t_{(27)} = 1.922$, $p = 0.065$]. It has to be noted though, that the effects reported here are based on the possible influence of all amplitude modulated sounds as well as unmodulated "control" sounds and this secondary analysis serves safety and feasibility purposes.

4. DISCUSSION

Acoustic stimulation or sound therapy is proposed as a main treatment option for chronic subjective tinnitus (Langguth et al.,

TABLE 6 | Differences in tinnitus loudness and questionnaire scores before (pre) and after (post) experimental procedures.

Measure	Mean score pre	SD[a] pre	Mean score post	SD post	df	*t*-value	*p*-value
VAS loudness (mm)	54.46	17.39	48.25	17.48	27	2.774	0.01
TQ total score (0–84)	38.36	13.09	35.07	14.78	27	2.062	0.049
THI total score (0–100)	42.25	16.3	38.29	16.95	27	1.922	0.065

[a]*SD, Standard Deviation.*

2013). Numerous approaches for acoustic stimulation exist, be it in experimental studies (e.g., Roberts et al., 2006; Reavis et al., 2012; Hoare et al., 2014), longitudinal clinical trials (e.g., Okamoto et al., 2010; Adamchic et al., 2014), fitted hearing aids or sound players (e.g., Vernon and Meikle, 2003), mobile apps or webpages, and various user-driven self-administered forms. As of yet, there is neither an established general-purpose acoustic stimulation to abolish or reduce tinnitus nor a working strategy for subtypization of responder profiles. To further probe the field of acoustic stimulations for tinnitus therapy, the purpose of this exploratory study was to compare 10 Hz AM sounds (pure tones, noise, music and FM sounds) and unmodulated sounds (pure tone, noise) regarding their temporary suppression of tinnitus loudness in participants with tonal tinnitus.

First we found that all sounds elicit a short-term suppression of tinnitus loudness (seconds to minutes) with strongest suppression right after stimulus offset . Adding to this, feasibility of the overall procedure could be confirmed as scores of both tinnitus questionnaires as well as the VAS for tinnitus loudness were lower after the experiment . Furthermore, no adverse events or persisting increase in tinnitus loudness or distress during and after the experimental procedure were noted. Second, akin to the findings of Reavis et al. (2012), while not directly comparable (due to higher presentation loudness in our study, frequency ranges instead of matched tinnitus pitch and white noise instead of pink noise in the former study), we found that AMTinnitus and AMFM produced a significantly stronger tinnitus loudness suppression than noise.

Furthermore, both AMTinnitus and AMFM produced superior suppression than AMMusic condition with the amplitude modulated notch filter.

Finally, AMTinnitus resulted in a clearly more pronounced suppression than AMLow.

Taken together, these results imply that AM sounds, especially in or around the tinnitus frequency (i.e., AMTinnitus and AMFM, Schaette et al., 2010), may produce larger suppression than unmodulated sounds. Yet, the direct contrast between AMTinnitus and Tinnitus pure tone did not result in a significant difference, but the direction and the size of the statistical values may point to a significant contrast in future studies (see **Figure 2** and **Table 3**). Possible cumulative effects of tinnitus suppression over the entire acoustic stimulation procedure in block 1 can be largely ruled out as there were no order effects . Third, with the manipulations of the AMTinnitus stimulus in block 2 either increasing the stimulus duration to 6 min, or reducing either overall sound level (30 dB), or fading of the stimulus in the last minute, we could partly show that these manipulations led to an altered tinnitus suppression: Standard AMTinnitus produced significantly more tinnitus suppression than both of the sound level-manipulated variations according to our expectations (i.e., reduced sound level, see **Figure 3** and **Table 5**), yet the longer version of the very same stimulus failed to show increased overall tinnitus suppression . However, comparing the loudness growth curves of the standard AMTinnitus with the version longer in duration, there may be a difference in suppression depth from 90 s onwards after stimulation offset. While the initial suppression at 0 s seems to be in similar range in both stimuli, the longer version may sustain the suppression for a longer time as reflected in the flatter curve. This effect could be topic of possible future studies where stimulation duration undergoes respective manipulation.

Looking at the AMFM stimulus we noticed both a good suppression potential second to AMTinnitus and a promising tolerance as participants clearly preferred AMFM over all other stimuli for block 2 (10/28 chose AMFM out of the 7 alternative options). On the other hand, it is challenging to interpret these results given the lack of a direct control sound (i.e., 10 Hz FM without AM). Finally, the sounds with amplitude modulated notch filter (AMPinknotch and AMMusic) were designed to test possible short-term suppression effects of the established long-term sound therapy with notch-filtered music (Pantev et al., 2012). AMMusic clearly exhibited the least overall suppression probably due to missing energy of the sounds in and around the filtered frequency range inherent to the presented songs, as music is both spectrally and temporally highly variable in amplitude (see **Figure 1E**). To a lesser degree, this is also true of (pink) noise so that both of the notch-filtered AM sounds are not straightforwardly comparable in acoustic morphology and putative suppression effects to the pure tone sounds. Furthermore, given the tonal nature of the tinnitus in participants, this result certainly was expectable. All in all, the weaker suppression effect of these filter gain modulated sounds may be due to missing energy in the critical frequency bands of the notch filter, which is not surprising given the long-term application and its putatively induced reversal of maladaptive map plasticity through residual inhibition (Pantev et al., 2012; Tass et al., 2012).

Generally, between 90 and 120 s after stimulus offset, or even earlier in some stimuli (i.e., AMMusic, Pink noise, AMPinknotch, AMLow), tinnitus loudness reaches 90% of the baseline loudness and tends to reach 100% after 180 s, which equals the stimulation duration. A similar pattern was observed by Reavis et al. (2012) in representative, individual suppression profiles, while group statistics are not performed in a comparable manner to our study. First, we did not focus on responders for statistical analyses like the previous study as all subjects, conditions and time points were included in our study. Second, no transformation on the

variables or other adjustments to the raw data were performed. Yet, given the various differences in the study design of Reavis et al. (2012), namely measuring suppression during acoustic stimulation, having reference tones in and after the stimulation and applying a loudness regime slightly below matched tinnitus, results are still deemed comparable and we may substantiate the former findings that AM (and partly FM) sounds elicit better tinnitus suppression than traditional maskers (i.e., unmodulated white noise and pure tones).

4.1. Limitations

In the following we would like to consider some issues, which may be regarded as shortcomings of our study, while not being detrimental given the exploratory scope of this study. First, looking at the sound stimuli, unlike Reavis et al. (2012) we did not use white noise as (control) masking sounds, which may limit the interpretation of especially the contrast to AMTinnitus, as in white noise there is more sound energy in the high frequency bands where tinnitus usually manifests. Besides, there also was no direct, unmodulated control sound to AMLow and noise was not amplitude modulated over the entire audible frequency range. Future studies should therefore define respective a priori contrasts with only a single or few parameters manipulated in the stimuli to ensure optimal comparability. Second, sound presentation may be updated with consideration of tinnitus laterality (contra- vs. ipsi- vs. bilateral presentation) (Feldmann, 1971) and with related adjustments for asymmetrical hearing loss (Roberts et al., 2008), loudness weighting reconsidered (i.e., application of more detailed loudness contour curves (ISO 226) to the stimuli instead of dbA weighting), and finally matching sounds alongside the active stimuli to evaluate loudness growth independent from tinnitus (Reavis et al., 2012). Third, to both identify and analyze tinnitus subgroups as well as responder profiles, it would be advantageous to include further questionnaires to probe comorbidities and (general) quality of life (Langguth et al., 2007) and, more importantly, questionnaires elucidating personal profiles, like the NEO-PI-R (Costa and McCrae, 2008), possibly related to tolerance and acceptance of sound therapy in tinnitus. Fourth, given the behavioral nature of the current study, both neurophysiological models for cortical and subcortical responses to these stimuli and possible beneficial effects for tinnitus have to be specifically tested in fitting paradigms in future studies. Finally, in block 2, we could not test for order effects because the conditions with the stimuli chosen by the participants were deliberately left out in the analysis of the data. Given the inexistence of such order effects in block 1 and identical randomization strategies used in both blocks, we do not expect an order effect in the trimmed analysis of block 2.

4.2. Conclusion and Outlook

Given the results of the present study in the context of previous findings, we conclude (and partly replicate) that amplitude modulated sounds with various carrier sounds in and around tinnitus frequency are feasible for short-term tinnitus suppression. With a modulation rate of 10 Hz in the EEG α band, we expect indirect neuromodulation and normalization of the endogeneous (also: individual) α rhythm which has been shown to be reduced in patients with tinnitus. Exact mechanisms of this auditory entrainment should therefore be investigated by means of respective neurophysiological methods (MEG/EEG) to test if and how auditory entrainment and possibly related tinnitus suppression is reflected by neural oscillations. Beyond that, longitudinal studies in real life should be performed to evaluate the envisioned long-term goal of this approach, namely to develop individually-customized mobile tinnitus sound therapies with aesthetically appealing sounds.

AUTHOR CONTRIBUTIONS

PN and WS: substantial contribution to the design of the study, data analysis, drafted and revised the manuscript. JM: substantial contribution to the design of the study and data acquisition. MM: substantial contribution to the discussion of the approach and paradigm. MS and BL: drafted and revised the manuscript.

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REFERENCES

Adamchic, I., Langguth, B., Hauptmann, C., and Tass, P. A. (2012). Psychometric evaluation of visual analog scale for the assessment of chronic tinnitus. *Am. J. Audiol.* 21, 215–225. doi: 10.1044/1059-0889(2012/12-0010)

Adamchic, I., Toth, T., Hauptmann, C., and Tass, P. A. (2014). Reversing pathologically increased EEG power by acoustic coordinated reset neuromodulation. *Hum. Brain Mapp.* 35, 2099–2118. doi: 10.1002/hbm.22314

Adjamian, P., Sereda, M., and Hall, D. A. (2009). The mechanisms of tinnitus: perspectives from human functional neuroimaging. *Hear. Res.* 253, 15–31. doi: 10.1016/j.heares.2009.04.001

Adjamian, P., Sereda, M., Zobay, O., Hall, D. A., and Palmer, A. R. (2012). Neuromagnetic indicators of tinnitus and tinnitus masking in patients with and without hearing loss. *J. Assoc. Res. Otolaryngol.* 13, 715–731. doi: 10.1007/s10162-012-0340-5

Becher, A. K., Höhne, M., Axmacher, N., Chaieb, L., Elger, C. E., and Fell, J. (2015). Intracranial electroencephalography power and phase synchronization changes during monaural and binaural beat stimulation. *Eur. J. Neurosci.* 41, 254–263. doi: 10.1111/ejn.12760

Costa, P. T., and McCrae, R. R. (2008). The revised neo personality inventory (neo-pi-r). *SAGE Handb. Pers. Theory Assess.* 2, 179–198. doi: 10.4135/9781849200479.n9

De Ridder, D., Elgoyhen, A. B., Romo, R., and Langguth, B. (2011). Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8075–8080. doi: 10.1073/pnas.1018466108

De Ridder, D., Vanneste, S., Weisz, N., Londero, A., Schlee, W., Elgoyhen, A. B., et al. (2014). An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci. Biobehav. Rev.* 44, 16–32. doi: 10.1016/j.neubiorev.2013.03.021

Draganova, R., Ross, B., Wollbrink, A., and Pantev, C. (2008). Cortical steady-state responses to central and peripheral auditory beats. *Cereb. Cortex* 18, 1193–1200. doi: 10.1093/cercor/bhm153

Eggermont, J. J., and Komiya, H. (2000). Moderate noise trauma in juvenile cats results in profound cortical topographic map changes in adulthood. *Hum. Audit. Neuroimaging* 142, 89–101. doi: 10.1016/S0378-5955(00)00024-1

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Elgoyhen, A. B., Langguth, B., De Ridder, D., and Vanneste, S. (2015). Tinnitus: perspectives from human neuroimaging. *Nat. Rev. Neurosci.* 16, 632–642. doi: 10.1038/nrn4039

Erlandsson, S., and Dauman, N. (2013). Categorization of tinnitus in view of history and medical discourse. *Int. J. Qual. Stud. Health Well Being* 8:55. doi: 10.3402/qhw.v8i0.23530

Feldmann, H. (1971). Homolateral and contralateral masking of tinnitus by noise-bands and by pure tones. *Audiology* 10, 138–144. doi: 10.3109/00206097109072551

Gilles, A., Schlee, W., Rabau, S., Wouters, K., Fransen, E., and Van de Heyning, P. (2016). Decreased speech-in-noise understanding in young adults with tinnitus. *Front. Neurosci.* 10:288. doi: 10.3389/fnins.2016.00288

Goebel, G., and Hiller, W. (1994). The tinnitus questionnaire. A standard instrument for grading the degree of tinnitus. results of a multicenter study with the tinnitus questionnaire. *HNO* 42, 166–172.

Hallam, R., Jakes, S., and Hinchcliffe, R. (1988). Cognitive variables in tinnitus annoyance. *Br. J. Clin. Psychol.* 27, 213–222. doi: 10.1111/j.2044-8260.1988.tb00778.x

Hazell, J. W. P., and Wood, S. (2009). Tinnitus masking-a significant contribution to tinnitus management. *Br. J. Audiol.* 15, 223–230. doi: 10.3109/03005368109081442

Henry, J. A., Frederick, M., Sell, S., Griest, S., and Abrams, H. (2015). Validation of a novel combination hearing aid and tinnitus therapy device. *Ear Hear.* 36, 42–52. doi: 10.1097/AUD.0000000000000093

Henry, J. A., and Meikle, M. B. (2000). Psychoacoustic measures of tinnitus. *J. Am. Acad. Audiol.* 11, 138–155.

Henry, J. A., Rheinsburg, B., and Zaugg, T. (2004). Comparison of custom sounds for achieving tinnitus relief. *J. Am. Acad. Audiol.* 15, 585–598. doi: 10.3766/jaaa.15.8.6

Hoare, D. J., Searchfield, G. D., El Refaie, A., and Henry, J. A. (2014). Sound therapy for tinnitus management: practicable options. *J. Am. Acad. Audiol.* 25, 62–75. doi: 10.3766/jaaa.25.1.5

Hoffman, H. J., and Reed, G. W. (2004). "Epidemiology of tinnitus," in *Tinnitus: Theory and Management*, ed J. B. Snow (Hamilton, BC: Decker), 16–41.

Hyvärinen, P., Mendonça, C., Santala, O., Pulkki, V., and Aarnisalo, A. A. (2016). Auditory localization by subjects with unilateral tinnitus. *J. Acoust. Soc. Am.* 139, 2280–2289. doi: 10.1121/1.4946897

Jastreboff, M. M. (2007). Sound therapies for tinnitus management. *Prog. Brain Res.* 166, 435–440. doi: 10.1016/S0079-6123(07)66042-7

Jastreboff, P. J. (1990). Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci. Res.* 8, 221–254. doi: 10.1016/0168-0102(90)90031-9

Langguth, B., Goodey, R., Azevedo, A., Bjorne, A., Cacace, A., Crocetti, A., et al. (2007). Consensus for tinnitus patient assessment and treatment outcome measurement: tinnitus research initiative meeting, regensburg, july 2006. *Prog. Brain Res.* 166, 525–536. doi: 10.1016/S0079-6123(07)66050-6

Langguth, B., Kreuzer, P. M., Kleinjung, T., and De Ridder, D. (2013). Tinnitus: causes and clinical management. *Lancet Neurol.* 12, 920–930. doi: 10.1016/S1474-4422(13)70160-1

Lockwood, A. H., Salvi, R. J., and Burkard, R. F. (2002). Tinnitus. *N. Engl. J. Med.* 347, 904–910. doi: 10.1056/NEJMra013395

Mazurek, B., Olze, H., Haupt, H., and Szczepek, A. J. (2010). The more the worse: the grade of noise-induced hearing loss associates with the severity of tinnitus. *Int. J. Environ. Res. Public Health* 7, 3071–3079. doi: 10.3390/ijerph7083071

Meyer, M., Luethi, M. S., Neff, P., Langer, N., and Büchi, S. (2014). Disentangling tinnitus distress and tinnitus presence by means of EEG power analysis. *Neural Plast.* 2014:468546. doi: 10.1155/2014/468546

Moazami-Goudarzi, M., Michels, L., Weisz, N., and Jeanmonod, D. (2010). Temporo-insular enhancement of EEG low and high frequencies in patients with chronic tinnitus. *BMC Neurosci.* 11:40. doi: 10.1186/1471-2202-11-40

Moon, I. J., Won, J. H., Kang, H. W., Kim, D. H., An, Y. H., and Shim, H. J. (2015). Influence of tinnitus on auditory spectral and temporal resolution and speech perception in tinnitus patients. *J. Neurosci.* 35, 14260–14269. doi: 10.1523/JNEUROSCI.5091-14.2015

Newman, C. W., Jacobson, G. P., and Spitzer, J. B. (1996). Development of the tinnitus handicap inventory. *Arch. Otolaryngol. Head Neck Surg.* 122, 143–148. doi: 10.1001/archotol.1996.01890140029007

Nondahl, D. M., Cruickshanks, K. J., Huang, G.-H., Klein, B. E. K., Klein, R., Tweed, T. S., et al. (2012). Generational differences in the reporting of tinnitus. *Ear Hear.* 33, 640–644. doi: 10.1097/AUD.0b013e31825069e8

Okamoto, H., Stracke, H., Stoll, W., and Pantev, C. (2010). Listening to tailor-made notched music reduces tinnitus loudness and tinnitus-related auditory cortex activity. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1207–1210. doi: 10.1073/pnas.0911268107

Pantev, C., Okamoto, H., and Teismann, H. (2012). Music-induced cortical plasticity and lateral inhibition in the human auditory cortex as foundations for tonal tinnitus treatment. *Front. Syst. Neurosci.* 6:50. doi: 10.3389/fnsys.2012.00050

Penner, M. J., and Bilger, R. C. (1992). Consistent within-session measures of tinnitus. *J. Speech Hear. Res.* 35, 694–700. doi: 10.1044/jshr.3503.694

Picton, T. W., John, M. S., Dimitrijevic, A., and Purcell, D. (2003). Human auditory steady-state responses: respuestas auditivas de estado estable en humanos. *Int. J. Audiol.* 42, 177–219. doi: 10.3109/14992020309101316

Reavis, K. M., Rothholtz, V. S., Tang, Q., Carroll, J. A., Djalilian, H., and Zeng, F.-G. (2012). Temporary suppression of tinnitus by modulated sounds. *J. Assoc. Res. Otolaryngol.* 13, 561–571. doi: 10.1007/s10162-012-0331-6

Roberts, L. E., Moffat, G., Baumann, M., Ward, L. M., and Bosnyak, D. J. (2008). Residual inhibition functions overlap tinnitus spectra and the region of auditory threshold shift. *J. Assoc. Res. Otolaryngol.* 9, 417–435. doi: 10.1007/s10162-008-0136-9

Roberts, L. E., Moffat, G., and Bosnyak, D. J. (2006). Residual inhibition functions in relation to tinnitus spectra and auditory threshold shift. *Acta oto-laryngologica Suppl.* 126, 27–33. doi: 10.1080/03655230600895358

Schaette, R., and Kempter, R. (2006). Development of tinnitus-related neuronal hyperactivity through homeostatic plasticity after hearing loss: a computational model. *Eur. J. Neurosci.* 23, 3124–3138. doi: 10.1111/j.1460-9568.2006.04774.x

Schaette, R., König, O., Hornig, D., Gross, M., and Kempter, R. (2010). Acoustic stimulation treatments against tinnitus could be most effective when tinnitus pitch is within the stimulated frequency range. *Hear. Res.* 269, 95–101. doi: 10.1016/j.heares.2010.06.022

Schaette, R., and McAlpine, D. (2011). Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J. Neurosci.* 31, 13452–13457. doi: 10.1523/JNEUROSCI.2156-11.2011

Schecklmann, M., Vielsmeier, V., Steffens, T., Landgrebe, M., Langguth, B., Kleinjung, T., et al. (2012). Relationship between audiometric slope and tinnitus pitch in tinnitus patients: insights into the mechanisms of tinnitus generation. *PLoS ONE* 7:e34878. doi: 10.1371/journal.pone.0034878

Schlee, W., Schecklmann, M., Lehner, A., Kreuzer, P. M., Vielsmeier, V., Poeppl, T. B., et al. (2014). Reduced variability of auditory alpha activity in chronic tinnitus. *Neural Plast.* 2014:436146. doi: 10.1155/2014/436146

Schwarz, D. W. F., and Taylor, P. (2005). Human auditory steady state responses to binaural and monaural beats. *Clin. Neurophysiol.* 116, 658–668. doi: 10.1016/j.clinph.2004.09.014

Stein, A., Engell, A., Lau, P., Wunderlich, R., Junghoefer, M., Wollbrink, A., et al. (2015). Enhancing inhibition-induced plasticity in tinnitus–spectral energy contrasts in tailor-made notched music matter. *PLoS ONE* 10:e0126494. doi: 10.1371/journal.pone.0126494

Tass, P. A., Adamchic, I., Freund, H.-J., Stackelberg, T. V., and Hauptmann, C. (2012). Counteracting tinnitus by acoustic coordinated reset neuromodulation. *Restorat. Neurol. Neurosci.* 30, 137–159. doi: 10.3233/RNN-2012-110218

Tyler, R., Stocking, C., Secor, C., and Slattery, W. H. (2014). Amplitude modulated S-tones can be superior to noise for tinnitus reduction. *Am. J. Audiol.* 23, 303–308. doi: 10.1044/2014_AJA-14-0009

Vanneste, S., and De Ridder, D. (2012). The auditory and non-auditory brain areas involved in tinnitus. An emergent property of multiple parallel overlapping subnetworks. *Front. Syst. Neurosci.* 6:31. doi: 10.3389/fnsys.2012.00031

Vernon, J. (1977). Attempts to relief tinnitus. *Ear Hear.* 2:124.

Vernon, J. A., and Meikle, M. B. (2003). Masking devices and alprazolam treatment for tinnitus. *Otolaryngol. Clin. North Am.* 36, 307–320.

Watanabe, K., Kamio, T., Ohkawara, D., Aoki, H., Baba, S., and Yagi, T. (1997). [Suppression of tinnitus by band noise masker–a study of 600 cases]. *Nihon Jibiinkoka Gakkai kaiho* 100, 920–926. doi: 10.3950/jibiinkoka.100.920

Weisz, N., Hartmann, T., Dohrmann, K., Schlee, W., and Norena, A. (2006). High-frequency tinnitus without hearing loss does not mean absence of deafferentation. *Hear. Res.* 222, 108–114. doi: 10.1016/j.heares.2006.09.003

Weisz, N., Moratti, S., Meinzer, M., Dohrmann, K., and Elbert, T. (2005). Tinnitus perception and distress is related to abnormal spontaneous brain activity as measured by magnetoencephalography. *PLoS Med.* 2:e153. doi: 10.1371/journal.pmed.0020153

Wunderlich, R., Lau, P., Stein, A., Engell, A., Wollbrink, A., Rudack, C., et al. (2015a). Impact of spectral notch width on neurophysiological plasticity and clinical effectiveness of the tailor-made notched music training. *PLoS ONE* 10:e0138595. doi: 10.1371/journal.pone.0138595

Wunderlich, R., Stein, A., Engell, A., Lau, P., Waasem, L., Shaykevich, A., et al. (2015b). Evaluation of iPod-based automated tinnitus pitch matching. *J. Am. Acad. Audiol.* 26, 205–212. doi: 10.3766/jaaa.26.2.9

Xiong, H., Chen, L., Yang, H., Li, X., Qiu, Z., Huang, X., et al. (2013). Hidden hearing loss in tinnitus patients with normal audiograms: implications for the origin of tinnitus. *J. Clin. Otorhinolaryngol. Head Neck Surg.* 27, 362–365.

Zeng, F.-G., Tang, Q., Dimitrijevic, A., Starr, A., Larky, J., and Blevins, N. H. (2011). Tinnitus suppression by low-rate electric stimulation and its electrophysiological mechanisms. *Hum. Audit. Neuroimaging* 277, 61–66. doi: 10.1016/j.heares.2011.03.010

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REVIEW
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Acoustic Coordinated Reset Neuromodulation: A Systematic Review of a Novel Therapy for Tinnitus

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Background: There are growing technological advances in the development of sound-based methods for the treatment of tinnitus. Most of these methods intend to affect the speculated underlying neurological causes of tinnitus. Acoustic coordinated reset (CR) neuromodulation is one of them. A novel method that as of yet seems inadequately reviewed.

Purpose: To evaluate the current evidence on acoustic CR neuromodulation as a method for the treatment of tinnitus and to assess whether the method can be implemented in daily clinical practice.

Methods: A systematic literature search was performed in 13 databases in the period from February 1, 2015 to May 1, 2016. Studies regarding acoustic CR neuromodulation as a treatment method for tinnitus were included in the present review.

Results: A total of 8 studies were eligible for being reviewed comprising a total of 329 patients. Overall, the evidence level of the published literature was low. The main findings in the included studies were that acoustic CR neuromodulation was safe and well tolerated and most patients reported reduction of tinnitus symptoms. The neurophysiological basis of the method was claimed to be desynchronization, anti-kindling, and change of abnormal frequency couplings in a widespread tinnitus network comprising both auditory and non/auditory brain areas based on EEG analyses.

Conclusion: The available evidence is insufficient for clinical implementation of acoustic CR neuromodulation. The limited level of evidence suggests that acoustic CR neuromodulation may have positive effects on tinnitus symptoms. Preliminary electroencephalographic data are compatible with the claim that tinnitus reduction after CR treatment is mediated by a desynchronizing effect. However, a proof for this claim is still lacking.

Keywords: acoustic coordinated reset neuromodulation, desynchronizing, anti-kindling, tinnitus, systematic review

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INTRODUCTION

Subjective tinnitus (ST) is an auditory phantom phenomenon, where an auditory perception is not related to a physical, external or internal, and sound source (1, 2). Approximately 10–15% of the adult population experience tinnitus (3). Tinnitus occurs in varying degrees and may have different etiologies (2). Up to 85% of tinnitus cases are accompanied by hearing loss related to external

noise trauma (4). Other risk factors include longevity, ototoxic medication, otological diseases, head injury, cerebral diseases, and mandibular joint disorders (5, 6).

There are several theories regarding the mechanisms of ST generation such as phantom auditory perception (7), stimulated acoustic emissions (8), the dorsal cochlear nucleus hypothesis (9–11), and increased spontaneous firing rate (SFR) (12, 13). Tinnitus is most frequently related to damage of the peripheral hearing system, thereby leading to a deafferentiation of neurons influencing more central parts of the auditory system. One of the tinnitus generation theories is that cochlear damage results in deafferentiation-induced cortical map reorganization (1). When central auditory neurons are deprived of their normal input, they begin to show responsiveness to the characteristic frequency of neighboring, less affected regions in the tonotopic map (14). Also included among the neuronal changes are increased SFRs, abnormal synaptic connectivity, and synchronization (15).

The varying causes of tinnitus generation have led to development of a wide variety of treatments. Several methods have been studied, although the benefit has not been conclusively demonstrated. The efficacy of cognitive behavioral therapy, however, is well established, and studies have shown a beneficial effect on tinnitus distress.[1] Other methods include medical treatments (e.g., intravenous Lidocaine) and psychological approaches in combination with therapeutic sound, such as tinnitus retraining therapy. Sound therapies alone include the use of music, white noise generators, and hearing aids (16). Potential mechanisms through which sound therapy may act involve masking, habituation, and reversing of cortical reorganization through lateral inhibition.

There are growing technological advances in sound-based approaches of which the majority is designed to target the maladaptive plasticity perceived as the underlying mechanism of ST (16). Acoustic coordinated reset (CR) neuromodulation® is one of them (6, 14).

[1]Dinces EA. *Tinnitus*. Available from: http://www.uptodate.com/contents/treatment-of-tinnitus?source=machineLearning&search=tinnitus&selectedTitle=2~150&sectionRank=1&anchor=H2#H2

The concept of CR neuromodulation was initially developed as a treatment method for Parkinson's disease, where pathological neural synchronization was counteracted by desynchronizing, electrical deep brain stimulation. Since the inventors considered the pathological nerve activity of both Parkinson's and ST to be characterized by hyperactivity and pathological synchronization, acoustic CR neuromodulation was introduced as a non-invasive counterpart (14).

Coordinated reset neuromodulation has been examined in several animal (17, 18) and human studies (19). Over the past few years, studies regarding the effects and mechanisms of acoustic CR neuromodulation have also been presented.

Acoustic CR neuromodulation is a patterned stimulation with tones adjusted to the patient's dominant tinnitus frequency, which aims at counteracting pathological neuronal synchronization. Phase reset is proposed to be achieved by a repetitive stimulus delivery of tones with different frequencies gathered around the dominant tinnitus pitch (2).

To facilitate understanding of CR neuromodulation in general, some principles should be addressed (**Figure 1**):

1. CR neuromodulation (**Figure 1**) is based on the phase reset of oscillatory neuronal activity through desynchronization. It is proposed to counteract the deafferentiation-induced upregulated synchrony and connectivity through desynchronizing and anti-kindling effects (20, 21).
2. Neural populations display spike timing-dependent plasticity (STDP), i.e., they continuously regulate the strength of their synaptic connectivity relative to the pre- and postsynaptic firing (spikes). In other words, neural activity and the strength of their connections are related.
3. CR neuromodulation is designed to cause long-lasting anti-kindling effects; desynchronizing stimulation of neuronal populations causes the neurons to unlearn their pathological connectivity. The method attempts to change spike firing timing networks using series of tones and to force firing from a pathological state with abnormally synchronized synapses to a desynchronized state with weaker synapses. It achieves this

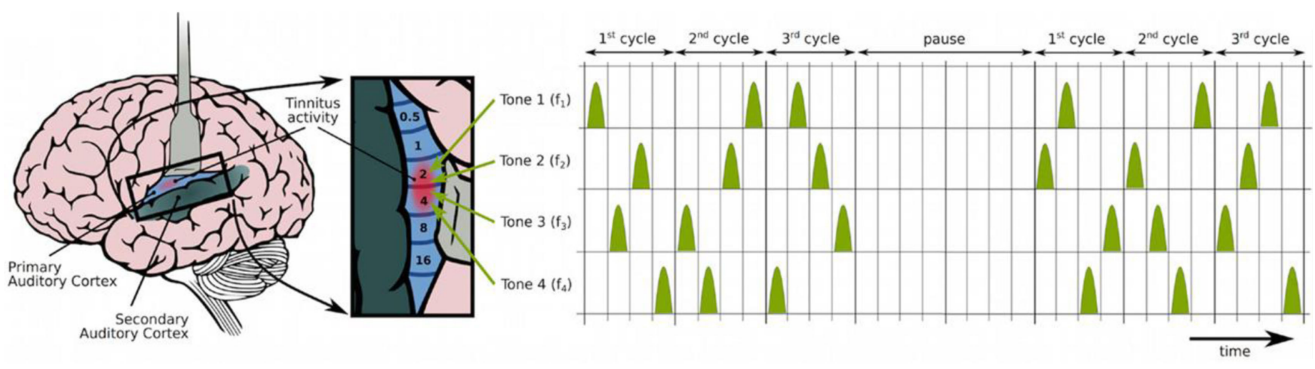

FIGURE 1 | Acoustic coordinated reset neuromodulation: the spatiotemporal organization of stimuli (23). The figure shows how stimuli are comprised by four different tones (triangles) gathered around the dominant tinnitus pitch in a randomized sequence during three cycles, followed by two silent cycles in a 3:2 ON–OFF pattern, to ideally enhance the desynchronizing effect (20, 24). Adapted from Chittka and Brockmann (25), with permission from Tass et al.

through tones played through hearing aid style earphones. The patients are supposed to listen to the tones 4–6 h per day for, e.g., 12 weeks.
4. The phenomenon when such states are present at the same time is known as STDP-induced multistability (2, 22).

The present review provides a systematic overview of studies of acoustic CR neuromodulation as a novel treatment method for ST. The purpose was to establish the current level of evidence available for the intervention and to assess whether the method can be recommended in the clinical setting.

METHODS

Literature search was performed in 13 databases in the period from 1st February to 1st May 2016, using the following search strings: (i) "Tinnitus" AND "acoustic coordinated reset neuromodulation," (ii) Tinnitus AND acoustic coordinated reset neuromodulation, (iii) Tinnitus AND acoustic CR neuromodulation, and (iv) Tinnitus and acoustic coordinated reset (CR) neuromodulation. MeSH terms were not available for either tinnitus or neuromodulation. The highest number of search results was found using search string (ii). None of the other search strings resulted in additional articles. The reference lists of articles identified by this search strategy were reviewed, and one article was considered relevant and selected (26). Trial registers were also searched. A complete list of searched resources is included in **Table 1**.

The present systematic review was intended to follow the PRISMA 2009 guideline. No case control studies could be found, and only one randomized controlled trial (RCT) was available. Therefore, with regards to inclusion and exclusion criteria no restrictions were placed on study design, sample size, or date of release. Study language was restricted to English, German, Danish, Norwegian, or Swedish. Unpublished data were excluded from the review. The intention was to evaluate all studies of patients suffering from subjective tonal tinnitus treated with acoustic CR neuromodulation. Hence, the *a posteriori* inclusion criteria for suitable articles were any original research article concerning tinnitus and acoustic CR neuromodulation, its effect, and/or mechanisms of action. All articles were read by all the three authors.

The following outcomes parameters were identified: visual analog scale (VAS) score (VAS-L: loudness and VAS-A: annoyance), Tinnitus Handicap Inventory, Tinnitus Questionnaire (TQ), Tinnitus Handicap Questionnaire (THQ), The World Health Organization Quality of Life-BREF, tinnitus frequency, spontaneous EEG analysis [including changes regarding connectivity and cross-frequency coupling (CFC) in the tinnitus network], tinnitus pitch change, Tinnitus Functional Index questionnaire, TQ (TBF-12), Global Clinical Improvement-Impression Scale (CGI-I7), and Numeric Rating Scale.

TABLE 1 | Resources searched in following databases.

Resources	No. hits
PubMed	14
Embase	18
Scopus	14
Web of Science	9
SveMed+	0
Cochrane	8
TRIP database	9
ProQuest	22
EBSCOhost	7
Biotechnology research abstract	1
BMJ journal	0
BIBSYS (Oria.no)	1
AMED alternative medicine	0
Others	
http://Clinicaltrials.gov	3
http://Controlled-trials.com	0
http://Clinicaltrialsregister.eu	0

RESULTS

A total of 8 publications were eligible for the review comprising a total of 329 patients in 3 main study populations (**Figure 2**). Details with regard to study population, design, and outcome measures are listed in **Table 2**. Due to the low number of studies, heterogeneous designs, overlap between study populations, and incomparability, each of the studies is commented in the following.

In 2012, Tass et al. published a computational study (27) where they illustrated the simplified neuronal model concept of CR anti-kindling and desynchronizing: an algorithm that was also applicable to the concept of non-invasive acoustic CR neuromodulation. In addition, they pointed to some important features of CR: (1) the pitches of the CR tones should be grouped around the patient-specific tinnitus frequency; (2) CR was effective no matter whether the CR stimulus was confined to the deafferentiated region (most effective) or if both the deafferentiated and non-deafferentiated regions were stimulated; and (3) an optimized spacing of the different CR tone pitches was present, neither too narrow, to ideally stimulate distinct subpopulations, nor too wide, to primarily affect the deafferentiated region. Acoustic CR stimulation requires sufficient hearing ability in the deafferentiated region, which can be achieved by hearing aids. The study suggested that acoustic CR neuromodulation may be a reliable method for the control of synchronization and abnormal interactions in affected neuronal populations.

Subsequently, in a single blind RCT (RESET study) (23), Tass et al. examined the safety and effects of acoustic CR neuromodulation in 63 patients with chronic tonal ST, randomly allocated into 5 different treatment arms (G1–G5). They found the intervention to be safe and well tolerated. Acoustic CR neuromodulation resulted in a significant reduction of tinnitus symptoms, as well as VAS loudness and annoyance, improvement in TQ severity levels, reduction in mean TQ scores, and a reduction of tinnitus frequency. Effects gained in 12 weeks of treatment persisted through a 4-week therapy pause. After a long-term extension period (24 weeks), already gained treatment effects were sustained or improved further. Placebo treatment (G5) did not lead to any significant changes in outcome measures. A comparison between groups revealed that CR therapy was more efficacious

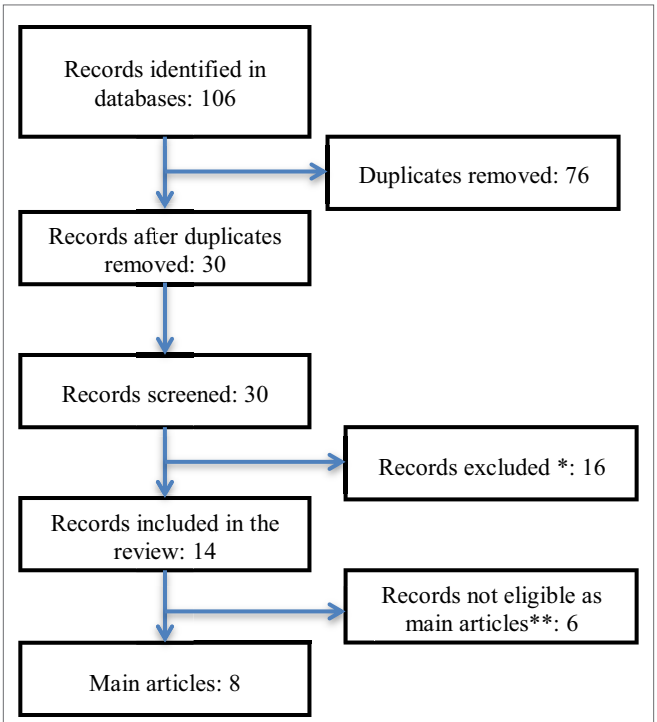

FIGURE 2 | Flowchart of the study selection. *Abstracts, conference–proceedings, reviews, editorials, dissertations and theses, and records not on subject. **Articles assessing endpoints, Hungarian article (language restrictions), not published data, and letters.

when used 4–6 h per day as opposed to 1 h per day. After 12 weeks of therapy, tinnitus associated EEG alterations were reversed. Pathologically elevated δ and γ activity were both decreased in the primary and secondary auditory cortex, as well as in frontal brain areas. Tinnitus-related reduction of α activity was reversed, leading to an enhancement in auditory and prefrontal areas. Thus, CR-induced neuronal changes comprised both auditory and non-auditory brain areas.

Based on existing data from 59 of the 63 patients from the RESET study, Adamchic et al. (28) found a significant correlation between the absolute value of the tinnitus pitch (frequency) change induced by acoustic neuromodulation, and absolute changes of VAS loudness and annoyance scores. The study also found that changes of brain synchrony patters, induced by CR neuromodulation, were associated with pitch change. These changes included the decreases in γ power and increases in α activity in distinctive brain regions, as well as alterations in functional connectivity in the γ frequency band between brain areas of this network. Brain areas found to be associated with these changes involved left temporal cortex, right and left frontal areas, the dorsolateral prefrontal region, and the anterior cingulate cortex. The study discovered that acoustic CR neuromodulation induced tinnitus pitch change, with a simultaneous reduction in tinnitus symptoms and positive changes in oscillatory brain activity.

In another evaluation of the data from the RESET study, Adamchic et al. (29) compared the EEG data from 28 patients with bilateral ST with the spontaneous EEG data from healthy controls. The study showed that acoustic CR neuromodulation shifted the abnormal brain activity associated with tinnitus toward physiological levels. Hence, in a group of "good responders" (TQ improvement >12 points), acoustic CR neuromodulation significantly normalized the patient's brain oscillations and even led to a complete abolishment of pathological power in several brain regions and frequency bands. These changes were significantly correlated with a reduction of tinnitus severity.

In 2013, using the same study population, Silchenko et al. (30) investigated if acoustic CR neuromodulation induced alterations of effective connectivity in the neuronal network underlying tinnitus perception. The effective connectivity in gamma, delta, and alpha frequency bands between brain areas comprising the primary auditory cortex, posterior cingulate cortex, dorsolateral prefrontal areas, and temporal areas were found to be significantly different in ST patients as opposed to healthy controls. When analyzing the types of interactions, they found a significant imbalance of excitation and inhibition in the tinnitus network of brain sources. Acoustic CR therapy significantly altered the strength of these connections so that they approached or even became indistinguishable to the healthy state network structure. Such restoration of effective connectivity was not seen in the group of non-responders.

To further investigate the communicative pathways within the tinnitus network, Adamchic et al. (31) performed a re-analysis of the existing dataset from the RESET study. They found that abnormal CFC[2] in tinnitus patients might coordinate tinnitus-relevant activity and thus provide effective communication between nodes of the tinnitus network. Reduction of tinnitus severity after acoustic CR stimulation led to a partial normalization of abnormal CFC. Treatment-induced tinnitus pitch change significantly modulated changes in CFC.

In 2015, Hauptmann et al. (26) published a prospective, non-randomized, non-controlled, multicenter, and clinical study with 200 chronic tinnitus patients. TQ "Tinnitus-Beeinträchtigungs-Fragebogen TBF12" (TBF-12) and CGI-I7 were used to study the safety and efficacy of acoustic CR neuromodulation. The treatment was found to be well tolerated and with no adverse events. Acoustic CR neuromodulation caused a statistically and clinically significant decrease in TBF-12 scores as well as in CGI-I7 after 12 months of therapy.

The same year, Williams et al. (32), published a clinical case study, where they described the quantitative treatment outcomes of patients undergoing acoustic CR neuromodulation. In line with the abovementioned clinical study, they showed a statistically and clinically significant improvement in tinnitus symptoms after 22–26 weeks of treatment, measured by VAS scores (loudness and annoyance) and THQ.

A protocol for a double-blind RCT study on the evaluation of acoustic CR neuromodulation was suggested by Hoare et al. (34). Although the behavioral results of the study have not been published, they are available on a trial

[2]Cross-frequency coupling (CFC) is a phenomenon proposed to coordinate neural dynamics across spatial and temporal scales (33).

TABLE 2 | Overview of the eight studies included in the review.

Reference, country	Study design	Sample	Outcome measures	Main results
Tass et al. (27), Germany	Computer analysis	None	Coordinated reset (CR) neuromodulation: model presentation illustrating the concept of CR in a simplified neuronal model, considering neurons with spike timing-dependent plasticity transformation of the concept of deep brain stimulation into non-invasive, acoustic CR stimulation	Non-invasive acoustic CR neuromodulation may be a novel therapy for tinnitus
Tass et al. (23), Germany	Prospective, randomized, single blind, placebo-controlled trial: RESET	63	Visual analog scale (VAS) Tinnitus Questionnaire (TQ) Tinnitus frequency Spontaneous EEG	CR neuromodulation caused a significant decrease of tinnitus loudness and symptoms, and reversed tinnitus-related EEG alterations
Adamchic et al. (28), Germany	Part of RESET	59	Tinnitus pitch change versus tinnitus loudness and/or annoyance (VAS score) Changes of brain synchrony induced by CR neuromodulation versus tinnitus pitch change	VAS scores significantly correlated with the absolute value of the CR neuromodulation-induced tinnitus pitch change ($r = 0.92$ baseline to 12 weeks, $p < 0.01$) Significant changes in brain activity were associated with a pronounced tinnitus pitch change
Adamchic et al. (29), Germany	Part of RESET	28	EEG pattern in the tinnitus patients after CR neuromodulation versus EEG pattern in healthy controls EEG in tinnitus patients before and after acoustic CR neuromodulation Relationship between CR neuromodulation-induced changes of different resting EEG parameters and tinnitus symptoms	Tinnitus patients significantly deviated from healthy controls concerning oscillatory brain activity CR neuromodulation significantly normalized patient's brain oscillations in all frequency bands CR neuromodulation-induced normalization of EEG power was significantly associated with reduction of tinnitus severity
Silchenko et al. (30), Germany	Part of RESET	28	Comparison of EEG in tinnitus patients before and after CR neuromodulation Comparison of EEG in tinnitus patients with healthy controls	CR neuromodulation significantly normalized both power and causal interactions within a tinnitus-related network CR neuromodulation specifically counteracted an imbalance of excitation and inhibition in tinnitus patients CR neuromodulation qualitatively changed the spectral response of the tinnitus network by modifying the shape of the averaged transfer function, so that the latter became similar to the control group
Adamchic et al. (31), Germany, USA	Re-analysis of existing dataset from RESET	59	To investigate how the oscillations in the various frequency bands interact	Identification of changes of cross-frequency coupling (CFC) Phase–amplitude CFC increased in tinnitus patients within the auditory cortex and the dorsolateral prefrontal regions between the phase of delta-theta and the amplitude of gamma oscillations Theta phase in the anterior cingulate region modulated gamma in the auditory and dorsolateral prefrontal regions
Hauptmann et al. (26), Germany, UK, USA	Prospective open-label, non-randomized, non-controlled multicenter clinical study	200 23 study centers	TQ (TBF-12) Global Clinical Improvement-Impression Scale (CGI-I7) Numeric Rating Scale (NRS) (0–100)	TBF-12 (total score) showed a mean reduction of 4.1 points (–37.9%) compared to baseline ($p < 0.01$) CGI-I7 revealed that 66.9% of the patients reported an improvement of tinnitus [very much improved (8.7%), much improved (25%), or slightly improved (33.2%)] ($p < 0.01$) Tinnitus-related loudness and annoyance were reduced by 11.1 points (18.9%) and 14.7 points (25.2%), respectively, compared to baseline ($p < 0.01$) on the NRS

(*Continued*)

TABLE 2 | Continued

Reference, country	Study design	Sample	Outcome measures	Main results
Williams et al. (32), UK, Germany	Clinical case study, open-label, non-randomized, non-controlled	66	Tinnitus Handicap Questionnaire (THQ) score VAS for tinnitus annoyance and loudness	VAS scores were significantly improved: 25.8% mean reduction in tinnitus loudness, 32% mean reduction in tinnitus annoyance ($p < 0.01$ compared to baseline) A clinically significant reduction in tinnitus loudness and annoyance was recorded in 59.1 and 72.7% of the patient group, respectively THQ scores were significantly improved by an average of 19.4% ($p < 0.01$) 58.8% of patients experienced a clinically significant reduction in THQ score

outcomes website (https://clinicaltrials.gov/ct2/show/results/NCT01541969?sect=X0125#all). The efficacy of acoustic CR neuromodulation was compared to placebo (tinnitus masking), with crossover of the placebo group to receive the proprietary intervention. The study was completed on February 24, 2016. However, deviations from trial protocol and lack of compliance with the manufacturer's fitting instructions led to doubts about the validity of the results. Hence, results could not be published with evidence, and therefore, it was not included in the present review (35, 36).

DISCUSSION

This review was performed to provide a view over the existing evidence and use of acoustic CR neuromodulation in the treatment of ST. Overall, relatively few publications were found through the search, and the general evidence level was low except for a single RCT. The technique demands assessment of the specific tinnitus frequency and that the patient has (aided) hearing capacity within the given frequency area. A total of 329 patients with chronic tinnitus were included in basically 3 various study populations. Outcome measures were psycho-acoustic tests and EEG. The majority of the included patients reported reduction of tinnitus that was associated with EEG changes toward normalization. The method intends to counteract abnormal synchronization and connectivity through desynchronizing and anti-kindling effects. More specifically, acoustic CR neuromodulation induced pitch change and partially reversed abnormal CFC in a widespread tinnitus network comprising both auditory and non-auditory brain areas. However, several shortcomings of the studies impede generalization of the obtained results and the neurophysiological basis of the method may also be questioned (see below). Thus, the available evidence is insufficient for confident clinical implementation of acoustic CR neuromodulation as a treatment modality for tinnitus at the present moment.

We only identified 8 original reports about acoustic CR neuromodulation based on 3 different populations, i.e., a maximum of 329 patients. Different scopes of the studies, designs, and outcome parameters impede comparison between them as well as use of mutual quality and bias assessment methods. Moreover, many complicated statistical analyses were applied making it difficult to decipher and interpret the results and overall conclusions. Hence, PRISMA guidelines could not be followed in all aspects.

The same authors have generated most of the studies and articles, using the same populations. Independent replication of the positive pilot results is still lacking.

The proof-of-concept study performed by Tass et al. (23) was a RCT—a study design with the greatest evidential value. However, as also pointed out by Rücker and Antes in their reply (37) to Tass et al., the study had several shortcomings: a small number of participants, use of five treatment arms, and most importantly, the lack of direct comparison between the different treatment arms, which is the main idea of RCT studies. Besides baseline to post treatment comparisons within each treatment arm, no direct comparisons between groups were conducted. Tass et al. argued that the study showed quality features not often encountered

in tinnitus research (38). A sample size calculation in order to ensure inclusion of sufficient numbers of participants in the different treatment schedules is recommended prior to such studies: the more subgroups, the more participants. A pilot study is also advantageous, for instance, to determine variation of the outcome measures.

In an additional analysis, different treatment arms were *post hoc* pooled and a comparison between an "effective" versus an "ineffective" group was performed. Such pooling results in loss of information and confounders are introduced due to use of different stimuli within the same group, and a false treatment effect may be the result (37, 39). And finally, the applied statistics were rather complex and unusual (e.g., Euclidean distance).

Clinical series or reports, such as the two clinical case studies (26, 32), are associated with a low level of evidence and the main reason is the absence of a control group. A clinical case study, such as the one conducted by Hauptman et al., is meant to consolidate the results from studies with higher level of evidence, e.g., RESET. With an intention to reinforce previously conducted studies, the risk of bias due to the desire for an effect can be comprised. It should also be mentioned that the use of fee-paying participants in the study by Williams et al. might have affected the results due to selection bias. Despite the low level of evidence, both Hauptman and Williams stated that acoustic CR neuromodulation caused a significant decrease of tinnitus. This trend was seen in different outcome measures.

The clinical case studies differ with respect to inclusion criteria, providing heterogeneous study populations. Future studies should be based on precise inclusion criteria to make them more homogenous. This includes consensus regarding the definition of chronic tinnitus, inclusion of both unilateral and bilateral cases, hearing thresholds, etc. Information about confounding factors such as age, psychiatric disorders, medication, and previously received tinnitus therapy should be addressed. Consensus regarding exclusion criteria is important as well. And finally, use of the same reliable and valid outcome measures in different studies is mandatory for comparing results, e.g., VAS and TQ scores (40, 41).

The fundamental challenge in tinnitus research goes far beyond the abovementioned problems with clinical designs and choice of outcome measures because we still know only little about the underlying pathophysiology. As a consequence hereof, the optimal objective parameters are chosen more or less in the dark, whether EEG or other neuroimaging techniques. In an opinion article by Elgoyhen et al. (42), they critically assessed the results of recent neuroimaging studies of ST. The authors concluded that neuroimaging results are highly variable, but interpretation of available data indicates ST to follow auditory deafferentiation leading to lack of sensory information. Elgoyhen et al. suggested that increased activity in auditory pathways could explain the perception of the sound itself, maybe permitted or facilitated by interactions or communication with non-auditory brain areas. The relevance of cortical map reorganization as the cause of tinnitus was questioned, and the authors pointed out that the apparent distortion of the tonotopic map associated with tinnitus may merely be a compensatory response and not the cause of tinnitus. If so, cortical reorganization becomes less relevant to understanding the mechanisms eliciting the perception of phantom sounds and questions the theoretical basis of acoustic CR neuromodulation. In the light of the suggestions by Elgoyhen et al., the obvious questions are: what do the apparent findings in the reviewed reports represent and what is the background for them? Can listening to series of tones be able to interfere with a complex brain disorder involving several brain structures and functions like tinnitus? Regardless of the physiological mechanisms, the RESET study had limitations in design.

Future studies of acoustic CR neuromodulation should comprise RCTs with strict inclusion and exclusion criteria and using several neuroimaging techniques.

CONCLUSION

Acoustic CR neuromodulation has been introduced as a novel treatment for ST. The current evidence level is low, and the assumed underlying physiological mechanisms have been questioned. Therefore, further studies are needed before the method can be recommended as a tinnitus treatment modality.

DEFINITIONS (22)

Phase reset (43) – Phase resetting in neurons is when the dynamical behavior of an oscillation is shifted. This occurs when a stimulus perturbs the phase within an oscillatory cycle and a change in period occurs.

Synchrony – The relation that exists when things occur at the same time, e.g., when neurons fire simultaneously.

Desynchronization – The reverse of or absence of synchrony. Initially synchronized oscillating (fluctuating) systems desynchronize as parameters (existing conditions) changes, or might do so under influence of external stimulation.

Anti-kindling – Unlearning of pathologically strong interactions and/or connectivity of neural networks. The intention is to approach or even regain physiological activity.

Spike timing-dependent plasticity (STDP) (44) – A process by which neurons continuously regulate the strength of their synaptic connections. The neurons adjust the connection strengths based on the relative timing of a particular neuron's firing (or spikes).

Multistability (45) – Multistability is the characteristic of a system that presents two or more mutually exclusive stable states (attractors) for a given set of parameters or conditions. If a system has multiple coexisting attractors and stimulus is sufficiently strong to cause switching among stable states, it may be said to be multistable.

AUTHOR CONTRIBUTIONS

MW: design, data analysis, drafting, acquisition of data final approval, and responsibility for content of manuscript. TO and DL: data analysis, critically revision, final approval, and responsibility for content of manuscript.

REFERENCES

1. Eggermont JJ, Roberts LE. The neuroscience of tinnitus. *Trends Neurosci* (2004) 27(11):676–82. doi:10.1016/j.tins.2004.08.010
2. Eggermont JJ, Tass PA. Maladaptive neural synchrony in tinnitus: origin and restoration. *Front Neurol* (2015) 6:29. doi:10.3389/fneur.2015.00029
3. Hall DA, Láinez MJ, Newman CW, Sanchez TG, Egler M, Tennigkeit F, et al. Treatment options for subjective tinnitus: self reports from a sample of general practitioners and ENT physicians within Europe and the USA. *BMC Health Serv Res* (2011) 11:302. doi:10.1186/1472-6963-11-302
4. Axelsson A, Prasher D. Tinnitus induced by occupational and leisure noise. *Noise Health* (2000) 2(8):47–54.
5. Noreña AJ. Revisiting the cochlear and central mechanisms of tinnitus and therapeutic approaches. *Audiol Neurotol* (2015) 20(1):53–9. doi:10.1159/000380749
6. Baguley D, McFerran D, Hall D. Tinnitus. *Lancet* (2013) 382(9904):1600–7. doi:10.1016/S0140-6736(13)60142-7
7. Jastreboff PJ. Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci Res* (1990) 8(4):221–54. doi:10.1016/0168-0102(90)90031-9
8. Kemp DT. Stimulated acoustic emissions from within the human auditory system. *J Acoust Soc Am* (1978) 64(5):1386–91. doi:10.1121/1.382104
9. Kaltenbach JA, Zhang J, Finlayson P. Tinnitus as a plastic phenomenon and its possible neural underpinnings in the dorsal cochlear nucleus. *Hear Res* (2005) 206(1–2):200–26. doi:10.1016/j.heares.2005.02.013
10. Kaltenbach JA. The dorsal cochlear nucleus as a contributor to tinnitus: mechanisms underlying the induction of hyperactivity. *Prog Brain Res* (2007) 166:89–106. doi:10.1016/S0079-6123(07)66009-9
11. Levine RA. Somatic (craniocervical) tinnitus and the dorsal cochlear nucleus hypothesis. *Am J Otolaryngol* (1999) 20(6):351–62. doi:10.1016/S0196-0709(99)90074-1
12. Noreña AJ, Eggermont JJ. Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. *Hear Res* (2003) 183(1–2):137–53. doi:10.1016/S0378-5955(03)00225-9
13. Kaltenbach JA. Summary of evidence pointing to a role of the dorsal cochlear nucleus in the etiology of tinnitus. *Acta Otolaryngol Suppl* (2006) (556):20–6. doi:10.1080/03655230600895309
14. Hoare D, Sereda M, Adjamian P, Hall D. Recent technological advances in sound-based approaches to tinnitus treatment: a review of efficacy considered against putative physiological mechanisms. *Noise Health* (2013) 15(63):107. doi:10.4103/1463-1741.110292
15. Eggermont JJ, Roberts LE. The neuroscience of tinnitus: understanding abnormal and normal auditory perception. *Front Syst Neurosci* (2012) 6:53. doi:10.3389/fnsys.2012.00053
16. Hoare DJ, Searchfield GD, Refaie AE, Henry JA. Sound therapy for tinnitus management: practicable options. *J Am Acad Audiol* (2014) 30(1):62–75. doi:10.3766/jaaa.25.1.5
17. Tass PA, Silchenko AN, Hauptmann C, Barnikol UB, Speckmann E-J. Long-lasting desynchronization in rat hippocampal slice induced by coordinated reset stimulation. *Phys Rev E Stat Nonlin Soft Matter Phys* (2009) 80(1):11902. doi:10.1103/PhysRevE.80.011902
18. Tass PA, Qin L, Hauptmann C, Dovero S, Bezard E, Boraud T, et al. Coordinated reset has sustained aftereffects in parkinsonian monkeys. *Ann Neurol* (2012) 72(5):816–20. doi:10.1002/ana.23663
19. Adamchic I, Hauptmann C, Barnikol UB, Pawelczyk N, Popovych O, Barnikol TT, et al. Coordinated reset neuromodulation for Parkinson's disease: proof-of-concept study. *Mov Disord* (2014) 29(13):1679–84. doi:10.1002/mds.25923
20. Tass PA. A model of desynchronizing deep brain stimulation with a demand-controlled coordinated reset of neural subpopulations. *Biol Cybern* (2003) 89(2):81–8. doi:10.1007/s00422-003-0425-7
21. Tass PA, Majtanik M. Long-term anti-kindling effects of desynchronizing brain stimulation: a theoretical study. *Biol Cybern* (2006) 94(1):58–66. doi:10.1007/s00422-005-0028-6
22. Popovych OV, Tass PA. Control of abnormal synchronization in neurological disorders. *Front Neurol* (2014) 5:268. doi:10.3389/fneur.2014.00268
23. Tass PA, Adamchic I, Freund HJ, Von Stackelberg T, Hauptmann C. Counteracting tinnitus by acoustic coordinated reset neuromodulation. *Restor Neurol Neurosci* (2012) 30(2):137–59. doi:10.3233/RNN-2012-110218
24. Lysyansky B, Popovych OV, Tass PA. Desynchronizing anti-resonance effect of m: n ON–OFF coordinated reset stimulation. *J Neural Eng* (2011) 8(3):36019. doi:10.1088/1741-2560/8/3/036019
25. Chittka L, Brockmann A. Perception space—the final Frontier. *PLoS Biol* (2005) 3(4):e137. doi:10.1371/journal.pbio.0030137
26. Hauptmann C, Ströbel A, Williams M, Patel N, Wurzer H, von Stackelberg T, et al. Acoustic coordinated reset neuromodulation in a real life patient population with chronic tonal tinnitus. *Biomed Res Int* (2015) 2015:569052. doi:10.1155/2015/569052
27. Tass PA, Popovych OV. Unlearning tinnitus-related cerebral synchrony with acoustic coordinated reset stimulation: theoretical concept and modelling. *Biol Cybern* (2012) 106(1):27–36. doi:10.1007/s00422-012-0479-5
28. Adamchic I, Hauptmann C, Tass PA. Changes of oscillatory activity in pitch processing network and related tinnitus relief induced by acoustic CR neuromodulation. *Front Syst Neurosci* (2012) 6:18. doi:10.3389/fnsys.2012.00018
29. Adamchic I, Toth T, Hauptmann C, Tass PA. Reversing pathologically increased EEG power by acoustic coordinated reset neuromodulation. *Hum Brain Mapp* (2014) 35(5):2099–118. doi:10.1002/hbm.22314
30. Silchenko AN, Adamchic I, Hauptmann C, Tass PA. Impact of acoustic coordinated reset neuromodulation on effective connectivity in a neural network of phantom sound. *Neuroimage* (2013) 77:133–47. doi:10.1016/j.neuroimage.2013.03.013
31. Adamchic I, Langguth B, Hauptmann C, Tass PA. Abnormal cross-frequency coupling in the tinnitus network. *Front Neurosci* (2014) 8:284. doi:10.3389/fnins.2014.00284
32. Williams M, Hauptmann C, Nitesh P. Acoustic CR neuromodulation therapy for subjective tonal tinnitus: a review of clinical outcomes in an independent audiology practice setting. *Front Neurol* (2015) 6:54. doi:10.3389/fneur.2015.00054
33. Aru J, Aru J, Priesemann V, Wibral M, Lana L, Pipa G, et al. Untangling cross-frequency coupling in neuroscience. *Curr Opin Neurobiol* (2015) 31:51–61. doi:10.1016/j.conb.2014.08.002
34. Hoare DJ, Pierzycki RH, Thomas H, McAlpine D, Hall DA. Evaluation of the acoustic coordinated reset (CR®) neuromodulation therapy for tinnitus: study protocol for a double-blind randomized placebo-controlled trial. *Trials* (2013) 14(1):207. doi:10.1186/1745-6215-14-207
35. Hoare DJ, Pierzycki RH, Thomas H, Hall DA. *Evaluation of the CR Neuromodulation Treatment for Tinnitus – Study Results – ClinicalTrials*. (2015). Available from: https://clinicaltrials.gov/ct2/show/results/NCT01541969?sect=X01256#all
36. Hall DA, Hoare DJ, Pierzycki RH, et al. *59257caf5076ef0653477c-5964fa1512e468b0e8*. (2015). Available from: www.hearing.nihr.ac.uk; http://www.hearing.nihr.ac.uk/research/a-systematic-evaluation-of-the-acoustic-cr-neuromodulation-treatment-for-ti/
37. Rücker G, Antes G. Reply to Tass et al. on "Counteracting tinnitus by acoustic coordinated reset neuromodulation". *Restor Neurol Neurosci* (2012) 30(2); *Restor Neurol Neurosci* (2013) 31(3):235–7. doi:10.3233/RNN-121123
38. Tass PA, Adamchic I, Freund H-J, von Stackelberg T, Hauptmann C. Rebuttal to reply by G. Rücker and G. Antes on Tass et al. "Counteracting tinnitus by acoustic coordinated reset neuromodulation". *Restor Neurol Neurosci* (2012) 30(2); *Restor Neurol Neurosci* (2013) 31(3):235–7. doi:10.3233/RNN-121123
39. Senn S, Julious S. Measurement in clinical trials: a neglected issue for statisticians? *Stat Med* (2009) 28(26):3189–209. doi:10.1002/sim.3603
40. Adamchic I, Langguth B. Psychometric evaluation of Visual Analog Scale for the assessment of chronic tinnitus. *Am J Audiol* (2012) 21:215–26. doi:10.1044/1059-0889(2012/12-0010)impairment
41. Adamchic I, Tass PA, Langguth B, Hauptmann C, Koller M, Schecklmann M, et al. Linking the Tinnitus Questionnaire and the subjective Clinical Global Impression: which differences are clinically important? *Health Qual Life Outcomes* (2012) 10(1):79. doi:10.1186/1477-7525-10-79
42. Elgoyhen AB, Langguth B, De Ridder D, Vanneste S. Tinnitus: perspectives from human neuroimaging. *Nat Rev Neurosci* (2015) 16(10):632–42. doi:10.1038/nrn4003
43. Krogh-Madsen T, Butera R, Ermentrout GB, Glass L. Chapter 2: Phase resetting neural oscillators: topological theory versus the realworld. In: Schultheiss NW, Prinz AA, Butera RJ, editors. *Phase Response Curves in Neuroscience, Theory, Experience End Analysis*. Vol. 6. New York: Springer New York (2012).

44. Markram H, Gerstner W, Sjöström PJ. Spike-timing-dependent plasticity: a comprehensive overview. *Front Synaptic Neurosci* (2012) 4:2. doi:10.3389/fnsyn.2012.00002
45. Kelso JAS. Multistability and metastability: understanding dynamic coordination in the brain. *Philos Trans R Soc Lond B Biol Sci* (2012) 367:906–18. doi:10.1098/rstb.2011.0351

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OPINION
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Evaluation of the Acoustic Coordinated Reset (CR®) Neuromodulation Therapy for Tinnitus: Update on Findings and Conclusions

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BACKGROUND

The therapeutic effects of Coordinated Reset (CR®) Neuromodulation were originally discovered by researchers at the Forschungzentrum Juelich GmbH (FZJ) in Germany, under the supervision of Tass et al. (2012a).

Based on confirmatory research in primates, initial results of Prof. Tass' research suggested that the plastic modification of the behavior of neuron populations by induced desynchronization (i.e., a process of "unlearning" of both pathological neuronal synchrony and pathological synaptic connectivity) results in a substantial and long-lasting reduction of disease symptoms (Tass et al., 2012b; Adamchic et al., 2014a).

In this set of experiments, Parkinson's-induced monkey models were treated in independent laboratories with Coordinated Reset (CR®) Neuromodulation through the well-established deep brain stimulation (DBS) technique. Standard high frequency DBS was used as a control condition. The stimulation lasted 2 h a day for 5 consecutive days. In both experiments the primates remained symptom-free for over 30 days after CR stimulation was ceased (Tass et al., 2012b; Wang et al., 2016).

Subsequent trials in human patients showed alleviation of Parkinson's symptoms such as tremor, akinesia, dystonia, etc., for many hours after only short periods of CR® Neuromodulation (4 h for 3 consecutive days) (Adamchic et al., 2014a).

These results led to an expansion and further development of the CR® Neuromodulation program in different research centers worldwide. Additional focus was given to the application of CR® via other sensory inputs, e.g., acoustic, tactile, visual, etc. That eventually led to the development of a treatment program for tinnitus.

There is a general consensus that the percept of tinnitus is triggered by cochlear damage, which degrades the auditory input to central neural pathways. This initiates physiological and electrical changes within the auditory areas that result in aberrant patterns of neuronal activity interpreted as sound.

Acoustic CR® Neuromodulation is designed as a patient specific targeted sound therapy. The treatment has been provided to over 3,000 patients worldwide to date. Several peer-reviewed papers have reported reduction of tinnitus symptoms (from baseline) in populations of tinnitus sufferers by using a portable acoustic neurostimulator providing Acoustic CR® Neuromodulation; including tinnitus loudness measured using a visual analog scale (Tass et al., 2012a; Williams et al., 2015), tinnitus annoyance measured using a visual analog scale (Tass et al., 2012a; Bencsik et al., 2015; Williams et al., 2015), and various multi-attribute scales used to measure tinnitus symptom severity, i.e., Tinnitus Questionnaire (Tass et al., 2012a), Tinnitus Handicap Questionnaire (Williams et al., 2015), and Tinnitus Handicap Inventory (Bencsik et al., 2015; Hauptmann et al., 2015).

Both accurate tone "location" of the tinnitus percept on the auditory cortex, as well as the timing and sequence of the tone based impulses have been reported to be important factors in effective inducement of neuronal desynchronization. In the studies referred to above, such neural desynchronization led to a reduction of tinnitus symptoms. Because accurate tone location in the auditory cortex was found to be important, a proprietary tinnitus pitch matching procedure was developed and deployed in order to calibrate the patient specific sound therapy tones.

Because tinnitus is a highly subjective pathology, it has been proven to be difficult to assess with objective and standardized tests. In these circumstances, any placebo effects can have a great impact on treatment of tinnitus, as well as its personal percept.

In October 2011, Nottingham University Hospitals NHS Trust agreed to conduct an investigator-led evaluation of acoustic CR neuromodulation versus placebo effects, recruiting 100 participants with a 1:1 allocation ratio (Hoare et al., 2013) (ClinicalTrials.gov NCT01541969). Participants were adults aged $\geq$18 years of age, chronic subjective tinnitus for more than 3months with a minimum score of 18 points measured using the Tinnitus Handicap Inventory and a dominant tinnitus pitch corresponding to a frequency between 0.2 and 10 kHz, and with an average hearing loss no greater than 60 dB (0.5, 1, 2, and 4 kHz).

MAIN TRIAL RESULTS AND POTENTIAL EXPLANATION OF THE OBSERVATIONS

The formal results of the RESET2 trial (ClinicalTrials.gov identifier: NCT01541969) were non-conclusive, which was not expected, considering the past and current volumes of observational data showing substantial benefit (Hoare et al., 2013).

In Tass et al. (2012a) and in a number of later publications (Hauptmann et al., 2015; Williams et al., 2015) statistically and clinically significant improvements have been reported in visual analog scales, in tinnitus loudness and annoyance scores (reduction of 53 and 49% after 12 weeks of therapy, respectively, on stimulation), and in tinnitus questionnaire results (reduction of 29% after 12 weeks of therapy) results (Adamchic et al., 2012b, 2014b; Tass et al., 2012a). Furthermore, the measured tinnitus pitch was reduced by 28.5% in the first 12 weeks of treatment (Tass et al., 2012a), and the observed tinnitus pitch change was correlated with a treatment-induced reduction of tinnitus loudness and/or annoyance and changes in oscillatory brain activity (Adamchic et al., 2012a; Tass et al., 2012a). The EEG results indicated a substantial, CR-induced reduction of tinnitus-related auditory binding in a pitch-processing network associated with the therapeutic procedure, where a readjustment of stimulation parameters was performed at each visit, provided the matched tinnitus frequency had changed (Adamchic et al., 2012a).

Whilst the RESET2 trial at NHS did not observe a significant difference between treatment and placebo in experimental conditions, all the more recent above mentioned clinical studies report significant improvements in patient populations.

Tinnitus questionnaires could have reduced responsiveness to treatment-related change when a large proportion of participants respond at baseline at either extreme of the response scale. The so-called "floor effects" (scores at the minimum of the response scale) reduce sensitivity to detecting group-level improvement, while "ceiling effects" (scores at the maximum of the response scale) reduce sensitivity to detecting group-level worsening (Terwee et al., 2007). In a more recent analysis of the baseline TFI (Tinnitus Functional Index; Meikle et al., 2012) data from RESET2, Fackrell et al. (2016) found over 50% of questionnaire items scored at either floor or ceiling. This evidence certainly warrants caution in future studies with similar use of these tinnitus assessment questionnaires.

The exact application as defined of the proprietary tinnitus pitch matching procedure is paramount in order to observe any beneficial effect. Sub-optimal pitch matching will potentially lead to incorrectly computed patient specific therapy tones, which may not have any more effect than a placebo. The study-specific pitch matching procedure used in RESET2 differed from manufacturer's recommendations (Hall et al., 2016).

Professor Tass' team has since validated and launched an automated adaptive pitch-matching method (Hauptmann et al., 2016) to prevent deviations from a recommended pitch-matching procedure, which has since been utilized in all commercial applications of Acoustic CR® as well as in current and future clinical trials. The authors concluded that this new procedure offers more guidance to the audiologist and patient which is seen as of particular importance for a uniform and standardized application of pitch-matching in clinical trials. Such standardization is expected to improve the quality of measures in tinnitus therapy effectiveness.

It is reasonable to question whether only 12-weeks of device usage is sufficient for the benefit of therapy to accrue. It is noted that more recent clinical studies report alleviation of tinnitus after 6 and 12 months of therapy (Hauptmann et al., 2015; Williams et al., 2015), and data on tinnitus status at 3-, 6-, and 12-months post-fitting indicate a gradual decline in mean self-reported tinnitus severity measured using a shortened version of the Tinnitus Handicap Inventory (Hauptmann et al., 2015). The RESET2 trial design does not permit anything more than mere speculation, because all participants exited the RCT at 3 months and were unblinded to the intervention.

Abbreviations: CR®, coordinated reset; DBS, deep brain stimulation; TFI, Tinnitus Functional Index.

CONCLUSIONS

Recommendation

The authors therefore consider that controlled trials to test clinical effectiveness of Acoustic CR Neuromodulation for tinnitus are worthwhile. With the knowledge gained from the RESET2 trial, it is suggested that future trials should include a placebo or "usual standard of care" control group that is well characterized, should follow a well-defined and trained pitch matching protocol, should assess the status of tinnitus for longer than 12 weeks (we suggest at least 6 months), should better control the baseline characteristics to avoid floor and ceiling effects and should use an outcome instrument with known measurement properties for the target population.

What Else Could Be Learned from the RESET2 Trial?

Among the many other aspects learned another key fact was demonstrated by the RESET2 results:

During the trial 50 patients received stimulation from tones delivered at frequencies very different from the tinnitus frequency perceived by the patient and were therefore independent of used pitch-matching procedure. These stimulating tones were intended to be placebo control tones, as the trial was designed to be a double blind trial. The frequency of these tones was very different from the frequency of the tones which would have been deployed had these patients been receiving Acoustic CR® Neuromodulation treatment. Only a small "placebo-" benefit, i.e., <7.5% of an improvement in THQ (Tinnitus Handicap Questionnaire) was observed in these patients receiving the placebo treatments.

ETHICS, CONSENT, AND PERMISSIONS

Permission to conduct the study was granted by the National Research Ethics Service (NRES) Committee, East Midlands–Nottingham 1, Nottingham, UK. Written informed consent was obtained from each participant in accordance with the permissions granted. For further details see Hoare et al. (2013).

CONSENT FOR PUBLICATION

For details see Hoare et al. (2013).

AVAILABILITY OF DATA AND MATERIAL

See ClinicalTrials.gov NCT01541969.

AUTHOR CONTRIBUTIONS

Both authors read and approved the final manuscript. DH planned and conducted the trial. MH and DH performed analysis and discussed the results. Both authors drafted the manuscript and were involved in final editing.

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REFERENCES

Adamchic, I., Hauptmann, C., Barnikol, U. B., Pawelczyk, N., Popovych, O., Barnikol, T. T., et al. (2014a). Coordinated reset neuromodulation for Parkinson's disease: proof-of-concept study. *Mov. Disord.* 29, 1679–1684. doi: 10.1002/mds.25923

Adamchic, I., Hauptmann, C., and Tass, P. A. (2012a). Changes of oscillatory activity in pitch processing network and related tinnitus relief induced by acoustic CR neuromodulation. *Front. Sys. Neurosci.* 6:18. doi: 10.3389/fnsys.2012.00018

Adamchic, I., Langguth, B., Hauptmann, C., and Tass, P. A. (2014b). Abnormal cross-frequency coupling in the tinnitus network. *Front. Neurosci.* 8:284. doi: 10.3389/fnins.2014.00284

Adamchic, I., Tass, P. A., Langguth, B., Hauptmann, C., Koller, M., Schecklmann, M., et al. (2012b). Linking the tinnitus questionnaire and the subjective clinical global impression: which differences are clinically important? *Health Qual. Life Outcomes.* 10:79. doi: 10.1186/1477-7525-10-79

Bencsik, B., Gáborján, A., Harnos, A., László, K., Végso, P., and Tamás, L. (2015). Acoustic CR®-Neuromodulation-First experiences in Hungary with a novel method in the therapy of chronic subjective tinnitus. *Ideggyogy. Sz.* 68, 189–198. doi: 10.18071/isz.68.0189

Fackrell, K., Hall, D. A., Barry, J. G., and Hoare, D. J. (2016). Psychometric properties of the Tinnitus Functional Index (TFI): assessment in a UK research volunteer population. *Hear. Res.* 335, 220–235. doi: 10.1016/j.heares.2015.09.009

Hall, D. A., Pierzycki, R. H., Thomas, H., and Hoare, D. J. (2016). Designing and conducting a double-blind randomized placebo-controlled trial of a novel sound therapy for tinnitus: a commentary on medical device trials in ENT and Audiology. *Ann. Oto Rhinol.* 3:1101. Available online at: www.jscimedcentral.com/Otolaryngology/otolaryngology-3-1101.pdf

Hauptmann, C., Ströbel, A., Williams, M., Patel, N., Wurzer, H., von Stackelberg, T., et al. (2015). Acoustic coordinated reset neuromodulation in a real life patient population with chronic tonal tinnitus. *Biomed Res. Int.* 2015:569052. doi: 10.1155/2015/569052

Hauptmann, C., Wegener, A., Poppe, H., Williams, M., Popelka, G., and Tass, P. A. (2016). Validation of a mobile device for Acoustic Coordinated Reset Neuromodulation tinnitus therapy. *J. Am. Acad. Audiol.* 27, 720–731. doi: 10.3766/jaaa.15082

Hoare, D. J., Pierzycki, R. H., Thomas, H., McAlpine, D., and Hall, D. A. (2013). Evaluation of the acoustic coordinated reset (CR®) neuromodulation therapy for tinnitus: study protocol for a double-blind randomized placebo-controlled trial. *Trials* 14:207. doi: 10.1186/1745-6215-14-207

Meikle, M. B., Henry, J. A., Griest, S. E., Stewart, B. J., Abrams, H. B., McArdle, R., et al. (2012). The tinnitus functional index: development of a new clinical measure for chronic, intrusive tinnitus. *Ear. Hear.* 33, 153–176. doi: 10.1097/AUD.0b013e31822f67c0

Tass, P. A., Adamchic, I., Freund, H. J., von Stackelberg, T., and Hauptmann, C. (2012a). Counteracting tinnitus by acoustic coordinated reset neuromodulation. *Restor. Neurol. Neurosci.* 30, 137–159. doi: 10.3233/RNN-2012-110218

Tass, P. A., Qin, L., Hauptmann, C., Dovero, S., Bezard, E., Boraud, T., et al. (2012b). Coordinated reset has sustained aftereffects in Parkinsonian monkeys. *Ann Neurol*. 72, 816–820. doi: 10.1002/ana.23663

Terwee, C. B., Bot, S. D., de Boer, M. R., van der Windt, D. A., Knol, D. L., Dekker, J., et al. (2007). Quality criteria were proposed for measurement properties of health status questionnaires. *J. Clin. Epidemiol.* 60, 34–42. doi: 10.1016/j.jclinepi.2006.03.012

Wang, J., Nebeck, S., Muralidharan, A., Johnson, M. D., Vitek, J. L., and Baker, K. B. (2016). Coordinated Reset deep brain stimulation of subthalamic nucleus produces long-lasting, dose-dependent motor improvements in the 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine non-human primate model of parkinsonism. *Brain Stimul*. 9, 609–617. doi: 10.1016/j.brs.2016.03.014

Williams, M., Hauptmann, C., and Patel, N. (2015). Acoustic CR neuromodulation therapy for subjective tonal tinnitus: a review of clinical outcomes in an independent audiology practice setting. *Front. Neurol.* 6:54. doi: 10.3389/fneur.2015.00054

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ORIGINAL RESEARCH
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Heidelberg Neuro-Music Therapy Enhances Task-Negative Activity in Tinnitus Patients

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Background: Suffering from tinnitus causes mental distress in most patients. Recent findings point toward a diminished activity of the brain's default-mode network (DMN) in subjects with mental disorders including depression or anxiety and also recently in subjects with tinnitus-related distress. We recently developed a therapeutic intervention, namely the Heidelberg Neuro-Music Therapy (HNMT), which shows an effective reduction of tinnitus-related distress following a 1-week short-term treatment. This approach offers the possibility to evaluate the neural changes associated with the improvements in tinnitus distress. We previously reported gray matter (GM) reorganization in DMN regions and in primary auditory areas following HNMT in cases of recent-onset tinnitus. Here we evaluate on the same patient group, using functional MRI (fMRI), the activity of the DMN following the improvements tinnitus-related distress related to the HNMT intervention.

Methods: The DMN activity was estimated by the task-negative activation (TNA) during long inter-trial intervals in a word recognition task. The level of TNA was evaluated twice, before and after the 1-week study period, in 18 treated tinnitus patients ("treatment group," TG), 21 passive tinnitus controls (PTC), and 22 active healthy controls (AC). During the study, the participants in TG and AC groups were treated with HNMT, whereas PTC patients did not receive any tinnitus-specific treatment. Therapy-related effects on DMN activity were assessed by comparing the pairs of fMRI records from the TG and PTC groups.

Results: Treatment of the TG group with HNMT resulted in an augmented DMN activity in the PCC by 2.5% whereas no change was found in AC and PTC groups. This enhancement of PCC activity correlated with a reduction in tinnitus distress (Spearman Rho: -0.5; $p < 0.005$).

Conclusion: Our findings show that an increased DMN activity, especially in the PCC, underlies the improvements in tinnitus-related distress triggered by HNMT and identify the DMN as an important network involved in therapeutic improvements.

Keywords: fMRI, tinnitus, Heidelberg Model of Music Therapy, RSN, neuroplasticity, recent-onset tinnitus, precuneus

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INTRODUCTION

Suffering from tinnitus causes mental distress in most patients. Especially in situations with reduced external sensory input (e.g., at night, in silent rooms), the tinnitus percept comes to the fore and can cause emotional distress (Møller, 2016). Focused attention on the tinnitus can trigger sleeping disorders and impair concentration, regeneration as well as mood (Husain, 2016).

Over the past 15 years, great effort has been made to clarify the origin and tinnitus sensitivity of the so-called "task-negative activation" (TNA) during the brains "resting state" when less sensory input needs to be processed (Raichle et al., 2001; Buckner et al., 2008, 2013). Numerous experiments using functional Magnetic Resonance Imaging (fMRI) contrasted resting state brain activity during phases of less salient stimulation (TNA) vs. brain activity during active task performance (task-positive activation; Shulman et al., 1997; Buckner, 2013). Doing nothing also activates these regions of the brain's resting state (Fox et al., 2005). Whereas, the task-positive activity usually varies with study design, the task negative activity shows a very consistent task-independent pattern (Gusnard and Raichle, 2001). These TNAs in phases of no activity converge with the resting state network (RSN) in areas of the brain's "Default-mode Network" (DMN) comprising the medial prefrontal cortex (MPF), the posterior cingulate cortex (PCC) with an extension to the precuneus, and the lateral parietal cortex (LP; Raichle, 2015).

The functional meaning of the DMN is assigned to mental relaxation, introspective and autobiographical self-awareness, emotional regulation, and internal mental projections between past and future (Gusnard et al., 2001; Andrews-Hanna, 2012). The intrinsic DMN activation has been shown to be reduced in patients with psychiatric disorders (Broyd et al., 2009; Sripada et al., 2012) but also in patients experiencing tinnitus-related distress (De Ridder et al., 2014; Husain and Schmidt, 2014; Lanting et al., 2016; Leaver et al., 2016). However, opposite findings with respect to recent-onset tinnitus have been observed, though focused on the precuneus, too (Carpenter-Thompson et al., 2015). Thus, it seems consequential to further explore the relationship between precuneus and tinnitus distress.

Impaired resting state due to tinnitus perception influences the dorsal attention system which is related to top-down attention control during task execution (Husain, 2016). The impaired DMN function is also thought to contribute to the typical stress-related symptoms such as sleep disturbance, anxiety, depression, irritation, and concentration difficulties (Langguth, 2011; Schmidt et al., 2013; Vanneste and De Ridder, 2015; Møller, 2016).

Neural networks for tinnitus are present in auditory and non-auditory areas (De Ridder et al., 2014; Elgoyhen et al., 2015). Findings on TNAs and neural connectivity results from resting-state fMRI indicated that the PCC/precuneus region is also corrupted by tinnitus (Schmidt et al., 2013; Han et al., 2015; Vanneste and De Ridder, 2015; Lanting et al., 2016) due to its role in the brain's sleep control (Shannon et al., 2013) which in turn is impaired by tinnitus (Alster et al., 1993). However, the relationship between distress and precuneus involvement is still unclear. Following the observations of Lanting et al. (2016) and Schmidt et al. (2013), we argued that the state of mental rest may be corrupted by the tinnitus, since it induces a salient stimulus that in turn reduces DMN activation at rest. We measured task-positive and task-negative activity using fMRI in a visual word recognition task in tinnitus patients and healthy controls both treated with HNMT, as well as in untreated tinnitus patients. The levels of task-negative activity before and after the 1-week treatment period were contrasted to observe their specific alterations induced by HNMT. Since the HNMT has been shown to rapidly reduce the tinnitus distress, this design allowed us to observe the relationship between level of distress and TNAs. Our hypothesis argues that HNMT would allow tinnitus patients to reactivate their DMN after the treatment.

Recent findings from music therapy research revealed an inverse cortical reorganization in the DMN areas following a 1-week application of the Heidelberg Neuro-Music Therapy (HNMT): growth of gray matter (GM) density has been found in the PCC and the precuneus, suggesting a potential influence of HNMT onto DMN activity (Krick et al., 2015). Although, the neuroplastic changes can be deemed as an indirect indicator for a long-term alteration of the activity level, the best evidence for the activity behavior consists in examining the activity itself. This paper aims to evaluate changes in the DMN activity induced by HNMT.

MATERIALS AND METHODS

Participants

In this study, we included data from participants which were recorded in the context of a recent therapy control study where data from structural MRI measurements have already been reported (Krick et al., 2015). In this context, data for Blood Oxygen Level Dependency (BOLD) imaging were derived parallel to the structural measurements. However, whereas the previous study observed structural changes following HNMT longitudinally, the current study addressed changes in brain activity in the same participants. This convergence allowed complementary viewing both perspectives in the same brain regions.

Fifty patients with recent onset of tinnitus (between 6 and 12 weeks prior to the intervention) were invited to participate in the HNMT study after a treatment following the standard clinical protocol for acute tinnitus in the University Hospital for Ear, Nose, and Throat at the University of Heidelberg. Audiograms and psychological examination were conducted before the decision for study inclusion. All patients had age-appropriate hearing levels (audiograms are attached as Supplementary Material) and reported no otological or psychological co-morbidity. Severe hearing impairment was an exclusion criterion in this study. At the time of the pre-participation evaluation (T0), patients were randomly assigned to one of two groups: either a treatment group (TG) receiving a 5-day 1-week treatment with HNMT or a group of passive tinnitus controls (PTC) who did not receive any tinnitus specific treatment. The patient groups did not differ in biographical characteristics nor in tinnitus-related parameters (**Table 1**). For ethical reasons, PTC patients

TABLE 1 | Overview of participant groups and tinnitus parameters.

	TG ($n = 18$)	PTC ($n = 21$)	AC ($n = 22$)	Statistics
Tinnitus causation [acute hearing loss/noise trauma/distress/other] (*n*)	1/7/6/4	2/6/8/5		$\chi^2_{(df\ =\ 1)} = 0.489\ p = 0.484$
Type of tinnitus [tonal/non-tonal] (n)	10/8	12/9		$\chi^2_{(df=1)} = 0.170\ p = 0.680$
Tinnitus frequency (Hz) [mean (*SD*)]	5,112 (2,382)	6,369 (3,193)		$t_{(df\ =\ 41)} = -1.469\ p = 0.154$
Tinnitus localisation [right/left/bilateral/not determinable] (*n*)	5/7/4/2	4/8/5/4		$\chi^2_{(df\ =\ 1)} = 0.146\ p = 0.703$
TQ score from initial anamnestic diagnostics [mean (*SD*)]	38.50 (15.4)	36.20 (16.82)		$t_{(df\ =\ 41)} = 0.737\ p = 0.465$
Tinnitus duration up to initial anamnestic diagnostics (T_0) (weeks) [mean (*SD*)]	5.10 (2.14)	4.63 (2.01)		$t_{(df\ =\ 41)} = 0.567\ p = 0.575$
Tinnitus duration up to start of therapy (T_1) (weeks) [mean (*SD*)]	8.14 (1.85)	8.10 (1.45)		$t_{(df\ =\ 41)} = 0.082\ p = 0.935$
Patients' age (years) [mean (*SD*)]	43.9 (10.4)	42.6 (11.5)	38.9 (14.0)	Kruskal–Wallis test $\chi^2_{(df\ =\ 2)} = 1.54\ p = 0.464$
Patients' sex [male/female] (n)	10/8	13/8	12/10	$\chi^2_{(df\ =\ 2)} = 0.273\ p = 0.873$

also received therapeutic intervention, but this was delivered subsequent to the study period. Participants in both groups were informed about MRI measurements and the noise level of the scanner. All participants were insured for any health impairment and accidents. They gave written informed consent in accordance with the Declaration of Helsinki. The study was approved by the ethical review board of Saarland (ID-number 111/11).

Seven patients were excluded due to disappearance of tinnitus before the first MRI session. Two patients were excluded because of claustrophobia during the first MRI session. One patient was excluded due to image artifacts in the anatomical scan. Thus, the effective sample comprised 19 patients in the TG group and 22 patients in the PTC group. The mean delay between onset of tinnitus and first MRI session (T1) was 8.14 (*SD* 1.85) weeks in the TG group and 8.10 (*SD* 1.45) weeks in the PTC group [$t_{(df\ =\ 41)} = 0.082; p = 0.935$].

A group of 22 healthy participants were recruited to match the sex and age profile of the tinnitus patients. This group served as active controls (AC) as they also underwent HNMT as implemented in the TG group. Due to motion artifacts, two fMRI data sets had to be discarded from analysis (one from TG and one from PTC). Thus, the sample size comprised 61 participants in the present report in contrast to the original 63 reported in the preceding paper (Krick et al., 2015). The groups and the respective parameters are summarized in **Table 1**.

Intervention (HNMT)

The study protocol consisted of nine consecutive sessions of individualized therapy, comprising acoustic training for frequency discrimination, auditory attention control tasks, and guided exercises for mindfulness and distress regulation. Therapy took place on 5 consecutive days (from Monday to Friday) with two therapy sessions per day (except Friday with one session). Music therapy can be divided into two main categories, receptive (music listening based) and active (music making). Each morning and each afternoon session lasted for 50 min, with 25 min of active music therapy and 25 min of receptive music therapy. Two trained music therapists carried out the therapy. One therapist performed the active modules, the other the receptive modules. The interventions were structured into treatment modules of directive counseling, habituation training, and stress management. A more detailed description can be found in Argstatter et al. (2014) and Grapp et al. (2013).

Distress Evaluation

Psychological complaints were assessed using the German version of the "Tinnitus Questionnaire" (TQ) as described by Goebel and Hiller (1998). This well-validated inventory comprises 52 items and records tinnitus related complaints. The items can be aggregated to variables representing the dimensions of mental distress: emotional and cognitive load, tinnitus duration, hearing impairments, sleep disturbance, and somatoform disorders. The global TQ-score ranges between the minimum score of 0 and the maximum score of 84, where high values indicate high tinnitus-related distress. Four levels of severity have been established for mild (0–30), middle (31–46), severe (47–59), and very severe (60–84) distress.

TQ scores were obtained at start of the study period (T1), and after the study week (T2). These two measurements were compared to quantify subjective alterations in the level of tinnitus-related distress over the study week.

The results of distress evaluation for the participants of the current study have been reported in the context of structural findings in Krick et al. (2015). In the TG and PTC groups, the initial TQ scores at T0 were similar at 38.50 ± 15.4 (*SD*) and 36.20 ± 16.82 (*SD*), respectively, [$t_{(df\ =\ 41)} = 0.737; p = 0.465$]. The HNMT application resulted in a significant difference in TQ scores ($T = -5.7$, $df = 18$, $p < 0.0001$) between the TG and PTC groups. In the TG group, the tinnitus distress score decreased by 17.7 TQ scale points (*SD* 13.6). In the PTC group, the TQ scores did not significantly change. The therapy effect on the subjective distress was further confirmed by a 2 × 2 repeated measures ANOVA ($df = 1$; $F = 22.9$; MSE = 1374; $p < 0.00005$).

Imaging Paradigm

DMN activity was assessed by means of event-related fMRI observing activation for rest conditions in contrast to a visual word recognition task. The task consisted in meaningful words (task condition) and non-sense strings (rest condition). The

visual stimuli were presented by a projector behind the MR cabinet through a shielded window on a semitransparent screen. The screen was viewable over a mirror system placed on the top of the head coil. The rest condition consisted of non-sense letter strings for visual compensation, but no reaction was required from the participants. The length of the letter strings was matched for those of the meaningful words used.

Since in general the MRI technique is accompanied with a considerable noise emission, there was need for noise cancellation, especially for tinnitus sufferers. The visual presentation allowed for use of earplugs in addition to noise-cancelling headphones.

Each 24-min fMRI session comprised 960 stimuli, including 160 meaningful words and 800 non-sense strings. Stimuli were presented with a frequency of 40 stimuli per minute for continuous visual stimulation. Presentation time was each 1 s for words and non-sense strings. Participants were required to press a button when they recognized a meaningful word. Between two meaningful words, non-sense strings were presented. The participants were asked to monitor the non-sense strings without any action but to wait for the next meaningful word. For the purpose of analyzing the TNA, the brain response during the rather long inter-trial intervals (ITI) of 8.8 $\pm$ 7.65 s (*SD*) as task-negative periods was contrasted with the word recognition condition. Functional scan parameters of the BOLD imaging were set to time-to-repeat (TR) of 2.2 s, echo time (TE) to 30 ms, and a flip angle of 90°. Thirty slices of 3 mm thickness with a 0.75 mm gap to the next slice covered the whole brain. Each slice was scanned into a 94 $\times$ 94 matrix resulting in a voxel volume of 2 $\times$ 2 $\times$ 3 mm^3. For each run, 655 scans were acquired including 4 preceding prescans for compensating the magnetization saturation effects. The prescans were later discarded from data analysis.

In addition, two high-resolution anatomical whole-brain scans were obtained at T1 and T2, using a Magnetization Prepared Rapid Acquisition of Gradient Echoes (MPRAGE; Mugler and Brookeman, 1990) sequence. These 3D brain images resulted in isometric voxel dimensions of 0.9 $\times$ 0.9 $\times$ 0.9 mm^3.

Preprocessing and modeling of MRI measurements were executed according to standard procedures detailed in the software "Statistical Parametric Mapping" (SPM8, Wellcome Trust Centre for Neuroimaging, London). The preprocessing of the functional scans consisted of slice time correction, motion correction, segmentation of the anatomical scan and co-registration the resulting gray matter (GM) compartment to the mean image of corrected functional scans, co-registering the anatomical-image to the position of the functional mean image, determination of normalization parameters using the anatomical image, normalization of functional scans to the template of the Montreal Neurological Institute (MNI space), and final Gaussian smoothing using a 8 mm radius in each direction.

Both the functional and the anatomical MRI sequences were conducted twice, before (T1) and after (T2) the study period. During this time span, participants from the TG and AC groups were treated with HNMT. The first-level task-negative activity of both time points per participant was included in a second-level "flexible factorial model" (2 $\times$ 2 ANOVA) as implemented in SPM. This model used "subject," "time," and "group" as factors with an assumption of interaction between "time" and "group" to predict the influence of HNMT on the subjects' brain activity.

RESULTS

DMN Activity

The general task-negative activity was measured by contrasting the brain activity during the ITI period against the brain activity during the word recognition condition using scans from T1. The task-negative activities from all participants ($n = 61$) were compared using a one-sample *t*-test as implemented in SPM. As expected, the DMN network was activated, including MPF, LP, PCC, and PCC/precuneus (**Figure 1** and **Table 2**). The DMN activation has been found highly significant on cluster level [$p < 0.001$, family-wise error (FWE) corrected for multiple comparisons].

A flexible factorial model was used to calculate the "group $\times$ time" differences of brain activity alterations. Three conditions were of interest: (1) tinnitus-related effect on the DMN, (2) therapy-related effects on the DMN and (3) a possible intersection between therapy-related and tinnitus-related effects.

To assess therapy related effects, the activity change among the tinnitus patients was compared using "group" and "time" as factors of a 2 $\times$ 2 ANOVA. The TG group revealed an increase in task-negative activity from T1 to T2, especially in PCC/precuneus, LP, and MPF (**Figure 2**), while the untreated patients in the PTC group displayed no clusters of enhanced DMN activity in the same time span. The contrast "TG > PTC"

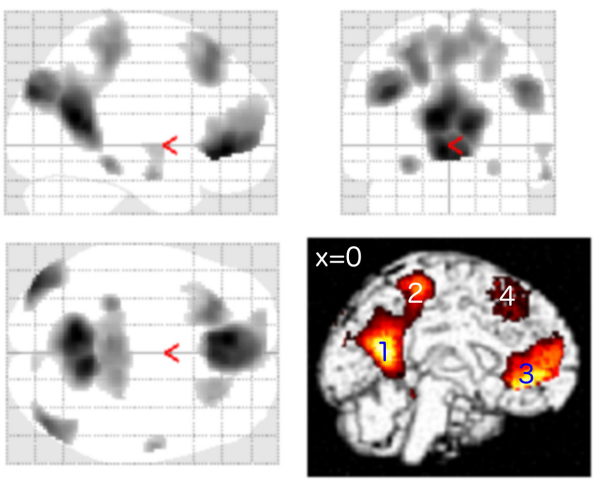

FIGURE 1 | One-sample *t*-test from first fMRI scan of all participants ($n = 61$) contrasting the inter-stimulus interval (ITI) against meaningful words. The figure presents the "glass brain" output of SPM as well as clusters overlaid on the median plane of a T1-weighted brain image. The task-negative condition comprised clusters in LP, PCC (1), precuneus (2), superior parietal lobe, ventro-medial PFC (3), supplemental motor area (4), and dorsolateral prefrontal cortex ($p < 0.001$ uncorrected for visualization; for more details see **Table 2**). These patterns were closely matched to the Default Mode Network (DMN).

TABLE 2 | Location of the activated DMN clusters (**$p < 0.01$; *$p < 0.05$ after FWE correction for multiple comparisons).

Contrast/location	MNI space (x/y/z)	*Z*-value	*p* (cluster)
General task-negative activation: inter-trial interval > word condition ($n = 61$; $p < 0.05$; FWE corrected; 25 voxels extent threshold)			
Left precuneus/posterior cingulate cortex	−8/−58/16	>8	<0.001**
Right precuneus/posterior cingulate cortex	10/−52/10	>8	
Ventromedial orbitofrontal cortex	−6/38/−6	>8	<0.001**
Left postcentral gyrus	−2/−36/56	6.45	<0.001**
Right postcentral gyrus	8/−38/58	5.77	
Left superior frontal sulcus	−22/28/46	6.88	<0.001**
Right superior frontal sulcus	22/30/46	5.21	<0.001**
Left inferior parietal gyrus	−40/−84/30	>8	<0.001**
Right inferior parietal gyrus	46/−78/32	7.26	<0.001**
Left hippocampus	−28/−36/−14	7.48	<0.001**
TG > PTC; after > before HNMT; masked with general task-negative activation ($n = 18/21$; $p < 0.001$; uncorrected; 25 voxels extent threshold)			
Precuneus/posterior cingulate cortex	−8/−62/16	4.06	<0.001**
Left inferior parietal gyrus	−48/−76/26	3.95	<0.1
Right inferior parietal gyrus	54/−66/28	3.98	n.s.
Left medial frontal gyrus	−8/50/6	3.59	n.s.
Right medial frontal gyrus	10/56/14	3.65	n.s.
TG > AC; after > before HNMT; masked with general task-negative activation ($n = 18/22$; $p < 0.001$; uncorrected; 25 voxels extent threshold)			
Precuneus/posterior cingulate cortex	−2/−54/28	4.28	<0.001**
Left inferior parietal lobe	−44/−78/28	3.55	n.s.
Precuneus	−6/−50/−8	3.54	n.s.
TG > AC & TG > PTC; after > before HNMT; masked with gen. task-negative activation ($n = 18/22$ & $18/21$; $p < 0.001$; uncorrected; 25 voxels extent threshold)			
Precuneus/posterior cingulate cortex	−2/−54/26	4.26	<0.001**
Left inferior parietal gyrus	−44/−76/26	3.55	n.s.

was further used as spatial mask (region of interest, ROI) for general therapy-related effects on the DMN.

Tinnitus-related effects were evaluated by comparing the two groups of "treated" participants: the TG and AC groups. Results indicated that the treated tinnitus patients in TG exhibited an increase in the DMN activity compared to the treated healthy participants in AC (**Table 2**). In contrast to the results from structural data (Krick et al., 2015), there were no HNMT-related effects on DMN in the AC group compared to the PTC group. The individual changes in the PCC/precuneus region were further analyzed by exporting them from SPM and using the statistic tool SPSS (IBM). Scaling the activity change in PCC/precuneus among all participants, the HNMT induced rising activation levels in the TG group solely of 2.5% (**Figure 3B**). Mann–Whitney *U*-tests revealed significant differences between the TG and PTC groups ($Z = -3.2$; $p < 0.001$) as well as between the TG and AC groups ($Z = -3.6$; $p < 0.001$).

The conjunction of therapy-related and tinnitus-related effects was assessed by contrasting task-negative activity between the TG and AC groups within the spatial mask for therapy-related effects (TG > PTC). It focused on a cluster in the PCC/precuneus area (**Figure 3A**) and isolated the specific tinnitus-related effect among the therapy-related effects.

Connection between DMN-Activity and Tinnitus-Distress

Using SPSS, the therapy-specific change in the DMN activity was further consolidated by a significant correlation with improvements in tinnitus distress: lower levels of tinnitus distress led to rising DMN activity ($n = 39$; Spearman-Rho: −0.5; $p < 0.005$; **Figure 4**).

DMN: Functional and Structural Alterations due to HNMT

For this synoptic presentation, structural alterations due to HNMT (Krick et al., 2015) and the functional findings regarding the therapy-related effect reported here were each exported from SPM and analyzed by SPSS. The initial assumption of a general relationship between the DMN activity and the HNMT-induced structural alterations in this network was shown in the correlation between the dynamics of the precuneus activity and structural GM alterations ($n = 61$; Spearman-Rho: 0.36; $p < 0.005$) over the study period (**Figure 6**). The anatomical convergence of structural and functional effects was able to figure out by an overlap in the precuneus/PCC region (**Figure 5**).

DISCUSSION

We used TNAs to observe the DMN in the context of the HNMT tinnitus-therapy control task. The general DMN pattern was similar to findings from previous studies (Fox et al., 2005; Buckner et al., 2008; Raichle, 2015). Since we used twice the identical measurements in the same participants in a longitudinal design, comparable results at T1 and T2 were expected. We used both the HNMT participation (TG vs. PTC) and tinnitus occurrence (TG vs. AC) conditions for analyzing the influence on the DMN activity. Two fMRI measurements were recorded at the beginning and end of the 1-week study period to record longitudinal changes of the BOLD effect. We yielded tinnitus-related effects among general therapy effects due the HNMT approach in the PCC/precuneus region.

The PCC is one of the most interlinked anatomical areas of the brain (Hagmann et al., 2008). Although, its role remains unclear, it seems to play an important role in a variety of cognitive tasks (Leech and Sharp, 2014): The PCC region is involved in internally directed cognition (Buckner et al., 2008). It also seems to control the focus of attention, especially between internally

and externally focused thoughts (Leech et al., 2011; Trevis et al., 2016). The PCC region is not homogenous but separated into ventral and dorsal parts (Leech et al., 2011). Regarding both location and behavior of the tinnitus-related therapy effect, the found activation could be assigned to the ventral PCC that in turn is considered to be part of the DMN (Raichle, 2015). The ventral PCC section is strongly connected to the ventral medial PFC as well as the temporal lobes (Choi et al., 2006). The known interplay between ventral PCC and temporal lobes may explain the effect of HNMT on tinnitus, since the structural effects of HNMT showed increase of GM density in temporal areas (Krick et al., 2015).

Lanting et al. (2016) observed suppressed addressability of DMN by TNAs in tinnitus patients in comparison to healthy controls. Ueyama et al. (2013) measured reduced global connectivity in the DMN-specific RSN regions, including the PCC, with increasing loudness of tinnitus. This means that suffering from tinnitus can cause a degradation of the PCC/precuneus functions in task-negative phases (Lanting et al., 2016). Independent component analysis using resting-state fMRI also partly indicated a reduced engagement of this DMN region in tinnitus patients due to a more task-based state due to perception of the internal noise (Schmidt et al., 2013). However, there are contradictory results that showed increased connectivity to the precuneus in pulsatile tinnitus (Han et al., 2015). These relations between tinnitus strength and DMN alterations led to our hypothesis of the HNMT effects on DMN and especially on the PCC/precuneus region.

The modulation of the tinnitus percept by a short-term intervention lasting 5 days only afforded an opportunity to have

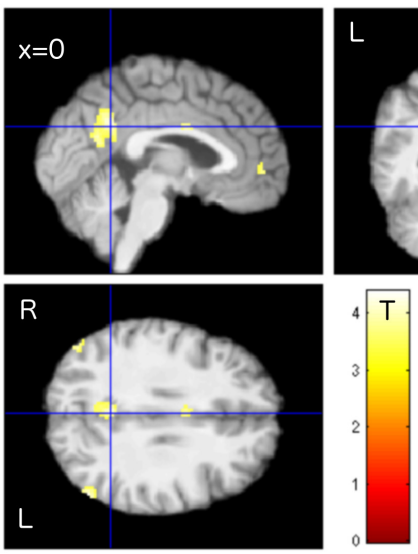

FIGURE 2 | Enhanced activity in the precuneus/PCC region was observed in treated patients (TG) compared to untreated patients (PTC) after the study week. The location of PCC activity by this contrast matched with the DMN pattern as shown in **Figure 1**.

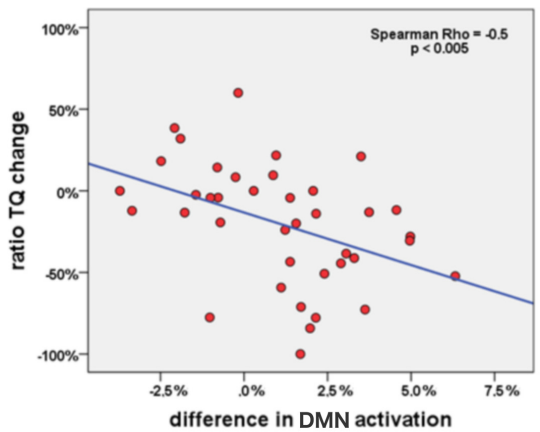

FIGURE 4 | Correlation between change of DMN activation in PCC (percent signal change) and change of tinnitus distress (in percent) in all tinnitus patients ($n = 39$) during the study week.

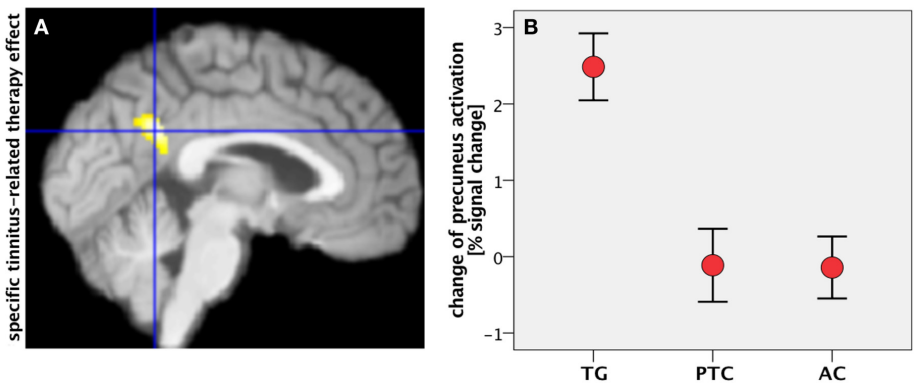

FIGURE 3 | (A) Specific tinnitus-related therapy effect by contrasting task-negative activity between treated patients (TG) and "treated" healthy controls (AC) within the mask for therapy-related effects (median plane). **(B)** Change of precuneus activity (in percent signal change) in all groups after the study week. Error bars: standard error of mean.

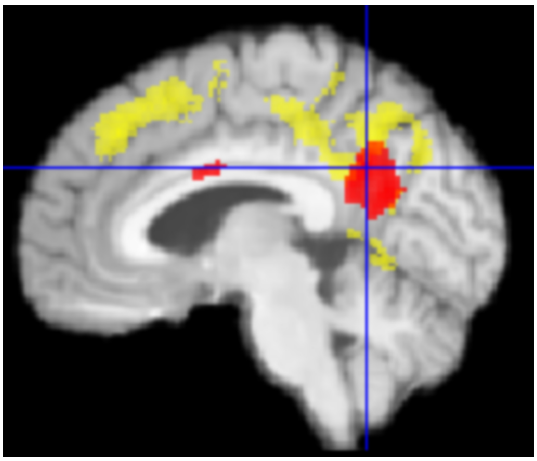

FIGURE 5 | Graphical overlap (median plane) of change in PCC activity (red) und structural gray matter (GM) change from Krick et al. (2015) (yellow) in TG vs. PTC. Both effects converged in the precuneus/PCC-region.

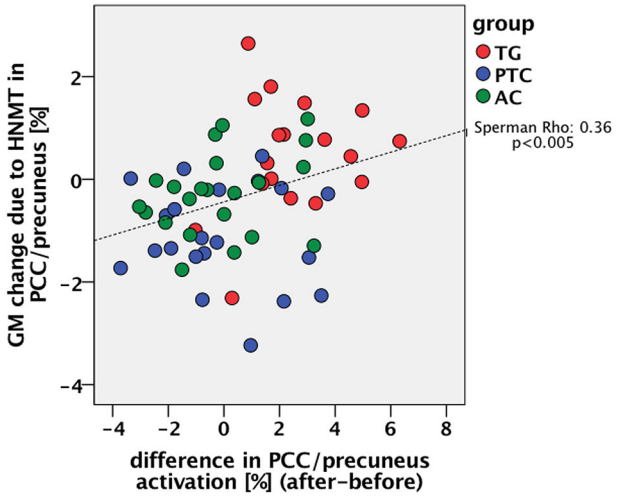

FIGURE 6 | Relation between the previously reported change of GM density in the PCC/precuneus by the HNMT application (Krick et al., 2015) and the measured change of PCC activity.

an insight into the relationship between therapy-induced tinnitus modulation and related DMN activity. The data revealed a rising task-negative activity in tinnitus patients following HNMT, especially in the ventral PCC. The increase of PCC activity was accompanied by rising GM density in this region (Krick et al., 2015). One could assume that HNMT initiated a neural reorganization, reversely to the tinnitus influence. The initial question about the relation between structural GM alterations and their underlying dynamics of brain activity must be resolved toward the convergence between both processes. This effect on tissue level correlated with the clinical therapy effect as measured by TQ. Argstatter et al. (2012) were able to demonstrate the long-term character of clinical therapy success. For this reason, one could assume some kind of neural rehabilitation of the DMN as a result of HNMT.

Since it is known that precuneus and PCC are also involved in the perception of musically induced emotions (Blood et al., 1999) and auditory imagery (Yoo et al., 2001), HNMT incorporates a module aimed at re-conditioning the emotional negative reaction toward the tinnitus percept by using auditory-based mental imagination. Due to the established positive emotional connotation in course of the therapy, the patients usually succeed in decoupling their negative reactions toward the tinnitus sound or even manage to completely ignore the acoustic interference. A reduced activity and a connectivity pattern of the PCC/precuneus region are known to be related to tinnitus distress (Maudoux et al., 2012; Lanting et al., 2016). The tinnitus-reconditioning is extended to tinnitus-prone situations by means of mental imagination. Eventually, patients report less subjective salience of their tinnitus percept. It seems plausible to explain these mechanisms with the activation of an effective noise-cancellation system (Rauschecker et al., 2010).

On the level of auditory processing and attention control, there is evidence that music based stimulation has positive effects on the tinnitus percept. Sound therapies that focus on "notched music listening" distributed lateral inhibition into the tinnitus region and have demonstrated an increase in responsiveness of the PCC (Pape et al., 2014). The "acoustic coordinated reset neuromodulation" (CR; Tass et al., 2012) makes use of computer based auditory stimuli presented as short tones in a random varying sequence above and below the tinnitus frequency and aims at a kind of "anti-kindling" of the underlying neuronal circuits. In CR, a desynchronization of the excitatory connection between PCC and AC has been observed (Tass et al., 2012). Whereas, these approaches are based on passive interventions using computer-modified musical stimuli, HNMT pursues an active involvement by means of repeated stimulation of the central auditory pathway with natural sounds in the range of the individual's tinnitus frequency. Thus, the therapeutic sounds are processed in conscious interaction. Since the PCC/precuneus region has been shown to be involved with the discrimination of musical chords (Fujisawa and Cook, 2011), it seems plausible to attribute some of the changes found in this region to changes in the effect of HNMT due to the corresponding training for frequency discrimination. The harmonic chord perception and the HNMT effect resulted in neighboring activations within the ventral PCC/precuneus region. However, whether the HNMT's effect relies on the explicit perception of frequency relations or emotional relaxation-related effects currently remains an unsolved question.

Limitations

Functional measurements of brain activity can only depict the relative levels of hemodynamic blood flow between defined conditions, in this case in task-positive and task-negative states. Although, we observed some changes with the interaction of participant group and time, it is safe to say that this cannot originate from TNA. One can only argue that identical tasks performed at two time points may primarily evoke similar responses in all participants. This led to the logical consequence that the task-negative responses might be changed.

Although, the DMN-specific RSN seems to interplay with the so-called "tinnitus core" (Husain and Schmidt, 2014), our findings were not able to depict its relation to the network for tinnitus maintenance itself (Husain, 2016).

CONCLUSION

Unlike other auditory procedures, HNMT commits the patients to take an active role in overcoming their tinnitus distress. HNMT aims to target some the neuronal hubs that possibly trigger tinnitus distress. These assumed mechanisms were reflected by a distinct correlation between reduced tinnitus distress and increased DMN activity. As a diminished connectivity to the precuneus seems to be a characteristic pattern for non-pulsatile tinnitus patients (Han et al., 2015; Lanting et al., 2016), we were able to confirm a relationship between the role of PCC/precuneus and the effects of HNMT in recent-onset tinnitus.

ETHICS STATEMENT

This study was carried out in accordance with the recommendations of the ethic board of Saarland/Germany with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the ethic board of Saarland.

AUTHOR CONTRIBUTIONS

CK: MRI measurements, analysis of MRI data, data interpretation. HA: Development of Heidelberg Neuro-Music Therapy, therapy management, statistics. MG: therapist, analysis of clinical data, statistics. PP: tinnitus diagnostics, clinical therapy control. WR: neuroradiological screening, coordinator between the facilities, study coordinator.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fnins.2017.00384/full#supplementary-material

REFERENCES

Alster, J., Shemesh, Z., Ornan, M., and Attias, J. (1993). Sleep disturbance associated with chronic tinnitus. *Biol. Psychiatry* 34, 84–90. doi: 10.1016/0006-3223(93)90260-K

Andrews-Hanna, J. R. (2012). The brain's default network and its adaptive role in internal mentation. *Neuroscientist* 18, 251–270. doi: 10.1177/1073858411403316

Argstatter, H., Grapp, M., Hutter, E., Plinkert, P. K., and Bolay, H. V. (2014). The effectiveness of neuro-music therapy according to the Heidelberg model compared to a single session of educational counseling as treatment for tinnitus: a controlled trial. *J. Psychosom. Res.* 78, 285–292. doi: 10.1016/j.jpsychores.2014.08.012

Argstatter, H., Grapp, M., Hutter, E., Plinkert, P., and Bolay, H. V. (2012). Long-term effects of the "Heidelberg Model of Music Therapy" in patients with chronic tinnitus. *Int. J. Clin. Exp. Med.* 5, 73–288.

Blood, A. J., Zatorre, R. J., Bermudez, P., and Evans, A. C. (1999). Emotional responses to pleasant and unpleasant music correlate with activity in paralimbic brain regions. *Nat. Neurosci.* 2, 382–387.

Broyd, S. J., Demanuele, C., Debener, S., Helps, S. K., James, C. J., and Sonuga-Barke, E. J. S. (2009). Default-mode brain dysfunction in mental disorders: a systematic review. *Neurosci. Biobehav. Rev.* 33, 279–296. doi: 10.1016/j.neubiorev.2008.09.002

Buckner, R. L. (2013). The brain's default network: origins and implications for the study of psychosis. *Dialogues Clin. Neurosci.* 15, 351–358.

Buckner, R. L., Andrews-Hanna, J. R., and Schacter, D. L. (2008). The brain's default network: anatomy, function, and relevance to disease. *Ann. N. Y. Acad. Sci.* 1124, 1–38. doi: 10.1196/annals.1440.011

Buckner, R. L., Krienen, F. M., and Yeo, B. T. (2013). Opportunities and limitations of intrinsic functional connectivity MRI. *Nat. Neurosci.* 16, 832–837. doi: 10.1038/nn.3423

Carpenter-Thompson, J. R., Schmidt, S. A., and Husain, F. T. (2015). Neural plasticity of mild tinnitus: an fMRI investigation comparing those recently diagnosed with tinnitus to those that had tinnitus for a long period of time. *Neural Plast.* 2015:161478. doi: 10.1155/2015/161478

Choi, H. J., Zilles, K., Mohlberg, H., Schleicher, A., Fink, G. R., Armstrong, E., et al. (2006). Cytoarchitectonic identification and probabilistic mapping of two distinct areas within the anterior ventral bank of the human intraparietal sulcus. *J. Comp. Neurol.* 495, 53–69. doi: 10.1002/cne.20849

De Ridder, D., Vanneste, S., Weisz, N., Londero, A., Schlee, W., Elgoyhen, A. B., et al. (2014). An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci. Biobehav.* 44, 16–32. doi: 10.1016/j.neubiorev.2013.03.021

Elgoyhen, A. B., Langguth, B., De Ridder, D., and Vanneste, S. (2015). Tinnitus: perspectives from human neuroimaging. *Nat. Rev. Neurosci.* 16, 632–642. doi: 10.1038/nrn4003

Fox, M. D., Snyder, A. Z., Vincent, J. L., Corbetta, M., Van Essen, D. C., and Raichle, M. E. (2005). The human brain is intrinsically organized into dynamic, anticorrelated functional networks. *Proc. Natl. Acad. Sci. U.S.A.* 102, 9673–9678. doi: 10.1073/pnas.0504136102

Fujisawa, T. X., and Cook, N. D. (2011). The perception of harmonic triads: an fMRI study. *Brain Imaging Behav.* 5, 109–125. doi: 10.1007/s11682-011-9116-5

Goebel, G., and Hiller, W. (1998). *Tinnitus-Fragebogen: (TF); ein Instrument zur Erfassung von Belastung und Schweregrad bei Tinnitus; Handanweisung*. Göttingen: Hogrefe Verl. für Psychologie.

Grapp, M., Hutter, E., Argstatter, H., Plinkert, P. K., and Bolay, H. V. (2013). Music therapy as an early intervention to prevent chronification of tinnitus. *Int. J. Clin. Exp. Med.* 6, 589–593.

Gusnard, D. A., Akbudak, E., Shulman, G. L., and Raichle, M. E. (2001). Medial prefrontal cortex and self-referential mental activity: relation to a default mode of brain function. *Proc. Natl. Acad. Sci. U.S.A.* 98, 4259–4264. doi: 10.1073/pnas.071043098

Gusnard, D. A., and Raichle, M. E. (2001). Searching for a baseline: functional imaging and the resting human brain. *Nat. Rev. Neurosci.* 2, 685–694. doi: 10.1038/35094500

Hagmann, P., Cammoun, L., Gigandet, X., Meuli, R., Honey, C. J., Wedeen, V. J., et al. (2008). Mapping the structural core of human cerebral cortex. *PLoS Biol.* 6:e159. doi: 10.1371/journal.pbio.0060159

Han, L., Pengfei, Z., Zhaohui, L., Fei, Y., Ting, L., Cheng, D., et al. (2015). Resting-state functional connectivity density mapping of etiology confirmed unilateral

pulsatile tinnitus patients: altered functional hubs in the early stage of disease. *Neuroscience* 310, 27–37. doi: 10.1016/j.neuroscience.2015.09.032

Husain, F. T. (2016). Neural networks of tinnitus in humans: elucidating severity and habituation. *Hear. Res.* 334, 37–48. doi: 10.1016/j.heares.2015.09.010

Husain, F. T., and Schmidt, S. A. (2014). Using resting state functional connectivity to unravel networks of tinnitus. *Hear. Res.* 307, 153–162. doi: 10.1016/j.heares.2013.07.010

Krick, C. M., Grapp, M., Daneshvar-Talebi, J., Reith, W., Plinkert, P. K., and Bolay, H. V. (2015). Cortical reorganization in recent-onset tinnitus patients by the Heidelberg Model of Music Therapy. *Front. Neurosci.* 9:49. doi: 10.3389/fnins.2015.00049

Langguth, B. (2011). A review of tinnitus symptoms beyond 'ringing in the ears': a call to action. *Curr. Med. Res. Opin.* 27, 1635–1643. doi: 10.1185/03007995.2011.595781

Lanting, C., Wozniak, A., van Dijk, P., and Langers, D. R. (2016). Tinnitus- and task-related differences in resting-state networks. *Adv. Exp. Med. Biol.* 894, 175–187. doi: 10.1007/978-3-319-25474-6_19

Leaver, A. M., Turesky, T. K., Seydell-Greenwald, A., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2016). Intrinsic network activity in tinnitus investigated using functional MRI. *Hum. Brain Mapp.* 37, 2717–2735. doi: 10.1002/hbm.23204

Leech, R., and Sharp, D. J. (2014). The role of the posterior cingulate cortex in cognition and disease. *Brain* 137, 12–32. doi: 10.1093/brain/awt162

Leech, R., Kamourieh, S., Beckmann, C. F., and Sharp, D. J. (2011). Fractionating the default mode network: distinct contributions of the ventral and dorsal posterior cingulate cortex to cognitive control. *J. Neurosci.* 31, 3217–3224. doi: 10.1523/JNEUROSCI.5626-10.2011

Maudoux, A., Lefebvre, P., Cabay, J. E., Demertzi, A., Vanhaudenhuyse, A., Laureys, S., et al. (2012). Auditory resting-state network connectivity in tinnitus: a functional MRI study. *PLoS ONE* 7:e36222. doi: 10.1371/journal.pone.0036222

Møller, A. R. (2016). Sensorineural tinnitus: its pathology and probable therapies. *Int. J. Otolaryngol.* 2016:2830157. doi: 10.1155/2016/2830157

Mugler, J. P. III., and Brookeman, J. R. (1990). Three-dimensional magnetization-prepared rapid gradient-echo imaging (3D MP RAGE). *Magn. Reson. Med.* 15, 152–157. doi: 10.1002/mrm.1910150117

Pape, J., Paraskevopoulos, E., Bruchmann, M., Wollbrink, A., Rudack, C., and Pantev, C. (2014). Playing and listening to tailor-made notched music: cortical plasticity induced by unimodal and multimodal training in tinnitus patients. *Neural Plast.* 2014:516163. doi: 10.1155/2014/516163

Raichle, M. E. (2015). The brain's default mode network. *Annu. Rev. Neurosci.* 38, 433–447. doi: 10.1146/annurev-neuro-071013-014030

Raichle, M. E., MacLeod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., and Shulman, G. L. (2001). A default mode of brain function. *Proc. Natl. Acad. Sci. U.S.A.* 98, 676–682. doi: 10.1073/pnas.98.2.676

Rauschecker, J. P., Leaver, A. M., and Mühlau, M. (2010). Tuning out the noise: limbic-auditory interactions in tinnitus. *Neuron* 66, 819–826. doi: 10.1016/j.neuron.2010.04.032

Schmidt, S. A., Akrofi, K., Carpenter-Thompson, J. R., and Husain, F. T. (2013). Default mode, dorsal attention and auditory resting state networks exhibit differential functional connectivity in tinnitus and hearing loss. *PLoS ONE* 8:e76488. doi: 10.1371/journal.pone.0076488

Shannon, B. J., Dosenbach, R. A., Su, Y., Vlassenko, A. G., Larson-Prior, L. J., Nolan, T. S., et al. (2013). Morning-evening variation in human brain metabolism and memory circuits. *J. Neurophysiol.* 109, 1444–1456. doi: 10.1152/jn.00651.2012

Shulman, G. L., Fiez, J. A., Corbetta, M., Buckner, R. L., Miezin, F. M., Raichle, M. E., et al. (1997). Common blood flow changes across visual tasks: 11. Decreases in Cerebral Cortex. *J. Cogn. Neurosci.* 95, 648–663. doi: 10.1162/jocn.1997.9.5.648

Sripada, R. K., King, A. P., Welsh, R. C., Garfinkel, S. N., Wang, X., Sripada, C. S., et al. (2012). Neural dysregulation in posttraumatic stress disorder: evidence for disrupted equilibrium between salience and default mode brain networks. *Psychosom. Med.* 74, 904–911. doi: 10.1097/PSY.0b013e318273bf33

Tass, P. A., Adamchic, I., Freund, H.-J., Stackelberg, T., von, and Hauptmann, C. (2012). Counteracting tinnitus by acoustic coordinated reset neuromodulation. *Restor. Neurol. Neurosci.* 30, 137–159. doi: 10.3233/RNN-2012-110218

Trevis, K. J., McLachlan, N. M., and Wilson, S. J. (2016). Cognitive mechanisms in chronic tinnitus: psychological markers of a failure to switch attention. *Front. Psychol.* 7:1262. doi: 10.3389/fpsyg.2016.01262

Ueyama, T., Donishi, T., Ukai, S., Ikeda, Y., Hotomi, M., Yamanaka, N., et al. (2013). Brain regions responsible for tinnitus distress and loudness: a resting-state fMRI study. *PLoS ONE* 8:e67778. doi: 10.1371/journal.pone.0067778

Vanneste, S., and De Ridder, D. (2015). Stress-Related functional connectivity changes between auditory cortex and cingulate in tinnitus. *Brain Connect.* 5, 371–383. doi: 10.1089/brain.2014.0255

Yoo, S. S., Lee, C. U., and Choi, B. G. (2001). Human brain mapping of auditory imagery: event-related functional MRI study. *Neuroreport* 12, 3045–3049. doi: 10.1097/00001756-200110080-00013

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Heidelberg Neuro-Music Therapy Restores Attention-Related Activity in the Angular Gyrus in Chronic Tinnitus Patients

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Background: Tinnitus is the perception of a phantom sound without external acoustic stimulation. Recent tinnitus research suggests a relationship between attention processes and tinnitus-related distress. It has been found that too much focus on tinnitus comes at the expense of the visual domain. The angular gyrus (AG) seems to play a crucial role in switching attention to the most salient stimulus. This study aims to evaluate the involvement of the AG during visual attention tasks in tinnitus sufferers treated with Heidelberg Neuro-Music Therapy (HNMT), an intervention that has been shown to reduce tinnitus-related distress.

Methods: Thirty-three patients with chronic tinnitus, 45 patients with recent-onset tinnitus, and 35 healthy controls were tested. A fraction of these (21/21/22) were treated with the "compact" version of the HNMT lasting 1 week with intense treatments, while non-treated participants were included as passive controls. Visual attention was evaluated during functional Magnet-Resonance Imaging (fMRI) by a visual Continous Performance Task (CPT) using letter-based alarm cues ("O" and "X") appearing in a sequence of neutral letters, "A" through "H." Participants were instructed to respond via button press only if the letter "O" was followed by the letter "X" (GO condition), but not to respond if a neutral letter appeared instead (NOGO condition). All participants underwent two fMRI sessions, before and after a 1-week study period.

Results: The CPT results revealed a relationship between error rates and tinnitus duration at baseline whereby the occurrence of erroneous "GO omissions" and the reaction time increased with tinnitus duration. Patients with chronic tinnitus who were treated with HNMT had decreasing error rates (fewer GO omissions) compared to treated recent-onset patients. fMRI analyses confirmed greater activation of the AG during CPT in chronic patients after HNMT treatment compared to treated recent-onset patients.

Conclusions: Our findings suggest that HNMT treatment helps shift the attention from the auditory phantom percept toward visual cues in chronic tinnitus patients and that this shift in attention may involve the AG.

Keywords: tinnitus, fMRI neuroimaging, Heidelberg model of music therapy, tinnitus distress, tinnitus treatment, attention

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INTRODUCTION

Tinnitus is the perception of a phantom sound, such as a pure tone, ringing, buzzing, or other noise, without external acoustic stimulation. However, while the occurrence of tinnitus may be often disturbing, it does not necessarily cause the disease pattern of a mental suffering (Malouff et al., 2011). Conversely, the duration since the tinnitus onset and the gender seem influence the individually reported mental distress (Seydel et al., 2013). Thus, the etiology of tinnitus-related distress depends on a number of poorly understood factors (Møller, 2016) that has led to several models and theories on the pathophysiology of tinnitus (Henry et al., 2014). Findings from Positron Emission Tomography (PET) measurements showed an involvement of auditory and non-auditory networks for maintaining the tinnitus percept (Mirz et al., 1999). The awareness of tinnitus activates those brain centers that are also involved in conscious acoustic perception (Dehaene and Changeux, 2011). Presumably prefrontal and parietal attention networks play a crucial role in conscious perception. The inferior parietal lobe has been found to be a key region for the awareness of an acoustic percept (Sadaghiani et al., 2009). Recent tinnitus research suggested a relationship between these attention processes and maintenance of tinnitus (Roberts et al., 2013). Husain et al. (2015) observed that tinnitus patients' attention networks showed more activation during visual than auditory tasks. These findings suggested a need for rising engagement of the attention system in tinnitus patients while focusing on visual stimuli. It is unclear, however, whether these perceptual changes arose immediately with the tinnitus onset or whether they were gradually provoked by the persisting tinnitus percept. Recent findings on tinnitus distress suggested that the distress-related higher mental effort for attention control may arise primarily from focusing on self-referred emotional signals that were triggered by a network consisting of the amygdala, the anterior cingulate cortex, the insula, and the parahippocampal area (Vanneste et al., 2010). Patients' attention thus shifts from the outside world toward the inner "mental pain" caused by the tinnitus distress (Malouff et al., 2011). Mohamad et al. (2016) summarized attention-related studies in tinnitus sufferers: That review did not tend to generally impaired attention due to tinnitus but some evidence that tinnitus patients performed poorer on selective attention in visual tasks compared to normal hearing controls (Hallam et al., 2004; Stevens et al., 2007), but non-significant findings were mentioned, too (Heeren et al., 2014). Hence, the observations so far remained unclear whether and how the tinnitus percept may claim resources for the visual attention. Selective attention in the visual domain and the respective brain responses can be measured by a Continuous Performance Task (CPT) which requires sustained attention to a sequence of visual stimuli (Hester et al., 2004). In this context, reaction time (RT) and error rates for responses can be measured to assess effects of a musical intervention (Guo et al., 2015).

The inferior parietal lobe plays a key role in conscious awareness of acoustic and visual cues (van Gaal et al., 2011; Igelström et al., 2016). Humphreys and Chechlacz (2015) described a network for attentive visual search which includes the angular gyrus (AG), the middle occipital gyrus, superior and middle temporal gyri, and the insula. The latter is also known as part of the tinnitus network, whereas the temporal regions and the AG are involved in self-awareness and self-perception (De Ridder et al., 2014). Hester et al. (2004) found these regions to be systematically involved in self-monitoring during visual CPT. The AG appears to play a crucial role for binding the attention to the individually most salient sensory stream (Taylor et al., 2011). Visual attention can be disrupted by suppressing AG activity using Transcranial Magnet Stimulation (TMS; Taylor and Thut, 2012). If the AG is involved in driving awareness toward the most salient sensory modality, the attention focus may be strongly driven toward the auditory system in case of tinnitus (De Ridder et al., 2014; Husain et al., 2015). Resting-state functional MRI scans revealed higher amplitude of low-frequency fluctuations in the AG and other attention-related areas (Chen et al., 2015). PET measurements confirmed these findings showing higher AG activity in chronic tinnitus patients (Song et al., 2012). Binding the attention to the auditory sensation in turn may explain the attention deficits in visual tasks as well as the attention conflict in the auditory domain in tinnitus patients (Araneda et al., 2015; Li et al., 2016). Using PET measurements, Plewnia et al. (2007) observed both tinnitus-related hyperactivity in the right AG and TMS-related attenuation of tinnitus after stimulation of this area. This finding indicated that the AG is a key region in understanding the erroneous focus on the phantom noise in clinically decompensated tinnitus patients.

A number of therapy options have been developed for tinnitus treatment, comprising Tinnitus Retraining Therapy, Cognitive Behavioral Therapy, Progressive Tinnitus Management, Biofeedback, Education, and Relaxation Therapies (Herraiz et al., 2007; Hesse and Laubert, 2010; Hesser et al., 2011; Folmer et al., 2014; Grewal et al., 2014; Myers et al., 2014). Most therapies aim to dissuade patients auditory self-monitoring. In those studies, however, there was little consistency in the therapeutic effects and their scientific verifiability. The statistically weak or inconsistent observations may reflect variation in the aberrant mental states of tinnitus patients. With respect to long-lasting neuroplastic changes, starting with the onset of tinnitus (Mühlau et al., 2006; Landgrebe et al., 2009; Schneider et al., 2009; Husain et al., 2011; Leaver et al., 2012; Boyen et al., 2013; Schecklmann et al., 2013), one can assume that the duration of tinnitus is one of several factors for the variable effects in tinnitus relief (Malouff et al., 2011). Relief is not directly related to the loudness of the phantom sound, but to the level of "catastrophizing" its affective experience (Møller, 2016). This in turn corresponds to the strength of an emotionally driven attention shift in tinnitus' pathophysiology causing tinnitus-related distress (Vanneste et al., 2010; Ueyama et al., 2013). The compact approach of the Heidelberg Neuro-Music Therapy (HNMT) has been shown to reduce tinnitus-related distress as assessed by a controlled trial (Argstatter et al., 2015). Its effects have also been shown to be maintained over a long time (Argstatter et al., 2012).

In the current study, we implemented a task that has previously been used in our research unit for examination of adults with attention deficit syndrome, the visual CPT (Schneider et al., 2010). The CPT was implemented as GO/NOGO task, allowing the observation of attention and inhibition control.

Attention difficulties can be detected by omitted GO stimuli, whereas decreased inhibition control results in erroneous NOGO reactions. The current paper aims to show the effects of tinnitus duration on the control of visual attention and the effects of HNMT on the visual attention network in tinnitus patients. Thus, we assessed the attention binding to the visual cue that required the participant's decision for action or inhibition. We postulated that HNMT will improve the visual attention in those patients with initial attention impairments.

MATERIALS AND METHODS

Participants

Both recent-onset patients unsuccessfully completing the standard clinical treatment (tinnitus persisting for a maximum of 6 months) and patients with chronic tinnitus (duration of at least 6 months) were invited for participation. Thirty-three patients with chronic tinnitus (12 females), 45 patients with recent-onset tinnitus (19 females), and 35 healthy controls (18 females) participated in the trial (**Table 1**). The group of healthy controls was enrolled after recruitment of the tinnitus groups, according to the patients' gender ratio and age distribution. Mean age was 47.6 ± *SD* 10.4 years in the chronic tinnitus group, 43.1 ± *SD* 10.5 years in the recent-onset tinnitus patients, and 43.4 ± *SD* 14.5 years in healthy controls (**Table 1**). In the group of recent-onset patient there was less variance with respect to the tinnitus duration (mean 8.1 weeks ± *SD* 1.6 weeks), since those participants were systematically included immediately after the standard treatment by otorhinolaryngology. In contrast to this, tinnitus duration in the chronic cases ranged from 1 to 14 years (mean 5.26 years ± *SD* 4.1 years). All participants underwent clinical examination and audiometric testing. The tinnitus patients were additionally examined by a pre-participation evaluation based on the TRI-recommendations (audiological testing, otolaryngological examination, and psychological intake interview). Patients were excluded if the tinnitus was related to anatomic lesions of the ear, retro-cochlear lesions, or to cochlear implants. Further exclusion criteria included clinical diagnosis of a comorbid severe mental disorder, clinical diagnosis of Menière's Disease, severe hyperacusis, or severe hearing impairment (>40 dB) in the tinnitus frequencies region. The latter criterion was defined to allow the participation of HNMT without hearing aids.

The study was conducted in accordance with the Declaration of Helsinki and approved by the local Saarland (Germany) ethics committee (ID-number 111/11). A complete clinical study protocol was compiled according to the ICH guidelines for good clinical practice.

Tinnitus Questionnaire (TQ)

Psychological complaints were assessed using the German version of the "Tinnitus Questionnaire" (TQ) as described by Goebel and Hiller (1998). This well-validated inventory comprises 52 items and records tinnitus related complaints. The items can be aggregated to variables representing the dimensions of mental distress: emotional and cognitive load, tinnitus duration, hearing impairments, sleep disturbance, and somatoform disorders. The global TQ-score ranges between the minimum score of 0 and the maximum score of 84, in which high values indicate high tinnitus related distress. Four levels of severity include: mild (0–30), middle (31–46), severe (47–59), and very severe (60–84) affliction. Cross-correlation between TQ and Tinnitus Handicap Inventory (THI) was reported by Zeman et al. (2012) yielding a Pearson's correlation coefficient of 0.87.

The patients' distress was examined by TQ at the time point of the pre-participation evaluation (T0) as well as before (T1) and after (T2) the study period. In the tinnitus groups, we included adult patients who were diagnosed with disturbing tinnitus (TQ-score > 30), but not completely decompensated tinnitus (TQ-score < 64). At T0, the tinnitus distress as measured by TQ was 43.2 ± 9.6 *SD* in chronic patients and 37.3 ± 15.8 *SD* in recent-onset patients. Since the tinnitus duration is regarded in the TQ score, there was a systematic difference between recent-onset and chronic tinnitus.

Intervention (HNMT)

All participants from the three groups, chronic and recent-onset tinnitus patients as well as healthy controls, were included in the examination of visual attention, but the groups were randomly divided in different treatment cohorts, comprising the participation in the "compact" HNMT in each group. The other option consisted in either no treatment or weekly sessions ("standard" protocol) instead of the compact treatment over 5 days. So, in each group of participants, treatment cohorts with comparable sample sizes were selected for undergoing the compact HNMT application. Thus, 21 chronic tinnitus patients (9 females, tinnitus duration 250 weeks ± 187 *SD*), 21 recent-onset tinnitus patients (9 females, tinnitus duration 8.14 weeks ± 1.85 *SD*), and 22 healthy controls (10 females, no tinnitus) underwent the compact HNMT.

For examination of interaction between tinnitus duration and visual performance, the initial behavioral measurements at T1 from all participants, comprising both treated and non-treated subgroups, were regarded. At this time the conditions were comparable for all participants, since there wasn't yet an influence from the subsequent therapy option. Hence, evaluations from this time point were independent from the therapy option and, hence, used to generally assess influences from the individually pre-existing or not existing tinnitus to the visual attention.

The subgroups treated with the compact HNMT (**Table 1**, marked in bold text) were compared to reveal the duration-specific effect among therapy-related effects on the attention network. The sub-groups of recent-onset tinnitus patients and the "treated" healthy controls have been already included in a previously reported study on structural effects from HNMT, in which we found that HNMT caused a major improvement of 17.7 points on the TQ scale in treated tinnitus patients (**Figure 1**, Krick et al., 2015). In the current study, we analogously evaluated the effects of HNMT in chronic tinnitus patients. As the HNMT has been found to rapidly reduce tinnitus distress (Grapp et al., 2013; Krick et al., 2015), this therapy can be used to assess distress-related effects on brain activation during a short study period.

TABLE 1 | Participants ($n = 113$) and therapy participation.

		Tinnitus			Sum
		Controls	Recent-onset	Chronic	
Music therapy and tinnitus profile	None (*n*)	13 (8f/5m)	24 (10f/14m)		37
	Frequency (*SD*)	None	6,376 (3,176)		
	Duration (*SD*)		0.16 y (0.03)		
	Standard (*n*)			12 (3f/9m)	12
	Frequency *(SD)*			6,200 (3,615)	
	Duration *(SD)*			6.09 y (4.05)	
	Compact (*n*)	**22 (10f/12m)**	**21 (9f/12m)**	**21 (9f/12m)**	**64**
	Frequency *(SD)*	None	5,102 (2,332)	6,785 (2,547)	
	Duration (*SD*)		0.16 y (0.04)	4.80 y (3.56)	
Sum		35	45	33	113

The HNMT was offered weekly (standard) or condensed in 1 week (compact). For fMRI comparison of HNMT effect only participants undergoing the compact treatment were included (n = 64; bold). Behavior observations, however, comprised reaction data of all participants at first measurement (before the study period).

The study protocol of the compact version of HNMT consisted of 9 consecutive 50-min sessions of individualized therapy, comprising acoustic training for frequency discrimination, auditory attention control tasks, and guided exercises for mindfulness and distress regulation. Therapy took place on 5 consecutive days (Monday to Friday) with two therapy sessions per day. Music therapy can be divided into two main categories: receptive (music listening based) and active (music making). Each morning and each afternoon session lasted for 50 min, in which there were 25 min of active music therapy and 25 min of receptive music therapy. Two trained music therapists carried out the therapy, in which one therapist performed the active modules and the other performed the receptive modules. The interventions were structured into treatment modules of directive counseling, habituation training, and stress management. A more detailed description can be found in Argstatter et al. (2015) and Grapp et al. (2013).

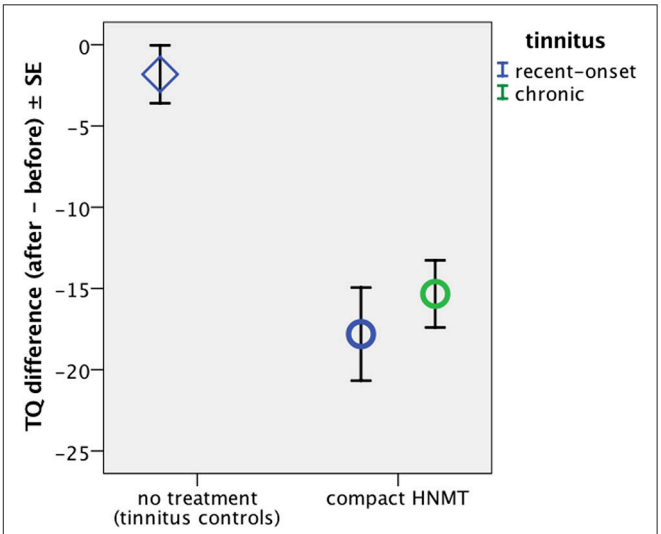

FIGURE 1 | Change in subjective tinnitus distress as measured by TQ. Krick et al. (2015) found that HNMT caused a major improvement in tinnitus distress in recent-onset tinnitus patients (blue circle), as measured by a reduction of near 18 on the TQ scale, an improvement which was not observed in non-treated passive control patients (rhomb). Here we measured the HNMT effect in chronic tinnitus sufferers (green circle), revealing a similar decrease on the TQ score by 15.3 ± *SD* 9.5 ($n = 21$; $Z = -4.3$; $p < 0.0001$). There was no significant difference in the therapy-induced TQ reduction between treated chronic and treated recent-onset patients ($n = 21/21$; $Z = -0.4$; $p > 0.6$).

Experimental Setup

Independently from the assignment to the treatment cohorts, all participants performed a letter-based CPT twice, during two fMRI sessions at T1 and at T2. Capital letters "A" through "H" were used as "neutral" stimuli in a quasi-random order. Letters "O" and "X" were used as alerting events inserted in the letter stream. Each letter was presented for 420 ms on a screen. Participants were instructed to watch the rapid sequence of letters and to look out for the letter "O" as an alarm stimulus. If the next letter following the "O" was an "X," they were asked to press a button (GO condition). In all other cases, the participants were instructed not to press the button. Especially if the letter "O" was followed by a letter different from "X," no reaction (NOGO condition) was required. The response button was counterbalanced for left and right hand in all groups.

Each condition consisted of 30 trials of GO or NOGO events which were randomly inserted among the presentation of 1800 letters over 12.6 min. The letters were projected through a window of the Magnet-Resonance (MR) cabin on a screen placed behind the head-side opening of the scanner. A mirror on the top of the head coil allowed viewing the letters in a light square on black background. The presentation was controlled by a computer synchronized with the imaging pulses.

Data Acquisition and Data Analysis

The 3 tesla MR scanner "Skyra" (Siemens) was used for imaging. Functional scan parameters of the Blood Oxygen Dependent (BOLD) imaging were set to time-to-repeat (TR) of 2.2 s, echo

time (TE) to 30 ms, and flip angle of 90°. Thirty slices of 3 mm thickness with a 0.75 mm gap to the next slice covered the whole brain. Each slice was scanned into a 94 × 94 matrix resulting in a voxel volume of 2 × 2 × 3 mm^3. For each run, 348 scans were acquired, including 4 prescans to compensate for magnetization saturation effects. The prescans were later discarded from data processing.

The functional BOLD imaging was conducted at T1 and T2. Two high-resolution anatomical scans over the whole brain were also measured at T1 and at T2, using a Magnetization Prepared Rapid Acquisition of Gradient Echoes (MPRAGE; Mugler and Brookeman, 1990) sequence. This 3D brain image resulted in isometric voxel dimensions of 0.9 × 0.9 × 0.9 mm^3.

Thus, functional and anatomical MRI (fMRI) scans were each conducted in two consecutive MRI sessions before and after the 1-week study period, meaning that all participants underwent the GO/NOGO CPT twice, at T1 and T2. The same hand and the same procedure were used in both consecutive measurements. During the MRI sessions, double ear protection (earmuffs and head phones) was applied to reduce scanner noise. For compensating ametropia, MRI-compatible glasses with adapted diopter for each eye were offered if necessary.

Preprocessing and modeling of MRI measurements were executed using standard procedures of *Statistical Parametric Mapping* (SPM8, Wellcome Trust Centre for Neuroimaging, London). Preparation of the functional scans included:

- slice time and motion correction of functional scans;
- segmentation of the anatomical scan;
- co-registration of the resulting gray matter (GM) compartment to the mean image of corrected functional scans;
- co-registration of the anatomical image to the position of the functional mean image;
- determination of normalization parameters using the anatomical image;
- application of normalization parameters to the functional scans with fitting to the template of the Montreal Neurological Institute (MNI space);
- and Gaussian smoothing using an 8-mm radius in each direction.

For general activation regarding to the alarm stimulus, the visual cue "O" from both GO and NOGO trials of the first run were pooled by a conjunction analysis. The effect of HNMT with respect to the tinnitus duration was estimated using both MRI sessions by a flexible factorial model (2 × 2 ANOVA) with "Time" (T1, T2) as within-subject factors and "Group" (chronic, recent-onset, control) as between-subject factors to explore the effect of treatment in the various participant groups. An assumption of interaction between "Time" and "Group" was predicted due to the effect of HNMT and tinnitus duration on the subjects' brain activity.

The GO and the NOGO conditions were modeled using the canonical hemodynamic response function and its first derivative as evoked by the visual alarm stimulus "O." This first-level "O"-responses of both measurement times (T1 and T2) per participant was included in the flexible factorial model.

Behavioral accuracy and RT from button responses were evaluated separately using SPSS Statistics (IBM). As the error rates from both GO and NOGO conditions did not correspond to a Gaussian distribution, non-parametric tests (Spearman Rho correlation and Wilcoxon test for paired data) were used to investigate the influence from tinnitus duration on the subjects' attention.

RESULTS

Therapy Effect on Subjective Distress Score (TQ)

Participation in HNMT yielded a reduction of 15.3 ± *SD* 9.5 scale points on the TQ score in chronic tinnitus sufferers ($n = 21$; Mann–Whitney-*U* test: $Z = -4.3$; $p < 0.0001$) compared to non-treated tinnitus patients ($n = 22$) which showed only a small test-retest effect of −1.8 score points. In chronic tinnitus patients, the subjective tinnitus distress was similarly reduced after HNMT as previously reported for treated recent-onset patients (Krick et al., 2015; **Figure 1**). When comparing the findings from recent-onset patients with the TQ reduction in treated chronic patients, the effects did not significantly differ between both patient groups ($n = 21/21$; $Z = -0.4$; $p > 0.6$).

Visual Attention Task and Behavioral Accuracy

Regarding the individual tinnitus anamnesis, there was a significant interaction between tinnitus duration and error rates from GO/NOGO task. At T1, error rates for both the GO condition (Spearman-Rho: +0.27; $p < 0.005$) and the NOGO condition (Spearman-Rho: −0.23; $p < 0.05$) depended on the type of tinnitus: chronic or recent-onset. Error rates from GO trials increased with tinnitus duration, whereas error rates from NOGO trials were diminished with chronic tinnitus (**Figure 2A**).

Latencies of the observed reactions also yielded effects of tinnitus duration (**Figure 2B**). Patients with chronic tinnitus exhibited longer RTs compared to healthy controls (Mann–Whitney-U; $Z = -5.9$; $p < 0.0001$), whereas there was no significant RT difference between controls and patients with recent-onset tinnitus (Mann–Whitney-U; $Z = -0.82$; $p > 0.4$). A linear regression model showed that the RT latency was delayed by about 42 ms with each year of tinnitus duration ($T = 7.3$; $p < 0.0001$).

Non-parametric paired Wilcoxon tests were performed to assess differences between HNMT treated and untreated participants. In the non-treated patients, error rates did not differ between start and end of the study period, neither in the GO condition (Wilcoxon Z: −0.01; $p > 0.99$) nor in the NOGO condition (Wilcoxon Z: −1.4; $p > 0.15$). For both groups participating in HNMT, "treated" controls and treated tinnitus patients, there were a measurable effect by the HNMT application in each case, showing each diminishing error rates between T1 and T2. This effect was similar between "treated" controls (GO: Wilcoxon *Z*: −1.68; $p < 0.1$; NOGO: Wilcoxon

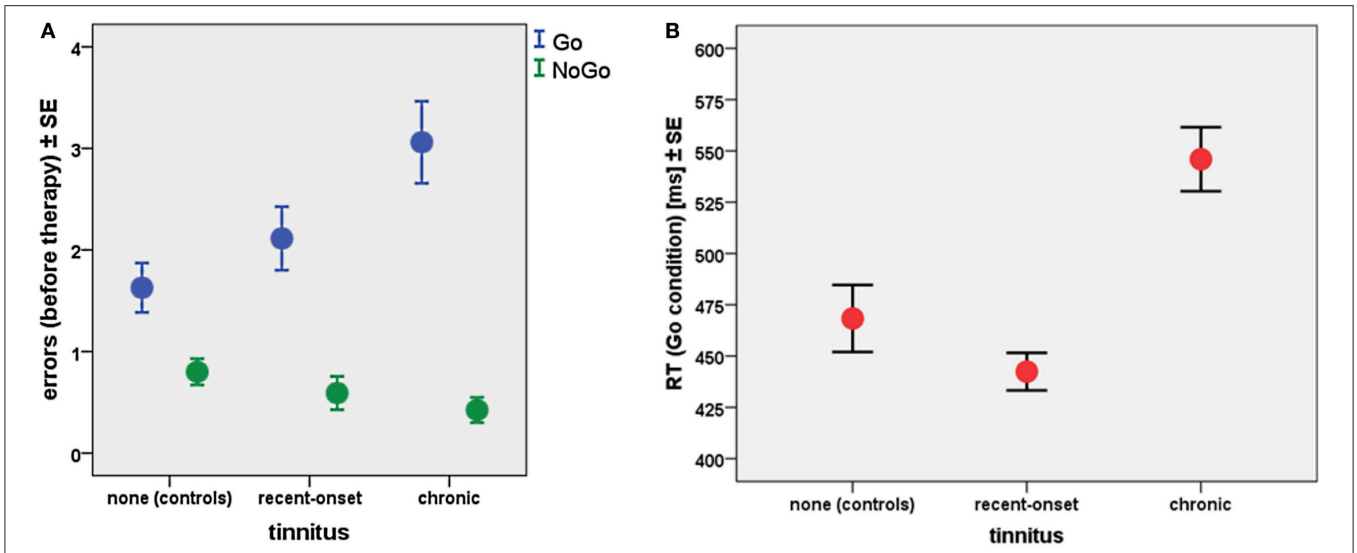

FIGURE 2 | (A) Error rates and **(B)** reaction times (RT) related to tinnitus type at T1 for all participants (35 healthy controls, 45 recent-onset tinnitus patients, and 33 chronic tinnitus patients).

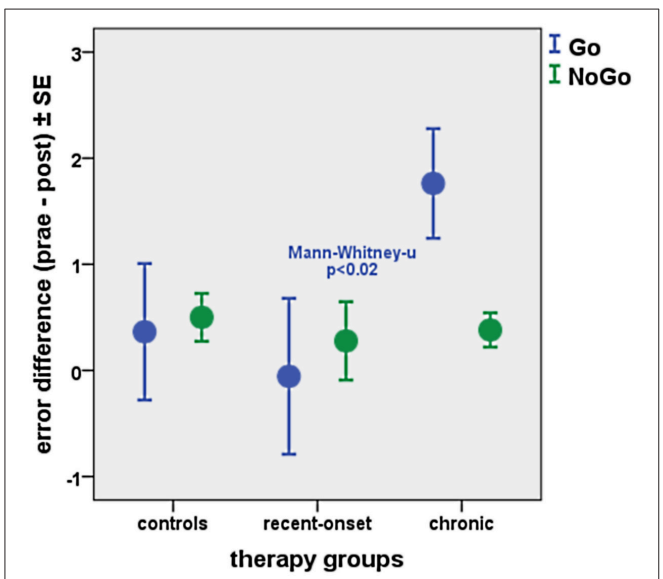

FIGURE 3 | Difference of error rates for GO and NOGO trials before and after HNMT in treated subsamples of healthy controls ($n = 22$), recent-onset tinnitus patients ($n = 21$), and chronic tinnitus sufferers ($n = 21$).

Z: −2.00; $p <$ 0.05) and treated patients (GO: Wilcoxon Z: −1.83; $p <$ 0.1); NOGO: Wilcoxon Z: −1.74; $p <$ 0.1). However, comparing the subgroups of treated tinnitus patients, the reduction of GO errors was significantly more pronounced in chronic compared to recent-onset tinnitus patients (Mann–Whitney Z: −2.3; $p < 0.02$) as shown in **Figure 3**. In contrast to this, there was no effect on the attention-related errors in non-treated participants (GO: Wilcoxon Z: −0.01; $p > 0.9$; NOGO: Wilcoxon Z: −1.41; $p > 0.15$).

Brain Activation

BOLD contrast by the effect of visual attention on the cue "O" was derived from the first MRI session (T1) for all participants ($n = 113$). This analysis was realized by a conjunction (comparable with the statistical intersection by an AND operation) of both GO and NOGO conditions, since both conditions were based on the visual cue "O." We aimed to observe here the brain network for conscious processing and attention binding (Dehaene and Changeux, 2011). Thus, the common activation by the visual cue for both GO and NOGO trials was relevant for observing its interdependency with tinnitus. The brain activations covered dorsolateral inferior frontal areas, the insula, superior and middle temporal regions, the premotor cortex, inferior parietal lobes, and inferior occipital areas in both hemispheres (**Figure 4**). In this network, activation in the right (MNI: +60/−42/+18) and left (MNI: −26/−62/+46) AG scored highest (**Table 2**).

Based on the fact of AG involvement in conscious attention control, a region of interest (ROI) including the left and the right AG was derived from the brain atlas for Automatic Anatomical Labeling (AAL, Tzourio-Mazoyer et al., 2002) using the WFU PickAtlas (Maldjian et al., 2003). The neural base of the observed behavioral differences between long-term tinnitus sufferers and recent-onset tinnitus patients was investigated within this ROI by utilization of the HNMT effects on the distress symptoms. A flexible factorial model for repeated measures with "Time" as within-subject factor and "Group" as between-subject factor was performed in treated patients to reveal effects from the individual tinnitus duration on change in AG activation. Contrasts of the respective activation levels at T1 and T2 exhibited higher activation differences in the right AG (MNI: +50/−64/+28) and left AG (MNI: −50/−68/+24) in chronic patients compared to recent-onset patients (**Figure 5**). The maximum peak regarding this activation difference was located in the right AG, even for a whole-brain analysis (**Table 2**). When reversely contrasting

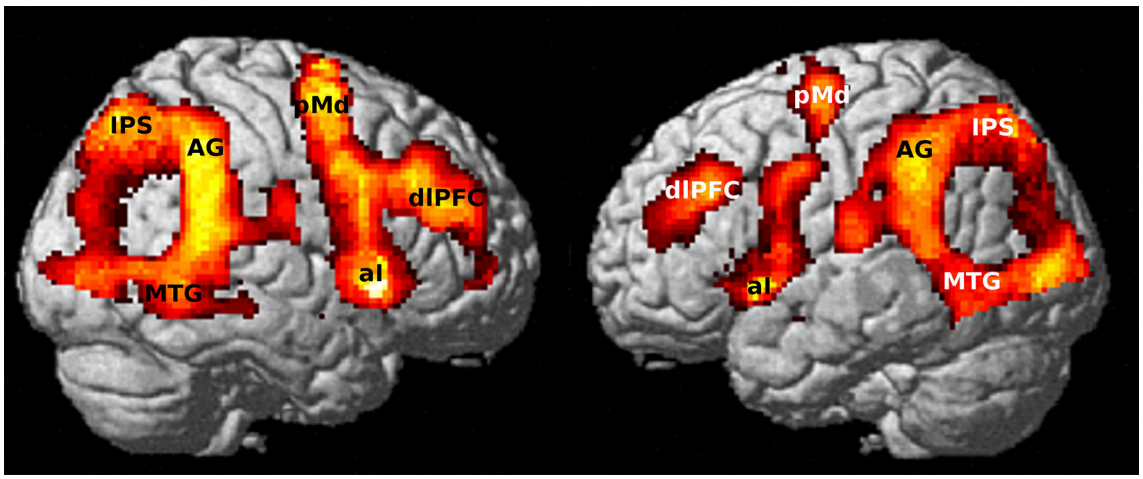

FIGURE 4 | Activated network for to the alarm stimulus ("O") from the first fMRI session (T1) by conjunction of conditions GO & NOGO ($n = 113$). Maximum activation peaked in the right AG ($p < 10^{-10}$ FWE corrected; 20 voxels extent threshold).

the differences in the opposite direction, there were no clusters peaking higher in recent-onset than in chronic patients.

DISCUSSION

The effect of auditory discrimination training and sound-based stimulation have often been discussed in tinnitus therapy (Flor et al., 2004; Herraiz et al., 2010; Hoare et al., 2013) yielding inhomogeneous outcome. While most music-based approaches are passive interventions which are based on listening to computer-modified musical stimuli, the HNMT pursues an active engagement by means of repeated stimulation of the central auditory pathway with sounds in the harmonic spectrum of the individual tinnitus frequency. Thus, the therapeutic sounds are actively performed in aware attention, not as passive listening. Evidence seems to indicate that the amount of attention dedicated to the auditory input is essential (Argstatter et al., 2015). The HNMT combines complementary receptive and active based music interventions to intervene at different levels of the neural tinnitus network. The "Neuroauditive Cortex Training" is a targeted and active auditory training focusing on tone sequences centered around the frequency of the patients' tinnitus frequency. A systematic and targeted training of inaccurately intonated musical sounds enables the patients to exert influence on their auditory processes since they learn to actively filter out irrelevant information and to concentrate on relevant acoustic stimuli. Music-based exercises for mindfulness then promotes desensitization of tinnitus perception. The individual tinnitus sound is embedded in relaxation music and the patients learn to decouple possible negative reactions toward the tinnitus sound or even to completely ignore the acoustic on-top-interference. Being able to disengage from emotional stimuli may reduce the tendency to experience negative affect and redeploying attention has been postulated to lead to a "flexibility of attention" which may free up cognitive resources (Linehan et al., 2007).

Jonker et al. (2013) were able to show that auditory distractors did not provoke a general influence on the visual attention as measured by CPT. This may mean that neither the tinnitus percept nor the MRI noise themselves would be sufficient to induce attention-related effects on the visual perception. However, our results from the visual GO/NOGO attention task yielded rising frequency of omission errors (GO errors) with the duration of tinnitus. In addition, erroneous NOGO responses were reduced with the duration of tinnitus. This observation can be explained in the context of a tinnitus-duration-dependent attention loss relating to the visual task (Husain et al., 2015) due to the shift toward the auditory tinnitus percept (Alpini and Cesarani, 2006). One can then assume that difficulties in monitoring the visual stream concerned both GO and NOGO trials. If the "alarm stimulus" was perceived to a lesser degree in chronic tinnitus patients, the reduced number of false alarms can be interpreted as due to a lack of visual attention rather than from better inhibition control. Following this line of argument, the conjunction of both conditions as a common predictor for visual attention seemed justifiable for the functional analyze regarding the visual cue "O."

Since the attention-related effects as observed by the GO/NOGO task were different with the tinnitus duration, we compared the visual attention and its change due to HNMT in recent-onset and chronic tinnitus patients. HNMT led to a decrease of omission errors in the chronic patients whose attention was most impaired before therapy. The error reduction can be explained in terms of a reinforcement of visual attention. According to this reduction of GO errors response times for the GO condition were also most reduced in chronic tinnitus patients after HNMT (**Figure 6**). The therapy-induced acceleration in cases of chronic tinnitus differed significantly from "treated" controls (Mann–Whitney-U tests; $Z = -2.99$; $p < 0.005$) as well as from treated patients with recent-onset tinnitus ($Z = -2.25$;

TABLE 2 | Activated Clusters for the visual cue "O": (A) general brain activations for the cue "O" and (B) HNMT-induced alterations in relation to tinnitus duration.

Localization	MNI (x y z)			*p* cluster
(A) ACTIVATION DUE TO "O" CUE; CONJUNCTION GO & NOGO, *N* = 113 WHOLE BRAIN ANALYSIS; *P* < 0.00001 FWE CORRECTED				
R angular Gyrus (AG)	60	−42	18	$p < 0.001$ corr
L angular Gyrus (AG)	−26	−62	46	$p < 0.001$ corr
R intraparietal sulcus (IPS)	34	−62	48	$p < 0.001$ corr
L intraparietal sulcus (IPS)	−32	−50	38	$p < 0.001$ corr
R insula	34	20	4	$p < 0.001$ corr
L insula	−32	18	4	$p < 0.001$ corr
R inferior frontal gyrus (IFG)	46	8	22	$p < 0.001$ corr
L inferior frontal gyrus (IFG)	−32	36	10	$p < 0.001$ corr
R premotor cortex (pMd)	46	8	32	$p < 0.001$ corr
L premotor cortex (pMd)	−28	−12	68	$p < 0.001$ corr
R middle frontal gyrus (MFG)	40	34	32	$p < 0.001$ corr
L middle frontal gyrus (MFG)	−44	32	32	$p < 0.001$ corr
posterior medial frontal gyrus	4	8	52	$p < 0.001$ corr
R middle temporal gyrus (MTG)	50	−52	−6	$p < 0.001$ corr
L middle temporal gyrus (MTG)	−50	−66	−2	$p < 0.001$ corr
(B) "O" CUE; T2 > T1; CHRONIC (21) > RECENT-ONSET PATIENTS (21) MASKED WITH ROI COVERING THE AG; SMALL-VOL. CORR.; *P* < 0.001 UNCORR.				
R AG	50	−64	28	$p < 0.05$ corr
L AG	−50	−68	24	n.s.
Localization	**MNI (x y z)**			**Cluster size**
(C) "O" CUE; T2 > T1; CHRONIC (21) > RECENT-ONSET PATIENTS (21) WHOLE-BRAIN ANALYSIS; *P* < 0.001 UNCORR.				
R angular gyrus (AG)	50	−64	28	236 voxels
L angular gyrus (AG)	−50	−68	24	41 voxels
L middle temporal gyrus (MTG)	−50	−18	−14	55 voxels
R precuneus	10	−56	20	25 voxels
(D) "O" CUE; T2 > T1; CHRONIC (21) > CONTROLS (22) WHOLE-BRAIN ANALYSIS; *P* < 0.001 UNCORR.				
L frontal eye field (FEF)	−16	−16	64	79 voxels
R frontal eye field (FEF)	−22	−8	68	191 voxels
R premotor cortex (pMC)	10	26	72	81 voxels
(E) "O" CUE; T2 > T1; RECENT-ONSET (21) > CONTROLS (22) WHOLE-BRAIN ANALYSIS; *P* < 0.001 UNCORR.				
no suprathreshold clusters				

The cluster-related significance (p cluster) was calculated after Family-wise Error (FEW) correction for multiple comparisons (abbr. "corr").

$p < 0.05$). Hence, the therapy-induced alteration of attention parameters was dependent on the tinnitus duration. In contrast to this, the therapy-induced TQ change showed no influence from tinnitus duration (**Figure 1**).

The behavioral effect of HNMT coincidentally matched with a higher task-related activation level of the bilateral AG with a right-sided maximum. The role of the AG has previously been established in visual attention control (Taylor et al., 2011; Humphreys and Chechlacz, 2015) and it has been recognized as part of the "tinnitus core" (De Ridder et al., 2014). Dehaene and Changeux (2011) assumed its role as part of a "Global Neural Workspace" for conscious processing. Thus, one can assume that the structural overlap between visual attention network, awareness network, and tinnitus core network mediated between the duration of tinnitus and a diminished awareness in visual attention (Roberts et al., 2013). The evidence of a HNMT-induced reinforcement of AG activation in chronic tinnitus patients underlined the role of this brain area in the context of the central attention control. Therefore, the reduction of omission errors can be explained as a relaxation of the erroneous attention binding to the auditory sensation in chronic tinnitus patients.

In general, the AG is considered to be a cerebral cross-modal hub for multisensory information and for reorienting attention to relevant information (Seghier, 2013). This means that the AG acts as a central gate for reorienting or shifting the attention toward those sensations that gain the highest salience in terms of emotional value or individual meaning (Gottlieb, 2007). The AG seems to include at least two centers: a dorsal and a ventral part (Uddin et al., 2010) which are involved either in multisensory bottom-up tasks, such as reading (Price, 2010; Segal and Petrides, 2013), or in top-down control of self-referential mental concepts (Bahnemann et al., 2010; Kim, 2010). The reactivation of AG found in this study can be anatomically assigned to the ventral part of AG. This region additionally overlaps with parietal areas of the "default-mode network" (Raichle, 2015) and it plays a role in self-relevant memory retrieval (Kim, 2010) as well as in emotional and

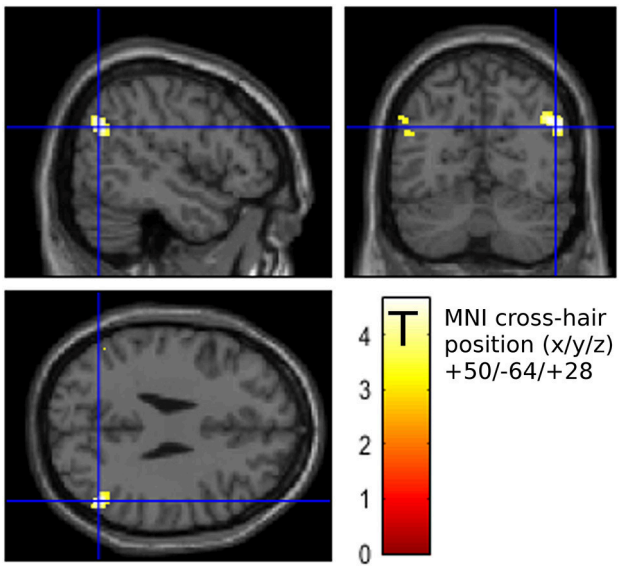

FIGURE 5 | Activation difference (after – before HNMT) yielded stronger effects in chronic ($n = 21$) compared to recent-onset ($n = 21$) patients in the mask covering the left and the right angular gyrus (AG) ($p < 0.001$ uncorrected; 20 voxels extent threshold). The maximum peak was situated in the right AG (crosshairs, neurological view). This cluster peaked in a T score of 4.7 that was significant after FWE correction on cluster level ($p < 0.05$).

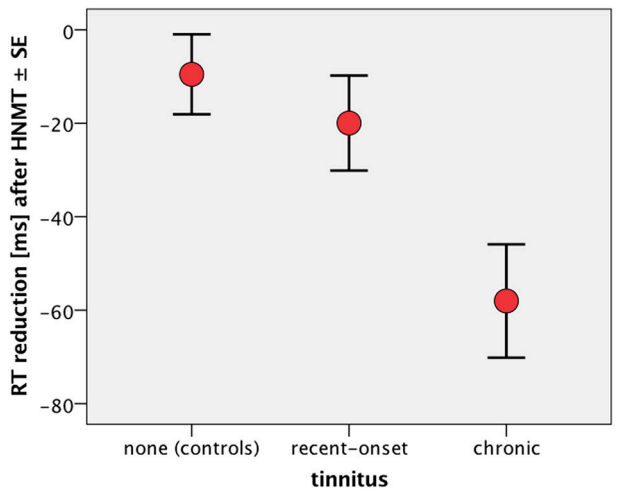

FIGURE 6 | RT difference (after – before) over the 1-week study period in the GO condition. "Treated" healthy participants served as active controls ($n = 22$) to compare the mental acceleration due to the compact HNMT in treated patients with recent-onset ($n = 21$) and chronic tinnitus ($n = 21$). RT was significantly reduced in chronic patients compared to both recent-onset patients (Mann–Whitney-U tests; $p < 0.05$) and active controls ($p < 0.005$). There was no significant RT difference between active controls and recent-onset patients ($p > 0.63$).

social perception (Bahnemann et al., 2010; Igelström et al., 2016). Plewnia et al. (2007) located the tinnitus-related activation of right AG (MNI: 52/−66/32) neighboring our findings of task-related reinforcement of the AG activity (MNI: 50/−64/28). This means that the same region is addressed by both monitoring the tinnitus and visual attention control, however, the attention is driven toward different sensory modalities. When suffering from chronic tinnitus, paying attention to a visually presented alarm stimuli seems to be impaired due to a failure to switch the attention toward the visual input. Trevis et al. (2016) recently conformed this finding also using a letter-based behavioral experiment. The long-term effects from the tinnitus duration causing the difficulties in attention switching may be based on structural change among attention-related and self-referencing brain networks (Lanting et al., 2016; Leaver et al., 2016). HNMT has been found to partially redirect these structural brain alterations (Krick et al., 2015). This in turn may explain the sensitivity of HNMT regarding the restored ability to switching attention between auditory and visual perception in chronic tinnitus sufferers.

Since we used temporal jittered ITI, the occurrence of the rare alarm stimuli (60 cues/1,800 letters = 3.33%) was not predictable by the participants. Following the dual-attention model (Corbetta and Shulman, 2002), the participants had to monitor the visual stream by internally guided attention ("top-down control"), whereas the occurrence of an "O" did not match the participant's expectance but happened externally driven ("bottom-up process;" Serences et al., 2005). Hence, regarding more the alarm stimuli rather than the GO/NOGO conditions, this task was similar to an oddball paradigm (Clark et al., 2000). However, activation of the inferior parietal cortex in an oddball task presupposes a relevance to the main task (Downar et al., 2001; Cabeza et al., 2012) that had been defined in turn by the required GO/NOGO decisions. In most studies on perceptual reorienting tasks the naming of the respective inferior parietal areas was traditionally named "tempero-parietal junction" (TPJ) applying a less exact anatomical assignment by subsuming parts of AG, marginal gyrus (MG), and superior temporal areas (STG; Cabeza et al., 2012). In contrast, language-related studies tend to call the same area "angular gyrus" (Cabeza et al., 2012). In this study, the assignment of relevant meaning to the oddball stimulus "O" was realized by letters that in turn may explain the focus on AG but not MG or STG when performing a whole brain analysis (**Table 2**).

The combination of abnormal attention control due to mental focus on an unpleasing percept has been also observed in adults with ADHD symptoms that augmented the predisposition for chronic pain, especially in coincidence with further mental disorders (Stickley et al., 2016). The association between suffering from aversive sensations and attention difficulties may play a fundamental role in chronic tinnitus sufferers as well (Husain et al., 2015). Hence, the interaction between conscious tinnitus experience and deviant attention control may also become relevant in both future tinnitus research and tinnitus treatment.

Limitations

The compared samples of participants, suffering from either chronic or recent-onset tinnitus, differed slightly in age which was 4.5 years higher on average in chronic patients compared to recent-onset participants. However, the age of chronic patients

was not correlated with the respective tinnitus duration. The age difference is due to the circumstance that chronic patients suffered from tinnitus over a comparable mean time span. When acting on the assumption of a comparable mean age of onset in both groups, the cohorts seemed representative.

CONCLUSION

Our observations and the hitherto existing knowledge about the AG lead to the conclusion that this region plays a crucial role in both chronic tinnitus and suitable therapies. In particular, chronic tinnitus impaired the patients' attention for a visual task. HNMT has been shown to reinforce visual attention in chronic tinnitus patients by reorienting the activity in the AG to the cognitively demanding task. These effects regarding the role of AG in tinnitus were dependent on the time since the onset of tinnitus. The observed duration-related attention shift indicates a variable of tinnitus diversity that should be considered in therapy concepts.

AUTHOR CONTRIBUTIONS

CK: MRI measurements, analysis of MRI data, data interpretation. HA: Development of Heidelberg Neuro-Music Therapy, therapy management, statistics. MG: therapist, analysis of clinical data, statistics. PK: tinnitus diagnostics, clinical therapy control. WR: neuroradiological screening, coordinator between the facilities, study coordinator.

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REFERENCES

Alpini, D., and Cesarani, A. (2006). Tinnitus as an alarm bell: stress reaction tinnitus model. *ORL* 68, 31–37. doi: 10.1159/000090488

Araneda, R., De Volder, A. G., Deggouj, N., Philippot, P., Heeren, A., Lacroix, E., et al. (2015). Altered top-down cognitive control and auditory processing in tinnitus: evidences from auditory and visual spatial stroop. *Restor. Neurol. Neurosci.* 33, 67–80. doi: 10.3233/RNN-140433

Argstatter, H., Grapp, M., Hutter, E., Plinkert, P. K., and Bolay, H. V. (2015). The effectiveness of neuro-music therapy according to the Heidelberg model compared to a single session of educational counseling as treatment for tinnitus: a controlled trial. *J. Psychosom. Res.* 78, 285–292. doi: 10.1016/j.jpsychores.2014.08.012

Argstatter, H., Grapp, M., Hutter, E., Plinkert, P., and Bolay, H. V. (2012). Long-term effects of the "Heidelberg Model of Music Therapy" in patients with chronic tinnitus. *Int. J. Clin. Exp. Med.* 5, 273–288.

Bahnemann, M., Dziobek, I., Prehn, K., Wolf, I., and Heekeren, H. R. (2010). Sociotopy in the temporoparietal cortex: common versus distinct processes. *Soc. Cogn. Affect. Neurosci.* 5, 48–58. doi: 10.1093/scan/nsp045

Boyen, K., Langers, D. R., de Kleine, E., and van Dijk, P. (2013). Gray matter in the brain: differences associated with tinnitus and hearing loss. *Hear. Res.* 295, 67–78. doi: 10.1016/j.heares.2012.02.010

Cabeza, R., Ciaramelli, E., and Moscovitch, M. (2012). Cognitive contributions of the ventral parietal cortex: an integrative theoretical account. *Trends Cogn. Sci.* 16, 338–352. doi: 10.1016/j.tics.2012.04.008

Chen, Y. C., Xia, W., Luo, B., Muthaiah, V. P., Xiong, Z., Zhang, J., et al. (2015). Frequency-specific alternations in the amplitude of low-frequency fluctuations in chronic tinnitus. *Front. Neural Circuits* 9:67. doi: 10.3389/fncir.2015.00067

Clark, V. P., Fannon, S., Lai, S., Benson, R., and Bauer, L. (2000). Responses to rare visual target and distractor stimuli using event-related fMRI. *J. Neurophysiol.* 83, 3133–3139.

Corbetta, M., and Shulman, G. L. (2002). Control of goal-directed and stimulus-driven attention in the brain. *Nat. Rev. Neurosci.* 3, 201–215. doi: 10.1038/nrn755

De Ridder, D., Vannestea, S., Weisz, N., Londero, A., Schlee, W., Elgoyhen, A. B., et al. (2014). An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci. Biobehav. Rev.* 44, 16–32. doi: 10.1016/j.neubiorev.2013.03.021

Dehaene, S., and Changeux, J. P. (2011). Experimental and theoretical approaches to conscious processing. *Neuron* 70, 200–227. doi: 10.1016/j.neuron.2011.03.018

Downar, J., Crawley, A. P., Mikulis, D. J., and Davis, K. D. (2001). The effect of task relevance on the cortical response to changes in visual and auditory stimuli: an event-related fMRI study. *Neuroimage* 14, 1256–1267. doi: 10.1006/nimg.2001.0946

Flor, H., Hoffmann, D., Struve, M., and Diesch, E. (2004). Auditory discrimination training for the treatment of tinnitus. *Appl. Psychophysiol. Biofeedback* 29, 113–120. doi: 10.1023/B:APBI.0000026637.77671.f4

Folmer, R. L., Theodoroff, S. M., Martin, W. H., and Shi, Y. (2014). Experimental, controversial, and futuristic treatments for chronic tinnitus. *J. Am. Acad. Audiol.* 25, 106–125. doi: 10.3766/jaaa.25.1.7

Goebel, G., and Hiller, W. (1998). *Tinnitus-Fragebogen: (TF); ein Instrument zur Erfassung von Belastung und Schweregrad bei Tinnitus; Handanweisung.* Göttingen: Hogrefe Verlag für Psychologie.

Gottlieb, J. (2007). From thought to action: the parietal cortex as a bridge between perception, action, and cognition. *Neuron* 53, 9–16. doi: 10.1016/j.neuron.2006.12.009

Grapp, M., Hutter, E., Argstatter, H., Plinkert, P. K., and Bolay, H. V. (2013). Music therapy as an early intervention to prevent chronification of tinnitus. *Int. J. Clin. Exp. Med.* 6, 589–593.

Grewal, R., Spielmann, P. M., Jones, S. E., and Hussain, S. S. (2014). Clinical efficacy of tinnitus retraining therapy and cognitive behavioural therapy in the treatment of subjective tinnitus: a systematic review. *J. Laryngol. Otol.* 128, 1028–1033. doi: 10.1017/S0022215114002849

Guo, W., Ren, J., Wang, B., and Zhu, Q. (2015). Effects of relaxing music on mental fatigue induced by a continuous performance task: behavioral and ERPs evidence. *PLoS ONE* 10:e0136446. doi: 10.1371/journal.pone.0136446

Hallam, R. S., McKenna, L., and Shurlock, L. (2004). Tinnitus impairs cognitive efficiency. *Int. J. Audiol.* 43, 218–226. doi: 10.1080/14992020400050030

Heeren, A., Maurage, P., Perrot, H., De Volder, A., Renier, L., Araneda, R., et al. (2014). Tinnitus specifically alters the top- down executive control sub-component of attention: evidence from the attention network task. *Behav. Brain Res.* 269, 147–154. doi: 10.1016/j.bbr.2014.04.043

Henry, J. A., Roberts, L. E., Caspary, D. M., Theodoroff, S. M., and Salvi, R. J. (2014). Underlying mechanisms of tinnitus: review and clinical implications. *J. Am. Acad. Audiol.* 25, 5–22. doi: 10.3766/jaaa.25.1.2

Herraiz, C., Diges, I., Cobo, P., Aparicio, J. M., and Toledano, A. (2010). Auditory discrimination training for tinnitus treatment: the effect of different paradigms. *Eur. Arch. Otorhinolaryngol.* 267, 1067–1074. doi: 10.1007/s00405-009-1182-6

Herraiz, C., Hernandez, F. J., Toledano, A., and Aparicio, J. M. (2007). Tinnitus retraining therapy: prognosis factors. *Am. J. Otolaryngol.* 28, 225–229. doi: 10.1016/j.amjoto.2006.09.004

Hesse, G., and Laubert, A. (2010). Zur Pharmakotherapie des akuten und chronischen Tinnitus. *HNO* 10, 990–998. doi: 10.1007/s00106-010-2179-6

Hesser, H., Weise, C., Westin, V. Z., and Andersson, G. (2011). A systematic review and meta-analysis of randomized controlled trials of

cognitive-behavioral therapy for tinnitus distress. *Clin. Psychol. Rev.* 31, 545–553. doi: 10.1016/j.cpr.2010.12.006

Hester, R., Fassbender, C., and Garavan, H. (2004). Individual differences in error processing: a review and reanalysis of three event-related fMRI studies using the GO/NOGO task. *Cereb. Cortex* 14, 986–994. doi: 10.1093/cercor/bhh059

Hoare, D. J., Adjamian, P., Sereda, M., and Hall, D. A. (2013). Recent technological advances in sound-based approaches to tinnitus treatment: a review of efficacy considered against putative physiological mechanisms. *Noise Health* 15, 107–116. doi: 10.4103/1463-1741.110292

Humphreys, G. W., and Chechlacz, M. (2015). A neural decomposition of visual search using voxel-based morphometry. *J. Cogn. Neurosci.* 27, 1854–1869. doi: 10.1162/jocn_a_00828

Husain, F. T., Akrofi, K., Carpenter-Thompson, J. R., and Schmidt, S. A. (2015). Alterations to the attention system in adults with tinnitus are modality specific. *Brain Res.* 1620, 81–97. doi: 10.1016/j.brainres.2015.05.010

Husain, F. T., Medina, R. E., Davis, C. W., Szymko-Bennett, Y., Simonyan, K., Pajor, N. M., et al. (2011). Neuroanatomical changes due to hearing loss and chronic tinnitus: a combined VBM and DTI study. *Brain Res.* 1369, 74–88. doi: 10.1016/j.brainres.2010.10.095

Igelström, K. M., Webb, T. W., Kelly, Y. T., and Graziano, M. S. (2016). Topographical organization of attentional, social, and memory processes in the human temporoparietal cortex. *eNeuro* 3:ENEURO.0060-16.2016. doi: 10.1523/ENEURO.0060-16.2016

Jonker, T. R., Seli, P., Cheyne, J. A., and Smilek, D. (2013). Performance reactivity in a continuous-performance task: implications for understanding post-error behavior. *Conscious. Cogn.* 22, 1468–1476. doi: 10.1016/j.concog.2013.10.005

Kim, H. (2010). Dissociating the roles of the default-mode, dorsal, and ventral networks in episodic memory retrieval. *Neuroimage* 50, 1648–1657. doi: 10.1016/j.neuroimage.2010.01.051

Krick, C. M., Grapp, M., Daneshvar-Talebi, J., Reith, W., Plinkert, P. K., and Bolay, H. V. (2015). Cortical reorganization in recent-onset tinnitus patients by the Heidelberg Model of Music Therapy. *Front. Neurosci.* 9:49. doi: 10.3389/fnins.2015.00049

Landgrebe, M., Langguth, B., Rosengarth, K., Braun, S., Koch, A., Kleinjung, T., et al. (2009). Structural brain changes in tinnitus: grey matter decrease in auditory and non-auditory brain areas. *Neuroimage* 46, 213–218. doi: 10.1016/j.neuroimage.2009.01.069

Lanting, C., Woźniak, A., van Dijk, P., and Langers, D. R. (2016). Tinnitus- and task-related differences in resting-state networks. *Adv. Exp. Med. Biol.* 894, 175–187. doi: 10.1007/978-3-319-25474-6_19

Leaver, A. M., Seydell-Greenwald, A., Turesky, T. K., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2012). Cortico-limbic morphology separates tinnitus from tinnitus distress. *Front. Syst. Neurosci.* 5:21. doi: 10.3389/fnsys.2012.00021

Leaver, A. M., Turesky, T. K., Seydell-Greenwald, A., Morgan, S., Kim, H. J., and Rauschecker, J. P. (2016). Intrinsic network activity in tinnitus investigated using functional MRI. *Hum. Brain Mapp.* 37, 2717–2735. doi: 10.1002/hbm.23204

Li, Z., Gu, R., Zeng, X., Zhong, W., Qi, M., and Cen, J. (2016). Attentional bias in patients with decompensated tinnitus: prima facie evidence from event-related potentials. *Audiol. Neurootol.* 21, 38–44. doi: 10.1159/000441709

Linehan, M. M., Bohus, M., and Lynch, T. R. (2007). "Dialectical behavior therapy for pervasive emotion dysregulation," in *Handbook of Emotion,* ed J. Gross (New York, NY: Guildford Press), 581–605.

Maldjian, J. A., Laurienti, P. J., Burdette, J. B., and Kraft, R. A. (2003). An automated method for neuroanatomic and cytoarchitectonic atlas-based interrogation of fMRI data sets. *Neuroimage* 19, 1233–1239. doi: 10.1016/S1053-8119(03)00169-1

Malouff, J. M., Schutte, N. S., and Zucker, L. A. (2011). Tinnitus-related distress: a review of recent findings. *Curr. Psychiatry Rep.* 13, 31–36. doi: 10.1007/s11920-010-0163-1

Mirz, F., Pedersen, B., Ishizu, K., Johannsen, P., Ovesen, T., Stødkilde-Jørgensen, H., et al. (1999). Positron emission tomography of cortical centers of tinnitus. *Hear. Res.* 134, 133–144. doi: 10.1016/S0378-5955(99)00075-1

Mohamad, N., Hoare, D. J., and Hall, D. A. (2016). The consequences of tinnitus and tinnitus severity on cognition: a review of the behavioural evidence. *Hear. Res.* 332, 199–209. doi: 10.1016/j.heares.2015.10.001

Møller, A. R. (2016). Sensorineural tinnitus: its pathology and probable therapies. *Int. J. Otolaryngol.* 2016:2830157. doi: 10.1155/2016/2830157

Mugler, J. P. III, and Brookeman, J. R. (1990). Three-dimensional magnetization-prepared rapid gradient-echo imaging (3D MP RAGE). *Magn. Reson. Med.* 15, 152–157. doi: 10.1002/mrm.1910150117

Mühlau, M., Rauschecker, J. P., Oestreicher, E., Gaser, C., Rottinger, M., Wohlschlager, A. M., et al. (2006). Structural brain changes in tinnitus. *Cereb. Cortex* 16, 1283–1288. doi: 10.1093/cercor/bhj070

Myers, P. J., Griest, S., Kaelin, C., Legro, M. W., Schmidt, C. J., Zaugg, T. L., et al. (2014). Development of a progressive audiologic tinnitus management program for Veterans with tinnitus. *J. Rehabil. Res. Dev.* 51, 609–622. doi: 10.1682/JRRD.2013.08.0189

Plewnia, C., Reimold, M., Najib, A., Brehm, B., Reischl, G., Plontke, S. K., et al. (2007). Dose-dependent attenuation of auditory phantom perception (tinnitus) by PET-guided repetitive transcranial magnetic stimulation. *Hum. Brain Mapp.* 28, 238–246. doi: 10.1002/hbm.20270

Price, C. J. (2010). The anatomy of language: a review of 100 fMRI studies published in 2009. *Ann. N. Y. Acad. Sci.* 1191, 62–88. doi: 10.1111/j.1749-6632.2010.05444.x

Raichle, M. E. (2015). The brain's default mode network. *Annu. Rev. Neurosci.* 38, 433–447. doi: 10.1146/annurev-neuro-071013-014030

Roberts, L. E., Husain, F. T., and Eggermont, J. J. (2013). Role of attention in the generation and modulation of tinnitus. *Neurosci. Biobehav. Rev.* 37, 1754–1773. doi: 10.1016/j.neubiorev.2013.07.007

Sadaghiani, S., Hesselmann, G., and Kleinschmidt, A. (2009). Distributed and antagonistic contributions of ongoing activity fluctuations to auditory stimulus detection. *J. Neurosci.* 29, 13410–13417. doi: 10.1523/JNEUROSCI.2592-09.2009

Schecklmann, M., Lehner, A., Poeppl, T. B., Kreuzer, P. M., Rupprecht, R., Rackl, J., et al. (2013). Auditory cortex is implicated in tinnitus distress: a voxel-based morphometry study. *Brain Struct. Funct.* 218, 1061–1070. doi: 10.1007/s00429-013-0520-z

Schneider, M. F., Krick, C. M., Retz, W., Hengesch, G., Retz-Junginger, P., Reith, W., et al. (2010). Impairment of fronto-striatal and parietal cerebral networks correlates with attention deficit hyperactivity disorder (ADHD) psychopathology in adults - a functional magnetic resonance imaging (fMRI) study. *Psychiatry Res.* 183, 75–84. doi: 10.1016/j.pscychresns.2010.04.005

Schneider, P., Andermann, M., Wengenroth, M., Goebel, R., Flor, H., Rupp, A., et al. (2009). Reduced volume of Heschl's gyrus in tinnitus. *Neuroimage* 45, 27–939. doi: 10.1016/j.neuroimage.2008.12.045

Segal, E., and Petrides, M. (2013). Functional activation during reading in relation to the sulci of the angular gyrus region. *Eur. J. Neurosci.* 38, 2793–2801. doi: 10.1111/ejn.12277

Seghier, M. L. (2013). The angular gyrus: multiple functions and multiple subdivisions. *Neuroscientist* 19, 43–61. doi: 10.1177/1073858412440596

Serences, J. T., Shomstein, S., Leber, A. B., Golay, X., Egeth, H. E., and Yantis, S. (2005). Coordination of voluntary and stimulus-driven attentional control in human cortex. *Psychol. Sci.* 16, 114–122. doi: 10.1111/j.0956-7976.2005.00791.x

Seydel, C., Haupt, H., Olze, H., Szczepek, A. J., and Mazurek, B. (2013). Gender and chronic tinnitus: differences in tinnitus-related distress depend on age and duration of tinnitus. *Ear Hear.* 34, 661–672. doi: 10.1097/AUD.0b013e31828149f2

Song, J. J., De Ridder, D., Van de Heyning, P., and Vanneste, S. (2012). Mapping tinnitus-related brain activation: an activation-likelihood estimation metaanalysis of PET studies. *J. Nucl. Med.* 53, 1550–1557. doi: 10.2967/jnumed.112.102939

Stevens, C., Walker, G., Boyer, M., and Gallagher, M. (2007). Severe tinnitus and its effect on selective and divided attention. *Int. J. Audiol.* 46, 208–216. doi: 10.1080/14992020601102329

Stickley, A., Koyanagi, A., Takahashi, H., and Kamio, Y. (2016). ADHD symptoms and pain among adults in England. *Psychiatry Res.* 246, 326–331. doi: 10.1016/j.psychres.2016.10.004

Taylor, P. J. C., and Thut, G. (2012). Brain activity underlying visual perception and attention as inferred from TMSeEEG: a review. *Brain Stimul.* 5, 124–129. doi: 10.1016/j.brs.2012.03.003

Taylor, P. J. C., Muggleton, N. G., Kalla, R., Walsh, V., and Eimer, M. (2011). TMS of the right angular gyrus modulates priming of pop-out in visual search: combined TMS-ERP evidence. *J. Neurophysiol.* 106, 3001–3009. doi: 10.1152/jn.00121.2011

Trevis, K. J., McLachlan, N. M., and Wilson, S. J. (2016). Cognitive mechanisms in chronic tinnitus: psychological markers of a failure to switch attention. *Front. Psychol.* 7:1262. doi: 10.3389/fpsyg.2016.01262

Tzourio-Mazoyer, N., Landeau, B., Papathanassiou, D., Crivello, F., Etard, O., Delcroix, N., et al. (2002). Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 15, 273–289. doi: 10.1006/nimg.2001.0978

Uddin, L. Q., Supekar, K., Amin, H., Rykhlevskaia, E., Nguyen, D. A., Greicius, M. D., et al. (2010). Dissociable connectivity within human angular gyrus and intraparietal sulcus: evidence from functional and structural connectivity. *Cereb. Cortex* 20, 2636–2646. doi: 10.1093/cercor/bhq011

Ueyama, T., Donishi, T., Ukai, S., Ikeda, Y., Hotomi, M., Yamanak, N., et al. (2013). Brain regions responsible for tinnitus distress and loudness: a resting-state fMRI study. *PLoS ONE* 8:e67778. doi: 10.1371/journal.pone.0067778

van Gaal, S., Lamme, V. A., Fahrenfort, J. J., and Ridderinkhof, K. R. (2011). Dissociable brain mechanisms underlying the conscious and unconscious control of behavior. *J. Cogn. Neurosci.* 23, 91–105. doi: 10.1162/jocn.2010.21431

Vanneste, S., Plazier, M., der Loo, Ev., de Heyning, P. V., Congedo, M., and De Ridder, D. (2010). The neural correlates of tinnitus-related distress. *Neuroimage* 52, 470–480. doi: 10.1016/j.neuroimage.2010.04.029

Zeman, F., Koller, M., Schecklmann, M., Langguth, B., Landgrebe, M., and TRI Database Study Group (2012). Tinnitus assessment by means of standardized self-report questionnaires: psychometric properties of the Tinnitus Questionnaire (TQ), the Tinnitus Handicap Inventory (THI), and their short versions in an international and multi-lingual sample. *Health Qual. Life Outcomes* 10:128. doi: 10.1186/1477-7525-10-128

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frontiers in Human Neuroscience

ORIGINAL RESEARCH
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In Patients Undergoing Cochlear Implantation, Psychological Burden Affects Tinnitus and the Overall Outcome of Auditory Rehabilitation

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Cochlear implantation (CI) is increasingly being used in the auditory rehabilitation of deaf patients. Here, we investigated whether the auditory rehabilitation can be influenced by the psychological burden caused by mental conditions. Our sample included 47 patients who underwent implantation. All patients were monitored before and 6 months after CI. Auditory performance was assessed using the Oldenburg Inventory (OI) and Freiburg monosyllable (FB MS) speech discrimination test. The health-related quality of life was measured with Nijmegen Cochlear implantation Questionnaire (NCIQ) whereas tinnitus-related distress was measured with the German version of Tinnitus Questionnaire (TQ). We additionally assessed the general perceived quality of life, the perceived stress, coping abilities, anxiety levels and the depressive symptoms. Finally, a structured interview to detect mental conditions (CIDI) was performed before and after surgery. We found that CI led to an overall improvement in auditory performance as well as the anxiety and depression, quality of life, tinnitus distress and coping strategies. CIDI revealed that 81% of patients in our sample had affective, anxiety, and/or somatoform disorders before or after CI. The affective disorders included dysthymia and depression, while anxiety disorders included agoraphobias and unspecified phobias. We also diagnosed cases of somatoform pain disorders and unrecognizable figure somatoform disorders. We found a positive correlation between the auditory performance and the decrease of anxiety and depression, tinnitus-related distress and perceived stress. There was no association between the presence of a mental condition itself and the outcome of auditory rehabilitation. We conclude that the CI candidates exhibit high rates of psychological disorders, and there is a particularly strong association between somatoform disorders and tinnitus. The presence of mental disorders remained unaffected by CI but the degree of psychological burden decreased significantly post-CI. The implants benefitted patients in a number of psychosocial areas, improving the symptoms of depression and anxiety, tinnitus, and their quality of life and coping strategies. The prevalence of mental disorders in patients who are candidates for CI suggests the need for a comprehensive psychological and psychosomatic management of their treatment.

Keywords: tinnitus, cochlear implants, psychological disorders, CIDI, psychological comorbidities

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INTRODUCTION

Cochlear implants (CI) make it possible for many people with hearing disorders to regain auditory perception and an acoustic understanding of language. The degree of improvement any particular patient experiences is influenced by complex, individual factors, leading to great variations in outcomes. A better grasp of the causes of this variation would improve our ability to predict the outcome for individual CI candidates.

One area of discussion has been the influence of concurrent psychological conditions on the neuronal plasticity of the auditory cortex. Alongside quantitative improvements in hearing, CI is known to have a positive effect on patients' quality of life (Olze et al., 2011, 2012b, 2016; Blasco and Redleaf, 2014; Ramos-Macias et al., 2016). Other influential factors include the progression of deafness, whether and how effectively hearing aids are used in rehabilitation, and motivational and psychological factors among patients—which can only be determined through a quantitative and qualitative measurement of their psychological burden.

In our earlier study of 43 patients with unilateral deafness who had undergone the cochlear implantation, we showed that tinnitus and concurrent psychological conditions had a significant influence on their quality of life (Olze et al., 2011). This study took into account the need for a qualified assessment of the patients' psychological conditions prior to and following implantation. The following year, we demonstrated that the CI patients with a high level of tinnitus-related distress had low quality of life, experienced more stress and more difficulties in coping with their situation (Olze et al., 2012a,b). From 2,251 language tests carried out on post-lingually deafened adults, Blamey et al. concluded that the level of successful postoperative hearing was negatively correlated with the length of the period of deafness prior to Cl (Blamey et al., 2013). Lin et al. observed a 60% improvement of speech recognition among patients over 60 years of age when assessed 1 year post-implantation. Beyond 60, each additional year was accompanied by a drop in the level of speech understanding of 1.3% (Lin et al., 2012). Several of these studies also employed standardized interviews such as the Composite Diagnostic Interview (CIDI), which revealed a range of psychological disturbances.

Van der Werf et al. described an association between psychological disturbances and limitations in hearing in a study of 3,021 youths and adults ranging from 14 to 24 years of age; the subjects were interviewed at regular intervals over a period of 10 years (van der Werf et al., 2011). The younger members of this group (aged 14–17) exhibited a higher incidence of psychological disturbances than those in the 18–24 age group. Their conclusion was that younger patients were going through a more sensitive period of development and as a result, their deafness had a greater impact on their psychological state and social interactions.

Mance and Edwards showed that a young Cl patient's psychological well-being correlated positively with the degree to which they considered themselves similar to people of their own age with normal hearing (Mance and Edwards, 2012). Fellinger et al. (2005) indicated that deaf patients are more commonly affected by somatic disturbances, fear and stress, than those with normal hearing. The deaf subjects had more social contact with other hearing-impaired people than with the unimpaired. The same group of hearing-impaired individuals generally had poorer social relationships than their deaf counterparts (Fellinger et al., 2007). All the scales that were employed yielded worse scores; in contrast to the truly deaf study participants, they were not simply members of a peer group with similar problems. This indicated that their quality of life depends on the level of contentment and success they feel within a system in which hearing plays an important role. The study concludes that the hearing-impaired are generally more isolated than those who are completely deaf, and that Cl can represent a successful form of assistance for this group.

In the present study, we aim to determine the occurrence of psychosocial burden, including the manifestation of psychological diagnoses, using questionnaires and a standardized interview in a subset of patients scheduled to undergo cochlear implantation. We formulated the following hypotheses:

(H1) that patients undergoing CI would suffer from a higher psychological burden than the general population;
(H2) that psychological burden is significantly reduced by the provision of a Cl;
(H3) that a lower-level of post-operative speech recognition improvement is associated with a higher level of psychological burden;
(H4) that certain dimensions of psychosocial limitations decrease the benefits of Cl.

METHODS

Data Collection

Between October 2010 and January 2012, within the framework of the Cochlear Implant Program of the Charité University Hospital in Berlin, 52 post-lingually, bilaterally deafened adults were interviewed prior to CI; 47 subjects were consecutively included in the study (**Table 1**), approved by a local Ethics Committee. All investigations were conducted according to the principles expressed in the Declaration of Helsinki. All patients gave their informed written consent.

The mean value of FB MS on the implanted ear was for all 47 patients 3.08; $SD = 8.8$. On the contralateral ear, 36 patients were hard of hearing with a mean value of FB MS 16.1; $SD = 24.0$, whereas the remaining 11 patients had a mean value of FB MS 77.3; $SD = 16.9$.

The following procedures were applied to all patients prior to implantation and then half a year after they had received the Cl. The CIDI was carried out on a laptop computer. As questionnaires on paper, patients were administered the Oldenburg Inventory (OI), the Nijmegen Questionnaire (NCIQ), and the general perceived quality of life questionnaire (SF36). The following additional questionnaires were completed using a pocket computer: General- Anxiety Disorder (GAD), General Depression Scale (ADS), the COPE Inventory, the tinnitus questionnaire (TQ), and the Perceived Stress Questionnaire. Audiometric measurements were performed in the Audiometry Unit of the ORL, Head and Neck Surgery Department.

TABLE 1 | Descriptive statistics.

Total number of patients	47	
Gender	Women	Men
	30	17
Mean age (years)	56.09	63.08
Mean duration of deafness* on the implanted side (in years)	15.4 ($SD = 18.8$)	
Side of implantation	21 left and 26 right	
Mean use of CI per day (in hours)	13.1 ($SD = 3.7$)	

**According to subjective patient's reports.*

The consent of the Ethics Commission was obtained prior to the study. All patients consented to their participation in the interviews and questionnaires.

Composition of the Patient Sample

The cohort for this study was comprised of 30 women and 17 men who averaged 58.62 years of age and a period of deafness lasting 15.4 years averaged over the group. The cause of deafness varied between the patients: unknown cause of deafness was stated by 19 patients; recurrent otitis media in the childhood was reported by 6 patients; sudden sensorineural hearing loss was reported by 4 patients; noise was reported by 3 patients; meningitis in 2 patients. Meniére's disease, otosclerosis, hypoxia at birth, cholesteatoma, autoimmune diseases, familial hearing loss, and childhood violence were given once as a reason for deafness. Six patients did not answer this question. The period of deafness was determined based on the statement about the time when a patient felt that wearing a hearing aid stopped being of significant benefit. Most of the patients had secondary school education and had completed professional training, they were in a long-term relationship and, as expected given the high average age, were retired. The causes of their hearing loss correspond to the types and distribution generally found in data from literature on this topic.

Composite International Diagnostic Interview

The CIDI was developed in the context of epidemiological studies by the World Health Organization (WHO) (Robins et al., 1988). The interview is used in both a Short Form and the DIA- X/M-CIDI Münchner Composite International Diagnostic Interview (Wittchen, 1994). For this project the latter was used, specifically version 1.2 from 13.08.1999. Its goal is to assess psychological and behavioral disturbances according to ICD-10 and DSM-IV, and its contents are divided into several sections (A through X) and three supplementary sections, listed in **Table 2**. Not all of the sections were employed in this study because to do so would have represented an undue burden on patients' time. Single interviews lasted between 30 and 90 min. Questions covered time frames ranging from the previous 2 weeks to 12 months and extended to the patient's entire lifespan. The interview can be conducted by anyone; no prior clinical or diagnostic expertise is required. The

TABLE 2 | Sections of CIDI.

Section	Description	Included in this study	ICD10 code
A	General questions	X	
B	Disorders resulting from the use of tobacco		F17
C	Somatoform disorders	X	F45
D	Phobic disorders	X	F40- F41
E	Depression (affective disorders F30- F39)	X	F32- F33
F	Manic episode (affective disorders F30)		F30
G	Schizophrenia and schizoaffective disorders		F20- F29
H	Eating disorders	X	F50
I	Psychological and behavioral disorders caused by alcohol	X	F10
K	Obsessive-compulsive disorder	X	F42
L	Disorders resulting from the use of medications other substance abuse	X	F11- F19
M	Organic disorders (including symptomatic mental disorders)		F0- F09
N	Post-traumatic stress disorder	X	F43
ML	Munich task list		
Q	Final questions		
P	Interview observations		
X	Interview judgment		
FR	Family genetics		
SQ	Personal and other questions		
RL	Restless-leg syndrome Questionnaire		

Interrater Reliability of the CIDI lies at 0.81–1.0. The reliability of retesting for single diagnoses of somatic conditions lies between 0.49 and 0.67; for affective disturbances the value lies at 0.77, for single diagnoses between 0.45 and 0.69, and for anxiety diagnoses between 0.57 and 0.72.

Audiometric Testing Procedure: Freiburg Monosyllable Test in Quiet (FB MS)

The Freiburg monosyllable speech discrimination test (Hahlbrock, 1953) involves presenting patients with 2 × 20 monosyllabic words at a volume of 65 dB HL under quiet conditions. The patient's task is to repeat each word. The FB MS can be selected from 10 test lists; here lists 9 and 10 were used. Each correctly repeated word counts for 5% of the comprehension score. The test was carried out on each ear separately prior to the CI procedure (noted as the "ear to be implanted," and "opposite ear"). Following Cl implantation, measurements were carried out using the same lists of words. As on the ear to be implanted, the average speech comprehension prior to CI was only about 3%, the learning bias was excluded. On the non-CI ear, the average speech comprehension was 16.1% for 36 patients and 77.3% for the remaining 11 patients.

Any patients who wore a hearing aid in the "opposite ear" had to remove it prior to the test. The words were presented *via* speakers and the non-CI ears were plugged.

Testing the Quality of Hearing by Questionnaire

Oldenburg Inventory

The OI (Kollmeier and Holube, 1991) originally consisted of 21 items with 5 subscales. The current study used the shortened version of the questionnaire with 12 questions and 3 scales: Listening in a quiet setting (Questions 1, 3, 5, 7), listening with noise interference (4, 6, 8, 11, 12) and directional listening (2, 9, 10). The 12 closed questions about everyday situations were marked with points from 1 to 5. The range for each item of the 3 subscales of the Ol was chosen from 1 to 5 which adds up to a total range from 12 to 60 for the 12 questions. Average scores for each subscale was used as well as the overall result ranging from 1 to 5. The higher the score, the better the subjective hearing.

Nijmegen Cochlear Implantation Questionnaire

The purpose of the NCIQ (Hinderink et al., 2000) is to establish the quality of a patient's life prior to being outfitted with a CI and again afterwards. It is based on 6 scales and consists of 60 items, listed in **Table 3**. The NCIQ scores of the subscales as well as the total score are normalized to percentages. When used in studies of patients with CI, questions about contentment and the time the device has been worn are added to the NCIQ.

TABLE 3 | Domains of the Nijmegen Cochlear Implant Questionnaire (NCIQ).

Domains	Content	
Basic sound perception	Background sounds	NCIQ1
Advanced sound perception	Ability to communicate	NCIQ2
Speech production	Voice monitoring	NCIQ3
Self-esteem (psychological domain	Communicative skills	NCIQ4
activity	Personal and professional	NCIQ5
Social interactions	Communication with family, friends, peer groups	NCIQ6
NCIQ total	Score for health-related quality of life	NCIQ total

Psychometric Questionnaire to Establish Psychological Burden

SF36: Questions Regarding General Health

SF36 (Piehlmeier et al., 1996) is a German translation of the American SF36 Health Survey (Jenkinson et al., 1993) and consists of 8 scales. The questions relate to a patient's state of health and query an individual's overall perception of his or her state of health for the period of 1 week. Estimations of psychological and bodily health are scored separately and appear as individual values in the results provided here.

Tinnitus Questionnaire

The German version of TQ (Goebel and Hiller, 1994) aims to establish the degree of severity of a patient's tinnitus and consists of 52 items distributed in 6 scales. Scores can range from 0 to 84 and are evaluated as follows: a 1st degree burden falls within the range 0–30; 2nd degree, 31–46; 3rd degree, 47–59; and 4th degree from 60 to 84. Scores under 47 are considered "compensated" tinnitus and all higher scores are termed "decompensated." The compensatory type represents a form of tinnitus that is chronic and yet does not impose grave restrictions on a patient's life.

Perceived Stress Questionnaire

The German version of PSQ (Fliege et al., 2005) delivers a patient's subjective perception of stress factors. It comprises 30 items grouped into 7 scales designated as the following: Harassment, Overload, Fatigue/Irritability, Lack of Joy, Worries, and Tension. This study relies on the shortened version of the scale. Every scale can have values from 0 to 1. A score above 0.45 is considered to represent a moderate level of stress; anything above 0.6 is termed high stress.

COPE Inventory

COPE (Carver et al., 1989) is intended to investigate a patient's personal resources and modes of coping with stress. The shortened German Brief-COPE version used in this study contains 4 scales: avoidance, active problem solving, positive thinking, support-seeking behavior (compare with Knoll et al., 2005), whereas the answer scores may range between 0 and 30.

General Anxiety Disorder 7 Questionnaire

GAD-7 (Spitzer et al., 2006) investigates the frequency and degree to which a patient has experienced fear within the 2-week period preceding the questionnaire. Seven items are measured and scored based on patients' responses, using the following scale: 0 = not at all; 1 = on some days; 2 = more often than every other day; 3 = almost every day. A sum of the scores provides a value for estimating the degree of the burden presented by fear (small, mild, medium or strong). The answer scores range between 0 and 21.

General Depression Scale

The ADS (Mohiyeddini et al., 2002) aims to determine the presence, degree, and length of depression a person has experienced within week immediately prior to the questionnaire. 20 items are covered. The points are added to achieve a total score that ranges from 0 to 60. A score over 23 is considered to describe a condition of serious depression. When the ADS is administered to members of the general public, the average score is 14.30 (SD 9.7). The median score achieved by patients diagnosed with depression is 36.70 (SD 8.4), compared to those who experience states of fear or social phobias, who have on the average a median score of 36.60 (SD 8.71).

Statistical Procedure

Forty-seven patients were included in this study. The statistical evaluation was carried out with the software program SPSS (IBM Corp. Released 2015. IBM SPSS Statistics for Windows, Version 23.0.0.2, Armonk, NY: IBM Corp.). Frequency tables, mean

values and standard deviations were calculated descriptively (**Table 2**). The questionnaires and CIDI were evaluated using a nonparametric ANOVA (Friedman Test of relevant samples), once the requirements for the *t*-test of samples were achieved, to test the significance of differences before and following implantation (H2). To clarify the type of association and the influence of various parameters on progressive hearing ability, regression models were calculated, in which the performance in pre- and post-language audiograms were defined as dependent variables.

RESULTS

Hypothesis 1: That Patients Undergoing CI Would Suffer from a Higher Psychological Burden than the General Population (As Measured with CIDI)

Eighty one percent of patients had psychological disturbances before and/or after CI, which is in contrast to the incidence of psychological disturbances in general German population (32.1% in people between 18 and 65 years). In 13% of the cases, patients were diagnosed with a disturbance only prior to CI but not afterwards, while 11% received this diagnosis only afterwards (**Figure 1**).

Prior to implantation, 11% were diagnosed with affective disturbances, 32% suffered from anxiety and 53% from psychosomatic disturbances. Following CI, 11% of the patients exhibited one or more affective disturbances, 30% had anxiety and 34% a psychosomatic illness (**Figure 2**). One patient was diagnosed with obsessive-compulsive disorder before and after CI. Another exhibited a post-traumatic stress disorder prior to CI that was no longer apparent post-implantation based on the CIDI diagnosis.

Hypothesis 1 is regarded as confirmed based on the overall higher psychological comorbidity of relevant diagnoses in the patient group for the areas of anxiety and psychosomatic disturbances, when compared to the general population. These values change only marginally after cochlear implantation. A moderate decrease of psychosomatic disturbances is observed, although it does not reach significance.

Hypothesis 2: That the Psychological Burden Is Significantly Reduced by the Provision of a CI

Here we do not present results related to social data and age, sex, length of wearing the device, contentment, or length of deafness that fail to reach significance, with the exception of the stress level (PSQ). Descriptive results for all of the significant changes in the variables that were evaluated are summarized in **Table 4**.

All of the results related to hearing capacity show a highly significant degree of improvement. They include improvements of the auditory abilities measured by objective (audiometry in quiet and noise and directional hearing) and subjective evaluations (NCIQ, Ol).

All subscores of the Tinnitus Questionnaire and thus the combined total score indicating tinnitus-related distress (TQ) showed significant improvement. The average TQ score decreased significantly from 27.66 prior to CI to 18.70 post-CI ($p > 0.01$; for the subscales see **Table 4**). No significant changes were seen in overall stress perception (PSQ), or in its subscores. The coping mechanisms in COPE changed in the direction of a lower utilization of health care resources. Anxiety (GAD7) and depression (ADS) revealed significant statistical improvement.

All of the self-evaluated and audiometrically measured parameters exhibited significant improvements following CI. Accordingly, we regard hypothesis 2 as confirmed.

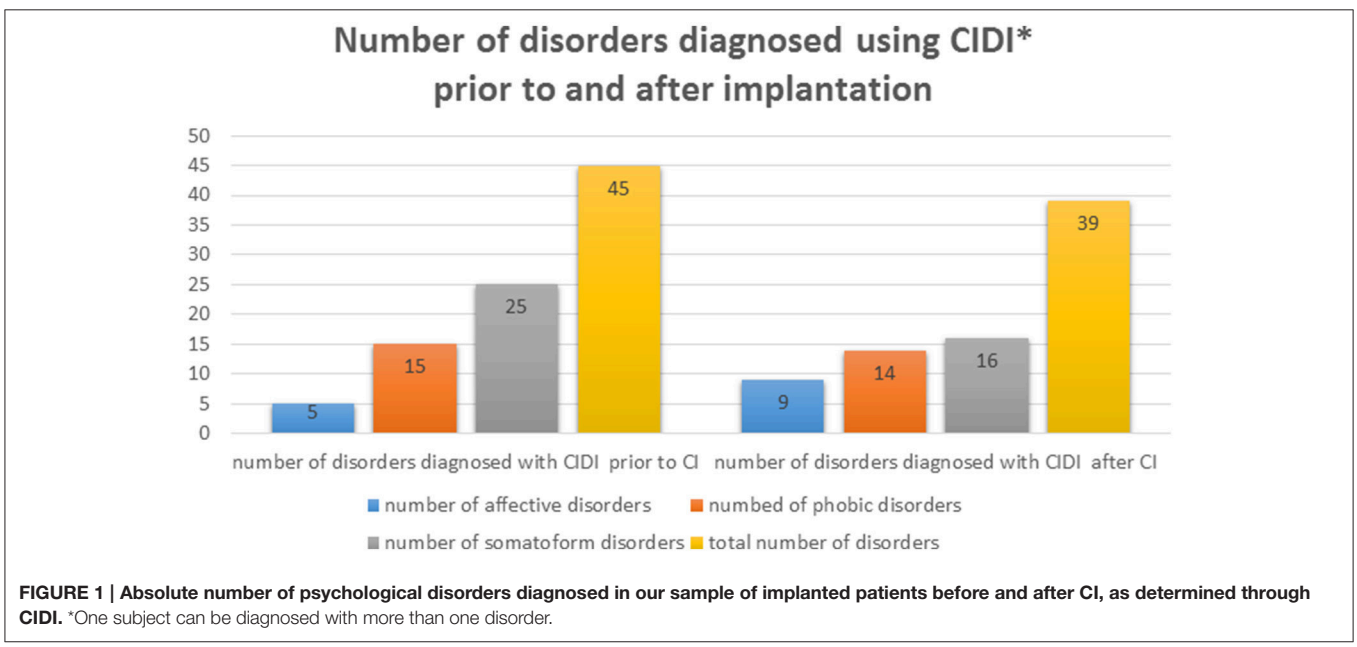

FIGURE 1 | Absolute number of psychological disorders diagnosed in our sample of implanted patients before and after CI, as determined through CIDI. *One subject can be diagnosed with more than one disorder.

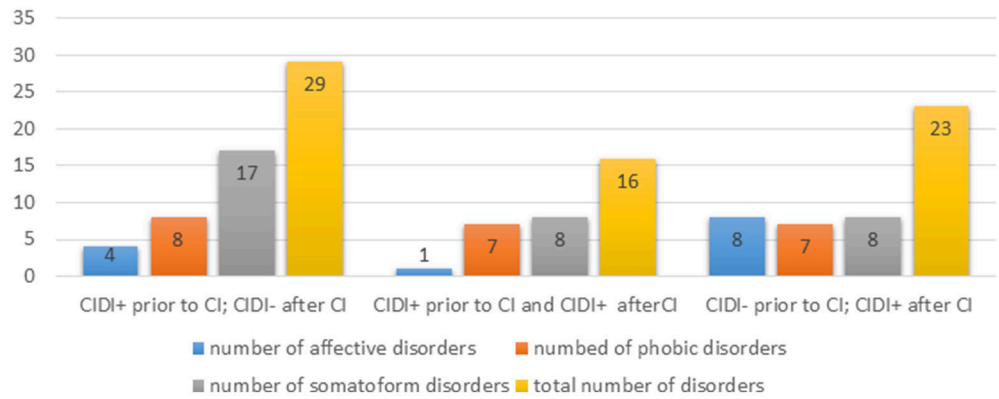

FIGURE 2 | Differences between the numbers of diagnoses occurring in three subgroups of patients. First subgroup represents positive diagnoses of psychological disorders prior to CI and their disappearance after CI. Second subgroup represents positive diagnoses prior to and after CI. The third subgroup represents presence of diagnoses following CI. *One subject can be diagnosed with more than one disorder.

Hypothesis 3: That a Lower-Level of Post-operative Speech Recognition Improvement Is Associated with a Higher Level of Psychological Burden

The analysis of how the psychological disturbances affect the measured parameters, revealed that the patients *without* psychosomatic, anxious or depressive illnesses had significantly better scores in almost all subcategories of the questionnaires before and after cochlear implantations. Across the board in these groups, there was significant improvement in speech comprehension.

Independently of the presence of psychosomatic conditions, changes in the TQ scores indicated significant effect of CI on the emotional burden, hearing problems, sleep disorders or psychosomatic complaints related to tinnitus. Their experience of stress underwent no significant change, with the exception of a significant drop in their level of concern (PSQ). No significant changes were noticed in any subscore of the COPE. Patients without an affective disturbance did not experience a significant change as measured either PSQ or COPE scores between their preoperative and postoperative measurements. This was also the case for patients with anxiety.

Group with Psychological Illness Prior to CI but None Afterwards

Patients who exhibited a psychological disturbance prior to CI but none after implantation could be characterized as follows. Those who were relieved of a psychosomatic illness had better results in FB MS, on all subscores of the OI, basic sound perception (NCIQ1), advanced sound perception (NCIQ2), activity (NCIQ5), emotional and cognitive burden through tinnitus with a significantly poorer performance in SF36 KSS. Patients with anxiety whose condition vanished after CI achieved better scores in directional hearing (OI), active behavior (NCIQ5), the establishment of social interactions (NCIQ6) and active stress management (COPEac).

Group with Psychological Illness before and after CI (Including New Conditions)

Patients whose psychological illness remained after CI, or who exhibited a new condition following the procedure, exhibited notably fewer significant changes overall in the parameters that were measured. The psychosomatic patients improved only in the parameters of hearing accompanied by distracting noise (OI), the establishment of social interactions (NCIQ6), psychological well-being (SF36 PSS), avoidance behavior (COPE ec) and assistance-seeking (COPE sc). The group with anxiety before and after CI showed significant improvement only in hearing accompanied by distracting noise (OI) and in basic sound perception (NCIQ1). This group had, however, significantly poorer results in measurements of physical well-being (SF 36 KSS) and experience of joy (PSQ joy).

The group in which new psychosomatic disturbances appeared had significantly better scores in the subareas: hearing accompanied by distracting noise (OI), speech production (NCIQ3), self-esteem (NCIQ4), activity (NCIQ5), social interactions (NCIQ6), and assistance-seeking behavior (COPEsc), while at the same time achieving significantly lower results for total bodily health (SF36 KSS).

Patients who exhibited new affective illnesses after CI had better results in the Freiburg monosyllable test, hearing against a quiet background (OI), advanced sound perception (NCIQ2), active behavior (NCIQ5) and social interactions (NCIQ6). They had significantly poorer results in total physical scores in SF36.

TABLE 4 | Test scores prior to and following CI (means, significance levels).

	N	Range	mean before CI	Standard deviation	mean after CI	Standard deviation	*Z*	*p* (<0.05)
FB MS	47	0–87.5	3.08	8.81	28.27	28.72	4.394	0.00001
OI quiet setting	45	1.0–4.6	2.56	0.77	3.34	0.85	4.114	0.00004
OI noise interference	45	1.0–4.4	1.93	0.65	2.74	0.80	4.939	0.000001
OI directional listening	45	1.0–4.5	2.10	0.73	2.78	0.95	3.749	0.0002
OI total	45	1.0–4.6	2.22	0.62	3.07	0.70	5.246	0.000000
NCIQ 1	46	0–92.5	45.32	20.95	61.23	17.44	3.74	0.0002
NCIQ 2	47	12.5–78	45.48	16.62	58.26	17.14	4.19	0.00003
NCIQ 3	47	25–82.5	64.02	18.11	71.51	15.86	2.912	0.004
NCIQ 4	46	20–82.5	43.79	16.94	51.54	18.12	2.68	0.007
NCIQ 5	47	0–94.2	41.56	18.29	53.57	18.27	4.342	0.000014
NCIQ 6	46	10–100	44.42	19.37	57.58	22.46	3.960	0.00008
NCIQ total	46	11.4–94	47.28	13.43	59.00	14.94	4.518	0.000006
SF36 physical health	46	21.5–64	52.07	7.48	45.21	10.51	4.357	0.00001
SF36 psychological health	46	15.5–62	42.91	10.72	48.33	9.87	3.414	0.0006
TQ emotional distress	47	0–20	7.19	6.11	5.15	5.77	2.414	0.016
TQ cognitive distress	47	0–14	5.19	4.86	3.808	4.84	3.472	0.0005
TQ intrusiveness	47	0–15	6.34	4.74	4.49	4.53	2.738	0.06
TQ auditory perceptual difficulties	47	0–14	5.70	4.91	3.85	4.26	2.451	0.01
TQ sleep disturbance	47	0–6	1.70	2.21	0.97	1.79	2.259	0.02
TQ somatic complaints	47	0–5	1.00	1.47	0.62	1.21	1.980	0.04
TQ total	47	0–72	27.66	22.01	18.70	19.34	3.001	0.002
PSQ worries	47	0–0.67	0.31	0.18	0.28	0.18	1.87	0.06
PSQ tension	47	0–0.93	0.36	0.22	0.34	0.23	0.726	0.468
PSQ joy	47	0.07–1	0.62	0.23	0.58	0.27	1.400	0.161
PSQ demands	47	0–0.8	0.28	0.18	0.27	0.20	0.570	0.569
PSQ total	47	0–0.69	0.34	0.17	0.33	0.18	0.638	0.524
COPE avoidance	43	7–22	11.74	2.74	10.21	2.97	2.346	0.02
COPE seeking support	43	7–21	13.98	3.4	12.74	3.62	2.718	0.007
COPE positive thinking	43	6–22	13.85	3.04	13.40	3.82	0.705	0.481
COPE active problem solving	43	4–16	10.53	2.59	9.33	2.80	2.056	0.04
Depression (ADSL)	47	5–41	17.93	10.84	14.40	9.24	2.336	0.02
GAD7	47	0–21	5.64	4.69	3.81	4.01	3.057	0.002

Patients diagnosed with anxiety disorders after but not prior to CI, showed significant improvement only in directional hearing (OI), social interactions (NCIQ6) and psychosomatic complaints through tinnitus (TQ SO).

Associations between Psychological Burden and Effects of CI in the Group as a Whole

Overall our work shows that the high-grade anxiety or depression present before (and after) CI lead to poorer estimates of acoustic perception (OI and NCIQ) than in patients who had low-grade of anxiety or depression (**Table 5**). Subjective estimates of a good quality of life (SF36) correlate positively with estimates regarding auditory perception. The benefits of speech recognition (FB MS) demonstrate a negative correlation with the length of deafness. The physical aspects of quality of life are initially reported at a lower level than these benefits, but over time this trend seems to reverse itself. Overall, older patients don't seem to profit as quickly as younger patients with regard to the perception of the quality of hearing, and they estimate their physical quality of life at lower levels before the implantation; the same is true of their estimates of contentment.

We were able to partially confirm hypothesis 3: that the level of psychological burden is accompanied by a decrease in the profit perceived/obtained from CI. This is particularly true in cases of symptoms of anxiety and depression, which potentially influence the target improvement in hearing capacity through their effects on quality of life.

Hypothesis 4: That Certain Dimensions of Psychosocial Limitations Decrease the Benefits of CI

To determine what influence CIDI-diagnosed conditions have on the effects of CI in terms of audiometrically determined speech recognition, we calculated a linear regression. As predictors, the

TABLE 5 | Correlations for pre- and post-implantation results.

	N	Pearson's correlation	Significance (<0.05)
DELTA MS (changes language hearing) and SF 36 psychological	45	−0.394	0.021
DELTA MS (changes language hearing) and duration deafness	45	−0.308	0.059
age and NCIQ (acoustic aspects quality of life) pre	46	−0.321	0.051
age and NCIQ (acoustic aspects quality of life) post	46	−0.321	0.051
age und satisfaction with CI	45	−0.488	0.005
age und SF 36 (bodily health) pre	46	−0.671	0.013
time after implantation and SF 36 PSS (psychological health) pre	45	0.275	0.030
NCIQ total (acoustic QOL) pre and satisfaction	46	0.468	0.007
NCIQ total (acoustic QOL) pre und OL (hearing quality) pre CI	46	0.441	0.011
NCIQ total (acoustic QOL) pre und OL(hearing quality) post CI	46	0.565	0.001
NCIQ total (acoustic QOL) pre and GAD pre CI	46	−0.434	0.012
NCIQ total (acoustic QOL) pre and ADS pre CI	46	−0.357	0.034
NCIQ total (acoustic QOL) post and satisfaction with CI	46	0.453	0.009
NCIQ total (acoustic QOL) post and duration deafness r	46	0.429	0.013
NCIQ total (acoustic QOL) post und OL (hearing quality) pre CI	46	0.549	0.002
NCIQ total (acoustic QOL) post und OL (hearing quality) post CI	46	0.440	0.011
NCIQ total (acoustic QOL) post and GAD (anxiety) pre CI	46	−0.368	0.030
NCIQ total (acoustic QOL) post and ADS (depression) post CI	46	−0.317	0.053
NCIQ total (acoustic QOL) post and SF 36 KSS (somatic aspects quality of life) post	46	0.388	0.013
NCIQ total (acoustic QOL) post and SF 36 PSS (psychological aspects quality of life) post	46	0.521	0.002
OI (hearing quality) pre CI and duration of deafness	46	0.406	0.018
OI (hearing quality) pre CI and satisfaction	46	0.385	0.024
OI (hearing quality) post CI and duration of deafness	46	0.390	0.022
OI (hearing quality) post CI and satisfaction	46	0.399	0.019
OI (hearing quality) post CI and ADS (depression) post CI	46	−0.327	0.048
OI (hearing quality) post CI and TF pre CI	46	0.418	0.015
OI (hearing quality) post CI and time after implantation	46	0.312	0.057
TQ (tinnitus distress) post and duration of deafness	47	0.316	0.054
TQ (tinnitus distress) post and satisfaction	47	−0.313	0.056
TQ (tinnitus distress) post and GAD (anxiety) post CI	47	0.638	0.000
TQ (tinnitus distress) post and ADS (depression) pre CI	47	0.393	0.021
TQ(tinnitus distress) post and ADS (depression) post CI	47	0.406	0.018
GAD (anxiety) pre CI and ADS (depression) pre CI	47	0.650	0.000
GAD (anxiety) pre CI and ADS (depression) post CI	47	0.357	0.034
GAD (anxiety) pre CI and SF 36 KSS (bodily health)	47	−0.364	0.026
GAD (anxiety) pre CI and SF 36 PSS (psychological health)	47	−0.426	0.010
GAD (anxiety) pre CI and stress pre CI	47	0.800	0.000
GAD (anxiety) pre CI and stress post CI	47	0.678	0.000
GAD (anxiety) post CI and duration deafness	47	0.444	0.010
GAD (anxiety) post CI and TQ(tinnitus distress) post	47	0.638	0.000
GAD (anxiety) post CI and stress post CI	47	0.535	0.002
GAD (anxiety) post CI and depression post CI	47	0.589	0.001
ADS (depression) pre CI and stress pre CI	47	0.787	0.000
ADS (depression) pre CI and stress post CI	47	0.744	0.000
ADS (depression) pre CI and SF 36 PSS (psychological aspects quality of life)	47	−0.620	0.002
ADS (depression) post CI and stress pre CI	47	10.53	0.04
ADS (depression) post CI and stress post CI	47	17.93	0.02
ADS (depression) post CI and SF 36 PSS (psychological health)	47	5.64	0.002

Shown are only significant results, with no correlations between tests.

TABLE 6 | Regression model: coefficients of predictors for speech perception after CI.

Model		Unstandardized coefficients		Standardized Coefficients	*t*	Significance
		B	Std. Error	Beta		
1	(Constant)	28.834	24.786		1.163	0.254
	Satisfaction	0.002	0.203	0.002	0.011	0.991
	Compliance (duration of CI per day)	1.116	1.559	0.123	0.716	0.479
	Affective disorder before but not after CI	20.601	19.737	0.197	1.044	0.305
	No affective disorder before but after CI	21.656	15.895	0.247	1.362	0.183
	Phobic disorder before but not after CI	−25.313	15.896	−0.373	−1.592	0.121
	No phobic disorder before but after CI	−25.820	14.500	−0.313	−1.781	0.085
	Phobic disorder before and after CI	28.958	24.032	0.306	1.205	0.237
	Somatoform Disorder before but not after CI	−14.527	12.848	−0.230	−1.131	0.267
	No somatoform disorder before but after CI	−31.122	18.030	−0.378	−1.726	0.094
	Somatoform disorder before and after CI	−28.909	18.616	−0.330	−1.553	0.131

Dependent Variable: Delta in speech perception (Freiburg monosyllable speech perception test).

presence of symptoms of affective, psychosomatic and anxiety disturbances were considered; the time of wearing a hearing aid and level of contentment were used as controls. The overall regression model was determined not to be significant ($F = 1.175$, $p < 0.344$).

Post-operative statistics reveal a tendency toward effects related to psychosomatic and anxiety disturbances. Patients who suffered from these types of conditions before and after the implantation exhibited less of a gain in audiometrically measured speech recognition. This effect was already evident in correlations related to the questionnaires (see **Table 6**), but only shows a tendency toward significance. Overall, in explaining the significant improvements in all areas of auditory perception and particularly language perception of the group of patients who underwent cochlear implantation, the presence of a psychological disturbance does not appear to be relevant.

Hypothesis 4 regarding the influence of diagnoses related to psychological factors cannot be confirmed, but psychosomatic effects and anxiety before the surgery seem to have an influence on the negative effect hearing specifics after CI.

DISCUSSION

Psychological Diagnosis and Hearing Deficiencies

In Germany, approximately 32.1% of the population aged 18–65 is affected by psychological disturbances. Figures from the Federal German Retirement Insurance system state that in the year 2014, 43% of the population reported having been affected by such problems at least once in their lives; the results are a reduction in performance and a retreat from social life. These disturbances are the most common reason for work-related disabilities in Germany, which is a key motivation to develop effective diagnoses and treatment. In our survey of 47 deaf patients, 31 had a psychological diagnosis before the implantation, a figure which is significantly higher than the average in the general population. Following the intervention, 25 patients (over half of the measured group) had such a diagnosis, which is still much higher than the standard reference. We regard this as relevant despite methodological criticisms which have been raised concerning the form of the interview that was used.

It is impossible to say how reliable and valid the results of the diagnoses captured with CIDI are in relation to clinical diagnoses. Becker et al. regard the use of CIDI as insufficient; in a study of the degree of consistency between clinical diagnoses and the use of CIDIs, they found Kappa values between 0.0 and 0.33 for all diagnoses (Becker et al., 2006). Such unsatisfactory values for the validity and reliability of the CIDI are partially confirmed by the literature (Semler et al., 1987). The important coping disturbances in deaf patients are not covered in these estimates (Hund et al., 2014). Kessler et al. reported good rates of correspondence between clinical diagnoses and standard interviews (Kessler et al., 2009). Knappe et al. consider the use of standardized interviews such as CIDI the "gold standard" (Knappe et al., 2008). In spite of these methodological issues, in agreement with data, we believe that the higher level of psychological diagnoses in our sample is an accurate representation of the patients' situation. In this context, we do not consider the burden of biopsychological and social factors related to deafness and potential side effects of the treatment to be the only causes of the higher level of psychological diagnoses. Some of the problems may be neurological; animal experiments have shown that there are direct connections between the acoustic cortex and the limbic system, and these also may play a role (Kraus and Canlon, 2012).

Our results are in agreement with data from the literature. In the current patient cohort, CIDI diagnoses could be broken down into three major groups: affective, anxiety-related, and psychosomatic disturbances. The diagnoses were commonly the following: for affective disturbance, dysthymia (coping disturbances); in anxiety: agoraphobia and other phobias of an indistinct nature; psychosomatic disturbances: pain and undefined psychosomatic conditions (possibly including tinnitus). Using CIDIs, we diagnosed frequent affective,

psychosomatic and anxiety disorders in patients with chronic tinnitus (Zirke et al., 2013). This is in agreement with high prevalence of psychological disorders diagnosed in patients with other chronic somatic diseases, such as cardiovascular diseases (Baumeister and Harter, 2011) or kidney dysfunction (McClellan et al., 2010).

Improvement of Burden Following CI

The loss of binaural hearing is associated with a decreased speech comprehension in noise and problems with sound localization, even when the unaffected ear is healthy. Additionally, in CI patients diagnosed with unilateral hearing loss and tinnitus, they showed improvement in speech understanding in noise following implantation (Blasco and Redleaf, 2014). Our study revealed a significant improvement in speech discrimination following CI (measured with FB MS, which is a standard component of measuring the speech recognition). Subjective hearing abilities increased in all subscales (OI), as was the case for all the subdomains of the NCIQ. Parallel use of audiometric and psychometric evaluations leads to consistent measurement of the CI effects.

Today, the improvement of speech recognition that is achieved through CI is presumed to be linked at least in part to the neuronal plasticity of the cortex. The ultrastructural organization of each component of the auditory system (periphery, cochlear nucleus and auditory midbrain and the auditory cortex) undergoes degradation if it is not stimulated (Baizer et al., 2015). Anatomical and physiological changes have all been shown to be at least partly reversible with renewed stimulation of the cochlear nerve, even considering that certain periods are more sensitive than others. This reorganization occurs in many areas: those that are directly connected to the cochlear nerve, those anatomically linked to it, more distant regions, and in homologically related areas of the other brain hemisphere (Hotting and Roder, 2013). In a review, Kral also addressed the issue of sensitive periods and visual/auditory cross-modal reorganization in adults who become deaf post-lingually (Kral, 2007). In imaging studies, a hypometabolism of the auditory cortex was predictive of better speech perception following CI (Lee et al., 2007). Our present study deals with the connectivity between the auditory cortex (possibly undergoing plastic changes) with other cortical areas like limbic system or the cortical areas responsible for attention.

Here TQ, COP, PSQ, ADS, and GAD7 revealed significant improvements, also confirming the results of previous studies. All of the subscales of the TQ achieved better scores. Earlier we reported a 39.2% drop in the burden of tinnitus after 2 years, and none of the patients without tinnitus exhibited worsening in this area (Olze et al., 2012b, 2016).

The effects of tinnitus are also considered highly connected to basic principles of synaptic and cortical plasticity (Mazurek et al., 2010; Georgiewa et al., 2016). Synaptic plasticity is defined as the degree to which the strength of synaptic transmission changes through activity. The repeated stimulation of afferent processes can lead to changes through long-term potentiation (LTP), whereas decreases in stimulation can cause long-term depression (LTD). Damage to the central or peripheral auditory systems can lead to an imbalance between LTP and LTD, and consequently to changes in the activity of ion channels, receptors, and neuronal transmission. Cellular hyperactivity may induce hyperactivation of the central auditory pathways and change cortical plasticity in ways that lead to tinnitus. A restoration of acoustic stimulation can be achieved through cochlear implants, and in the best-case-scenario this may lead to a reorganization of synaptic plasticity in ways that are accompanied by a reduction in tinnitus, which we observed in our sample using the TQ.

The COPE Inventory revealed a decrease in the degree to which patients rely on coping strategies following CI. In other words, a significant drop in efforts required to master a situation was observed after CI. This finding is consistent with Kobosko et al. (2012), who found that 78 post-lingually deafened adult patients employed less active coping strategies and more avoidance following CI.

The values we obtained here for HRQoL (SF-36) showed significant drops in total scores for physical factors, whereas psychological scores rose. However, our overall results suggest general improvements in the quality of life, corroborating similar findings of Arnoldner et al., who also reported inconsistencies in SF 36 with regard to the quality of life in a 10-year study following cochlear implantations (Arnoldner et al., 2014).

Perceived stress (PSQ) revealed no significant changes following CI but the scores of depression (ADS) and anxiety (GAD7) demonstrated statistically significant improvements. We speculate that this could reflect stronger connectivity between the auditory and limbic systems (amygdala) than this between the auditory system and HPA axis. At both measurement time points, ADS scores lay higher than those for the general population ($M = 14.30$), but clearly below the cut-off of 23, which is considered the level at which a patient is considered manifestly depressive. The scores we obtained were 17.93 ± 10.84 (prior to CI) and 14.40 ± 9.24 (post-CI).

The Effects of Psychological Disturbances on the Success of Treatment

The significant improvements in auditory and psychosocial parameters that we measured were higher for patients without psychological disturbances than for those who had been diagnosed. The former group enjoyed greater benefits in terms of the quality of life, their perception of tinnitus and speech recognition. This does not, however, seem to be directly related to the presence of preexisting psychological diagnoses. We were unable to confirm hypothesis 4 using a regression model to account for changes in speech comprehension. We interpret the correlative data in **Table 6** as potential evidence for an indirect link between the degree of psychological burden and the success of treatment. One factor to consider in this regard is that the neuronal plasticity of the auditory cortex permits an improvement of hearing after CI even in patients with high levels of burden. The degree of improvement is clearly lower, however, in patients suffering from depression or anxiety. This too, in our opinion, may be due to connectivity within the hearing networks of the brain, which

are thought to directly link the auditory cortex with the amygdala (Kraus and Canlon, 2012). Existing emotional burdens could lead to defects in this network. Another issue that will require further reflection is whether affective disorders—or the limitations in learning and memory that accompany them—have a direct impact on the mechanisms underlying neuronal plasticity.

Clinicians have long recognized that patients burdened by both a physical illness and a psychological disturbance have a lower life expectancy, poorer compliance, and poorer quality of life than those who exhibit only physical symptoms. The identification of accompanying psychological disturbances is a basic requirement in establishing a patient's right to rehabilitative care and is also crucial in predicting the likely effect of therapies on individuals (Blasco and Redleaf, 2014). Past observations by our group also indicate that patients with chronic tinnitus achieved significantly better scores not only for the burden brought by tinnitus, but also for stress, anxiety and other parameters if there was no indication of an accompanying psychological disorder (Zirke et al., 2013).

A diagnosis of anxiety or psychosomatic disturbances seems to have a particularly negative effect on the degree to which speech recognition improves from the pre- to postoperative stages of CI. The effects of therapy are modulated by mechanisms of neuronal plasticity, as described above, but also by behavioral patterns and dysfunctional coping strategies. Anxiety promotes avoidance behavior and a tendency to withdraw, which can result in a failure to engage in essential types of communication. The trends seen in our psychosomatic observations suggest that a focus on models and therapies related to the physical causes of these conditions places a stronger emphasis on external factors in accounting for the benefits of therapy, while making patients less aware of and less likely to draw on their resources to influence the outcome. For instance, patients should be psychologically encouraged and supported in their use of CI. We assumed that some CI recipients are simply highly focused on the degree of their disability before the implantation, whether or not they are affected by a psychological disturbance (Olze et al., 2011). As their hearing improves, psychological and other types of burdens, which may have been masked by auditory and communication problems, move into the foreground.

Study Limitations

The major limitations of this study are small size and lack of homogeneity in our sample. Of 47 patients, 36 were bilaterally deaf. The remaining 11 patients had a significant hearing loss on the non-CI ear that could not be classified as deafness. This might have affected some of our results, for instance it could have lowered the average scores of anxiety post-implantation. In addition, there may be differences between the bilaterally- and single sided deafened patients with significant hearing loss on non-CI ear, in terms of CIDI psychological diagnoses. Future focus on homogenous groups and increasing the sample number will clarify this issue. The next limitation of our study is the heterogeneity of our sample regarding the duration of hearing loss. The last limitation is the known hypersensitivity of disease detection by CIDI, when compared to classical diagnostic process (Becker et al., 2006; Terber et al., 2010). Despite this fact, this is the first work when CIDI is used to diagnose the patients undergoing cochlear implantation.

Clinical Significance

Since deaf patients have been shown to suffer from a higher psychological stress than the normal-hearing people, psychometric diagnosis and a patient history extended by psychosomatic aspects are indispensable when planning CI. In the presence of manifest psychological disorder (depression, anxiety, somatization disorder) appropriate therapeutic co-operation should be initiated even before the implantation. Apart from establishing the psychological status of a patient before the CI, psychosomatic monitoring should also be carried out after the implantation because of possible occurrence of psychological disorders after the CI, which could make the auditory rehabilitation process more difficult. Over the entire course of treatment, close cooperation of the ORL, head and neck surgeons and audiologists with therapists specialized in psychosomatic medicine is indispensable in order to achieve an optimal result of cochlear rehabilitation.

SUMMARY

In general, patients suffering from deafness have higher levels of psychological burden than the population as a whole. The standardized tools of psychiatric diagnosis reveal that they are particularly subject to affective disorders and anxiety, as well as psychosomatic illnesses, which are strongly associated with tinnitus. Conditions diagnosed before the surgery do not directly change through the implantation—this can only occur through targeted therapies. According to the sample description (age, sex, and other social variables) none of these factors significantly correlated with the significant improvement in all aspects of hearing abilities measured during this study. However, duration of deafness correlated significantly with the hearing quality (measured by Ol) pre- and post-CI. In addition, CI did have positive influence extending beyond hearing, particularly in many psychosocial aspects of their lives, including self-evaluations of their levels of depression and anxiety, tinnitus, quality of life, and coping strategies, as measured through the questionnaires.

Our results align with current models of the mechanisms that underlie neuronal plasticity, which at least partially account for post-CI improvements in the auditory processing of patients, including reductions in the symptoms of tinnitus, independent of a person's degree of psychological burden. While here the influence of concurrent psychological illnesses and various aspects of a patient's quality of life seem to have a rather indirect effect, it will be crucial to collect further data, such as the degree of hearing loss in the contralateral ear, to capture a better picture of how these factors, which vary highly between individuals, should be

integrated into predictions about the potential effectiveness of therapies. This strongly suggests that through the entire process of cochlear implantation, from the original diagnosis through rehabilitation and follow-up care, there is a particular need to provide comprehensive psychosomatic care for patients, especially considering the negative effects that anxiety and other psychosomatic conditions have on the ultimate value of the therapy in their lives.

AUTHOR CONTRIBUTIONS

PB: study design; analysis and interpretation of data; drafting of manuscript. AS: analysis and interpretation of data; drafting of manuscript. KK: data acquisition; analysis and interpretation of data. SG: data acquisition; data analysis. BM: critical revision; HO: study conception and design; analysis and interpretation of data.

REFERENCES

Arnoldner, C., Lin, V. Y., Honeder, C., Shipp, D., Nedzelski, J., and Chen, J. (2014). Ten-year health-related quality of life in cochlear implant recipients: prospective SF-36 data with SF-6D conversion. *Laryngoscope* 124, 278–282. doi: 10.1002/lary.24387

Baizer, J. S., Wong, K. M., Manohar, S., Hayes, S. H., Ding, D., Dingman, R., et al. (2015). Effects of acoustic trauma on the auditory system of the rat: the role of microglia. *Neuroscience* 303, 299–311. doi: 10.1016/j.neuroscience.2015.07.004

Baumeister, H., and Harter, M. (2011). [Psychological comorbidity in patients with musculoskeletal diseases]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 54, 52–58. doi: 10.1007/s00103-010-1185-x

Becker, J., Kocalevent, R. D., Rose, M., Walter, O. B., Frommer, J., and Klapp, B. F. (2006). Standardized diagnosing: computer-assisted (CIDI) diagnoses compared to clinically-judged diagnoses in a psychosomatic setting. *Psychotherapie Psychosomatik Medizinische Psychologie* 56, 5–14. doi: 10.1055/s-2005-866997

Blamey, P., Artieres, F., Baskent, D., Bergeron, F., Beynon, A., Burke, E., et al. (2013). Factors affecting auditory performance of postlinguistically deaf adults using cochlear implants: an update with 2251 patients. *Audiol. Neurootol.* 18, 36–47. doi: 10.1159/000343189

Blasco, M. A., and Redleaf, M. I. (2014). Cochlear implantation in unilateral sudden deafness improves tinnitus and speech comprehension: meta-analysis and systematic review. *Otol. Neurotol.* 35, 1426–1432. doi: 10.1097/MAO.0000000000000431

Carver, C. S., Scheier, M. F., and Weintraub, J. K. (1989). Assessing coping strategies: a theoretically based approach. *J. Pers. Soc. Psychol.* 56, 267–283. doi: 10.1037/0022-3514.56.2.267

Fellinger, J., Holzinger, D., Gerich, J., and Goldberg, D. (2007). Mental distress and quality of life in the hard of hearing. *Acta Psychiatr. Scand.* 115, 243–245. doi: 10.1111/j.1600-0447.2006.00976.x

Fellinger, J., Holzinger, D., Schoberberger, R., and Lenz, G. (2005). [Psychosocial characteristics of deaf people: evaluation of data from a special outpatient clinic for the deaf]. *Nervenarzt* 76, 43–51. doi: 10.1007/s00115-004-1708-5

Fliege, H., Rose, M., Arck, P., Walter, O. B., Kocalevent, R. D., Weber, C., et al. (2005). The Perceived Stress Questionnaire (PSQ) reconsidered: validation and reference values from different clinical and healthy adult samples. *Psychosom. Med.* 67, 78–88. doi: 10.1097/01.psy.0000151491.80178.78

Georgiewa, P., Szczepek, A. J., Rose, M., Klapp, B. F., and Mazurek, B. (2016). Cerebral processing of emotionally loaded acoustic signals by tinnitus patients. *Audiol. Neurootol.* 21, 80–87. doi: 10.1159/000443364

Goebel, G., and Hiller, W. (1994). [The tinnitus questionnaire. A standard instrument for grading the degree of tinnitus. Results of a multicenter study with the tinnitus questionnaire]. *HNO* 42, 166–172.

Hahlbrock, K. H. (1953). [Speech audiometry and new word-tests]. *Arch. Ohren Nasen Kehlkopfheilkd.* 162, 394–431. doi: 10.1007/BF02105664

Hinderink, J. B., Krabbe, P. F., and Van Den Broek, P. (2000). Development and application of a health-related quality-of-life instrument for adults with cochlear implants: the Nijmegen cochlear implant questionnaire. *Otolaryngol. Head Neck Surg.* 123, 756–765. doi: 10.1067/mhn.2000.108203

Hotting, K., and Roder, B. (2013). Beneficial effects of physical exercise on neuroplasticity and cognition. *Neurosci. Biobehav. Rev.* 37, 2243–2257. doi: 10.1016/j.neubiorev.2013.04.005

Hund, B., Reuter, K., Jacobi, F., Siegert, J., Wittchen, H.-U., Härter, M., et al. (2014). Adaptation of the composite international diagnostic interview (CIDI) for the assessment of comorbid mental disorders in oncology patients: the CIDI-O. *Psychother Psych. Med.* 64, 101–107. doi: 10.1055/s-0033-1357174

Jenkinson, C., Coulter, A., and Wright, L. (1993). Short form 36 (SF36) health survey questionnaire: normative data for adults of working age. *BMJ* 306, 1437–1440. doi: 10.1136/bmj.306.6890.1437

Kessler, R. C., Avenevoli, S., Green, J., Gruber, M. J., Guyer, M., He, Y., et al. (2009). National comorbidity survey replication adolescent supplement (NCS-A): III. Concordance of DSM-IV/CIDI diagnoses with clinical reassessments. *J. Am. Acad. Child Adolesc. Psychiatry* 48, 386–399. doi: 10.1097/CHI.0b013e31819a1cbc

Knappe, S., Runge, J., Beesdo, K., Jacobi, F., and Wittchen, H. U. (2008). [Diagnosing mental disorders: gold or tin standard?–Critical comments on standardized diagnostic interviews and clinical routine diagnoses]. *Psychother. Psychosom. Med. Psychol.* 58, 72–75. doi: 10.1055/s-2007-986197

Knoll, N., Rieckmann, N., and Schwarzer, R. (2005). Coping as a mediator between personality and stress outcomes: a longitudinal study with cataract surgery patients. *Eur. J. Pers.* 19, 229–247. doi: 10.1002/per.546

Kobosko, J., Pankowska, A., and Skarżyński, H. (2012). Coping strategies in postlingually deafened adult cochlear implant users in comparison to the hearing population. *Otolaryngol. Pol.* 66, 132–137. doi: 10.1016/S0030-6657(12)70761-8

Kollmeier, B., and Holube, I. (1991). Ein Fragebogen zur Erfassung des subjektiven Hörvermögens: erstellung der Fragen und Beziehungen zum Tonschwellenaudiogramm. *Audiologische Akustik* 30, 48–64.

Kral, A. (2007). Unimodal and cross-modal plasticity in the 'deaf' auditory cortex. *Int. J. Audiol.* 46, 479–493. doi: 10.1080/14992020701383027

Kraus, K. S., and Canlon, B. (2012). Neuronal connectivity and interactions between the auditory and limbic systems. Effects of noise and tinnitus. *Hear. Res.* 288, 34–46. doi: 10.1016/j.heares.2012.02.009

Lee, J. C., Yoo, M. H., Ahn, J. H., and Lee, K. S. (2007). Value of the promontory stimulation test in predicting speech perception after cochlear implantation. *Laryngoscope* 117, 1988–1992. doi: 10.1097/MLG.0b013e318 13437e6

Lin, F. R., Chien, W. W., Li, L., Clarrett, D. M., Niparko, J. K., and Francis, H. W. (2012). Cochlear implantation in older adults. *Medicine (Baltimore).* 91, 229–241. doi: 10.1097/MD.0b013e31826b145a

Mance, J., and Edwards, L. (2012). Deafness-related self-perceptions and psychological well-being in deaf adolescents with cochlear implants. *Cochlear Implants Int.* 13, 93–104. doi: 10.1179/1754762811Y.0000000017

Mazurek, B., Olze, H., Haupt, H., Klapp, B. F., Adli, M., Gross, J., et al. (2010). [Molecular biological aspects of neuroplasticity: approaches for treating tinnitus and hearing disorders]. *HNO* 58, 973–982. doi: 10.1007/s00106-010-2177-8

McClellan, W. M., Abramson, J., Newsome, B., Temple, E., Wadley, V. G., Audhya, P., et al. (2010). Physical and psychological burden of chronic kidney disease among older adults. *Am. J. Nephrol.* 31, 309–317. doi: 10.1159/000285113

Mohiyeddini, C., Hautzinger, M., and Bauer, S. (2002). A latent state-trait analysis on assessing trait and state components of three instruments for measuring depression: ADS, BDI, and SDS. *Diagnostica* 48, 12–18. doi: 10.1026//0012-1924.48.1.12

Olze, H., Grabel, S., Forster, U., Zirke, N., Huhnd, L. E., Haupt, H., et al. (2012a). Elderly patients benefit from cochlear implantation regarding auditory rehabilitation, quality of life, tinnitus, and stress. *Laryngoscope* 122, 196–203. doi: 10.1002/lary.22356

Olze, H., Knopke, S., Grabel, S., and Szczepek, A. J. (2016). Rapid positive influence of cochlear implantation on the quality of life in adults 70 years and older. *Audiol. Neurootol.* 21(Suppl. 1), 43–47. doi: 10.1159/000448354

Olze, H., Szczepek, A. J., Haupt, H., Forster, U., Zirke, N., Grabel, S., et al. (2011). Cochlear implantation has a positive influence on quality of life, tinnitus, and psychological comorbidity. *Laryngoscope* 121, 2220–2227. doi: 10.1002/lary.22145

Olze, H., Szczepek, A. J., Haupt, H., Zirke, N., Graebel, S., and Mazurek, B. (2012b). The impact of cochlear implantation on tinnitus, stress and quality of life in postlingually deafened patients. *Audiol. Neurootol.* 17, 2–11. doi: 10.1159/000323847

Piehlmeier, W., Bullinger, M., Kirchberger, I., Land, W., and Landgraf, R. (1996). Evaluation of the quality of life of patients with insulin-dependent diabetes mellitus before and after organ transplantation with the SF 36 health survey. *Eur. J. Surg.* 162, 933–940.

Ramos-Macias, A., Falcon Gonzalez, J. C., Borkoski-Barreiro, S. A., Ramos De Miguel, A., Batista, D. S., and Perez Plasencia, D. (2016). Health-related quality of life in adult cochlear implant users: a descriptive observational study. *Audiol. Neurootol.* 21(Suppl. 1), 36–42. doi: 10.1159/000448353

Robins, L. N., Wing, J., Wittchen, H. U., Helzer, J. E., Babor, T. F., Burke, J., et al. (1988). The composite international diagnostic interview. An epidemiologic instrument suitable for use in conjunction with different diagnostic systems and in different cultures. *Arch. Gen. Psychiatry* 45, 1069–1077.

Semler, G., Wittchen, H. U., Joschke, K., Zaudig, M., Von Geiso, T., Kaiser, S., et al. (1987). Test-retest reliability of a standardized psychiatric interview (DIS/CIDI). *Eur. Arch. Psychiatry Neurol. Sci.* 236, 214–222. doi: 10.1007/BF00383851

Spitzer, R. L., Kroenke, K., Williams, J. B. W., and Lowe, B. (2006). A brief measure for assessing generalized anxiety disorder - The GAD-7. *Arch. Intern. Med.* 166, 1092–1097. doi: 10.1001/archinte.166.10.1092

Terber, S., Bernardy, K., Philippe, J., Untersinger, I., and Kollner, V. (2010). Clinical diagnosis adjustment disorder: what does a structured interview reveal? *J. Psychosom. Res.* 68, 669.

van der Werf, M., Thewissen, V., Dominguez, M. D., Lieb, R., Wittchen, H., and Van Os, J. (2011). Adolescent development of psychosis as an outcome of hearing impairment: a 10-year longitudinal study. *Psychol. Med.* 41, 477–485. doi: 10.1017/S0033291710000978

Wittchen, H. U. (1994). Reliability and validity studies of the WHO–composite international diagnostic interview (CIDI): a critical review. *J. Psychiatr. Res.* 28, 57–84. doi: 10.1016/0022-3956(94)90036-1

Zirke, N., Seydel, C., Szczepek, A. J., Olze, H., Haupt, H., and Mazurek, B. (2013). Psychological comorbidity in patients with chronic tinnitus: analysis and comparison with chronic pain, asthma or atopic dermatitis patients. *Qual. Life Res.* 22, 263–272. doi: 10.1007/s11136-012-0156-0

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Cochlear Implantation of Bilaterally Deafened Patients with Tinnitus Induces Sustained Decrease of Tinnitus-Related Distress

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Objective: Tinnitus is a common symptom of hearing impairment. Patients who are bilaterally hard of hearing are often affected by tinnitus. However, they cannot undergo any of the standard tinnitus therapies, since they rely on hearing. Cochlear implantation (CI) used to treat severe hearing disabilities, such as bilateral hearing loss, was also shown to reduce tinnitus. Our goal was to determine if CI induces sustained reduction of tinnitus. We performed prospective, longitudinal analyses of tinnitus-related distress in a uniform group of bilaterally deafened patients after CI.

Patients and Methods: The homogenous sample consisted of 41 patients who met the inclusion criteria and were consecutively included in this study. The impact of unilateral CI on tinnitus-related distress, health-related quality of life (HRQoL), and hearing abilities was studied with validated instruments. The follow-up appointments were scheduled at 6, 12, and 24 months after CI surgery. During the appointments, hearing abilities were estimated with monosyllabic Freiburg test, whereas the tinnitus-related distress, the HRQoL, and the subjective hearing were measured with standard questionnaires [Tinnitus Questionnaire (TQ), Nijmegen Cochlear Implantation Questionnaire, and Oldenburg Inventory, respectively].

Results: Tinnitus-related distress decreased significantly from the mean TQ score of 35.0 (SD = 19.6) prior to surgery to the mean TQ = 27.54 (SD = 20.0) 6 months after surgery and remained sustained low until the end of follow-up period. In addition, CI significantly improved the hearing abilities and the HRQoL of all patients.

Conclusion: The results from our prospective study suggest that in a homogenous sample of bilaterally deafened, implanted patients who report having tinnitus prior to surgery, CI alone not only improves the hearing abilities but also significantly reduces the tinnitus-related distress and improves the HRQoL in a sustained way.

Keywords: cochlear implantation, hearing impairment, health-related quality of life, tinnitus-related distress, depressive symptoms, anxiety

INTRODUCTION

Tinnitus is a common symptom of hearing impairment (1–3). Therapeutic use of hearing aids to treat mild-to moderate hearing loss was demonstrated to correlate with a decrease of tinnitus (4), although the data generated by clinical research neither support nor dismiss the use of hearing aids in tinnitus treatment (5). Of all types of hearing impairment, the most cumbersome is the severe bilateral hearing loss, which is often treated with cochlear implantation (CI) (6–10). Bilateral hearing impairment affects 12.7% (30 million) of the US Americans above 12 years of age, and the prevalence of bilateral hearing impairment increases with age (11). We and others have previously reported the incidence of tinnitus among the bilaterally hearing-impaired patients ranging between 70 and 90% (12–14) and making tinnitus a serious complaint in this particular group of patients.

Already decades ago, clinical observations linked the CI-mediated hearing recovery with the reduction in tinnitus (15–17). Ever since, various studies addressed the relationship between cochlear implants and tinnitus (12); however, the outcomes of the studies were somewhat conflicting. There are three main reasons for this: the first is varying sample size (from 1 to 26); the second is using different follow-up times (from 1 to 24 months) (18, 19); and the third is that despite recent recommendations to measure tinnitus-related distress before and after CI (10), the methods and the domains vary extremely from study to study (20). Furthermore, the design of clinical trials is often retrospective and the patients included have various types of hearing impairment (21–23). Moreover, the methods of treatment are frequently dissimilar and include unilateral hearing impairment treated with unilateral CI to bilateral hearing impairment treated with bilateral CI.

In our earlier studies, we concentrated on measuring the influence of CI on the quality of life (24), tinnitus-related distress, and psychological comorbidities (13, 14). We have demonstrated significant improvement of all domains measured following the CI. However, the follow-up time was rather short (13) and the patient sample was not homogenous (14).

The outstanding question in the field is how the cochlear implants affect tinnitus and tinnitus-related distress. The full answer to this question will be possible upon accumulation of high-quality evidence. This, in turn, can only be achieved by using specific batch of standardized validated instruments and by applying prospective longitudinal design to the studies.

Our present aim was to study tinnitus-related distress in a relatively homogenous group of patients over a longer period after CI. Our main question was if in this defined cohort, tinnitus-related distress improves solely upon auditory rehabilitation, and if yes, if this improvement is sustained over longer period.

PATIENTS AND METHODS

Inclusion Criteria

The patients of both genders were consecutively included in the study upon signing written consent. Following inclusion criteria were used:

- diagnosis of bilateral severe or profound hearing loss with speech recognition ≤40% in the Freiburg Monosyllabic Test in quiet and with hearing aid; 65-dB sound pressure level
- tinnitus
- meeting of the clinical criteria for CI:
  - possibility to use general anesthesia
  - exclusion of retrocochlear disorder (e.g., vestibular schwannoma)
  - unremarkable cochlear anatomy
  - motivation for postoperative audiological rehabilitation
  - post-lingual deafness.

Description of Study

Forty-one patients met the inclusion criteria and were followed for 2 years after CI. The data were collected between 2009 and 2016; the patients were admitted to the hospital between April 2009 and May 2014 for unilateral CI, and their last follow-up appointment was scheduled between July 2011 and February 2016. The appointments were scheduled at 6, 12, and 24 months after the surgery (see **Figure 1**). There were 22 women and 20 men in the sample—descriptive statistics are presented in **Table 1**.

Test Performed

All patients were audiologically examined. In addition, they were asked to complete psychometric questionnaires before surgery and during each consecutive appointment. The audiological tests and psychometric questionnaires used were previously described in detail (14, 25) and are presented in **Table 2**.

Statistical Evaluation

For the statistical analyses, SPSS version 23 was used. Normal distribution was tested prior to statistical analysis using the Shapiro–Wilk test and a histogram. Because of lack of normal distribution in the majority of dataset, the Wilcoxon signed-rank test was used to compare the scores before and after CI. Correlations between Tinnitus Questionnaire (TQ) and Nijmegen Cochlear Implantation Questionnaire (NCIQ) scores were performed by computing the Spearman's rank correlation coefficient.

RESULTS

Postimplantation Sustained Decrease of Tinnitus-Related Distress

Tinnitus was the main inclusion criterion. Prior to CI, the mean TQ score reflecting tinnitus-related distress was 35 (**Table 3**). TQ score decreased significantly already 6 months after CI, and this improvement was sustained over the 24-month follow-up period (**Figure 2**). Significant improvement of tinnitus-related distress was noted in 64.5% of all patients in the cohort. Regarding the individual TQ subscales, the emotional and cognitive distress were significantly reduced 12 and 24 months after implantation but the intrusiveness of tinnitus-related distress decreased already 6 months after surgery and stayed on a significantly lower level as compared to that before CI (**Table 3**). There was a trend in improvement regarding the subscales "auditory perception difficulties" and "somatic complaints," but this trend has not reached the statistical significance.

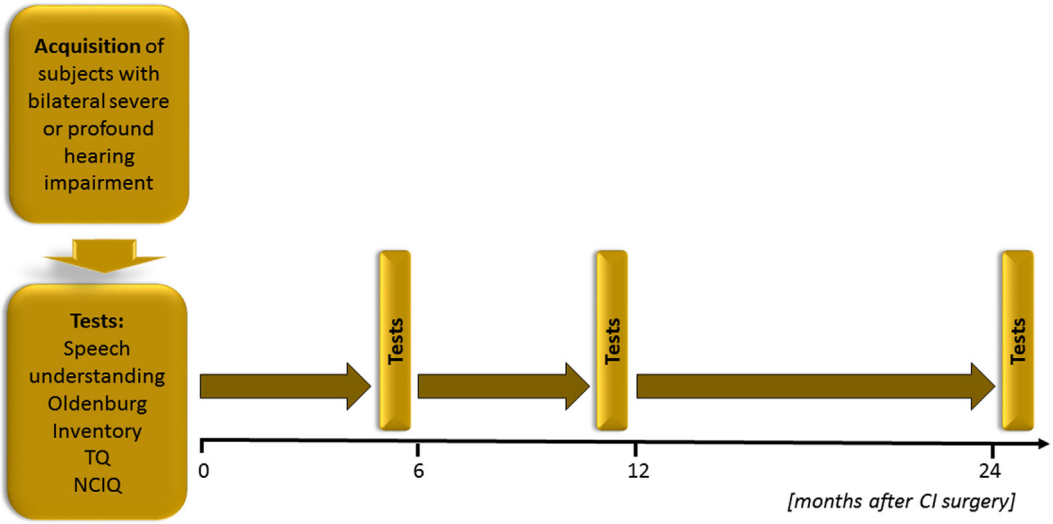

FIGURE 1 | **Study design**.

TABLE 1 | General patients' characteristics.

	Mean	Minimum	Maximum	SD
Age	61	25	81	13.45
Duration of hearing impairment (years)	18.82	1	67	18.84
Percent of speech recognition using monosyllabic Freiburg test 65-dB sound pressure level on the ear scheduled for implantation	7.86	0	40	13.21

Prior to CI, 13 patients were affected by a severe, decompensated, tinnitus-related distress (TQ score = 47 or more). Six months after surgery, four patients had TQ scores on the compensated level, 12 months after surgery, five patients were compensated, and 24 months later, seven patients were compensated. In two patients with compensated TQ scores prior to surgery, tinnitus-related distress progressed further to the severe, decompensated form after CI (**Figure 3**).

Post-Surgery Improvement of the Health-Related Quality of Life (HRQoL), Speech Perception, and Auditory Performance

The HRQoL measured by NCIQ also improved significantly, and the improvement was sustained over the period of study (**Figure 4**). In detail, the scales measuring basic sound perception, advanced sound perception, self-esteem, activity, and social interactions improved significantly 6 months after CI and remained so over the 24 months of the follow-up period. The only scale without statistically significant changes but with a trend toward improvement was "speech production" (**Table 3**).

Six months after CI, Oldenburg Inventory (**Figure 5**) demonstrated significant improvement in speech understanding in quiet and noise, as well speech localization at all measured time points of the follow-up period (**Table 3**). Similarly, monosyllabic Freiburg test indicated significant recovery of the hearing abilities by the implanted ear (**Table 3**). The speech recognition improved rapidly after surgery and was stable during the observation period of 2 years.

TABLE 2 | Questionnaires used in this study.

Health-related quality of life (HRQoL): Nijmegen Cochlear Implantation Questionnaire (NCIQ)	NCIQ is a validated tool designed to determine the HRQoL of implanted patients. The three main domains "physical," "psychological," and "social" are derived from six subdomains: 1 Basic sound perception 2 Advanced sound perception 3 Speech production 4 Self-esteem 5 Activity 6 Social interactions. The score ranges from 0 (very bad) to 100 (optimal).
Speech perception: Freiburg Monosyllabic Test	The Freiburg Monosyllabic Test was used to determine the preoperative speech recognition in silence at 65-dB sound pressure level with optimized hearing aid and postoperative with cochlear implant again as well
Subjective audiological assessment: Oldenburg Inventory (OI)	Data were collected preoperatively and postoperatively about the subjective hearing with the OI. The OI additionally includes a total score in 3 categories: "hearing in quiet," "hearing with background noise," and "localization." The 12 closed questions about everyday situations were marked with points from 1 to 5. The higher the score, the better the subjective hearing
Tinnitus distress: Tinnitus Questionnaire (TQ)	The tinnitus distress can be determined with TQ (26). Collected data represent 6 subdomains: emotional and cognitive distress, intrusiveness, auditory perceptual difficulties, sleeping disturbances, somatic complaints. The mean value is used to determine tinnitus grade: light (0–30 points), average (31–46 points), high (47–59 points), and very high (60–84 points). In addition, separation into compensated (≤46 points) and decompensated (47–84 points) tinnitus can be done based on the total score. The test–retest reliability is 0.94 for the total value and between 0.86 and 0.92 for the subscales. Cronbach's α is 0.94 for the total value of the TQ and between 0.74 and 0.92 for the subscales

TABLE 3 | Changes in parameters measured as compared to their values prior to cochlear implantation.

	Prior to surgery			6 months after surgery			12 months after surgery			24 months after surgery		
	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD	Mean	Median	SD
SR 65-dB sound pressure level (SPL)	7.9	0.0	13.2	40.4**	43.8	35.9	41.6**	35.0	34.2	43.2**	46.3	25.2
NCIQ 1	40.1	39.4	20.9	59.6**	60.0	20.0	62.1**	65.0	22.4	57.6**	60.0	18.1
NCIQ 2	42.4	40.0	19.2	56.1**	55.0	20.5	54.9**	55.0	21.0	53.2**	52.5	19.4
NCIQ 3	64.4	67.5	22.6	72.1	75.0	20.4	66.9	72.2	20.6	71.7	71.9	24.0
NCIQ 4	42.6	41.7	17.5	50.9**	45.0	19.7	52.9**	52.5	18.9	53.8**	50.0	21.4
NCIQ 5	37.9	37.5	18.2	49.1**	47.5	22.8	49.3**	47.5	23.0	47.5**	43.8	18.6
NCIQ 6	41.5	40.0	22.5	51.2**	50.0	25.7	53.0**	52.8	23.3	50.3**	50.0	18.9
NCIQ total	53.8	43.2	58.7	56.6**	55.1	18.5	57.5**	55.5	19.8	55.8**	56.8	15.2
TQ E	9.0	8.0	6.0	7.4	4.0	6.8	7.1*	5.0	6.6	7.2	7.0	6.4
TQ C	6.9	7.5	4.5	5.7	4.0	5.0	5.4*	4.5	4.4	5.6	4.5	4.5
TQ E + C	15.9	14.0	10.0	13.1	9.0	11.5	12.3*	9.5	10.9	12.7*	13.0	10.6
TQ I	8.2	9.0	4.1	6.1**	5.0	5.2	5.6**	5.5	4.7	5.8**	6.0	4.3
TQ A	6.6	6.0	4.8	5.2	5.0	4.9	5.1	5.5	4.7	5.2	5.5	4.5
TQ SI	2.5	2.0	2.4	1.8*	0.0	2.5	1.9	1.0	2.3	2.3	1.5	2.7
TQ SO	1.8	1.5	1.7	1.3	0.0	2.0	1.8	0.5	2.4	1.5	1.0	1.9
TQ total	35.0	33.5	19.6	27.5*	23.0	24.0	26.7**	26.5	22.8	27.6*	30.0	22.3
OI quiet	2.4	2.4	0.9	3.4**	3.6	1.0	3.4**	3.4	0.8	3.4**	3.4	0.9
OI noise	1.8	1.6	0.6	2.7**	2.6	1.0	2.7**	2.5	0.9	2.6**	2.4	0.8
OI localization	1.9	2.0	0.8	2.8**	3.0	1.0	2.7**	2.5	1.0	2.7**	2.5	1.0
OI total	2.1	1.9	0.7	3.0**	2.9	0.9	3.0**	2.9	0.8	2.9**	2.8	0.8

*Asterisks indicate significant differences between respective variables when compared to their values prior to surgery as per Wilcoxon signed-rank test; *$p \leq 0.05$, **$p \leq 0.01$. SR, speech recognition (Freiburg Monosyllabic Test, 65-dB SPL); NCIQ, German version of Nijmegen Cochlear Implantation Questionnaire (1 basic sound perception; 2 advanced sound perception; 3 speech production; 4 self-esteem; 5 activity; and 6 social interactions); TQ, Tinnitus Questionnaire (E, emotional distress; C, cognitive distress; E + C, combined psychological distress; I, intrusiveness; A, auditory perception difficulties; SI, sleep disturbances; SO, somatic complaints; total, total score); OI, Oldenburg Inventory.*

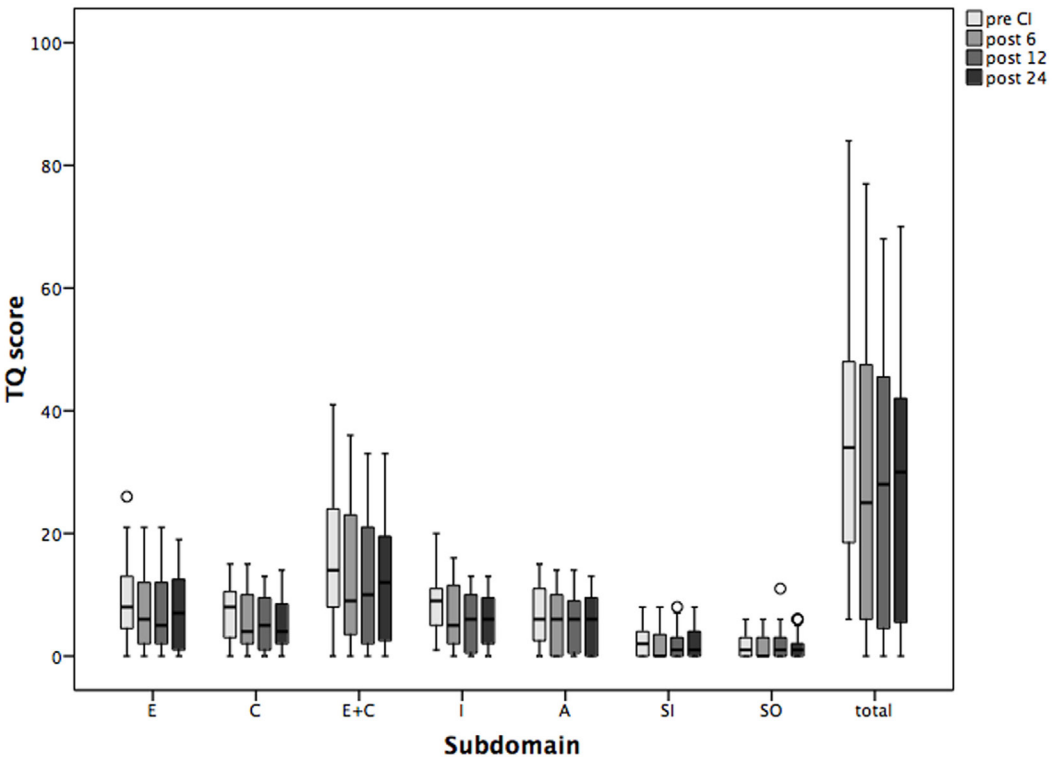

FIGURE 2 | Changes in tinnitus distress [Tinnitus Questionnaire (TQ)] and its subscales over the period of 2 years following cochlear implantation (CI). Shown are mean values and the range. Shown are mean values and the 95% CI. Pre CI, before CI; post 6, post 12, and post 24, 6, 12, and 24 months after surgery. E, emotional distress; C, cognitive distress; I, intrusiveness; A, auditory perceptual difficulties; SI, sleeping disturbances; SO, somatic complaints; total, total value.

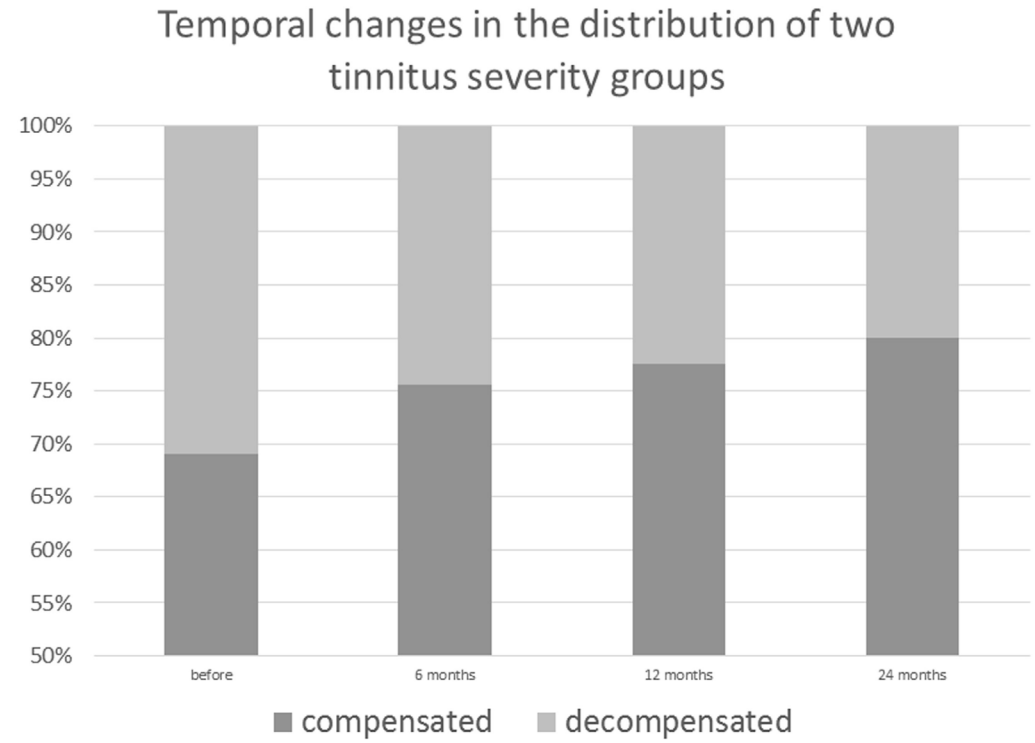

FIGURE 3 | **Post-cochlear implantation decrease in decompensated, severe form of tinnitus among implanted patients**.

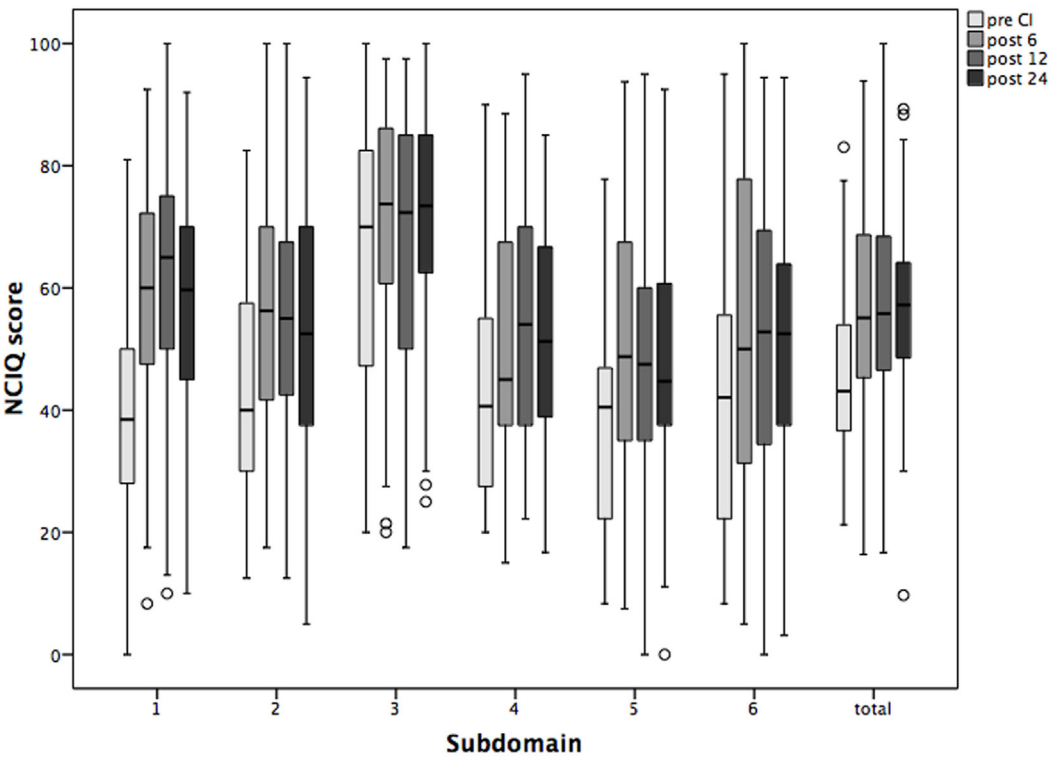

FIGURE 4 | **Changes in the health-related quality of life Nijmegen Cochlear Implantation Questionnaire (NCIQ) and its subdomains over the period of 2 years following cochlear implantation (CI)**. Shown are mean values and the 95% CI. Pre CI, before CI; post 6, post 12, and post 24, 6, 12, and 24 months after surgery. 1 basic speech perception; 2 advanced speech perception; 3 speech production; 4 self-esteem; 5 activity; and 6 social interactions.

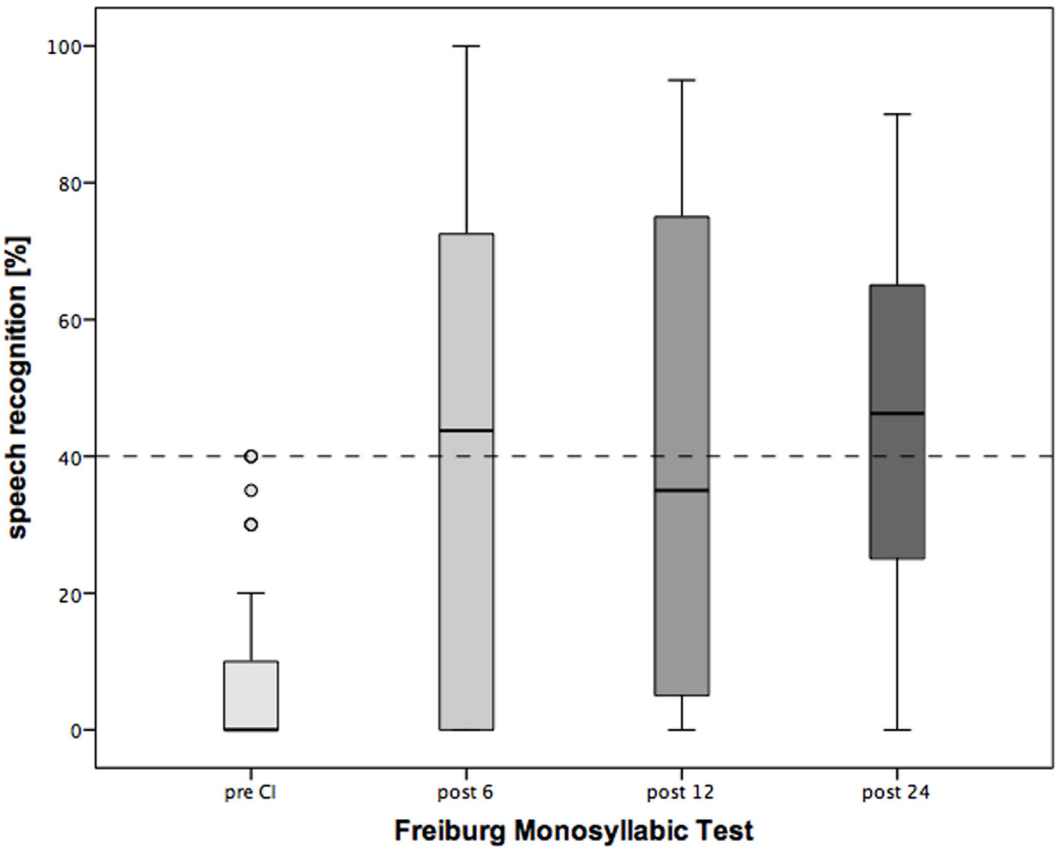

FIGURE 5 | Changes in speech recognition over the period of 2 years following cochlear implantation (CI). Shown are mean values and the range. Shown are mean values and the 95% CI. Dotted line: clinical criteria for CI.

Relationship between TQ and NCIQ

To determine if and how tinnitus-related distress affects the HRQoL, we computed the Spearman correlation coefficient for the respective variables. First, we analyzed the data obtained before CI (**Table 4**). We observed negative correlation between total TQ score and speech production (NCIQ3). The subscales indicating cognitive and emotional subscales as well as auditory difficulties reported by TQ were particularly affected. In addition, somatic complains correlated negatively with the background—and advanced sound perception as well as with self-esteem and social interactions (**Table 4**). Six months after CI, we found significant negative correlations between the total TQ score and all subdomains of NCIQ (**Table 5**), and 12 months after the CI, this was also the case (**Table 6**). Interestingly, 24 months after the CI, the correlations between total TQ score and NCIQ subscales "self-esteem" and "social interaction" were no longer significant (**Table 7**).

Compliance

Of 42 subjects originally included in this study, 41 patients filled the NCIQ questionnaire at the study onset, 39 patients after 12 months, and 40 patients after 2 years.

DISCUSSION

Tinnitus is often a symptom of hearing loss. Here, we demonstrated that in the bilaterally hearing-impaired patients with tinnitus, CI not only restores the auditory abilities but also reduces tinnitus-related distress and that this reduction was sustained for 2 years following surgery. To the best of our knowledge, our present study demonstrates for the first time the course of tinnitus-related distress in a homogenous cohort of bilaterally hard of hearing and tinnitus-positive patients, before and after CI. In addition, we show the relationship between tinnitus-related distress and the HRQoL and the postoperative auditory improvement over the 2-year course.

Prior to CI, the TQ score (total and subscales "emotional and cognitive distress" and "auditory difficulties") correlated significantly with the third subscale of the HRQoL NCIQ "speech production," whereas the total score of NCIQ correlated significantly (negative correlation) with the TQ subscale "somatic complaints" (**Table 4**). All correlations between NCIQ and TQ were negative, meaning that the decrease of tinnitus-related distress correlated with improvement of the quality of life and *vice versa*. Although these correlations decreased with time, they remained significant throughout the 24 months of the follow-up period (**Tables 5–7**),

TABLE 4 | Correlation between tinnitus and health-related quality of life *before* cochlear implantation.

			NCIQ1	NCIQ2	NCIQ3	NCIQ4	NCIQ5	NCIQ6	NCIQ total
Spearman-Rho	TQ E	Correlation coefficient	−0.202	−0.093	−0.313*	−0.097	−0.174	−0.146	−0.162
		Significance	0.205	0.561	0.046	0.548	0.276	0.362	0.313
		N	41	41	41	41	41	41	41
	TQ C	Correlation coefficient *t*	−0.118	−0.113	−0.315*	−0.144	−0.257	−0.240	−0.187
		Significance	0.464	0.483	0.045	0.370	0.105	0.131	0.241
		N	41	41	41	41	41	41	41
	TQ E + C	Correlation coefficient	−0.172	−0.108	−0.324*	−0.130	−0.232	−0.204	−0.191
		Significance	0.281	0.500	0.039	0.417	0.145	0.202	0.233
		N	41	41	41	41	41	41	41
	TQ I	Correlation coefficient	−0.287	−0.074	−0.300	−0.104	−0.210	−0.159	−0.199
		Sig. (2-seitig)	0.069	0.647	0.057	0.516	0.187	0.322	0.212
		N	41	41	41	41	41	41	41
	TQ A	Correlation coefficient	−0.258	−0.210	−0.437**	0.031	−0.044	0.043	−0.142
		Significance	0.104	0.187	0.004	0.849	0.784	0.791	0.378
		N	41	41	41	41	41	41	41
	TQ SI	Correlation coefficient	−0.354*	−0.315*	−0.263	−0.124	−0.279	−0.140	−0.259
		Significance	0.023	0.045	0.097	0.440	0.077	0.384	0.102
		N	41	41	41	41	41	41	41
	TQ SO	Correlation coefficient	−0.408**	−0.407**	−0.253	−0.274	−0.432**	−0.278	−0.413**
		Significance	0.008	0.008	0.111	0.083	0.005	0.078	0.007
		N	41	41	41	41	41	41	41
	TQ total	Correlation coefficient	−0.299	−0.205	−0.405**	−0.126	−0.243	−0.173	−0.260
		Significance	0.057	0.199	0.009	0.434	0.126	0.280	0.101
		N	41	41	41	41	41	41	41

*$p \leq 0.05$.
**$p \leq 0.01$.
Subscales of Nijmegen Cochlear Implantation Questionnaire (NCIQ): 1 basic sound perception; 2 advanced sound perception; 3 speech production; 4 self-esteem; 5 activity; and 6 social interactions.
Subscales of Tinnitus Questionnaire (TQ): E, emotional distress; C, cognitive distress; E + C, combined psychological distress; I, intrusiveness; A, auditory perception difficulties; SI, sleep disturbances; SO, somatic complaints.

TABLE 5 | Correlation between tinnitus and health-related quality of life *6 months after* cochlear implantation.

			NCIQ1	NCIQ2	NCIQ3	NCIQ4	NCIQ5	NCIQ6	NCIQ total
Spearman-Rho	TQ E	Correlation coefficient	−0.308	−0.397*	−0.421**	−0.331*	−0.553**	−0.365*	−0.462**
		Significance	0.050	0.010	0.006	0.034	0.000	0.019	0.002
		N	41	41	41	41	41	41	41
	TQ C	Correlation coefficient *t*	−0.347*	−0.399**	−0.446**	−0.421**	−0.586**	−0.413**	−0.524**
		Significance	0.026	0.010	0.003	0.006	0.000	0.007	0.000
		N	41	41	41	41	41	41	41
	TQ E + C	Correlation coefficient	−0.335*	−0.417**	−0.461**	−0.384*	−0.581**	−0.394*	−0.502**
		Significance	0.032	0.007	0.002	0.013	0.000	0.011	0.001
		N	41	41	41	41	41	41	41
	TQ I	Correlation coefficient	−0.378*	−0.466**	−0.486**	−0.368*	−0.613**	−0.444**	−0.535**
		Sig. (2-seitig)	0.015	0.002	0.001	0.018	0.000	0.004	0.000
		N	41	41	41	41	41	41	41
	TQ A	Correlation coefficient	−0.320*	−0.476**	−0.396*	−0.327*	−0.510**	−0.425**	−0.449**
		Significance	0.041	0.002	0.010	0.037	0.001	0.006	0.003
		N	41	41	41	41	41	41	41
	TQ SI	Correlation coefficient	−0.145	−0.185	−0.191	−0.357*	−0.527**	−0.240	−0.344*
		Significance	0.365	0.246	0.231	0.022	0.000	0.131	0.028
		N	41	41	41	41	41	41	41
	TQ SO	Correlation coefficient	−0.381*	−0.398**	−0.390*	−0.294	−0.457**	−0.413**	−0.449**
		Significance	0.014	0.010	0.012	0.062	0.003	0.007	0.003
		N	41	41	41	41	41	41	41
	TQ Total	Correlation coefficient	−0.331*	−0.435**	−0.430**	−0.359*	−0.589**	−0.404**	−0.497**
		Significance	0.035	0.004	0.005	0.021	0.000	0.009	0.001
		N	41	41	41	41	41	41	41

*$p \leq 0.05$.
**$p \leq 0.01$.
Subscales of Nijmegen Cochlear Implantation Questionnaire (NCIQ): 1 basic sound perception; 2 advanced sound perception; 3 speech production; 4 self-esteem; 5 activity; and 6 social interactions.
Subscales of Tinnitus Questionnaire (TQ): E, emotional distress; C, cognitive distress; E + C, combined psychological distress; I, intrusiveness; A, auditory perception difficulties; SI, sleep disturbances; SO, somatic complaints.

TABLE 6 | Correlation between tinnitus and health-related quality of life *12 months after* cochlear implantation.

			NCIQ1	NCIQ2	NCIQ3	NCIQ4	NCIQ5	NCIQ6	NCIQ total
Spearman-Rho	TQ E	Correlation coefficient	−0.377*	−0.545**	−0.362*	−0.329*	−0.409**	−0.386*	−0.400*
		Significance	0.018	0.000	0.024	0.041	0.010	0.015	0.010
		N	39	39	39	39	39	39	40
	TQ C	Correlation coefficient *t*	−0.505**	−0.593**	−0.372*	−0.408**	−0.477**	−0.447**	−0.488**
		Significance	0.001	0.000	0.020	0.010	0.002	0.004	0.001
		N	39	39	39	39	39	39	40
	TQ E + C	Correlation coefficient	−0.424**	−0.558**	−0.368*	−0.340*	−0.414**	−0.402*	−0.424**
		Significance	0.007	0.000	0.021	0.034	0.009	0.011	0.006
		N	39	39	39	39	39	39	40
	TQ I	Correlation coefficient	−0.454**	−0.598**	−0.490**	−0.332*	−0.472**	−0.446**	−0.490**
		Sig. (2-seitig)	0.004	0.000	0.002	0.039	0.002	0.004	0.001
		N	39	39	39	39	39	39	40
	TQ A	Correlation coefficient	−0.345*	−0.456**	−0.414**	−0.308	−0.348*	−0.306	−0.385*
		Significance	0.032	0.004	0.009	0.056	0.030	0.058	0.014
		N	39	39	39	39	39	39	40
	TQ SI	Correlation coefficient	−0.339*	−0.401*	−0.332*	−0.254	−0.350*	−0.307	−0.314*
		Significance	0.035	0.011	0.039	0.119	0.029	0.057	0.048
		N	39	39	39	39	39	39	40
	TQ SO	Correlation coefficient	−0.309	−0.356*	−0.331*	−0.323*	−0.358*	−0.433**	−0.312*
		Significance	0.056	0.026	0.039	0.045	0.025	0.006	0.050
		N	39	39	39	39	39	39	40
	TQ total	Correlation coefficient	−0.404*	−0.531**	−0.408**	−0.323*	−0.411**	−0.403*	−0.417**
		Significance	0.011	0.001	0.010	0.045	0.009	0.011	0.007
		N	39	39	39	39	39	39	40

*$p \leq 0.05$.
**$p \leq 0.01$.
Subscales of Nijmegen Cochlear Implantation Questionnaire (NCIQ): 1 basic sound perception; 2 advanced sound perception; 3 speech production; 4 self-esteem; 5 activity; and 6 social interactions.
Subscales of Tinnitus Questionnaire (TQ): E, emotional distress; C, cognitive distress; E + C, combined psychological distress; I, intrusiveness; A, auditory perception difficulties; SI, sleep disturbances; SO, somatic complaints.

TABLE 7 | Correlation between tinnitus and health-related quality of life *24 months after* cochlear implantation.

			NCIQ1	NCIQ2	NCIQ3	NCIQ4	NCIQ5	NCIQ6	NCIQ total
Spearman-Rho	TQ E	Correlation coefficient	−0.416**	−0.499**	−0.358*	−0.174	−0.384*	−0.346*	−0.328*
		Significance	0.008	0.001	0.023	0.282	0.014	0.029	0.039
		N	40	40	40	40	40	40	40
	TQ C	Correlation coefficient *t*	−0.408**	−0.452**	−0.359*	−0.170	−0.341*	−0.277	−0.353*
		Significance	0.009	0.003	0.023	0.296	0.031	0.084	0.026
		N	40	40	40	40	40	40	40
	TQ E + C	Correlation coefficient	−0.406**	−0.474**	−0.362*	−0.165	−0.351*	−0.308	−0.327*
		Significance	0.009	0.002	0.022	0.308	0.026	0.053	0.039
		N	40	40	40	40	40	40	40
	TQ I	Correlation coefficient	−0.434**	−0.496**	−0.357*	−0.215	−0.411**	−0.351*	−0.339*
		Sig. (2-seitig)	0.005	0.001	0.024	0.183	0.008	0.026	0.032
		N	40	40	40	40	40	40	40
	TQ A	Correlation coefficient	−0.301	−0.462**	−0.439**	−0.135	−0.393*	−0.280	−0.257
		Significance	0.060	0.003	0.005	0.405	0.012	0.080	0.109
		N	40	40	40	40	40	40	40
	TQ SI	Correlation coefficient	−0.415**	−0.434**	−0.522**	−0.176	−0.385*	−0.277	−0.396*
		Significance	0.008	0.005	0.001	0.278	0.014	0.083	0.011
		N	40	40	40	40	40	40	40
	TQ SO	Correlation coefficient	−0.413**	−0.460**	−0.357*	−0.210	−0.409**	−0.355*	−0.371*
		Significance	0.008	0.003	0.024	0.194	0.009	0.025	0.019
		N	40	40	40	40	40	40	40
	TQ total	Correlation coefficient	−0.419**	−0.526**	−0.416**	−0.169	−0.372*	−0.311	−0.334*
		Significance	0.007	0.000	0.008	0.297	0.018	0.051	0.035
		N	40	40	40	40	40	40	40

*$p \leq 0.05$.
**$p \leq 0.01$.
Subscales of Nijmegen Cochlear Implantation Questionnaire (NCIQ): 1 basic sound perception; 2 advanced sound perception; 3 speech production; 4 self-esteem; 5 activity; and 6 social interactions.
Subscales of Tinnitus Questionnaire (TQ): E, emotional distress; C, cognitive distress; E + C, combined psychological distress; I, intrusiveness; A, auditory perception difficulties; SI, sleep disturbances; SO, somatic complaints.

suggesting that the tinnitus-related emotional and cognitive distress as well as tinnitus-related auditory difficulties negatively influenced the life quality of the CI patients. Longer follow-up time should clarify if these correlations decay completely with time.

Before the CI, patients' quality of life (total score) was not affected by the tinnitus-induced auditory difficulties (**Table 4**) confirming our earlier observations (13). Six months after implantation, there was a large (Rho = −0.449) and significant ($p = 0.003$) negative correlation between these two variables (**Table 5**), very likely reflecting the fact that the process of regaining auditory abilities can be negatively affected by the tinnitus percept. In fact, this correlation and its significance declined 12 months after CI (Rho = −0.385, $p = 0.014$) (**Table 6**) and were no longer of significance 24 months after the surgery (**Table 7**).

Tinnitus is a complaint of 70–90% of hearing-impaired patients (12–14). In cases of patients who are bilaterally hard of hearing, tinnitus percept is a particularly disturbing symptom, because it is the only auditory input perceived by patients. In such cases, diverting the auditory attention from tinnitus to other sounds is problematic, making the therapeutic approaches difficult if not impossible. The two major therapies globally used for tinnitus treatment are tinnitus retraining therapy (TRT) and cognitive behavioral therapy (CBT). The neurophysiological model proposed by Jastreboff (27, 28) suggests the existence of auditory–limbic–sympathetic network responsible for negative effects of tinnitus sound and inducing the distress and inability to divert the attention of patients from the tinnitus sound. TRT, designed by Jastreboff based on the above theory, has since years been a frequent therapeutic choice of many clinicians (29–31). The second method widely used for tinnitus is the CBT, which was developed to treat anxiety disorders, depression, eating disorders, chronic low back pain, personality disorders, depression, and anxiety and successfully applied in the treatment for tinnitus (32–35). TRT, CBT, or a combination of both require at least some hearing abilities and can only be used to treat the patients who are hard of hearing *and* have tinnitus following successful auditory rehabilitation with CI.

The first positive effect of CI on tinnitus was reported in 1976 by House (15). Ever since, various studies with different sample sizes and inclusion criteria were performed. Corroborating our present results, the decrease of tinnitus-related distress after CI ranging from 46 to 95% was observed previously by others (12, 36, 37). In our present study, we also observed the reduction of tinnitus-related distress in half of the patients who had severe (decompensated) tinnitus prior to CI.

The central question addressing the mechanism in which CI reduces tinnitus-related distress remains open. The evidence collected in our present study suggests following possible scenarios:

- Following CI, the auditory abilities improve to the degree where the patients can focus their auditory attention on sounds other than tinnitus.
- Following CI, the improved auditory abilities increase the quality of life, thus decrease overall stress and positively affect the loop "stress-tinnitus."
- Following CI, the direct electrical stimulation of the auditory nerve induces plastic changes in the auditory reducing the tinnitus percept.

More quality evidence needs to be accumulated to determine, which of the presented scenarios is essential for tinnitus reduction after CI. It can also not be excluded that all three mechanisms play a role in tinnitus reduction and habituation. Future trials with specific tinnitus-oriented fitting of cochlear implants could shed more light on that topic.

While until recently, the clinical research involving cochlear implants was focused mainly on the audiological gain; at present, many authors are increasingly interested in changes of the quality of life and tinnitus-related distress (12, 38, 39). Quaranta et al. reported bilateral disappearance of tinnitus after unilateral CI in 65.8% of the patients (40), as measured by the Tinnitus Handicap Inventory—an instrument that is similar—but not identical—to TQ (41). Also, we have demonstrated earlier that the CI, in addition to having positive effect on hearing abilities, improves the life quality and decreases the tinnitus-related distress and psychological comorbidities (13, 42–45). There are several psychometric instruments measuring various parameters and domains used in tinnitus research and clinical routine. These instruments vary depending on the clinical orientation of the treating unit (audiology, ORL, clinical psychology or psychosomatic medicine) and on the country. Here, we propose creation of a specific set of standardized, validated, and internationally available instruments to measure CI-specific outcomes, which would include various aspects of tinnitus percept and tinnitus-related distress. In our present work, we used instruments that are widely available in the German-speaking countries. The Nijmegen Cochlear Implant Questionnaire NCIQ is an internationally validated, disease-specific instrument created for the assessment of the quality of life in patients with cochlear implants. OI is a popular, standardized instrument measuring perceived benefit of hearing aids. Also, the German version of TQ, which measures the tinnitus-related distress, is frequently used in the inpatient and outpatient settings to monitor the severity of tinnitus and its response to treatment. In order to study the influence of tinnitus on the outcome of CI, we suggest designing prospective, longitudinal clinical trials and using defined monitoring batch. Despite using such design, our present study is not free of pitfalls, as it could have included larger sample, and it was neither double blinded nor randomized. In addition, an appropriate control group is lacking. However, blinded and randomized design in the field of cochlear implant is difficult to be implemented because of specific features of the CI treatment, preventing the design of high-level evidence studies (19). Control group, which for instance could comprise patients who were implanted but their cochlear implants remain switched off, cannot be used because of obvious ethical reasons.

Previously reported high prevalence of tinnitus in the hearing-impaired patients puts the choice of tinnitus treatment in these patients up for discussion (12). In particular, the task of developing appropriate approach for the tinnitus treatment in bilaterally hearing-impaired patients remains open. The increasing incidence of hearing impairments, including the age-related hearing

loss in context of demographic changes in our society, emphasizes the need for improvement in the therapy guidelines (46).

In addition, although we observed the most pronounced decrease of tinnitus-related distress 12 months after the implantation, the maximal correlation between TQ score and speech recognition (**Table 3**) occurred 6 months after implantation. These results do not contradict each other; rather, they point at the dependence of auditory rehabilitation on tinnitus treatment. Since the auditory benefit is patient specific, it is difficult to measure. Speech recognition—a typical parameter that measures hearing improvement—when used alone is not enough to act as an adequate indicator of tinnitus suppression. This is reflected by the results obtained 2 years after CI. Similarly, the TQ scores suggest that a unilateral acoustic stimulation with noticeable postoperative asymmetry does not lead to an unfavorable influence on the tinnitus-related distress, even over several years. The bilateral CI could be an ultimate target of hearing rehabilitation. In fact, sustained improvement of TQ scores was observed in 40 patients subjected to sequential bilateral CI (25).

Final Conclusion

Taken together, our results suggest that patients who are bilaterally hard of hearing and have tinnitus profit from CI not only by regaining their auditory skills but also by a significant and sustained improvement of the HRQoL and reduction of tinnitus-related distress. Moreover, the negative correlation between tinnitus and the HRQoL indicates the importance of tinnitus as an obstacle in auditory rehabilitation of CI patients. It is tempting to speculate that therapy for tinnitus used after CI would further decrease tinnitus-related distress and, therefore, could increase the quality of life in this specific group of patients.

ETHICS STATEMENT

The local Ethics Committee (permit number EA2/030/13) approved this prospective, non-interventional, and longitudinal study. All investigations were conducted according to the principles expressed in the Declaration of Helsinki. All patients have given their informed written consent.

AUTHOR CONTRIBUTIONS

SK and HO designed the study. SK, SG, and SH collected the data. SK, AS, and HO interpreted the data. SK and AS drafted the manuscript. HO critically revised the manuscript.

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REFERENCES

1. Axelsson A, Sandh A. Tinnitus in noise-induced hearing loss. *Br J Audiol* (1985) 19:271–6. doi:10.3109/03005368509078983
2. Griest SE, Bishop PM. Tinnitus as an early indicator of permanent hearing loss. A 15 year longitudinal study of noise exposed workers. *AAOHN J* (1998) 46:325–9.
3. Mazurek B, Olze H, Haupt H, Szczepek AJ. The more the worse: the grade of noise-induced hearing loss associates with the severity of tinnitus. *Int J Environ Res Public Health* (2010) 7:3071–9. doi:10.3390/ijerph7083071
4. Saltzman M, Ersner MS. A hearing aid for the relief of tinnitus aurium. *Laryngoscope* (1947) 57:358–66. doi:10.1288/00005537-194705000-00005
5. Hoare DJ, Edmondson-Jones M, Sereda M, Akeroyd MA, Hall D. Amplification with hearing aids for patients with tinnitus and co-existing hearing loss. *Cochrane Database Syst Rev* (2014) (1):CD010151. doi:10.1002/14651858.CD010151.pub2
6. Fujimoto C, Ito K, Takano S, Karino S, Iwasaki S. Successful cochlear implantation in a patient with bilateral progressive sensorineural hearing loss after traumatic subarachnoid hemorrhage and brain contusion. *Ann Otol Rhinol Laryngol* (2007) 116:897–901. doi:10.1177/000348940711601205
7. Suzuki Y, Ogawa H, Baba Y, Suzuki T, Yamada N, Omori K. Cochlear implantation in a case of bilateral sensorineural hearing loss due to mumps. *Fukushima J Med Sci* (2009) 55:32–8. doi:10.5387/fms.55.32
8. Greenberg SL, Shipp D, Lin VY, Chen JM, Nedzelski JM. Cochlear implantation in patients with bilateral severe sensorineural hearing loss after major blunt head trauma. *Otol Neurotol* (2011) 32:48–54. doi:10.1097/MAO.0b013e3181ff73fd
9. Aoki M, Tanahashi S, Mizuta K, Kato H. Treatment for progressive hearing loss due to Paget's disease of bone – a case report and literature review. *J Int Adv Otol* (2015) 11:267–70. doi:10.5152/iao.2015.1572
10. Ramakers GG, Van Zon A, Stegeman I, Grolman W. The effect of cochlear implantation on tinnitus in patients with bilateral hearing loss: a systematic review. *Laryngoscope* (2015) 125:2584–92. doi:10.1002/lary.25370
11. Lin FR, Niparko JK, Ferrucci L. Hearing loss prevalence in the United States. *Arch Intern Med* (2011) 171:1851–2. doi:10.1001/archinternmed.2011.506
12. Baguley DM, Atlas MD. Cochlear implants and tinnitus. *Prog Brain Res* (2007) 166:347–55. doi:10.1016/S0079-6123(07)66033-6
13. Olze H, Szczepek AJ, Haupt H, Forster U, Zirke N, Grabel S, et al. Cochlear implantation has a positive influence on quality of life, tinnitus, and psychological comorbidity. *Laryngoscope* (2011) 121:2220–7. doi:10.1002/lary.22145
14. Olze H, Szczepek AJ, Haupt H, Zirke N, Graebel S, Mazurek B. The impact of cochlear implantation on tinnitus, stress and quality of life in postlingually deafened patients. *Audiol Neurootol* (2012) 17:2–11. doi:10.1159/000323847
15. House WF. Cochlear implants. *Ann Otol Rhinol Laryngol* (1976) 85:3–93. doi:10.1177/00034894760850S303
16. Thedinger B, House WF, Edgerton BJ. Cochlear implant for tinnitus. Case reports. *Ann Otol Rhinol Laryngol* (1985) 94:10–3. doi:10.1177/000348948509400102
17. House D. Tinnitus suppression via cochlear implants: review and remarks. *Int Tinnitus J* (1999) 5:27–9.
18. Arts RA, George EL, Stokroos RJ, Vermeire K. Review: cochlear implants as a treatment of tinnitus in single-sided deafness. *Curr Opin Otolaryngol Head Neck Surg* (2012) 20:398–403. doi:10.1097/MOO.0b013e3283577b66
19. Van Zon A, Peters JP, Stegeman I, Smit AL, Grolman W. Cochlear implantation for patients with single-sided deafness or asymmetrical hearing loss: a systematic review of the evidence. *Otol Neurotol* (2015) 36:209–19. doi:10.1097/MAO.0000000000000681
20. Hall DA, Haider H, Szczepek AJ, Lau P, Rabau S, Jones-Diette J, et al. Systematic review of outcome domains and instruments used in clinical trials of tinnitus treatments in adults. *Trials* (2016) 17:270. doi:10.1186/s13063-016-1399-9
21. Gordon KA, Jiwani S, Papsin BC. Benefits and detriments of unilateral cochlear implant use on bilateral auditory development in children who are deaf. *Front Psychol* (2013) 4:719. doi:10.3389/fpsyg.2013.00719
22. Gartrell BC, Jones HG, Kan A, Buhr-Lawler M, Gubbels SP, Litovsky RY. Investigating long-term effects of cochlear implantation in single-sided deafness: a best practice model for longitudinal assessment of spatial hearing abilities and tinnitus handicap. *Otol Neurotol* (2014) 35:1525–32. doi:10.1097/MAO.0000000000000437
23. Ramos Macias A, Falcon Gonzalez JC, Manrique M, Morera C, Garcia-Ibanez L, Cenjor C, et al. Cochlear implants as a treatment option for unilateral hearing

loss, severe tinnitus and hyperacusis. *Audiol Neurootol* (2015) 20(Suppl 1): 60–6. doi:10.1159/000380750
24. Hirschfelder A, Grabel S, Olze H. The impact of cochlear implantation on quality of life: the role of audiologic performance and variables. *Otolaryngol Head Neck Surg* (2008) 138:357–62. doi:10.1016/j.otohns.2007.10.019
25. Olze H, Grabel S, Haupt H, Forster U, Mazurek B. Extra benefit of a second cochlear implant with respect to health-related quality of life and tinnitus. *Otol Neurotol* (2012) 33:1169–75. doi:10.1097/MAO.0b013e31825e799f
26. Goebel G, Hiller W. [The Tinnitus Questionnaire. A standard instrument for grading the degree of tinnitus. Results of a multicenter study with the Tinnitus Questionnaire]. *HNO* (1994) 42:166–72.
27. Jastreboff PJ. Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci Res* (1990) 8:221–54. doi:10.1016/0168-0102(90)90031-9
28. Jastreboff PJ, Hazell JW, Graham RL. Neurophysiological model of tinnitus: dependence of the minimal masking level on treatment outcome. *Hear Res* (1994) 80:216–32. doi:10.1016/0378-5955(94)90113-9
29. Kroener-Herwig B, Biesinger E, Gerhards F, Goebel G, Verena Greimel K, Hiller W. Retraining therapy for chronic tinnitus. A critical analysis of its status. *Scand Audiol* (2000) 29:67–78. doi:10.1080/010503900424471
30. Bartnik G, Fabijańska A, Rogowski M. Effects of tinnitus retraining therapy (TRT) for patients with tinnitus and subjective hearing loss versus tinnitus only. *Scand Audiol* (2001) 30(Suppl 52):206–8. doi:10.1080/010503901300007542
31. Seydel C, Haupt H, Szczepek AJ, Hartmann A, Rose M, Mazurek B. Three years later: report on the state of well-being of patients with chronic tinnitus who underwent modified tinnitus retraining therapy. *Audiol Neurootol* (2015) 20:26–38. doi:10.1159/000363728
32. Andersson G. Psychological aspects of tinnitus and the application of cognitive-behavioral therapy. *Clin Psychol Rev* (2002) 22:977–90. doi:10.1016/S0272-7358(01)00124-6
33. Hesser H, Weise C, Westin VZ, Andersson G. A systematic review and meta-analysis of randomized controlled trials of cognitive-behavioral therapy for tinnitus distress. *Clin Psychol Rev* (2011) 31:545–53. doi:10.1016/j.cpr.2010.12.006
34. Jun HJ, Park MK. Cognitive behavioral therapy for tinnitus: evidence and efficacy. *Korean J Audiol* (2013) 17:101–4. doi:10.7874/kja.2013.17.3.101
35. Zenner HP, Vonthein R, Zenner B, Leuchtweis R, Plontke SK, Torka W, et al. Standardized tinnitus-specific individual cognitive-behavioral therapy: a controlled outcome study with 286 tinnitus patients. *Hear Res* (2013) 298:117–25. doi:10.1016/j.heares.2012.11.013
36. Quaranta N, Wagstaff S, Baguley DM. Tinnitus and cochlear implantation. *Int J Audiol* (2004) 43:245–51. doi:10.1080/14992020400050033
37. Pan T, Tyler RS, Ji H, Coelho C, Gehringer AK, Gogel SA. Changes in the tinnitus handicap questionnaire after cochlear implantation. *Am J Audiol* (2009) 18:144–51. doi:10.1044/1059-0889(2009/07-0042)
38. Andersson G, Freijd A, Baguley DM, Idrizbegovic E. Tinnitus distress, anxiety, depression, and hearing problems among cochlear implant patients with tinnitus. *J Am Acad Audiol* (2009) 20:315–9. doi:10.3766/jaaa.20.5.5
39. Contrera KJ, Betz J, Li L, Blake CR, Sung YK, Choi JS, et al. Quality of life after intervention with a cochlear implant or hearing aid. *Laryngoscope* (2016) 126:2110–5. doi:10.1002/lary.25848
40. Quaranta N, Fernandez-Vega S, D'elia C, Filipo R, Quaranta A. The effect of unilateral multichannel cochlear implant on bilaterally perceived tinnitus. *Acta Otolaryngol* (2008) 128:159–63. doi:10.1080/00016480701387173
41. Zeman F, Koller M, Schecklmann M, Langguth B, Landgrebe M; TRI Database Study Group. Tinnitus assessment by means of standardized self-report questionnaires: psychometric properties of the Tinnitus Questionnaire (TQ), the Tinnitus Handicap Inventory (THI), and their short versions in an international and multi-lingual sample. *Health Qual Life Outcomes* (2012) 10:128. doi:10.1186/1477-7525-10-128
42. Olze H, Grabel S, Forster U, Zirke N, Huhnd LE, Haupt H, et al. Elderly patients benefit from cochlear implantation regarding auditory rehabilitation, quality of life, tinnitus, and stress. *Laryngoscope* (2012) 122:196–203. doi:10.1002/lary.22356
43. Bruggemann P, Szczepek AJ, Rose M, Mckenna L, Olze H, Mazurek B. Impact of multiple factors on the degree of tinnitus distress. *Front Hum Neurosci* (2016) 10:341. doi:10.3389/fnhum.2016.00341
44. Knopke S, Grabel S, Forster-Ruhrmann U, Mazurek B, Szczepek AJ, Olze H. Impact of cochlear implantation on quality of life and mental comorbidity in patients aged 80 years. *Laryngoscope* (2016) 126:2811–6. doi:10.1002/lary.25993
45. Olze H, Knopke S, Grabel S, Szczepek AJ. Rapid positive influence of cochlear implantation on the quality of life in adults 70 years and older. *Audiol Neurootol* (2016) 21(Suppl 1):43–7. doi:10.1159/000448354
46. Davis A, Mcmahon CM, Pichora-Fuller KM, Russ S, Lin F, Olusanya BO, et al. Aging and hearing health: the life-course approach. *Gerontologist* (2016) 56(Suppl 2):S256–67. doi:10.1093/geront/gnw033

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Unilateral Cochlear Implantation Reduces Tinnitus Loudness in Bimodal Hearing: A Prospective Study

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Perceptive and receptive aspects of subjective tinnitus like loudness and tinnitus-related distress are partly independent. The high percentage of hearing loss in individuals with tinnitus suggests causality of hearing impairment particularly for the tinnitus percept, leading to the hypothesis that restoration of auditory input has a larger effect on tinnitus loudness than on tinnitus-related distress. Furthermore, it is assumed that high levels of depression or anxiety prevent reductions of tinnitus loudness and distress following restoration of activity in the cochlea. This prospective study investigated the influence of unilateral cochlear implant (CI) on tinnitus in 19 postlingually deafened adults during 6 months following implantation. All had bimodal provision with the other ear being continuously supported by a hearing aid. On the day before CI implantation (T1, T2), and at about 3 and 6 months postsurgery (T3, T4), participants were questioned about their current tinnitus. Loudness was rated on a Numeric Rating Scale, distress was assessed by the TQ12 Tinnitus Questionnaire, and depression and anxiety were recorded with the Hospital Anxiety and Depression Scale. At T2, 79% experienced tinnitus, one participant developed tinnitus after implantation. Following implantation, tinnitus loudness was reduced significantly by 42%, while reductions in tinnitus-related distress (–24%), depression (–20%), and anxiety (–20%) did not attain statistical significance. Significant correlations existed between tinnitus measures, and between postimplantation tinnitus-related distress and anxiety and depression scores. Moreover, improvement of hearing in the CI ear was significantly correlated with reduction in tinnitus loudness. A new aspect of this study is the particular influence of CI provision on perceptive aspects of preexisting tinnitus (hypothesis 1), with the effect size regarding postimplant reduction of perceived tinnitus loudness (1.40) being much larger than effect sizes on the reduction of tinnitus-related distress (0.38), depression (0.53), and anxiety (0.53). Contrary to expectation both tinnitus measures reduce even in the majority of CI recipients with increased levels of anxiety or depression. This suggests that reduction of the tinnitus signal by restoring activity in the cochlea cannot be entirely compensated for by central tinnitus mechanisms and results in a reduction of perceptive and less so of reactive aspects of subjective tinnitus.

Keywords: tinnitus, loudness reduction, cochlear implant, bimodal hearing, anxiety, depression, prospective study

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INTRODUCTION

This prospective study addresses changes in subjective tinnitus following cochlear implantation. A new aspect is the investigation of bimodal implantees, who hear with the help of a cochlear implant (CI) on one ear and an acoustic hearing aid (HA) on the contralateral ear. This combination of hearing substitution is rather common and provides significant real-world benefit as compared to unilateral CI (1). Moreover, influence on perceived loudness of the tinnitus and tinnitus-related distress are assessed separately and effect sizes are calculated.

Hearing loss is a major risk factor for tinnitus (2), and therefore, it is not surprising that tinnitus is common among CI candidates. On average, as many as 80% of CI candidates experience tinnitus (3, 4). It was realized early that CIs may reduce tinnitus (5) with several authors reporting on tinnitus reduction (6–11). However, the opposite, namely, exacerbation of a preexisting tinnitus, or development of tinnitus with CI use has also been observed (7, 12). The risk of developing tinnitus following CI ranges from 0 to 4%, while worsening of a preexisting tinnitus has been reported in 1–9% of cases (7). As tinnitus may lead to considerable suffering (13–15), the circumstances influencing its suppression, versus its worsening, or even the emergence of new tinnitus with CI use need to be explored.

The tinnitus signal or percept is thought to arise in the central auditory system in response to a hearing deficit, which in most cases can be attributed to impairments in the cochlea (16). The burden experienced by tinnitus extends beyond this percept and was shown to correlate with anxiety and depression (14, 17). Heterogeneity and severity in symptoms associated with tinnitus is reflected in a wide variety of proposed treatments (13) and may be the reason why overall effectiveness of currently available treatments is suboptimal.

Tinnitus is an auditory percept that varies in persistence, can be localized to one or both ears or is heard within the head, and that is perceived with variable loudness. Beyond this, people with tinnitus may suffer from their tinnitus and the amount of suffering cannot solely be determined by the perceptive qualities of the tinnitus, rather appearing to be associated more closely with mental health (14, 17, 18). In agreement, the reactive component, or distress related to the tinnitus percept, was found to be related to alterations in the emotional, attentional, and memory systems of the brain and to altered interactions between these systems and the auditory system: reportably leading to undue salience of the tinnitus signal (19).

We want to address the following hypotheses: first, we propose that because CIs restore input into the central auditory system, CI provision has a stronger effect on perceptive aspects of tinnitus than on reactive aspects. Thus, we expect CIs to primarily reduce tinnitus permanence and perceived loudness, and possibly change its localization if the hearing balance is altered between ears. At the same time, we expect a weaker effect of CI use on tinnitus-related distress, because this aspect of tinnitus is thought to depend mainly on interactions with the non-auditory brain [e.g., Ref. (15, 19)]. Second, we propose that high levels of depression and anxiety counteract CI-induced tinnitus attenuation, resulting in a lesser reduction of tinnitus-related distress and loudness in individuals with high depression and/or anxiety scores.

Moreover, as CIs are constantly improved in terms of type of implant, electrode insertion and positioning, and speech processing strategy, greater tinnitus suppression is expected by newer types of implants (6, 7). Therefore, it is important to explore the tinnitus reducing capacities of currently available CI technology.

The aims of this prospective study are therefore to (i) estimate the change of perceptive and (ii) reactive tinnitus measures separately, and (iii) examine the influence of mental health on the reduction of tinnitus symptoms following unilateral CI implantation in bimodal users.

MATERIALS AND METHODS

Procedure and Inclusion

Between 2014 and 2016, study participants were recruited from the patients at the CI Centre of the University Medical Centre Mannheim. Prospective participants had postlingual onset of profound hearing impairment. Recruitment was independent of reported tinnitus. Inclusion criteria comprised: first-time unilateral CI provision, an Advanced Bionics (HiRes 90K) implant as chosen by the patient, HA use at the other ear, and aged between 18 and 90 years. All patients who fulfilled these criteria were approached for inclusion. Exclusion criteria were assessed during an initial interview (T1) and included: insufficient knowledge of the German language, more than mild cognitive deficit, as assessed by the DemTect Test (20), and use of other implanted devices. Twenty-five patients were included in the study. Two participants discontinued the study following sequential bilateral implantation, one discontinued because of health conditions unrelated to the study or their tinnitus, one decided that study participation after T2 was too much effort, and two discontinued for reasons they did not disclose.

The initial interview, study inclusion (T1), and presurgery examination (T2) took place on the same day, usually the day before surgery (mean [SD]: 3 [7] days). Patients received a CI on their weaker ear while HA use was continued on the other ear. They left hospital on average 3 days postsurgery. Two to three weeks later, they participated in a week-long in-patient program with first fitting of the speech processor, several fitting sessions, and technical instruction on CI use. Until the first formal appointment at the CI Centre of the University Medical Centre Mannheim, 4 weeks following surgery, participants' mean daily processor use was 11 h. Postimplantation assessments T3 and T4 were scheduled for 3 and 6 months postimplantation (T3: 100 [18] days; T4: 221 [70] days).

Before T1, all subjects gave written informed consent in accordance with the Declaration of Helsinki. The protocol was approved by the Medizinische Ethikkommission II of the Medical Faculty Mannheim (approval no. 2014-527N-MA). Study participants were compensated for their participation.

Participant's Characteristics

Etiology of hearing loss varied greatly and was unknown in many cases. The decision which ear was to be implanted was based on

various audiological and anatomical criteria and was generally independent of reported tinnitus. Nineteen subjects completed the study. Before implantation, four did not experience tinnitus and three of them did not report tinnitus at any assessment, while one developed tinnitus between T2 and T3.

Study participants focused much more on their hearing problems than their tinnitus. When asked at pre-assessment (T1) what they expected of their CI, none mentioned tinnitus. No expectations were expressed by one participant, whereas the remaining 18 expected their hearing to improve in order to understand spoken language more easily and to participate in social situations again. Further characteristics of study participants are given in **Table 1**.

Measures

Pure tone audiometry (PTA) was conducted in sound field for both ears at 0.5, 1, 2, and 4 kHz both prior to and postsurgery with HA and if applicable CI. Measures across these frequencies were averaged separately for CI and HA ears (PTA-4). If a response could not be obtained because a frequency was not heard by the participant, values were set to 120 dB HL, i.e., 10 dB above the highest sound presentation level used during audiometry. The same questionnaires and audiological tests were used at the assessments preceding implantation (T2), as well as approximately 3 (T3) and 6 (T4) months postsurgery.

In addition to general background and expectations, questions about the presence, persistence, and location of tinnitus, a Numeric Rating Scale (NRS) (21) assessing current subjectively perceived tinnitus loudness (NRS 0–10: tinnitus audible only in silence—tinnitus louder than all other sounds) were used. Additionally, tinnitus-related distress was assessed with the 12-item version of the Tinnitus Questionnaire [TQ12, named MTQ in Ref. (14, 15)]. The TQ12 was developed by Hiller and Goebel (22) according to an optimal combination of high item-total correlations, reliability, and sensitivity for the assessment of changes in tinnitus-related distress. According to Zeman et al. (23), the TQ showed satisfying psychometric results, which were equally good for the long form and for the short TQ12 form. Internal consistency of TQ12 was $\alpha = 0.87$ (23). For a classification of tinnitus-related distress, Hiller and Goebel (22) proposed four grades with: scores 1–7 (grade 1) signifying no clinically relevant distress, a score of 8–12 (grade 2) representing moderate distress, a score of 13–18 (grade 3) representing severe distress, and a score of 19–24 representing the most severe distress due to tinnitus. Grades 3 and 4 are considered to require therapeutic intervention. Depression and anxiety were assessed with the Hospital Anxiety and Depression Scale [HADS (24)] at T2 and T4. For both subscales of the HADS, scores of 8 or above are considered to be indicative of potential problems in these areas (24). For tinnitus patients, internal consistencies of $\alpha = 0.83$ and $\alpha = 0.88$ were determined for the anxiety and depression subscales (25). One question with five options between "very good" (4) and "poor" (0) assessed the subjective impression of a participant regarding his/her general health condition at T2 and T4.

TABLE 1 | Participant's characteristics.

	Tinnitus at T2	No tinnitus at T2
Number	15	4
Gender: male (N)	2	2
Age mean (SD)	55.3 (14.0)	64.3 (19.3)
Cochlear implant (CI) left	9	2
CI ear: years with hearing impairment	25.5 (18.5)	24.0 (13.0)
Hearing aid (HA) ear: years with hearing impairment	21.3 (19.6)	17.0 (11.8)
Pre-OP HA use CI ear (excluding cross)	10	3
Pre-OP HA use other ear	15	4
PTA-4 (dB HL) of CI ear		
Preimplantation	95.9 (17.3)	91.3 (15.0)
Postimplantation	46.6 (12.8)	43.8 (9.7)
PTA-4 (dB HL) of HA ear		
Preimplantation	65.5 (18.6)	70.1 (15.1)
Postimplantation	61.1 (24.0)	72.3 (4.5)
Hospital Anxiety and Depression Scale (HADS) with at least 1 scale ≥8 at T2	6	0
Participants with relevant other health conditions	9	3

Shown are numbers per condition and means with respective SDs. PTA-4 thresholds of the CI ear decrease significantly with implantation indicating much better hearing postimplantation. Although the group without tinnitus is older, duration of hearing impairment at the HA ear is shorter, and hearing on the HA ear is worse, differences between tinnitus and non-tinnitus group are not statistically significant. Another potential difference between those affected by tinnitus and those without may be differences in mental wellbeing. Scores of 8 points or above in the HADS are seen as indicator of potential problems regarding depressiveness and anxiety, respectively, and are present only in the tinnitus group. Other relevant health conditions were, for instance, hypertension, thyroid, cardiologic, depression, and condition following a malignant tumor.

Statistical Analysis

Statistical analysis was performed with IBM SPSS Statistics, version 22 (SPSS/IBM, Chicago, IL, USA). Descriptive statistics included mean and SD. The effect of intracochlear electrical stimulation on tinnitus was assessed by comparing baseline values to the outcomes obtained at the end of the follow-up. Pearson correlation coefficients were calculated, with coefficients <0.5 being considered as weak, coefficients between 0.5 and 0.8 being considered as moderate, and >0.8 being considered as strong. In addition, a general linear model for repeated measurements with Bonferroni correction and in case of non-normality Friedman tests were performed prior to *post hoc* testing with two-sided *t*-tests or Wilcoxon signed-rank tests, respectively. Statistical significance was defined for *p* values smaller than 0.05, and *p*-values smaller than 0.01 were considered to be highly significant.

Effect sizes for repeated measures in dependent samples were calculated according to Bortz (26) [see also Ref. (27)] with the following equation:

$$\varepsilon = \frac{\mu_1 - \mu_2}{\hat{\sigma}\sqrt{1-r}}$$

The difference between means (μ_1, μ_2) is divided by the pooled variance ($\hat{\sigma}$). Introduction of the Pearson correlation coefficient (r) serves as a correction for the dependence among means. Effect sizes of >0.2 correspond to weak effects, of >0.5 to moderate, and of >0.8 to strong effects.

RESULTS

Pure Tone Audiometry

Preoperative aided thresholds at 0.5, 1, 2, and 4 kHz (PTA-4) in free sound field could not be determined for 10 of the 24 CI ears and for 1 HA ear due to no response in some or all of these frequencies. For calculation of overall improvement of hearing, values for measurements that did not yield a result were set to 120 dB HL. Pre- and postimplantation PTA-4 averages are shown in **Table 1**. Improvement of hearing thresholds of CI ears with CI use was highly significant ($t = 10.593$; $p < 0.001$), while average thresholds in the HA ear were unchanged ($t = 1.078$; $p = 0.301$). For participants with preexisting tinnitus, the CI ear remained the worse ear for three, became the better hearing ear for seven, and aided hearing levels were similar for both ears in five individuals. For all individuals without tinnitus, the CI ear became the better ear postsurgery.

Tinnitus before CI

Prior to implantation, tinnitus was reported by 79% (15/19) of the study participants. The four participants without tinnitus were older, duration of hearing impairment on the other ear was shorter, preimplantation hearing thresholds were more similar for the two ears, and they were less likely to report high scores in the anxiety and depression scales. Differences to the tinnitus group were not statistically significant, however. In contrast, reported duration of hearing impairment on the ear to be implanted was similar to the average in the tinnitus group (**Table 1**).

Preexisting tinnitus was permanent in 7 cases, and was localized across both ears, or was heard within the head in 10 individuals, while it localized to the future CI ear in 4 and to the HA ear in 1. Perceived loudness was very loud (≥8) for four participants and very low (≤2) for 2, with an average of 4.9 [(2.7), range 1–10] on the 0–10 NRS scale. According to the grading of Hiller and Goebel (22) with TQ12 scores above 12 indicating clinically significant tinnitus-related distress, two participants (13%) expressed clinically significant tinnitus-related distress, while the average score was 7.4 [(6.0), range 1–24], corresponding to low tinnitus-related distress (see **Figure 1**).

Changes in Tinnitus with CI Use

Of the 15 study participants with tinnitus at T2, all but 2 reported a subjective benefit following CI use. In two subjects with tinnitus prior to surgery, an additional tone arose for the CI ear or the HA ear, respectively. One subject developed new tinnitus at the CI ear, which was neither loud or distressing and not permanent by T4. At T2, seven participants reported permanent tinnitus, while this was true for six at T4.

Changes in tinnitus localization after cochlear implantation coincided with improvement of PTA-4 thresholds in the CI ear relative to the HA ear. Whereas tinnitus localization did not change if the CI ear remained the worse ear ($n = 3$), it changed in 50% of the other 12 in whom hearing balance between CI and HA ear was changed by CI use.

For calculations of average scores for the tinnitus and health variables, values obtained from the individual with tinnitus onset after surgery were omitted. On average, tinnitus loudness decreased by 42% between T2 and T4 resulting in an effect size of 1.40, which indicates a strong effect (**Figure 1**). Overall, this reduction was highly significant ($F = 9.161$; $p = 0.012$), and the reduction between T2 and T4 reached significance with *post hoc* testing ($p = 0.035$). Tinnitus loudness was at least halved in 47%. At the end of the study, mean perceived loudness was 2.9 (2.5) and was rated as 5 or below for all except one subject (**Figure 1**). This subject experienced a tinnitus of maximal loudness (10/10) at onset and at the end of the study.

On average, TQ12 scores decreased by 24% between T2 and T4 resulting in an effect size of 0.38 which indicates a weak effect of bimodal provision on tinnitus-related distress (**Figure 1**). The main effect just missed statistical significance ($chi^2 = 5.911$; $p = 0.052$), while *post hoc* tests clearly missed a significance level (T2–T3: $Z = -0.996$; $p = 0.319$; T2–T4: $Z = -1.646$; $p = 0.100$). Between T2 and T4, the TQ12 score decreased by at least 3 points (12.5% on the 0–24 TQ12 scale) for eight participants (53%), was unchanged for 5 (33%), and increased by at least 3 points for two participants. The latter both indicated severe stress independent of their hearing at the end of the study. At T4, all but three participants had tinnitus of the lowest category, grade 1, and one fell into grade 2, indicating mild to moderate tinnitus (**Figure 1**). The remaining two participants did not benefit from CI use in terms of tinnitus-related distress reduction. One of them reported maximal tinnitus-related distress (24/24) both at the beginning and at the end of the study, coinciding with maximal tinnitus loudness (10/10) at both assessments. This participant was diagnosed with an additional attack of sudden sensorineural hearing loss on the non-implanted ear during the study and expressed a high level of anxiety and depression symptoms at all times. The other participant had very low tinnitus loudness and distress prior to CI surgery. With CI use, tinnitus loudness increased from 2 to 3 points on the NRS, whereas the TQ12 score was increased by 17 points at T3 and by 16 points at T4, as compared to pre-CI, reaching a level of severe tinnitus distress (grade 3) at the post-CI assessments. Noteworthy in this participant were the continuously high levels of depression and anxiety. This was despite taking antidepressive medication since T3, prescribed independently of the study.

Anxiety, Depression, and General Health

On average, the levels of anxiety and depression were both reduced by 20% with an effect size of 0.53 indicating moderate effects on both factors (**Figure 1**). Reductions did not reach significance for either factor (anxiety: $t = 1.451$; $p = 0.169$; depression: $Z = -1.307$; $p = 0.191$). At the first assessment prior to implantation, six of the participants with preexisting tinnitus (40%) reported a score of 8 or above, either in one or in both HADS subscales, i.e., indicating potential problems in these areas. At T4, three participants reported a score of ≥8 in one of the HADS scales. During the study, scores dropped below 8 in 4 participants and increased in one, whose initial scores had been inconspicuous. The latter case could be related to events independent of the CI. Despite indications of mental health problems, tinnitus loudness and distress were reduced by more than 50% in four of the six participants who reported increased HADS scores

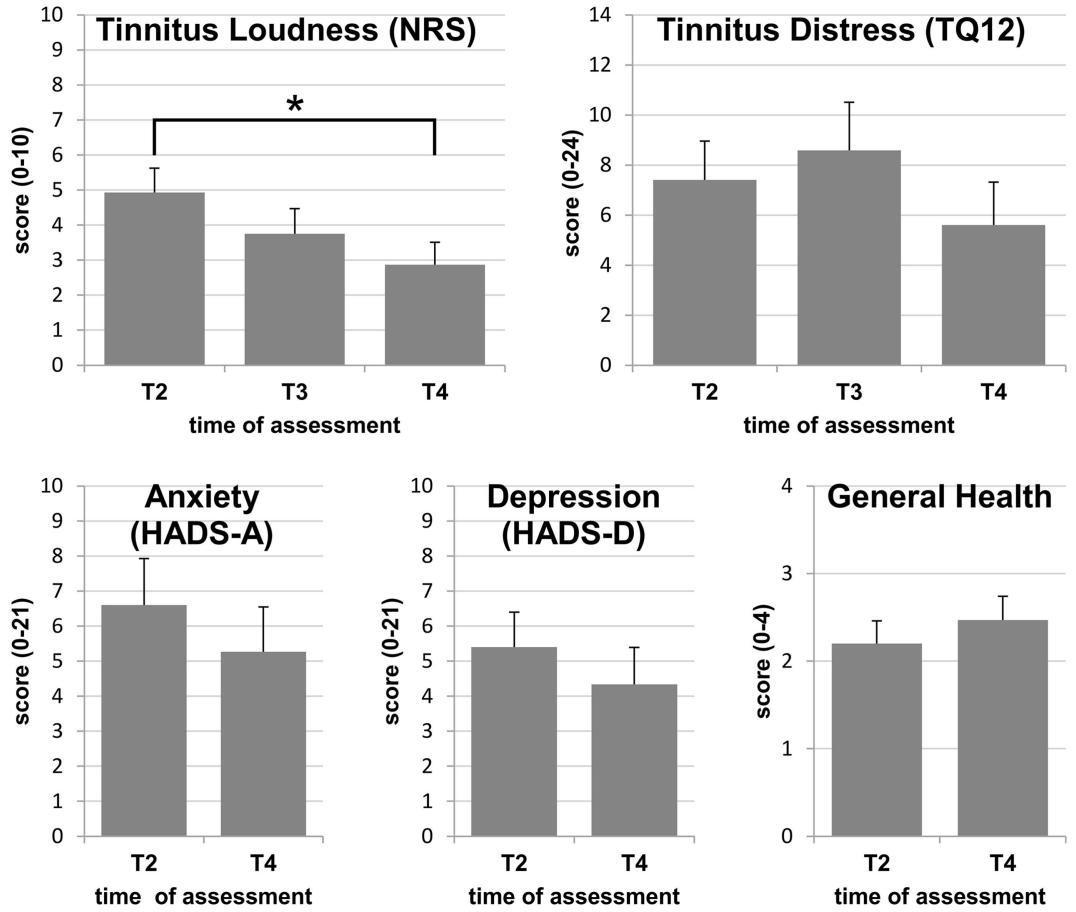

FIGURE 1 | Group means with SEs at assessments T2, T3, and T4: only the reduction of perceived tinnitus loudness reaches statistical significance, while differences between assessments did not attain statistical significance for tinnitus-related distress, anxiety, depression, or general health. Statistically significant differences are indicated by $^*p < 0.05$.

at T2, and also in the individual whose HADS scores increased above 8 during the study.

When asked for a judgment of their general health situation, only one individual reported a substantial improvement of 3 points on the 0–4 scale between T2 and T4. On average, general health was considered to be satisfactory at T2, and the majority did not report any improvement, often despite a statement that their hearing ability had improved considerably (**Figure 1**).

Correlation between Measures

In the correlation matrices presented in **Tables 2** and **3**, correlations between subjective tinnitus loudness and tinnitus-related distress peaked between significant to highly significant, but moderate correlations were found at all assessments. In addition, correlations between postimplantation tinnitus-related distress and the level of anxiety at T2 and T4 and also with the level of depression at T2 exhibited significant to highly significant correlations of moderate strength. When correlating changes during the study period, a significant correlation existed between improvement of hearing at the CI ear and the reduction of tinnitus loudness between T2 and T4 ($r = 0.584$; $p = 0.022^*$). In addition, time of hearing impairment showed a significant correlation with the improvement in anxiety scores between T2 and T4 (**Table 3**). Age at implantation did not show a significant correlation with any of the above variables, but a significant inverse correlation with general health indicating that older participants had more health problems. According to the reporting of chronic health conditions by the participants, these were mostly unrelated to their hearing (**Table 1**).

DISCUSSION

Tinnitus was common for the hearing-impaired participants of this study. Main findings are a significant reduction in subjective tinnitus loudness between preimplantation and 6 months postimplantation (T2 versus T4), and a significant correlation between improvement of hearing with CI use and the reduction in tinnitus loudness. As predicted by hypothesis 1, restoration of activity in the cochlea had a stronger effect on subjective tinnitus loudness, and less influence on tinnitus-related distress.

TABLE 2 | Correlations between measures.

	T2-TNRS	T3-TNRS	T4-TNRS	T2-TQ12	T3-TQ12	T4-TQ12	T2-HADS-A	T4-HADS-A	T2-HADS-D	T4-HADS-D	T2-Health	T4-Health
Age at implantation	0.044 0.875	0.206 0.521	0.055 0.846	0.253 0.363	0.195 0.544	0.027 0.925	0.068 0.810	0.239 0.392	0.295 0.285	0.083 0.768	−0.246 0.376	−0.647 0.009**
T2-TNRS	1	0.589 0.044*	0.697 0.004**	0.576 0.025*	0.528 0.078	0.274 0.322	−0.038 0.893	0.146 0.603	0.040 0.889	−0.121 0.667	−0.546 0.035*	−0.139 0.622
T3-TNRS		1	0.785 0.002**	0.826 0.001**	0.767 0.004**	0.624 0.030*	0.139 0.666	0.383 0.247	−0.009 0.978	−0.077 0.813	−0.043 0.894	−0.147 0.649
T4-TNRS			1	0.780 0.001**	0.760 0.004**	0.756 0.001**	0.375 0.168	0.482 0.069	0.237 0.395	0.089 0.752	−0.472 0.075	−0.220 0.432
T2-TQ12				1	0.651 0.022*	0.495 0.061	0.256 0.357	0.477 0.072	0.059 0.835	−0.176 0.529	−0.178 0.526	−0.244 0.381
T3-TQ12					1	0.851 <0.001**	0.587 0.045*	0.672 0.017*	0.510 0.090	0.498 0.100	−0.421 0.172	−0.537 0.072
T4-TQ12						1	0.681 0.005**	0.710 0.003**	0.558 0.031*	0.508 0.053	−0.411 0.128	−0.458 0.086
T2-HADS-A							1	0.725 0.002**	0.772 0.001**	0.654 0.008**	−0.365 0.180	−0.524 0.045*
T4-HADS-A								1	0.582 0.023*	0.616 0.014*	−0.197 0.482	−0.643 0.010**
T2-HADS-D									1	0.814 <0.001**	−0.541 0.037*	−0.648 0.009**
T4-HADS-D										1	−0.258 0.354	−0.482 0.069
T2-Health											1	0.505 0.055

Significant correlations exist between subjective tinnitus loudness and tinnitus-related distress. Further significant correlations are observed for postimplantation tinnitus-related distress with anxiety at T2 and T4 and with depression scores at T2. Furthermore, tinnitus loudness and depression at T2 and anxiety scores at T2 and T4 as well as age at implantation show significant inverse correlations with general health.

*Pearson correlation coefficients: **p < 0.01 (2-tailed); *p < 0.05 (2-tailed).*

HADS-A, Hospital Anxiety and Depression Scale for anxiety; HADS-D, Hospital Anxiety and Depression Scale for depression.

Moreover, magnitude of influence on tinnitus-related distress was lower than effects on anxiety and depression. Contrary to hypothesis 2 that high levels of anxiety or depression prevent reduction of tinnitus symptoms, these reduced in the majority of participants with high depression and anxiety scores, while failure to reduce or increases could be related to other current sources of distress.

To the best of our knowledge, this is the first longitudinal study on the influence of bimodal provision on tinnitus symptoms. Present findings are in general agreement with findings on unilateral electric amplification of sound by CI as reported in retrospective studies (11, 28–30), reviews (3, 4, 31–33), and a growing body of prospective studies [e.g., Ref. (7, 12, 34–39)]. Although total remission from tinnitus was not observed in the present study, most subjects noticed substantial reduction of their tinnitus, while worsening of a preexisting tinnitus was rare, predominantly pertained tinnitus-related distress, and appeared to be associated with increased levels of anxiety and depression. Emergence of tinnitus only after cochlear implantation was an exception, and as reported before (7, 12), resolved within a few weeks and was experienced as mild at the end of follow-up. In the sample by Pan et al. (12), those who acquired tinnitus had the shortest duration hearing loss and were the oldest implant recipients. This cannot be corroborated by the present results, however.

The effect size for loudness reduction indicates a strong effect comparable to or higher than effect sizes that were reported for generally accepted tinnitus therapies that, however, serve to reduce tinnitus-related distress as opposed to tinnitus loudness (40, 41). Two prospective studies on tinnitus following cochlear implantation addressed tinnitus loudness in a similar way, namely, by a visual analog scale (35, 38). The participants of these studies had severe to profound unilateral or bilateral hearing impairment and were provided unilaterally with a CI while hearing was not amplified at the other ear. Tinnitus loudness was reduced significantly and by a similar amount as in the present study, but effect sizes for the reduction of tinnitus loudness were not reported in these publications.

Bimodal provision was far less effective in reducing tinnitus-related distress. This finding was expected since tinnitus-related distress depends on further influences that cannot directly be influenced by restoration of cochlear activity (19). Furthermore, presurgery tinnitus-related distress was reported as mild to moderate by the majority of the study participants, even if the tinnitus was rather loud. This is in line with earlier studies on CI implantees (36, 39) and may have prevented findings of significant reductions. Taken together, present findings support the assumption that restoration of auditory input primarily reduces the tinnitus signal, whereas it has a weaker influence on tinnitus-related distress, and they support the distinction between perceived tinnitus loudness and distress (14, 15, 42). Furthermore, these findings corroborate the assumption that tinnitus-related distress is influenced by non-auditory factors as suggested previously (15, 17–19, 43).

Acquired hearing impairment represents a risk factor for increased levels of anxiety and depression (44, 45), especially in combination with distressing tinnitus (14, 18). A total of 40% of those with preexisting tinnitus indicated conspicuous levels of anxiety and/or depression before implantation, whereas average

TABLE 3 | Correlations between changes in measures with cochlear implant (CI) use.

	Pre- to postimplantation improvement					
	CI ear: PTA-4	**TNRS**	**TQ12**	**Hospital Anxiety and Depression Scale for anxiety (HADS-A)**	**Hospital Anxiety and Depression Scale for depression (HADS-D)**	**Health**
Age at implantation	−0.239	−0.008	0.211	−0.160	0.389	−0.422
	0.391	0.977	0.451	0.568	0.151	0.117
CI ear: years of hearing impairment	−0.338	−0.315	−0.250	0.580	−0.073	0.220
	0.218	0.253	0.368	0.023*	0.796	0.431
Hearing aid (HA) ear: years of hearing impairment	−0.466	−0.380	−0.319	0.613	−0.129	−0.058
	0.080	0.163	0.246	0.015*	0.647	0.839
CI ear: PTA-4 improvement	1	0.584	0.354	−0.273	−0.191	0.424
		0.022*	0.195	0.325	0.496	0.115
TNRS improvement		1	0.405	−0.312	−0.025	0.231
			0.134	0.258	0.929	0.408
TQ12 improvement			1	−0.364	0.082	−0.002
				0.182	0.772	0.994
HADS-A improvement				1	0.442	0.246
					0.099	0.377
HADS-D improvement					1	0.113
						0.688

Significant correlations are present between improvement of PTA-4 thresholds pre- to postimplantation of the CI ear and improvement, i.e., reduction of subjective tinnitus loudness between T2 and T4, and between years of hearing impairment of the CI and the HA ear and the reduction in anxiety scores between T2 and T4.
*Pearson correlation coefficients: *p < 0.05 (2-tailed).*

levels were low which is in accordance with earlier results from CI recipients [e.g., Ref. (28, 29, 35, 36)]. Depression and anxiety scores were reduced by 20% between baseline and the end of follow-up, but these differences did not attain statistical significance. Former reports differ in this aspect with some observing significant reductions whereas others do not, with discrepancies likely being dependent on sample characteristics (28–30, 36). Estimated magnitude of effects for the reduction of anxiety and depression were higher than for the reduction in tinnitus-related distress. This suggests that other aspects of life quality related to anxiety and depression may have been improved by bimodal provision (44, 45). Correlations of postimplant levels of tinnitus-related distress with anxiety and for fewer comparisons also with depression attained significance. An association between these measures has been reported for tinnitus populations with and without cochlear implants (8, 14, 28), and catastrophic interpretations of tinnitus have been associated with fear (46). But increased levels of anxiety or depression did not prevent a reduction in tinnitus loudness and tinnitus-related distress, except in two participants, indicating that a reduction in tinnitus is highly reliant on afferent auditory input. Despite a reduction in tinnitus-related complaints and better hearing in general, individuals with higher scores in the HADS scales were not satisfied with their quality of life. Although low in terms of percentage, CI recipients who do not experience tinnitus relief, or express compromised well-being, need to be taken care of as they might benefit from other types of therapies (13), and this in turn might improve performance with their CI.

The exact mechanism through which CI use suppresses tinnitus symptoms is unknown. Several mechanisms, such as masking, direct electrical nerve stimulation, habituation, and plastic reorganization in the brain have to be considered. Another aspect why CIs appear to be effective in reducing the tinnitus may be the intense auditory training required during rehabilitation. According to current knowledge, tinnitus is the result of maladaptive plasticity in the central auditory pathway in response to auditory deprivation. In the majority of cases, the trigger for tinnitus-related changes in the brain is impairment of the cochlea (16). Experimentally inducing auditory deprivation by exposing healthy subjects to complete silence triggers phantom sounds that are reversible upon restoration of the auditory input (47). In addition, continuous use of earplugs can lead to a reversible perception of tinnitus (48). This suggests that tinnitus can be induced by auditory deprivation and that it can be reversed by restoring input into the central auditory system. Restoration of peripheral input especially at the base of the cochlea which is important for activity in the tinnitus-relevant high-frequency areas of the auditory system can be achieved by CI use or by other types of electrical stimulation (8). Extracochlear (49–51) and intracochlear electrical stimulation (52–54) reduce tinnitus, even when the stimuli are "not audible." A study by Punte et al. (55) suggested that tinnitus suppression does only occur if the full length of the cochlea is electrically stimulated by a CI. On the other hand, continued presence of tinnitus during CI use may be due to the fact that hearing is not completely restored by the implant, or because a memory of the tinnitus has been established in the brain which is partly independent of external input.

Although electrical stimulation of the inner ear effectively suppresses tinnitus for many CI recipients, the reported percentages vary between studies and may depend on characteristics of the samples under study, particularly regarding non-auditory aspects (29). For conscious perception of the tinnitus signal and for the suffering arising from it, involvement of brain areas beyond the auditory system appears to be mandatory (19). Thus, tinnitus is not simply the result of defective auditory input but obviously requires further mechanisms. This may be the reason why some that are hard of hearing, or deaf, do not experience tinnitus: for instance, 21% of the participants of the present study.

Brain regions with alterations related to tinnitus have included the emotional, attentional, and memory systems. These systems are thought to influence processing in the auditory cortex and thus in the auditory system in a top-down manner (19, 56), particularly in those who suffer from their tinnitus (19, 57, 58). Behaviorally, this is evidenced for instance by enhanced levels of depression and anxiety in tinnitus populations especially, among those with distressing tinnitus (14, 15). Remarkably and similar to other studies (28, 35), average depression and anxiety scores are low in the present CI sample. This may be a favorable condition for allowing a reduction of the tinnitus signal in a bottom-up manner and may part explain the extent of tinnitus reduction by CIs. Although tinnitus with a major emphasis on non-auditory mechanisms (19) was thought to respond less to recovery of the auditory input, even individuals with enhanced levels of anxiety and depression responded to bimodal provision with a substantial reduction of tinnitus symptoms, given that there existed no other current sources of severe distress. In the latter, the tinnitus might even worsen despite attenuation of its trace within the auditory system. Noteworthy in this respect are the two individuals whose tinnitus was not positively influenced by bimodal hearing. Whereas in one, the tinnitus retained maximal loudness and distress throughout the study period, in the other negligent tinnitus-related distress before CI provision increased tremendously with CI use. Concomitantly high levels of anxiety and depression at pre- and postsurgical assessment in both individuals and the reporting of severe distress postimplantation are in agreement with the assumption that highly distressing tinnitus may predominantly be a consequence of central top-down processing and that it is therefore, less influenced by attenuation of the tinnitus signal in the ascending auditory pathway.

Taken together, CIs alone as well as in combination with a contralateral HA appear to be effective in reducing the tinnitus signal through influencing neurophysiological processes involved in the generation and maintenance of tinnitus, via compensation of peripheral deafferentation. In addition, enhanced attentiveness to environmental sounds following implantation may lower awareness of the tinnitus. Although psychological factors certainly contribute to the tinnitus relief obtained through implant use, restoring auditory input by electrical stimulation appears to be primary effect. In accordance, loudness is reduced to a larger extent than the tinnitus-related distress. Whether long-term CI use reverses reorganization in the central auditory system associated with peripheral deafferentation and with tinnitus (16) remains to be shown. Tinnitus reduction does not always result in increased well-being, however. In our study sample, individuals with increased levels of anxiety or depression still felt anxious or depressed, despite improvements in their tinnitus and their hearing in general. Such individuals might additionally require psychological help.

Limitations

As in all prospective studies with CI recipients, the sample under investigation is small and therefore, might not be representative. However, our results are in general agreement with the published literature. Study participants were observed in their first 6 months after cochlear implantation. This time may be too short as an endpoint since improvements in auditory comprehension tend to continue thereafter. However, others (35, 38) have shown that reductions of tinnitus loudness occur early after CI provision. Furthermore, as double-blind studies are not feasible in this patient group, this was an open study that is not completely free from bias. It is conceivable, however, that placebo-controlled studies are and will be exceptional in intracochlear electrical stimulation for tinnitus suppression. For our sample, it can be stated that, while amelioration of tinnitus was not the main focus of the participants' concerns or expectations, most experienced relieving tinnitus reduction.

CONCLUSION

Restoration of auditory input by bimodal provision (CI and contralateral HA) appears to be an efficient method of reducing tinnitus: primarily the perceived loudness. At the same time, the risk of worsening or developing tinnitus as a result of implant surgery is low. Therefore, restoration of auditory input in the high-frequency part of the cochlea, as achieved by a CI, can be regarded as an effective means for the reduction of tinnitus, but is only justified in patients with deeply compromised hearing ability. Electrical stimulation of the cochlea independent of CI use might represent a feasible alternative for those with better hearing yet experiencing loud tinnitus.

In addition to an effective stimulation of the tinnitus-relevant high-frequency range of the cochlea, and consequently of central auditory structures, effectiveness of CI use regarding tinnitus reduction might also be related to the low levels of tinnitus-related distress, depression, and anxiety commonly found prior to implantation. Based on our findings, we propose that central mechanisms exacerbating tinnitus have to be expected in individuals who exhibit increased levels of anxiety and depression in particular if they experience severe distress postimplantation. Although those who suffer from their tinnitus and show compromised mental health represent a small percentage of CI recipients with tinnitus, this aspect has to be taken care of. The impact on quality of life, possibly on acceptance of the CI, means that these individuals may require other types of specialized therapeutic interventions, interventions which however, are available (13).

AUTHOR CONTRIBUTIONS

JS: data acquisition, interpretation of data, and drafting of the manuscript. KH: critical revision. EW-F: study conception and design, data acquisition, analysis and interpretation of data, and drafting of the manuscript.

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REFERENCES

1. Fielden CA, Kitterick PT. Contralateral acoustic hearing aid use in adult unilateral cochlear implant recipients: current provision, practice, and clinical experience in the UK. *Cochlear Implants Int* (2016) 17:132–45. doi:10.1080/14670100.2016.1162382
2. Nondahl DM, Cruickshanks KJ, Huang GH, Klein BE, Klein R, Chappell R, et al. Tinnitus and its risk factors in the Beaver Dam offspring study. *Am J Audiol* (2011) 50:313–20. doi:10.3109/14992027.2010.551220
3. van Zon A, Peters JP, Stegeman I, Smit AL, Grolman W. Cochlear implantation for patients with single-sided deafness or asymmetrical hearing loss: a systematic review of the evidence. *Otol Neurotol* (2015) 36:209–19. doi:10.1097/MAO.0000000000000681
4. van Zon A, Smulders YE, Ramakers GG, Stegeman I, Smit AL, Van Zanten GA, et al. Effect of unilateral and simultaneous bilateral cochlear implantation on tinnitus: a prospective study. *Laryngoscope* (2016) 126:956–61. doi:10.1002/lary.25493
5. House WF. Cochlear implants. *Ann Otol Rhinol Laryngol* (1976) 85:1–93. doi:10.1177/00034894760850S303
6. Quaranta N, Wagstaff S, Baguley DM. Tinnitus and cochlear implantation. *Int J Audiol* (2004) 43:245–51. doi:10.1080/14992020400050033
7. Quaranta N, Fernandez-Vega S, D'elia C, Filipo R, Quaranta A. The effect of unilateral multichannel cochlear implant on bilaterally perceived tinnitus. *Acta Otolaryngol* (2008) 128:159–63. doi:10.1080/00016480701387173
8. Tyler RS, Rubinstein J, Pan T, Chang SA, Gogel SA, Gehringer A, et al. Electrical stimulation of the cochlea to reduce tinnitus. *Semin Hear* (2008) 29:326–32. doi:10.1055/s-0028-1095892
9. Punte AK, Vermeire K, Hofkens A, De Bodt M, De Ridder D, Van de Heyning P. Cochlear implantation as a durable tinnitus treatment in single-sided deafness. *Cochlear Implants Int* (2011) 12:S26–9. doi:10.1179/146701011X13001035752336
10. Mertens G, Kleine Punte A, De Bodt M, Van de Heyning P. Binaural auditory outcomes in patients with postlingual profound unilateral hearing loss: 3 years after cochlear implantation. *Audiol Neurootol* (2015) 20:67–72. doi:10.1159/000380751
11. Pierzycki RH, Edmondson-Jones M, Dawes P, Munro KJ, Moore DR, Kitterick PT. Tinnitus and sleep difficulties after cochlear implantation. *Ear Hear* (2016) 37:e402–8. doi:10.1097/AUD.0000000000000341
12. Pan T, Tyler RS, Ji H, Coelho C, Gehringer AK, Gogel SA. Changes in the tinnitus handicap questionnaire after cochlear implantation. *Am J Audiol* (2009) 18:144–51. doi:10.1044/1059-0889(2009/07-0042)
13. Langguth B. Treatment of tinnitus. *Curr Opin Otolaryngol Head Neck Surg* (2015) 23:361–8. doi:10.1097/MOO.0000000000000185
14. Wallhäusser-Franke E, Brade J, Balkenhol T, D'Amelio R, Seegmüller A, Delb W. Tinnitus: distinguishing between subjectively perceived loudness and tinnitus-related distress. *PLoS One* (2012) 7:e34583. doi:10.1371/journal.pone.0034583
15. Wallhäusser-Franke E, Repik I, Delb W, Glauner A, Hörmann K. Long-term development of acute tinnitus. *Laryngorhinootologie* (2015) 94:759–69. doi:10.1055/s-0035-1550039
16. Eggermont JJ, Roberts LE. The neuroscience of tinnitus: understanding abnormal and normal auditory perception. *Front Syst Neurosci* (2012) 6:53. doi:10.3389/fnsys.2012.00053
17. Langguth B, Landgrebe M, Kleinjung T, Sand GP, Hajak G. Tinnitus and depression. *World J Biol Psychiatry* (2011) 12:489–500. doi:10.3109/15622975.2011.575178
18. Brüggemann P, Szczepek AJ, Rose M, McKenna L, Olze H, Mazurek B. Impact of multiple factors on the degree of tinnitus distress. *Front Hum Neurosci* (2016) 10:341. doi:10.3389/fnhum.2016.00341
19. De Ridder D, Vanneste S, Weisz N, Londero A, Schlee W, Elgoyhen AB, et al. An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci Biobehav Rev* (2014) 44:16–32. doi:10.1016/j.neubiorev.2013.03.021
20. Kalbe E, Kessler J, Calabrese P, Smith R, Passmore AP, Brand M, et al. DemTect: a new sensitive cognitive screening test to support the diagnosis of mild cognitive impairment and early dementia. *Int J Geriatr Psychiatry* (2004) 19:136–43. doi:10.1002/gps.1042
21. Meikle MB, Stewart BJ, Griest SE, Henry JA. Tinnitus outcomes assessment. *Trends Amplif* (2008) 12:223–35. doi:10.1177/1084713808319943
22. Hiller W, Goebel G. Rapid assessment of tinnitus-related psychological distress using the mini-TQ. *Int J Audiol* (2004) 43:600–4. doi:10.1080/14992020400050077
23. Zeman F, Koller M, Schecklmann M, Langguth B, Landgrebe M. Tinnitus assessment by means of standardized self-report questionnaires: psychometric properties of the tinnitus questionnaire (TQ), the tinnitus handicap inventory (THI), and their short versions in an international and multi-lingual sample. *Health Qual Life Outcomes* (2012) 10:128. doi:10.1186/1477-7525-10-128
24. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. *Acta Psychiatr Scand* (1983) 67:361–70. doi:10.1111/j.1600-0447.1983.tb09716.x
25. Andersson G, Kaldo-Sandström V, Ström L, Strömgren T. Internet administration of the Hospital Anxiety and Depression Scale in a sample of tinnitus patients. *J Psychosom Res* (2003) 55:259–62. doi:10.1016/S0022-3999(02)00575-5
26. Bortz J. *Statistik für Sozialwissenschaftler*. Heidelberg: Springer (1993). 137 p.
27. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. Hillsdale, NJ: Lawrence Erlbaum (1988).
28. Andersson G, Freijd A, Baguley DM, Idrizbegovic E. Tinnitus distress, anxiety, depression, and hearing problems among cochlear implant patients with tinnitus. *J Am Acad Audiol* (2009) 20:315–9. doi:10.3766/jaaa.20.5.5
29. Kloostra FJ, Arnold R, Hofman R, Van Dijk P. Changes in tinnitus after cochlear implantation and its relation with psychological functioning. *Audiol Neurootol* (2015) 20:81–9. doi:10.1159/000365959
30. Olze H, Szczepek AJ, Haupt H, Förster U, Zirke N, Gräbel S, et al. Cochlear implantation has a positive influence on quality of life, tinnitus, and psychological comorbidity. *Laryngoscope* (2011) 21:2220–7. doi:10.1002/lary.22145
31. Blasco MA, Redleaf MI. Cochlear implantation in unilateral sudden deafness improves tinnitus and speech comprehension: meta-analysis and systematic review. *Otol Neurotol* (2014) 35:1426–32. doi:10.1097/MAO.0000000000000431
32. Gaylor JM, Raman G, Chung M, Lee J, Rao M, Lau J, et al. Cochlear implantation in adults: a systematic review and meta-analysis. *JAMA Otolaryngol Head Neck Surg* (2013) 139:265–72. doi:10.1001/jamaoto.2013.1744
33. Vlastarakos PV, Nazos K, Tavoulari EF, Nikolopoulos TP. Cochlear implantation for single-sided deafness: the outcomes. An evidence-based approach. *Eur Arch Otorhinolaryngol* (2014) 271:2119–26. doi:10.1007/s00405-013-2746-z
34. Bovo R, Ciorba A, Martini A. Tinnitus and cochlear implants. *Auris Nasus Larynx* (2011) 38:14–20. doi:10.1016/j.anl.2010.05.003
35. Kim DK, Moon IS, Lim HJ, Yoo SY, Heo KW, Bae SC, et al. Prospective, multicenter study on tinnitus changes after cochlear implantation. *Audiol Neurootol* (2016) 21:165–71. doi:10.1159/000445164
36. Knopke S, Gräbel S, Förster-Ruhrmann U, Mazurek B, Szczepek AJ, Olze H. Impact of cochlear implantation on quality of life and mental comorbidity in patients aged 80 years. *Laryngoscope* (2016) 126:2811–6. doi:10.1002/lary.25993
37. Kompis M, Pelizzone M, Dillier N, Allum J, DeMin N, Senn P. Tinnitus before and 6 months after cochlear implantation. *Audiol Neurootol* (2012) 17:161–8. doi:10.1159/000335126
38. Mertens G, De Bodt M, Van de Heyning P. Cochlear implantation as a long-term treatment for ipsilateral incapacitating tinnitus in subjects with unilateral hearing loss up to 10 years. *Hear Res* (2016) 331:1–16. doi:10.1016/j.heares.2015.09.016
39. Vallés-Varela H, Royo-López J, Carmen-Sampériz L, Sebastián-Cortés JM, Alfonso-Collado I. The cochlear implant as a tinnitus treatment. *Acta Otorrinolaringol Esp* (2013) 64:253–7. doi:10.1016/j.otorri.2012.11.008
40. Bauer CA, Brozoski TJ. Effect of tinnitus retraining therapy on the loudness and annoyance of tinnitus: a controlled trial. *Ear Hear* (2011) 32:145–55. doi:10.1097/AUD.0b013e3181f5374f
41. Zenner HP, Delb W, Kröner-Herwig B, Jäger B, Peroz I, Hesse G, et al. A multidisciplinary systematic review of the treatment for chronic idiopathic tinnitus. *Eur Arch Otorhinolaryngol* (2016). doi:10.1007/s00405-016-4401-y
42. Hiller W, Goebel G. When tinnitus loudness and annoyance are discrepant: audiological characteristics and psychological profile. *Audiol Neurootol* (2007) 12:391–400. doi:10.1159/000106482

43. Song JJ, Punte AK, De Ridder D, Vanneste S, Van de Heyning P. Neural substrates predicting improvement of tinnitus after cochlear implantation in patients with single-sided deafness. *Hear Res* (2013) 299:1–9. doi:10.1016/j.heares.2013.02.001
44. Contrera KJ, Wallhagen MI, Mamo SK, Oh ES, Lin FR. Hearing loss health care for older adults. *J Am Board Fam Med* (2016) 29:394–403. doi:10.3122/jabfm.2016.03.150235
45. Hsu WT, Hsu CC, Wen MH, Lin HC, Tsai HT, Su P, et al. Increased risk of depression in patients with acquired sensory hearing loss: a 12-year follow-up study. *Medicine (Baltimore)* (2016) 95:e5312. doi:10.1097/MD.0000000000005312
46. Cima RF, Crombez G, Vlaeyen JW. Catastrophizing and fear of tinnitus predict quality of life in patients with chronic tinnitus. *Ear Hear* (2011) 32:634–41. doi:10.1097/AUD.0b013e31821106dd
47. Heller MF, Bergman M. Tinnitus aurium in normally hearing persons. *Ann Otol Rhinol Laryngol* (1953) 62:73–83.
48. Schaette R, Turtle C, Munro KJ. Reversible induction of phantom auditory sensation through simulated unilateral hearing loss. *PLoS One* (2012) 7:e35238. doi:10.1371/journal.pone.0035238
49. Kuk FK, Tyler RS, Rustad N, Harker LA, Tye-Murray N. Alternating current at the eardrum for tinnitus reduction. *J Speech Hear Res* (1989) 32:393–400. doi:10.1044/jshr.3202.393
50. Mielczarek M, Michalska J, Polatyńska K, Olszewski J. An increase in alpha band frequency in resting state eeg after electrical stimulation of the ear in tinnitus patients – a pilot study. *Front Neurosci* (2016) 10:453. doi:10.3389/fnins.2016.00453
51. Rubinstein JT, Tyler RS, Johnson A, Brown CJ. Electrical suppression of tinnitus with high-rate pulse trains. *Otol Neurotol* (2003) 24:478–85. doi:10.1097/00129492-200305000-00021
52. Arts RA, George EL, Janssen M, Griessner A, Zierhofer C, Stokroos RJ. Tinnitus suppression by intracochlear electrical stimulation in single sided deafness – a prospective clinical trial: follow-up. *PLoS One* (2016) 11:e0153131. doi:10.1371/journal.pone.0153131
53. Chang JE, Zeng FG. Tinnitus suppression by electric stimulation of the auditory nerve. *Front Syst Neurosci* (2012) 6:19. doi:10.3389/fnsys.2012.00019
54. McKerrow WS, Schreiner CE, Snyder RL, Merzenich MM, Toner JG. Tinnitus suppression by cochlear implants. *Ann Otol Rhinol Laryngol* (1991) 100:552–8. doi:10.1177/000348949110000706
55. Punte AK, De Ridder D, Van de Heyning P. On the necessity of full length electrical cochlear stimulation to suppress severe tinnitus in single-sided deafness. *Hear Res* (2013) 295:24–9. doi:10.1016/j.heares.2012.08.003
56. Leaver AM, Turesky TK, Seydell-Greenwald A, Morgan S, Kim HJ, Rauschecker JP. Intrinsic network activity in tinnitus investigated using functional MRI. *Hum Brain Mapp* (2016) 37:2717–35. doi:10.1002/hbm.23204
57. Husain FT. Neural networks of tinnitus in humans: elucidating severity and habituation. *Hear Res* (2016) 334:37–48. doi:10.1016/j.heares.2015.09.010
58. Jastreboff PJ. Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci Res* (1990) 8:221–54. doi:10.1016/j.heares.2015.09.010

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frontiers in Neuroscience

ORIGINAL RESEARCH
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An Increase in Alpha Band Frequency in Resting State EEG after Electrical Stimulation of the Ear in Tinnitus Patients—A Pilot Study

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In our clinic invasive transtympanal promontory positive DC stimulations were first used, with a success rate of 42%. However, non-invasive hydrotransmissive negative DC stimulations are now favored, with improvement being obtained in 37.8% directly after the treatment, and 51.3% in a follow up 1 month after treatment. The further improvement after 1 month may be due to neuroplastic changes at central level as a result of altered peripheral input. The aim of the study was to determine how/whether a single electrical stimulation of the ear influences cortical activity, and whether changes observed in tinnitus after electrical stimulation are associated with any changes in cortical activity recorded in EEG. The study included 12 tinnitus patients (F–6, M-6) divided into two groups. Group I comprised six patients with unilateral tinnitus - unilateral, ipsilateral ES was performed. Group II comprised six patients with bilateral tinnitus—bilateral ES was performed. ES was performed using a custom-made apparatus. The active, silver probe—was immersed inside the external ear canal filled with saline. The passive electrode was placed on the forehead. The stimulating frequency was 250 Hz, the intensity ranged from 0.14 to 1.08 mA. The voltage was kept constant at 3 V. The duration of stimulation was 4 min. The EEG recording (Deymed QEST 32) was performed before and after ES. The patients assessed the intensity of tinnitus on the VAS 1-10.

Results: In both groups an improvement in VAS was observed—in group I—in five ears (83.3%), in group II—in seven ears (58.3%). In Group I, a significant increase in the upper and lower limit frequency of alpha band was observed in the central temporal and frontal regions following ES. These changes, however, were not correlated with improvement in tinnitus. No significant changes were observed in the beta and theta bands and in group II. Preliminary results of our research reveal a change in cortical activity after electrical stimulations of the ear. However, it remains unclear if it is primary or secondary to peripheral auditory excitation. No similar studies had been found in the literature.

Keywords: tinnitus, electrical stimulations, ear, neuromodulation, cortical activity

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INTRODUCTION

Tinnitus is one of the vaguest audiological and otological symptoms. It is defined as a phantom sound perception, which does not reflect any corresponding physical activity. Tinnitus had been a disturbing disorder since antiquity. Although, only peripheral—cochlear pathology was initially considered an ignition site, it appeared that tinnitus was not always relieved after cochlear nerve section (Baguley et al., 2002). Later, Jastreboff's neurophysiological model of tinnitus proposed that the cochlea played a role in phantom sound generation, with the wide involvement of the central nervous system (Jastreboff, 1990). Recently, the development of such medical diagnostic methods as neurophysiological analysis and neuroimaging has stimulated the formulation of new hypotheses concerning tinnitus generation (Kaltenbach, 2011) and consequently, new original treatment modalities for patients.

In our clinic electrical stimulation (ES) of the ear in tinnitus treatment was first used in the 1980s. Invasive transtympanal promontory positive DC stimulations were first used, with a success rate of 42% (improvement in questionnaire by at least 4 points in 10 point scale) (Konopka et al., 2001). However, non-invasive hydrotransmissive DC stimulations are now favored, with improvement being obtained in 37.8% directly after the treatment, and 51.3% in a follow up 1 month after treatment (improvement in questionnaire by at least 20%) (Mielczarek and Olszewski, 2014). The improvement in tinnitus was accompanied by an improvement in hearing threshold in pure tone audiometry, indicating the condition of the peripheral hearing organ had improved. We hypothesized that further improvement after 1 month may be due to neuroplastic changes at central level as a result of altered peripheral input. In consequence of abovementioned hypothesis we posed a question about the possible mechanisms in which ear ES influences tinnitus: is it only peripheral (cochlear) activity modulation, or central—secondarily to peripheral alternation, or maybe purely central? Hypothetically, for electric current applied to the ear, soft tissues work as a conductor, thus none of the mechanisms can be excluded. There are many studies on the effect of ear ES on peripheral structures, however, to our knowledge, there is no study exploring alternations at a central cortical level directly after peripheral non-invasive ear ES.

Many studies have reported the suppression of tinnitus after cochlear implantation (CI) (Quaranta et al., 2004; Todt et al., 2015; Mertens et al., 2016), but the exact mechanism which is behind tinnitus suppression remains unclear as well. Zeng et al. noticed an increase in alpha power after low-rate electric stimulation via CI (Zeng et al., 2011). High-rate stimulation (5000 pps), however, appeared to be inefficient in inducing central activity (Middlebrooks, 2008) although restored activity at the auditory nerve was observed (Rubinstein et al., 2003).

In the literature, there are few explanations of how ear ES influences tinnitus (an increase in the neurotransmitter's transmission in the synapses—Latkowski, 1981, modification of the hearing organ's electrical potentials—Portmann et al., 1979, or improvement in the blood flow in the inner ear and synchronization of spontaneous impulses in the auditory nerve fibers—Watanabe et al., 1997) but none of them point to central mechanisms.

Thanks to animal models of peripheral tinnitus (induced by acoustic trauma or ototoxic drugs) the mechanisms of direct cochlear stimulation had been explained (Nuttall and Ren, 1995; Ren and Nuttall, 1995). Furthermore, animal models show correlations between a change in central activity as a result of peripheral cochlear modulations. Norena et al. noticed that ES of the cochlea in guinea pigs using a positive current decreased the spontaneous firing rate in the high characteristic frequency neurons of the inferior colliculi, while a negative current had the opposite effect. Such an effect was absent or reverse for low characteristic frequency neurons (Noreña et al., 2015). Mulders et al. obtained reduction of pathological hyperactivity in inferior colliculi after intraperitoneal or cochlear injection of furosemide (Mulders et al., 2014a). What's more, the authors observed similar results after chronic furosemide treatment without hearing impairment (Mulders et al., 2014b). Reducing central hyperactivity authors claim to reduce behavioral signs of tinnitus decreasing spontaneous firing of auditory afferent.

Thanks to electrophysiological studies in tinnitus changes in cortical activity were widely explored (Shulman and Goldstein, 2002; Weisz et al., 2005; Adjamian et al., 2012; Meyer et al., 2014; Schlee et al., 2014). In consequence treatment methods aiming at neuromodulation are now "mainstream therapies." Thus neurophysiological measurements like EEG, MEG serve as an objective outcome measure to quantify therapeutic benefit.

The aim of the study was to determine how/whether a single electrical stimulation of the ear influences cortical activity, and whether changes observed in tinnitus after electrical stimulation are associated with any changes in cortical activity recorded in EEG.

MATERIALS AND METHODS

The study included 12 tinnitus patients (F–6, M-6) divided into two groups. Group I comprised six patients with unilateral tinnitus (six tinnitus ears, five—left, one—right ear), aged 36–73 (average 58.50 years, SD 11.83 years), unilateral, ipsilateral ES was performed. Group II comprised six patients with bilateral tinnitus (12 tinnitus ears), aged 38-78 years (average 53.50 years, SD 18.16 years), bilateral ES was performed starting with the right ear. The groups were balanced in terms of the age, and tinnitus duration.

Before the beginning of the study, ENT examination was performed, together with any necessary audiological and radiological diagnostics. The exclusion criteria included the following: pathology in the external and/or the middle ear, central nervous system disorders (e.g., epilepsy), positive history of neoplasms, implanted pacemaker. Patients with tinnitus in the head, not the ears, were disqualified from the research.

ES was performed using a custom-made apparatus supplied with four 1.5 V batteries. The device has an on / off button, frequency and current intensity buttons. The patient was in a lying position, on the side with the stimulated ear upwards. The

external ear canal was filled with saline solution. The active, silver probe—was immersed inside the external ear canal, avoiding contact with the skin of the canal. The passive electrode was placed on the forehead in the midline (glabella) after skin abrasion with a suitable sterile abrasive electrode paste and clean gauze. The two electrodes were placed in such a way that the current could flow throughout the hypothetical plane (longitudinal axis) of the cochlea. Direct rectangular negative current was applied via the active electrode. In all cases, the stimulating frequency was 250 Hz, the intensity ranged from 0.14 to 1.08 mA, and was applied according to the sensation experience by the patient. The voltage was kept constant at 3 V. The duration of each stimulation was 4 min.

EEG (Deymed QEST 32) was conducted in a room that conforms to EEG standards. Two EEG recordings of 5 min were performed before and after ES (in group I—before and after unilateral ES, in group II—before and after bilateral ES). A Deymed TruScan (10/20 system) electro-cap was fitted. The impedance of the electrodes didn't depend on the head position. The patients were asked to close their eyes and relax. They were asked to refrain from drugs, such as caffeine or alcohol, for 24 h preceding the EEG recording. None of the patients were using drugs influencing CNS activity (e.g., benzodiazepines). The upper and lower limit of a frequency band (Hz) and an averaged wave amplitude (μV) were assessed for alpha, beta and theta oscillations. These parameters were assessed before and after electrical stimulation, furthermore these results were assessed with regard to tinnitus change (assessed in VAS).

Comparisons of the limit frequencies of the bands and averaged amplitudes for alpha, beta and theta were made between reading taken before and after ES. In every recording three samples of 1–3 s were chosen and next marked. The minimal and maximal frequencies and amplitudes were automatically counted by the system. In order to assess potential changes in cortical activity, we compared between the following regions: central frontal (right F4-C4, left F3-C3), temporo –occipital (right T6-O2, left T5-O1), parieto—occipital (right P4-O2, left P3-O1), temporal anterior (right F8-T6, left F7-T5), central (right C4-T4, left T3-C3), posterior (right T4-T6, left T3-T5) and the left and right hemisphere.

In addition, the patients were asked to assess the intensity of tinnitus on the visual analog scale (VAS 1–10) directly before and after electrical stimulation.

The study was approved by the Institutional Review Board of the Medical University of Lodz. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Statistical Analysis

The exact Mann-Whitney rank-sum test for independent data and the exact Wilcoxon matched-pairs signed-ranks test were used to fit data. The exact procedures were chosen due to the small sizes of the samples. Generalized estimating equations (GEE) with robust standard errors also were used when dealing with more than one independent variable, and when inclusive of repeated measures. A level of $p < 0.05$ was considered statistically significant.

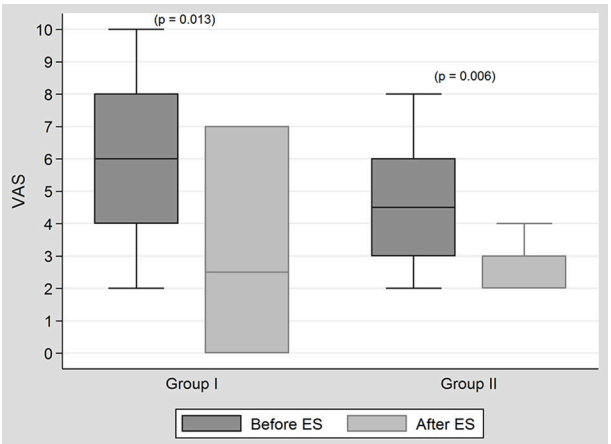

FIGURE 1 | The results of tinnitus treatment with ES in both groups. In both groups, significant changes in tinnitus, given according to VAS, were noted after ES. However, no significant difference was observed between the two groups in terms of tinnitus improvement according to VAS ($p = 0.699$).

RESULTS

Following ES, in Group I, an improvement in VAS was observed in five ears (83.3%)—tinnitus disappeared in two of them (33.3%) and no change was observed in the remaining ear (16.7%)—i.e., no deterioration in tinnitus was observed. In Group II, improvement was observed in seven ears (58.3%) and tinnitus disappeared in two of them (16.7%), while no change was observed in the other five ears (41.7%).

In both groups, significant changes in tinnitus, given according to VAS, were noted. However, no significant difference was observed between the two groups in terms of tinnitus improvement according to VAS ($p = 0.699$) (**Figure 1**).

In Group I, a significant increase in the upper and lower limit of frequency of the alpha band was observed in the left central temporal and left frontal regions following ES (**Figure 2**). These changes, however, were not correlated with any improvement in tinnitus. No significant changes were observed in the beta and theta bands and on the right side (hemisphere). In Group II, no significant changes were observed in the alpha, beta and theta bands. No significant changes were observed in the waves' amplitude (μV) before and after ES, and when right and left sides were compared. The intensity of current (mA) used in ES did not differ significantly ($p = 0.772$) between the groups (in both groups mean intensity was 0,47 mA). No pathological activity was observed in EEG after ES. We didn't observe gamma, nor delta oscillations in our patients.

DISCUSSION

Alpha oscillations are the dominant rhythm at rest in the sensory regions. This activity reflects excitatory—inhibitory balance, with decreased levels of alpha in excitatory conditions and increased in inhibition (Klimesch et al., 2007). Active stimulus processing or stimulus anticipation results in alpha power

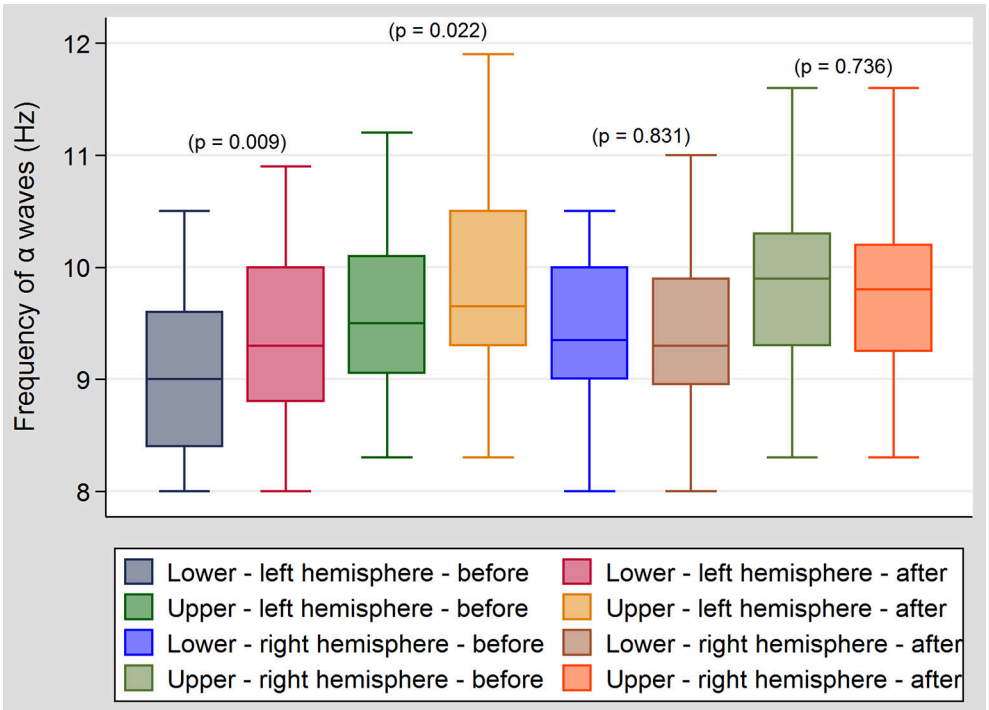

FIGURE 2 | Differences between upper and lower frequency of alpha band in group I before and after ES. In Group I, a significant increase in the upper and lower limit of frequency of the alpha band was observed in the left central temporal and frontal regions following ES. No significant changes were observed in the beta and theta bands and the right side in this group.

decrease which relates to decreased functional inhibition (Weisz et al., 2011). In consequence hyperexcitability within central auditory structures appears resulting from downregulation due to reduced peripheral—cochlear—activity. Increased spontaneous firing rate in neurons of central auditory pathway is hypothesized to be a crucial factor for tinnitus appearance (Noreña and Eggermont, 2003). Given the fact that changes in alpha band reflect shifts between excitatory—inhibitory processes and the view that tinnitus is a result of excitatory—inhibitory imbalance, in some research alpha rhythm and other frequency bands were assessed before and after tinnitus treatment. There are works on a change in alpha frequency, but most of the papers report changes in alpha power (reflecting neuronal synchronization).

In practice, two approaches in stimulations in tinnitus are possible (central vs. peripheral) and apparently both can directly or indirectly modify central activity. One approach - peripheral stimulation, aims to increase peripheral input, which results in spontaneous firing rate decrease and in consequence, in reducing central hyperactivity. The other - primarily aims to modify pathological central hyperactivity. Since tinnitus is known to be associated with excessive spontaneous activity in central auditory system (Mühlnickel et al., 1998; De Ridder et al., 2014), a spectrum of superficial brain stimulation techniques have been proposed to interfere with this maladaptive activity. EEG and MEG therefore would appear to be appropriate tools to quantify this pathological activity before and after implementation of therapeutic method.

Adamchic et al. Schlee et al. described reduced alpha frequency in tinnitus patients (Adamchic et al., 2014; Schlee et al., 2014). In the light of these results, our findings appear to be of advantage in tinnitus treatment. Some other studies, however, didn't repeat these findings (Shulman and Goldstein, 2002; Adjamian et al., 2012; Meyer et al., 2014). In terms of other oscillation bands the results are inconsistent as well. Schlee et al. demonstrated that shorter duration of tinnitus (less than 3 years) was correlated with a larger alpha variability than longer tinnitus duration (more than 3 years). The authors hypothesized that the latter may reflect decreased neuroplastic capacity of brain. Furthermore it was shown that alpha activity was variable and fluctuated with time in healthy subjects, while in tinnitus individuals this ability was significantly reduced. Interestingly, the authors interpreted alpha variability as an indicator for spontaneous tinnitus remission (Schlee et al., 2014). In our research, the changes in alpha frequency may hypothetically be indicative for better neuroplastic potential and thus better future therapeutic outcome. On the other hand, in the light of abovementioned research, the changes in alpha frequency might result just from physiological fluctuations (why they were absent in a group of bilateral tinnitus—remains for the authors unclear).

In our research significant changes in alpha band were observed in the frontal and temporal regions, both on the left side. This is probably due to ipsilateral ES (in most of the cases tinnitus was left sided). It is possible that a better balance between left and right sided tinnitus in the group would balance

lateralization of the results. Weisz et al. (2005) observed marked changes in alpha and delta power in temporal (right) and frontal (left) regions and suggested that these regions might be involved in tinnitus-related cortical activity. Temporal region would then be associated with perceptual aspects of the sound, and frontal—with distress and attention of tinnitus. In this research, like in our research, the most of the patients had left sided tinnitus. The authors concluded that the lateralization of the results might vanish if the study groups were balanced in terms of left and right-sided tinnitus. The justification for this assumption was Jastreboff's neurophysiological model of tinnitus which considered prefrontal cortex a place where sensory and emotional aspects of tinnitus are integrated, but without reference to the lateralization (Jastreboff, 1990).

Since the pathological cortical activity (increase in γ / δ and decrease in alpha band activity) appears to reflect the fact of tinnitus generation and relating distress, some studies demonstrated normalization of these activities in a course of tinnitus treatment (Müller et al., 2013; Adamchic et al., 2014).

Thus three promising neuromodulation methods emerged: transcranial direct (tDCS) and alternating current simulation (tACS), and transcranial magnetic stimulation (TMS) (Zaehle et al., 2010; Song et al., 2012; Vanneste et al., 2013a). Depending on the polarity, tDCS increases or decreases cortical excitability (Miranda et al., 2006). Cathodal tDCS induces hyperpolarization of the cortex, while anodal tDCS intensifies excitation through depolarization of the neurons (Monte-Silva et al., 2013; Joos et al., 2014). tASC increases alpha power (Zaehle et al., 2010; Vossen et al., 2015) and intracotrical inhibition. Random noise stimulation (tRNS) (Van Doren et al., 2014; Joos et al., 2015)—a new transcranial stimulation technique has recently emerged and appeared to be more effective in tinnitus reduction when compared to tDCS and tACS (Vanneste et al., 2013b; Claes et al., 2014).

In the light of inconsistent and non-replicable data on spontaneous EEG activity in tinnitus patients, two papers (Adjamian and Pierzycki et al.) have recently appeared shedding new light on EEG / MEG measurements in tinnitus (Adjamian, 2014; Pierzycki et al., 2016). Adjamian points to a careful analysis and interpretation of EEG / MEG data in tinnitus patients. Neglecting factors unrelated to tinnitus (e.g., the use of non-standardized tools, or protocols) and comorbidities such as hearing loss, hyperacusis, stress or depression might be sources for the flaws in EEG/MEG research results (Adjamian, 2014). In other paper, the author demonstrated that there are no differences due to tinnitus in any frequency band, except possibly delta (however it remains unclear if it was the effect of hearing loss or tinnitus together with hearing loss; Adjamian et al., 2012).

Vanneste et al. demonstrated correlation between psychoacoustic aspect of tinnitus and cortical activity. An increase in tinnitus loudness was associated with an increase in gamma-band activity in the auditory cortex (Vanneste et al., 2013c), which was attributed by the authors to a thalamo-cortical dysrhythmia model (De Ridder et al., 2015). This states that in the deafferented state, the dominant resting state alpha rhythm decreases to theta band activity (Llinás et al., 1999) and that constant abnormal coupled theta/gamma band activity occurs as a consequence of hyperpolarization in thalamus nuclei (De Ridder et al., 2011). In contrast, Pierzycki et al. found no relationship between whole scalp EEG band powers and psychoacoustic or psychosocial variables of tinnitus. The authors concluded that resting state whole scalp EEG should not be used as a biomarker for tinnitus (Pierzycki et al., 2016).

Preliminary results of our research revealed an increase in alpha frequency in the left central temporal and frontal regions. Although, this effect might suggest the treatment has potential, the results indicate that it was not correlated with improvement in tinnitus. Since the literature data on EEG / MEG activity in tinnitus has been inconsistent so far, the relevance of the present findings is not clear and needs further research. In previous research we obtained tinnitus relief after treatment which involved 15 ES. It is possible that the key factor contributing to improvement was repeated stimulation which was needed to evoke and consolidate changes in central auditory system. At this stage, it is unclear whether modulation of cortical activity is primary or secondary to peripheral auditory excitation. At the time of writing, no similar studies had been found in the literature.

In the light of research on peripheral electrical stimulation and central stimulation techniques (current or magnetic stimulation) it appears that both approaches may be efficient in tinnitus treatment.

The limitations of the study are the small size of the groups and the lack of a placebo and control group. In addition, the reason why EEG changes were obtained only in the unilateral tinnitus patients (group I), despite improvement in tinnitus having been observed in both groups, needs further investigation. The exact correlations between changes in cortical activity and tinnitus reduction will be the subject of future research.

CONCLUSIONS

Our results indicate that peripheral external ear ES changes cortical activity in tinnitus patients. One of the possible mechanisms in which ES influences tinnitus may therefore be a change in the cortical activity present within the left central temporal and frontal regions. However, whether this effect is primary or secondary to auditory system excitation remains to be investigated. Of the various forms of ES presented above, each may play a role in the tinnitus improvement observed in our study.

AUTHOR CONTRIBUTIONS

MM, JM, KP, and JO: Substantial contributions to the conception, design of the work, the acquisition, analysis, or interpretation of data for the work, drafting the work, revising it critically for important intellectual content, final approval of the version to be published, agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

Adamchic, I., Toth, T., Hauptmann, C., and Tass, P. A. (2014). Reversing pathologically increased EEG power by acoustic coordinated reset neuromodulation. *Hum. Brain Mapp.* 35, 2099–2118. doi: 10.1002/hbm.22314

Adjamian, P. (2014). The application of electro- and magneto-encephalography in tinnitus research - methods and interpretations. *Front. Neurol.* 5:228. doi: 10.3389/fneur.2014.00228

Adjamian, P., Sereda, M., Zobay, O., Hall, D. A., and Palmer, A. R. (2012). Neuromagnetic indicators of tinnitus and tinnitus masking in patients with and without hearing loss. *JARO* 13, 715–731. doi: 10.1007/s10162-012-0340-5

Baguley, D. M., Axon, P., Winter, I. M., and Moffat, D. A. (2002). The effect of vestibular nerve section upon tinnitus. *Clin. Otolaryngol. Allied Sci.* 27, 219–226. doi: 10.1046/j.1365-2273.2002.00566.x

Claes, L., Stamberger, H., Van de Heyning, P., De Ridder, D., and Vanneste, S. (2014). Auditory cortex tACS and tRNS for tinnitus: single versus multiple sessions. *Neural Plast.* 2014: 436713. doi: 10.1155/2014/436713

De Ridder, D., van der Loo, E., Vanneste, S., Gais, S., Plazier, M., Kovacs, S., et al. (2011). Theta-gamma dysrhythmia and auditory phantom perception. *J. Neurosurg.* 114, 912–921. doi: 10.3171/2010.11.JNS10335

De Ridder, D., Vanneste, S., and Freeman, W. (2014). The Bayesian brain: phantom percepts resolve sensory uncertainty. *Neurosci. Biobehav. Rev.* 44, 4–15. doi: 10.1016/j.neubiorev.2012.04.001

De Ridder, D., Vanneste, S., Langguth, B., and Llinas, R. (2015). Thalamocortical dysrhythmia: a theoretical update in tinnitus. *Front Neurol.* 6:124. doi: 10.3389/fneur.2015.00124

Jastreboff, P. J. (1990). Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci. Res.* 8, 221–254.

Joos, K., De Ridder, D., Van de Heyning, P., and Vanneste, S. (2014). Polarity specific suppression effects of transcranial direct current stimulation for tinnitus. *Neural Plast.* 2014:930860. doi: 10.1155/2014/930860

Joos, K., De Ridder, D., and Vanneste, S. (2015). The differential effect of low- versus high-frequency random noise stimulation in the treatment of tinnitus. *Exp. Brain Res.* 233, 1433–1440. doi: 10.1007/s00221-015-4217-9

Kaltenbach, J. A. (2011). Tinnitus: models and mechanisms. *Hear. Res.* 276, 52–60. doi: 10.1016/j.heares.2010.12.003

Klimesch, W., Sauseng, P., and Hanslmayr, S. (2007). EEG alpha oscillations: the inhibition-timing hypothesis. *Brain Res. Rev.* 53, 63–88. doi: 10.1016/j.brainresrev.2006.06.003

Konopka, W., Zalewski, P., Olszewski, J., Olszewska-Ziaber, A., and Pietkiewicz, P. (2001). Tinnitus suppression by electrical promontory stimulation (EPS) in patients with sensorineural hearing loss. *Auris Nasus Larynx* 28, 35–40. doi: 10.1016/S0385-8146(00)00086-9

Latkowski, B. (1981). Original improvement of the technique of implantation of microelectrodes in cochlear deafness.*Minerva Otolaringol.* 1, 69.

Llinás, R. R., Ribary, U., Jeanmonod, D., Kronberg, E., and Mitra, P. P. (1999). Thalamocortical dysrhythmia: a neurological and neuropsychiatric syndrome characterized by magnetoencephalography. *Proc. Natl. Acad. Sci. U.S.A.* 96, 15222–15227.

Mertens, G., De Bodt, M., and Van de Heyning, P. (2016). Cochlear implantation as a long-term treatment for ipsilateral incapacitating tinnitus in subjects with unilateral hearing loss up to 10 years. *Hear. Res.* 331, 1–6. doi: 10.1016/j.heares.2015.09.016

Meyer, M., Luethi, M. S., Neff, P., Langer, N., and Büchi, S. (2014). Disentangling tinnitus distress and tinnitus presence by means of EEG power analysis. *Neural Plast.* 2014:468546. doi: 10.1155/2014/468546

Middlebrooks, J. C. (2008). Cochlear-Implant high pulse rate and narrow electrode configuration impair transmission of temporal information to the auditory cortex. *J. Neurophysiol.* 100, 92–107. doi: 10.1152/jn.01114.2007

Mielczarek, M., and Olszewski, J. (2014). Direct current stimulation of the ear in tinnitus treatment: a double-blind placebo-controlled study. *Eur. Arch. Otorhinolaryngol.* 271, 1815–1822. doi: 10.1007/s00405-013-2849-6

Miranda, P. C., Lomarev, M., and Hallett, M. (2006). Modeling the current distribution during transcranial direct current stimulation. *Clin. Neurophysiol.* 117, 1623–1629. doi: 10.1016/j.clinph.2006.04.009

Monte-Silva, K., Kuo, M. F., Hessenthaler, S., Fresnoza, S., Liebetanz, D., Paulus, W., et al. (2013). Induction of late LTP-like plasticity in the human motor cortex by repeated non-invasive brain stimulation. *Brain Stimul.* 6, 424–432. doi: 10.1016/j.brs.2012.04.011

Mühlnickel, W., Elbert, T., Taub, E., and Flor, H. (1998). Reorganization of auditory cortex in tinnitus. *Proc. Natl. Acad. Sci. U.S.A.* 95, 10340–10343.

Mulders, W. H., Barry, K. M., and Robertson, D. (2014a). Effects of furosemide on cochlear neural activity, central hyperactivity and behavioural tinnitus after cochlear trauma in guinea pig. *PLoS ONE* 9:e97948. doi: 10.1371/journal.pone.0097948

Mulders, W. H., McMahen, C., and Robertson, D. (2014b). Effects of chronic furosemide on central neural hyperactivity and cochlear thresholds after cochlear trauma in Guinea pig. *Front Neurol.* 5:146. doi: 10.3389/fneur.2014.00146

Müller, N., Lorenz, I., Langguth, B., and Weisz, N. (2013). rTMS induced tinnitus relief is related to an increase in auditory cortical alpha activity. *PLoS ONE* 8:e55557. doi: 10.1371/journal.pone.0055557

Noreña, A. J., and Eggermont, J. J. (2003). Changes in spontaneous neural activity immediately after an acoustic trauma: implications for neural correlates of tinnitus. *Hear. Res.* 183, 137–153. doi: 10.1016/S0378-5955(03)00225-9

Noreña, A. J., Mulders, W. H., and Robertson, D. (2015). Suppression of putative tinnitus-related activity by extra-cochlear electrical stimulation. *J. Neurophysiol.* 113, 132–143. doi: 10.1152/jn.00580.2014

Nuttall, A. L., and Ren, T. (1995). Electromotile hearing: evidence from basilar membrane motion and otoacoustic emissions. *Hear. Res.* 92, 170–177.

Pierzycki, R. H., McNamara, A. J., Hoare, D. J., and Hall, D. A. (2016). Whole scalp resting state EEG of oscillatory brain activity shows no parametric relationship with psychoacoustic and psychosocial assessment of tinnitus: a repeated measures study. *Hear Res.* 331, 101–108. doi: 10.1016/j.heares.2015.11.003

Portmann, M., Cazals, Y., Negrevergne, M., and Aran, J. M. (1979). Temporary tinnitus suppression in man through electrical stimulation of the cochlea. *Acta Otolaryngol.* 87, 294–299

Quaranta, N., Wagstaff, S., and Baguley, D. M. (2004). Tinnitus and cochlear implantation. *Int. J. Audiol.* 43, 245–251. doi: 10.1080/14992020400050033

Ren, T., and Nuttall, A. L. (1995). Extracochlear electrically evoked otoacoustic emissions: a model for *in vivo* assessment of outer hair cell electromotility. *Hear. Res.* 92, 178–183.

Rubinstein, J. T., Tyler, R. S., Johnson, A., and Brown, C. J. (2003). Electrical suppression of tinnitus with high-rate pulse trains. *Otol. Neurotol.* 24, 478–485. doi: 10.1097/00129492-200305000-00021

Schlee, W., Schecklmann, M., Lehner, A., Kreuzer, P. M., Vielsmeier, V., Poeppl, T. B., et al. (2014). Reduced variability of auditory alpha activity in chronic tinnitus. *Neural Plast.* 2014:436146. doi: 10.1155/2014/436146

Shulman, A., and Goldstein, B. (2002). Quantitative electroencephalography: preliminary report–tinnitus. *Int Tinnitus. J.* 8, 77–86.

Song, J.-J., Vanneste, S., Van de Heyning, P., and De Ridder, D. (2012). Transcranial direct current stimulation in tinnitus patients: a systemic review and meta-analysis. *Sci. World J.* 2012:427941. doi: 10.1100/2012/427941

Todt, I., Rademacher, G., Mutze, S., Ramalingam, R., Wolter, S., Mittmann, P., et al. (2015). Relationship between intracochlear electrode position and tinnitus in cochlear implantees. *Acta Otolaryngol.* 135, 781–785. doi: 10.3109/00016489.2015.1024332

Van Doren, J., Langguth, B., and Schecklmann, M. (2014). Electroencephalographic effects of transcranial random noise stimulation in the auditory cortex. *Brain Stimul.* 7, 807–812. doi: 10.1016/j.brs.2014.08.007

Vanneste, S., Fregni, F., and De Ridder, D. (2013b). Head-to-Head comparison of transcranial random noise stimulation, transcranial AC stimulation, and transcranial DC stimulation for tinnitus. *Front Psychiatry.* 4:158. doi: 10.3389/fpsyt.2013.00158

Vanneste, S., van Dongen, M., De Vree, B., Hiseni, S., van der Velden, E., Strydis, C., et al. (2013c). Does enriched acoustic environment in humans abolish chronic tinnitus clinically and electrophysiologically? A double blind placebo controlled study. *Hear Res.* 296, 141–148. doi: 10.1016/j.heares.2012.10.003

Vanneste, S., Walsh, V., Van De Heyning, P., and De Ridder, D. (2013a). Comparing immediate transient tinnitus suppression using tACS and tDCS: a placebo-controlled study. *Exp. Brain Res.* 226, 25–31. doi: 10.1007/s00221-013-3406-7

Vossen, A., Gross, J., and Thut, G. (2015). Alpha Power Increase After Transcranial Alternating Current Stimulation at Alpha Frequency (α-tACS)

Reflects Plastic Changes Rather Than Entrainment. *Brain Stimul.* 8, 499–508. doi: 10.1016/j.brs.2014.12.004

Watanabe, K., Okawara, D., Baba, S., and Yagi, T. (1997). Electrocochleographic analysis of the suppression of tinnitus by electrical promontory stimulation. *Audiology* 36, 147–154.

Weisz, N., Hartmann, T., Müller, N., Lorenz, I., and Obleser, J. (2011). Alpha rhythms in audition: cognitive and clinical perspectives. *Front. Psychol.* 2:73. doi: 10.3389/fpsyg.2011.00073

Weisz, N., Moratti, S., Meinzer, M., Dohrmann, K., and Elbert, T. (2005). Tinnitus perception and distress is related to abnormal spontaneous brain activity as measured by magnetoencephalography. *PLoS Med.* 2:e153. doi: 10.1371/journal.pmed.0020153

Zaehle, T., Rach, S., and Herrmann, C. S. (2010). Transcranial alternating current stimulation enhances individual alpha activity in human EEG. *PLoS ONE* 5:e13766. doi: 10.1371/journal.pone.0013766

Zeng, F. G., Tang, Q., Dimitrijevic, A., Starr, A., Larky, J., and Blevins, N. H. (2011). Tinnitus suppression by low-rate electric stimulation and its electrophysiological mechanisms. *Hear. Res.* 277, 61–66. doi: 10.1016/j.heares.2011.03.010

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Excitation of the Auditory System as a Result of Non-invasive Extra-Cochlear Stimulation in Normal Subjects and Tinnitus Patients

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One of possible approach that may suppress tinnitus is electrical stimulation of the ear. At first invasive techniques were used (promontory or round window stimulation), nowadays a non-invasive method, namely hydrotransmissive electric stimulation (ES) through external acoustic canal, has been developed. The aim of the study is to investigate the effect of applying ES with positive and negative current polarities on the ears of healthy subjects and on the tinnitus ears of patients with tinnitus. This comparison further clarifies the mechanisms of operation of non-invasive extra-cochlear ear ES. A second aim is to assess the effects of ES on tinnitus in tinnitus patients. The material was composed of two groups: tinnitus group—49 patients suffering from tinnitus, and healthy students group—34 healthy individuals. ES was performed with the use of a custom-made apparatus. The active, silver probe–was immersed inside saline filling external ear canal. The passive electrode was placed on the forehead. Positive and next negative DC stimulation was provided with the use following frequencies: 0.25, 1, 2, 3, 4, 5, 6, 7, 8 kHz. We checked for the presence of the auditory percept (AP) and, if AP was present, the minimum current amplitude necessary to produce AP was measured. In our research both positive and negative polarities were efficient to evoke AP in the participants. This effect, however, was more pronounced for positive polarity in no tinnitus and normal hearing individuals (healthy students group). In the tinnitus group, current intensity needed to evoke AP was higher than in the healthy students group. However, comparing normal hearing vs. hearing loss patients within the tinnitus group, we did not observe the relationship between hearing threshold and current intensity evoking AP. Afterwards, we analyzed the effect of multi-frequency ES on tinnitus. It appeared to be effective in 75% of tinnitus ears (with a high score of disappearance–22%). Our study proved that extracochlear ES with positive and negative current was efficient to stimulate the auditory system. Stimulating tinnitus ears with two polarities we obtained a higher ratio of improvement (75%) comparing to positive stimulations.

Keywords: tinnitus, electric stimulation, cathodal stimulation, anodal stimulation, auditory percept, ear, sensorineural hearing loss

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INTRODUCTION

Tinnitus, an auditory percept that is not induced by any acoustic stimulation in the environment, is largely prevalent in the general population and can dramatically impair the quality of life. Tinnitus can be classified as peripheral or central: Peripheral tinnitus is defined as resulting from aberrant neural activity in the cochlear nerve propagating all the way up to the auditory centers, while central tinnitus is defined as resulting from aberrant neural activity generated in the auditory centers, when cochlear spontaneous activity is reduced or absent (in the case of severe hearing loss) (Noreña, 2015). However, according to some models of tinnitus, it is possible that tinnitus may have a mixed origin, i.e., peripheral and central (Noreña, 2011, 2015). It has been proposed that this mixed tinnitus can result from an amplification of residual cochlear nerve activity (Mulders and Robertson, 2009; Noreña, 2011). Various approaches to treatment can also be used. Central tinnitus can be treated by interfering with the tinnitus-related central mechanisms (Noreña and Eggermont, 2005; Pantev et al., 2012; Tass and Popovych, 2012); these approaches assume that tinnitus results from the central changes after hearing loss, and that these changes can be reversed by appropriate stimulation. Alternatively, peripheral or mixed tinnitus is treated by suppressing or reducing the spontaneous activity in the cochlea; more precisely, if tinnitus is caused by hyperactivity in the cochlear nerve, then a clinical approach should reduce this activity. This is the rationale for pharmacological treatment using NMDA antagonists (Puel, 2007; Ruel et al., 2008; van de Heyning et al., 2014).

Another approach that may suppress tinnitus-related cochlear activity is electrical stimulation (ES), first used when House reported total tinnitus suppression after cochlear implantation (House, 1976). Invasive techniques have been found to remove tinnitus in 43–60% (Aran and Cazals - transtympanal stimulations of the promontorium and round window respectively), 45% (Rubinstein–round window stimulation) in 69–77% (Ito and Sakakihara–promontory test and cochlear implant respectively) of cases depending on the study (Cazals et al., 1978; Portmann et al., 1979; Ito and Sakakihara, 1994; Rubinstein et al., 2003). Further non- invasive techniques have been developed, with success rates of 62.2% (Lee et al.—transcutaneous ES of the auricle), 50% (Kuk et al.—eardrum) and 40.4% (Maini and Deoganonkar – stimulation of the mastoid) (Portmann et al., 1979; Chouard et al., 1981; Shulman, 1987; Quaranta et al., 2004; Mielczarek and Olszewski, 2014). Nowadays electrical stimulation is used as a test to predict post-operative profits before cochlear implantation. During non-invasive extratympanic ear stimulation (via a ball-shaped electrode dipped in saline in external ear canal) sound perception is considered an evidence of acoustic nerve excitation, also confirming restored function (Bochenek et al., 1989; Skarzynski et al., 1999; Dehmel et al., 2008).

The first experiments with ES at the Medical University of Lodz date back to the 1980s. At first, invasive transtympanic promontory positive DC stimulations were used with a success rate reaching 42% (Konopka et al., 2001). Later, a non-invasive approach was used: hydrotransmissive stimulations through the external acoustic canal with the use of positive DC. Improvement was obtained directly after treatment in 37.8% of cases; however, the follow up after 1 month found the success ratio had increased to 51.3%. A comparison with a placebo group showed statistically significant differences indicating the value of the method (Mielczarek and Olszewski, 2014).

Although many studies have reported tinnitus improvement or tinnitus suppression after ES (Shulman, 1987; Bochenek et al., 1989; Skarzynski et al., 1999), the exact mechanism of this phenomenon remains unclear. It has been suggested that ES works by increasing the transmission of neurotransmitters in the synapses (Latkowski, 1981), modifying the electrical potentials of the hearing organ (Portmann et al., 1979), or by improving the blood flow in the inner ear and synchronizing the spontaneous impulses in the auditory nerve fibers (Watanabe et al., 1997). Another suggested mechanism of action is by stimulation of the C_2 dorsal root. Dehmel et al note that the C_2 fibers, supplying the skin of the retroauricular region and mucosal lining of the tympanic cavity, target cells in the dorsal column nuclei, which then send axons to the dorsal cochlear nuclei (Dehmel et al., 2008). This mechanism may account for the relief of tinnitus experienced after stimulation of the cochlea surface (Møller, 2016).

Despite the fact that ES have been used in tinnitus treatment since the1970s (studies testing different locations of the stimulating electrode and different parameters of the stimulating current) there are no recommendations on the adjustment of the stimulation conditions in tinnitus treatment. Two early studies on peripheral ES suggest that anodic (positive) ES, with an inhibitory potential should be used to suppress tinnitus; however, cathodic (negative) ES, bearing stimulating properties, should be used to excite the auditory nerve, evoking sound perception (Bochenek et al., 1989; Ren and Nuttall, 1995).

The aim of the study is to investigate the effect of applying positive and negative current polarities on the ear of a healthy subject and on the tinnitus ear of a patient with tinnitus. This comparison will further clarify the mechanisms of operation of non-invasive extra-cochlear ear ES. A second aim was to assess the effects of ES on tinnitus in tinnitus patients.

METHODS

The research was approved by Institutional Review Board of the Medical University of Lodz (RNN/251/05/KB) and was in accordance with the declaration of Helsinki. All patients gave their written, informed consent prior to inclusion in the study.

Study Sample

The research was conducted in the Department of Otolaryngology, Laryngological Oncology, Audiology and Phoniatrics, Medical University of Lodz. The material was composed of two groups (**Table 1**). Group I was a tinnitus group comprising 49 patients suffering from tinnitus ($n = 71$ tinnitus ears: 24 normal hearing ears and 47 sensorineural hearing loss ears). Twenty-eight of the participants were females and 21 were males, with an age range of 22–79 years (average = 53.4, $SD = 15.6$). In the case of unilateral tinnitus, only the tinnitus ear was

TABLE 1 | Patient characteristics.

	Tinnitus group (group I)	Healthy students group (group II)
Number of participants	49 (22 patients with bilateral, 27-unilateral tinnitus)	34 (in 33 students both ears were tested, in 1 person - one ear was tested)
Number of tested ears	71	67
Gender	28 F, 21 M	13 F, 21 M
Age in years (mean ± standard deviation), range	53.4 ± 15.6 range: 22–79 year	23.5 ± 2.9 range: 20–35 year
Visual Analog Scale for tinnitus loudness (range 0–10) Mean ± standard deviation	Before ES: 5.52 ± 1.70 After ES: 3.27 ± 2.37	

tested. The allocation to tinnitus group was randomized, done according to the order of admission to our department. Group II was formed of healthy subjects. This group comprised 34 healthy, normal hearing individuals without tinnitus: 13 female and 21 male, mean age 23.5 years, range 20–35 years ($SD = 2.9$) (n–67 ears). The study was not blinded.

Before the beginning of the study, otorhinolaryngological examination, and hearing tests (pure tone audiometry, speech audiometry, impedance audiometry, auditory brainstem responses) were conducted, as well as radiological diagnostics of head and cervical spine if necessary. Pathology in the external and/or the middle ear was an exclusion criterion, together with the presence of a pacemaker, CNS vascular malformation, epilepsy, or any history of head and neck neoplasm. Patients who reported tinnitus in the head but not in the ears were also excluded from the study. The patients from Group I were asked to assess tinnitus in visual analog scale (VAS) for loudness, directly before and directly after electrical stimulation. The scale ranged from 0 – no tinnitus, 1 – very quiet tinnitus to 10 – extremely loud tinnitus.

Experimental Procedure

ES was performed with the use of a custom-made apparatus supplied with four batteries of 1.5 V. The device allows for direct current stimulation within a frequency range of 0.25–8 kHz and an amplitude range of 0.01–2.24 mA. The external ear canal was filled with saline solution. The active silver probe was immersed inside external ear canal, avoiding contact with the skin of the canal. The passive electrode was placed on the forehead (in the midline) after skin abrasion with a suitable sterile abrasive electrode paste and clean gauze. The two electrodes were placed in such a way as to allow current transmission throughout the hypothetical plane (longitudinal axis) of the cochlea.

DC electrical stimulation was provided. The effects of positive current were first assessed. The series of tests with positive currents were completed then the same tests were carried out with negative currents. ES was performed using the following frequencies: 0.25, 1, 2, 3, 4, 5, 6, 7, and 8 kHz. For each stimulation frequency, the presence of an auditory precept (AP) was confirmed and, if AP was present, the minimum current amplitude necessary to produce AP was measured. ES treatment was started with a maximum well tolerated current intensity. If AP was present, the intensity of current was slowly decreased and the patient was asked to indicate the moment when the sound ceased to be audible. The protocol was performed first for positive (anodal), then for negative (cathodal) current in both groups, for each of the abovementioned frequencies.

The duration of the rectangular pulse depended on the frequency, e.g., for 250 Hz, one period lasted 4 ms (2 ms pulse and 2 ms pause). The voltage was constant and equaled 3 V. The maximum intensity was variable (ranged from 0.15 to 2.24 mA) and was applied according to the sensation of the patient. If the patient reported pain or other unpleasant sensation, the intensity of the current was smoothly decreased (with the intensity knob) to a level producing a tolerable sensation.

Next, the pitch of electrically-evoked AP was assessed in a sound proof chamber. A subgroup of 20 patients from the tinnitus group were selected. These perceptions were matched with free field sounds as pure tones from the audiometer. A pair of pure tones was delivered in a free field through the loudspeakers (Martin Audio C115), and the patient was asked to indicate the one which more closely resembled the sound perceived during ES. One tone was similar to the stimulating frequency and the other was next lower tone on the audiometer (Madsen Electronic Orbiter 922). If neither sound was similar, the next pair of tones was given. Matching was performed simultaneously with ES.

Statistical Testing

The numerical data was tested for normality of distribution using the Shapiro-Wilk W-test. Levene's test was performed in order to assess the homogeneity of variances in both compared groups. A p-level of 0.05 was assumed for all tests of significance. All the statistical procedures were conducted as two-tailed ones. Due to the small sample sizes and lack of normality, appropriate non-parametric tests were performed, and also robust standard errors or standard errors allowing for intragroup correlation were estimated when applicable.

Generalized estimating equations with clustered standard errors (i.e., allowing for intragroup correlation) were carried out for a numerical dependent variable (hearing threshold), a set of independent variables comprising current threshold values measured at selected frequencies, and subject hearing (hearing loss vs. normal hearing). All p-values obtained during the analysis approximated to a value of one.

RESULTS

Overall, 1,278 stimulations were conducted in the tinnitus group (71 ears × nine frequencies × two polarities), and 1,206 in

TABLE 2 | Logistic regression estimates for predictors of auditory perception (AP).

Investigated trait	OR (95% CI)	Level of statistical significance (*p*-value)
Type of tinnitus (tone vs. noise vs. tone + noise)	0.42 (0.15–1.13)	= 0.085
Age (1-year step)	0.97 (0.92–1.02)	= 0.172
Hearing level	0.65 (0.09–4.63)	= 0.664

the healthy student group (67 ears × nine frequencies × two polarities).

The Presence of AP During ES

In both groups, in the majority of cases, AP was produced during ES. Overall, it was present in 60 ears in the tinnitus group (84.5%) and in 66 ears in the healthy students group (98.5%). In the tinnitus group, from all the 1,278 stimulations, AP was evoked in 182 (14.24%) stimulations while no sound perception was evoked in the other 1,096 (85.76%). In the healthy students group, out of 1,206 stimulations, AP was evoked in 798 (66.2%) stimulations, while no AP was evoked in the other 408 (33.8%). The analysis showed that sound perception was less likely during ES in the tinnitus group than the healthy students group ($p < 0.001$). During ES temporary pain was reported by persons from both groups: in tinnitus group—by 25 patients (51%), and in healthy subjects group—by 14 individuals (41%). In all cases it was exclusively momentary and it disappeared as soon as current intensity was smoothly decreased, so the procedure was continued.

Furthermore, in the tinnitus group we tested whether the presence of the AP depended on the type of tinnitus (tone vs. noise vs. tone + noise), age or hearing level. Binary logistic regression with robust standard error was performed (**Table 2**). No relationship was confirmed (OR = 0.42; 95% CI: 0.15–1.13; $p = 0.085$). AP was present in 91.1% of ears with tone-like tinnitus, in 70.0% in noise-like tinnitus and 80.0% of ears when tinnitus was a mixture of tone and noise. Within the tinnitus group, the presence of AP did not depend on age (OR = 0.97; 95% CI: 0.92–1.02; $p = 0.172$), nor hearing level (OR = 0.65; 95% CI: 0.09–4.63; $p = 0.664$). It was present in 71.4% of normal hearing ears, and in 71.6% of hearing loss ears. The mean age of patients with AP was 51.5 years ($SD = 15.7$), and the mean age of those without was 60.5 years ($SD = 13.5$). The averaged pure tone audiograms in the tinnitus group (normal hearing and hearing loss subjects) and in the healthy students group are presented in **Figures 1**–**3**.

Analysis of Current Intensities Needed to Evoke AP

In the tinnitus group, the intensities of current evoking AP ranged from 0.16 to 1.67 mA (mean–0.614 mA), in the healthy students group—from 0.01 to 1.61 mA (mean–0.461 mA). The analysis showed that in the tinnitus group, ES needed higher intensities of current to evoke sound perception ($p < 0.003$). Moreover, in the tinnitus group we analyzed intensities of current evoking sound perception vs. averaged hearing thresholds at 0.25, 1, 2 kHz, respectively. We did not perform this analysis for higher stimulating frequencies since AP was practically absent for 4 kHz (present in five ears) and 8 kHz (present in three ears). There was no correlation between hearing threshold and current intensity needed to evoke AP during electrical stimulation.

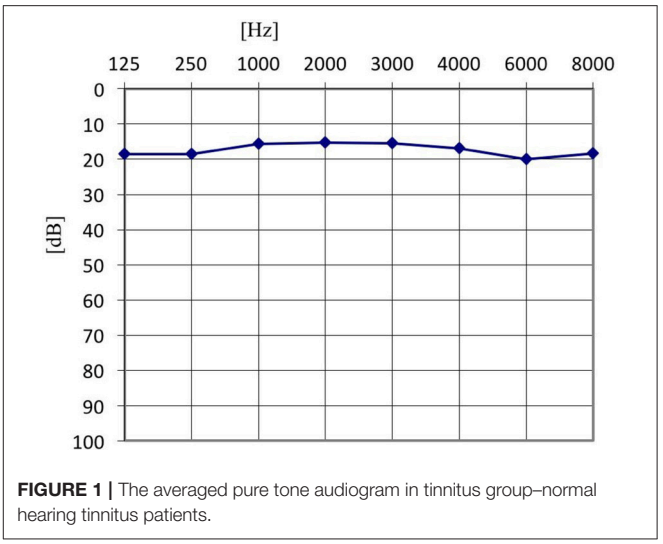

FIGURE 1 | The averaged pure tone audiogram in tinnitus group–normal hearing tinnitus patients.

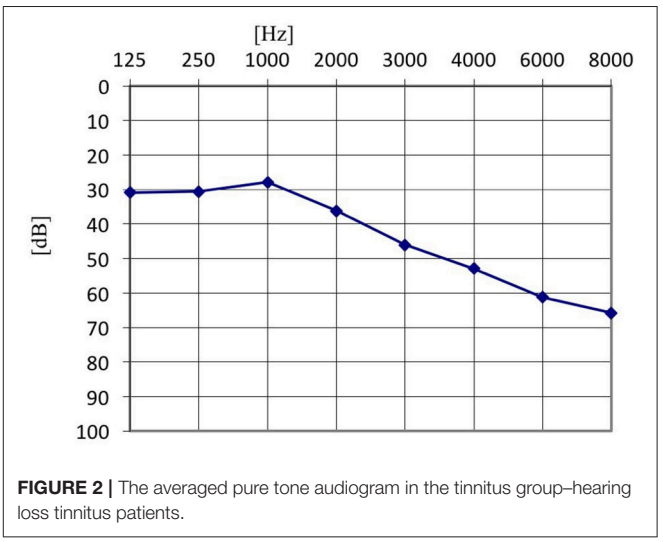

FIGURE 2 | The averaged pure tone audiogram in the tinnitus group–hearing loss tinnitus patients.

Analysis of Current Frequencies Needed to Evoke AP

In the tinnitus group, in the majority of cases (83.1%), AP was present for stimulating frequencies between 0.25 and 2 kHz (**Figure 4**).

In the healthy students group, AP was present for each stimulating frequency—0.25–8 kHz (**Figure 5**).

Correlation Between Frequency and Intensity of Current Needed to Evoke AP

Evaluation of the correlation between stimulating frequency and intensity of current indicated that higher stimulating frequencies

needed higher current intensities to evoke AP in the tinnitus group ($p < 0.001$) as well as the healthy students group ($p < 0.001$) (**Figure 6**).

Current Polarity vs. AP vs. Ear

Both current polarities (positive and negative) evoked AP during ES. In the majority of ears AP was evoked by both positive and negative current: in 57.7% of tinnitus ears in the tinnitus group and in 97% of ears in the healthy students group (**Figure 7**).

The binary logistic regression with robust standard error was performed. Furthermore, in tinnitus group, with respect to AP, positive polarity appeared to be significantly more efficient than negative. AP was present in nearly 82% of tinnitus ears when positive current was used, and in 61% during negative stimulations ($p < 0.001$). In healthy students group, both polarities were equally effective, evoking AP in 98.5% of ears ($p > 0.9$).

In the tinnitus group, ES needed higher intensities of current to evoke AP for positive polarity and for the left ear compared with negative polarity and the right ear (Pos. left vs. Pos. right: $t = 2.6041$, $p < 0.009$; Pos. left vs. Neg. left: $t = 2.511$, $p = 0.012$). All other comparisons (T-tests) were not significant ($p > 0.05$). In the healthy students group, positive polarity needed higher intensities to evoke AP ($p = 0.001$), without significant differences between left and right ear in this group ($p = 0.92$).

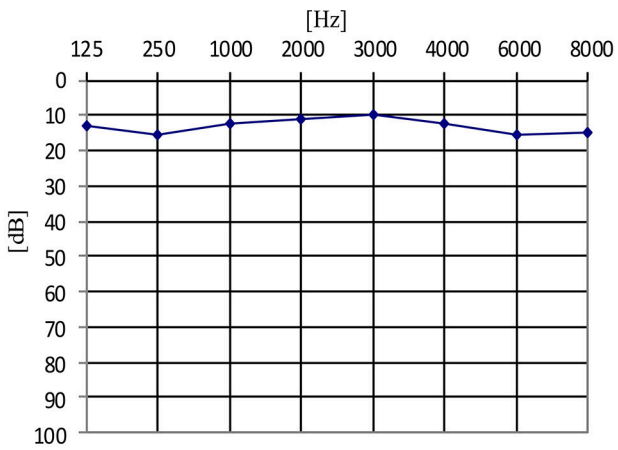

FIGURE 3 | The averaged pure tone audiogram in the healthy students group.

Matching Electrically Evoked AP With Free Field Sounds in Tinnitus Group

The matching was done in tinnitus group in 20 patients. A total of 448 matchings were performed. Electrically-evoked AP were matched with free field sounds (pure tones from the audiometer). The pitch of electrically-evoked AP changed with a change in stimulating current frequency. Sound perceptions evoked by low stimulating frequencies (0.25–2 kHz) had more exact matching to free field sounds. 1 kHz appeared to have the most adequate matchings. 3 and 4 kHz were mainly identified as lower sounds like 2 and 3 kHz, respectively. 6 and 8 kHz were identified as low (1 kHz) sounds (**Figure 8**).

Analysis of Tinnitus After ES in Tinnitus Group

Generalized estimating equations with robust standard errors were carried out for a categorical dependent variable (an

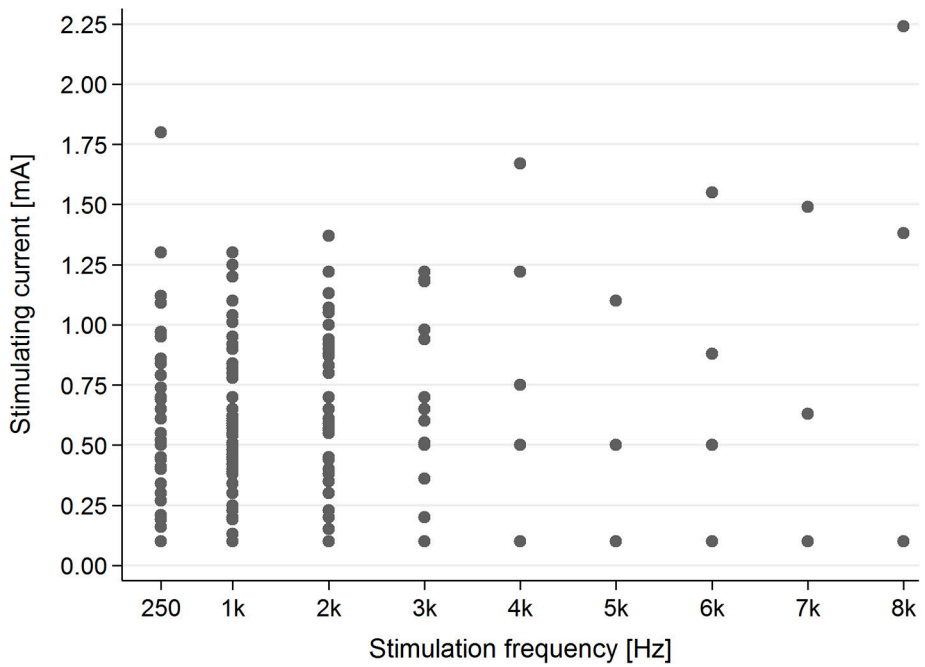

FIGURE 4 | The presence of the AP in the tinnitus group for particular stimulating current parameters (mA/kHz).

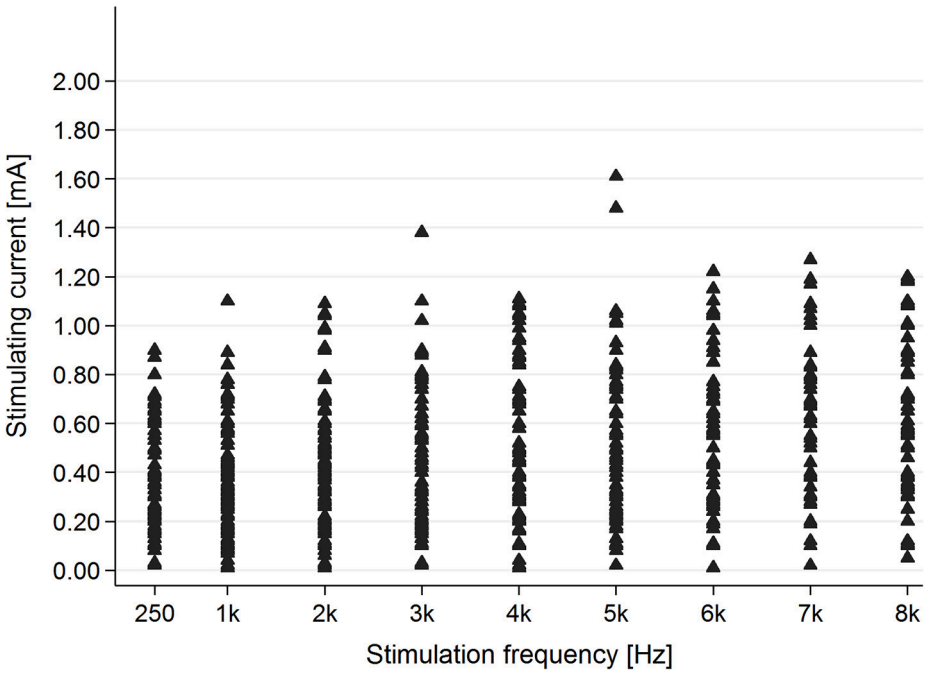

FIGURE 5 | The presence of the AP in the healthy students group for particular stimulating current parameters (mA/kHz).

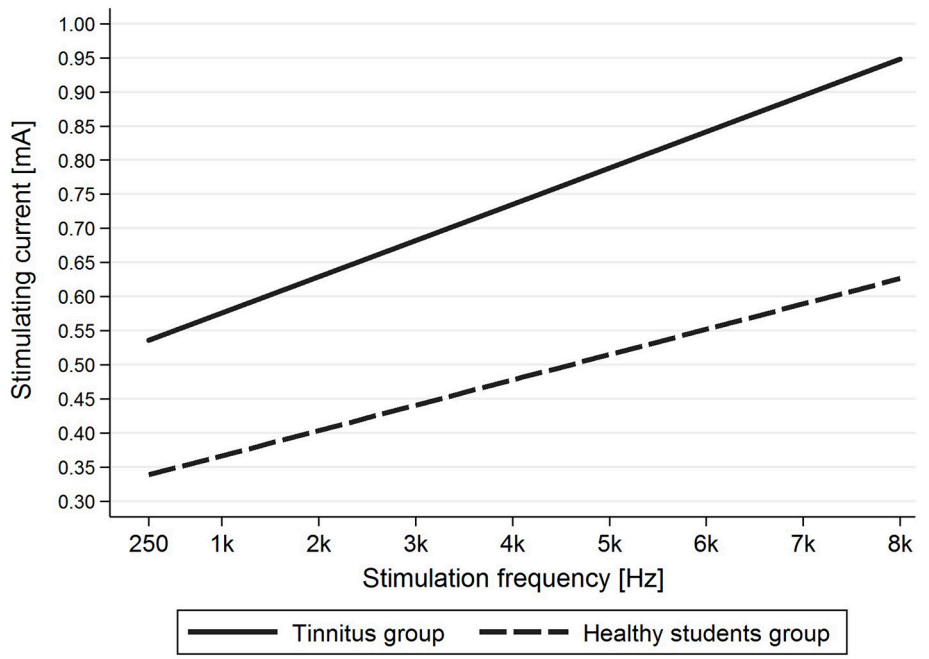

FIGURE 6 | The dependence of stimulating current intensity on stimulating frequency.

improvement in tinnitus) and a set of independent variables comprising sound perception and the subject status before and after stimulation.

Before and directly after ES, we asked 28 patients from the tinnitus group (46 tinnitus ears) to describe the loudness of tinnitus in VAS (visual analog scale for tinnitus loudness). The

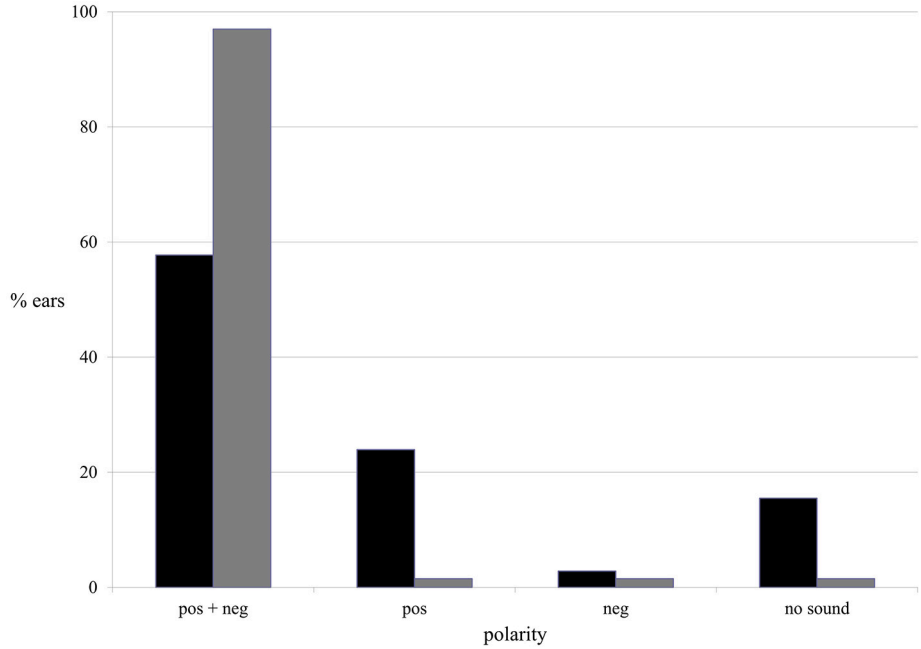

FIGURE 7 | The presence of the AP in terms of current polarity in the tinnitus group (black column) and the healthy students group (gray column).

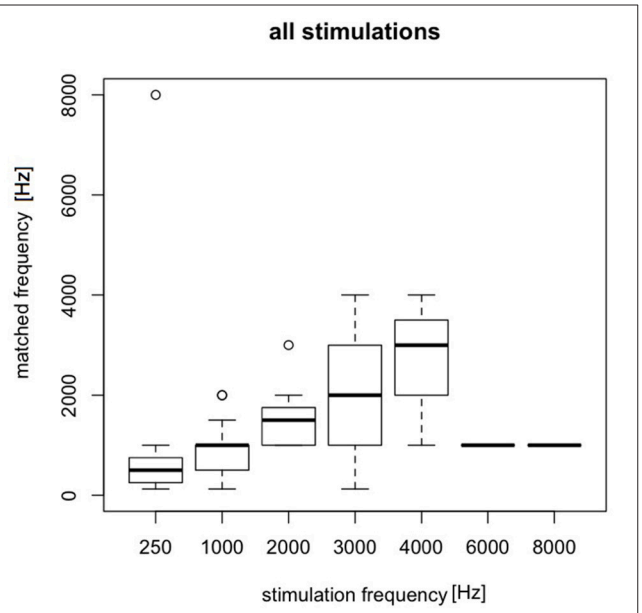

FIGURE 8 | Matching electrically evoked pitch of AP with free field sounds in the tinnitus group.

mean tinnitus loudness before stimulation was 5.52 (*SD* 1.70), and after 3.27 (*SD* 2.37; $p < 0.001$) **Figure 9**.

Directly after ES, there was improvement in 21 ears (75%), no change in five ears (18%), and worsening in two ears (7%). In 10 out of 46 ears (22%), tinnitus disappeared. Interestingly, there was no correlation between the improvement in tinnitus and the presence of electrically evoked AP ($p > 0.5$).

DISCUSSION

In our research both positive and negative polarities excited the auditory system evoking an AP in the study participants. This effect, however, was more pronounced for positive polarity in no tinnitus and normal hearing individuals (healthy students group). In the tinnitus group the AP was present for a much narrower range of stimulating frequencies (0.25–2 kHz) when compared to the no tinnitus and normal hearing healthy students group (0.25–8 kHz).

In the tinnitus group, the current intensity needed to evoke an AP was higher than in the healthy students group, which could suggest the effect of hearing threshold. However, comparing normal hearing vs. hearing loss patients within tinnitus group, again, we did not observe any relationship between hearing threshold and current intensity evoking AP. Furthermore, we matched electrically evoked AP pitch with free field sounds in tinnitus group. We saw that the pitch of AP changed with a change in stimulating frequency. Afterwards, we analyzed the effect of multi-frequency ES on tinnitus. It appeared to be effective in 75% of tinnitus ears (with a high score of disappearance – 22%). The interesting fact was that the improvement in tinnitus was not correlated with the presence of AP during the ES.

Animal studies showed that electrical current influences the micromechanics of the Corti organ. Nuttall and Ren stimulating the round window membrane and cochlear duct in guinea pigs reported movements in the basilar membrane and emission of sound from the cochlea. This phenomenon was possible only when some OHCs were intact so their movements displaced the basilar membrane. The authors indicated that any intra- or

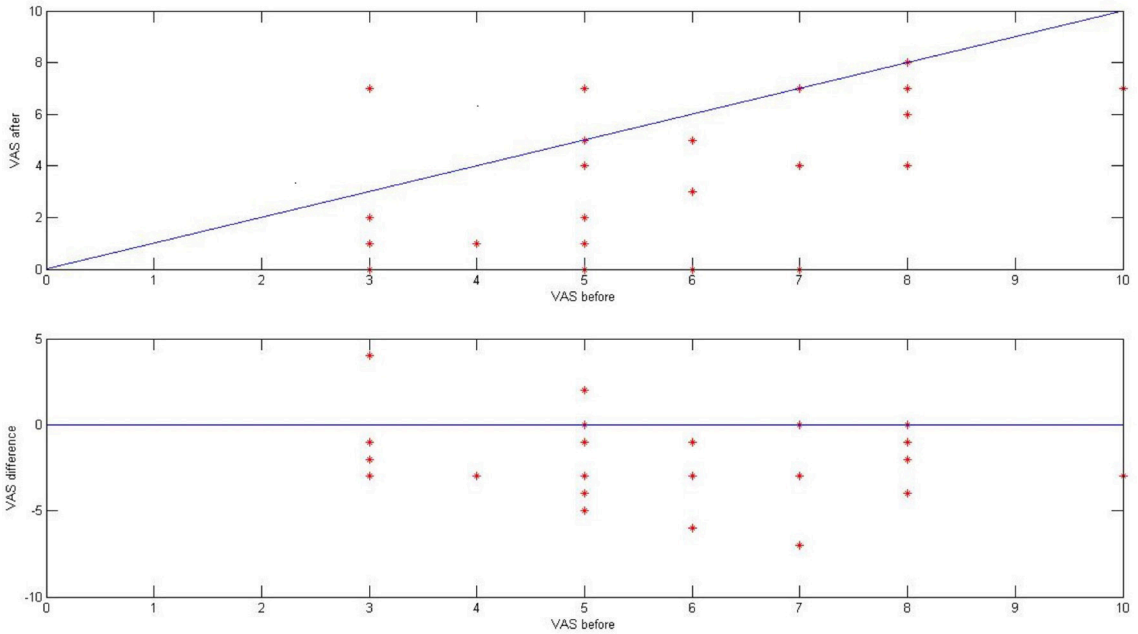

FIGURE 9 | The effect of ES on tinnitus in the tinnitus group presented as a result in VAS for tinnitus loudness (before and after stimulation).

extra-cochlear electric current stimulation affects the polarization of OHCs and could induce a traveling wave in the basilar membrane (Nuttall and Ren, 1995; Ren and Nuttall, 1995).

In our research, this mechanism of ES is also possible. Applying electric current to external ear canal we reach the cochlea (the conduction of the stimulus through the lining of the tympanic cavity and the round window) but since soft tissues have conductive properties, this might not be a single way in which electric impulse affects auditory system. Earlier research on ear ES demonstrated cortical potential alternations after ear ES, so we may alike influence central auditory system directly in the same time (Mielczarek et al., 2016). Furthermore, since the pitch of electrically evoked AP changed with a change of stimulating frequency, it is possible that we stimulate the cochlea (and auditory nerve) selectively, producing a normal traveling wave or directly changing (selectively) the OHC potential. However, the selectivity of the excitation is probably worst comparing to excitation of Corti organ with sound.

By applying negative pulses to the impaired cochlea, Portman et al. elicited auditory sensation, which was regarded as proof of auditory nerve excitation. This response, however, was not based on the stimulating frequency, as auditory sensation remained unchanged when the frequency was altered (Portmann et al., 1979).

Secondly, since in our research the AP was more pronounced in the group of healthy students (no tinnitus normal hearing young individuals) and since there were no differences between normal hearing and hearing loss patients in this regard within the tinnitus group, the authors assume that OHC function is not a determining factor for the AP phenomenon. We hypothesize that the condition of the synapsis and auditory neurons are responsible for these variations (Kujawa and Liberman, 2015; Viana et al., 2015).

Many studies describe the dependence of the ES effect on current polarity. Cazals et al. point to the direction of current flow applied to cochlea suggesting that positive polarity should be used to reduce tinnitus, and a negative polarity to evoke sound perception (Cazals et al., 1978). These findings have been confirmed elsewhere (Sun et al., 2000; Noreña et al., 2015), but the results seem to be inconsistent (Guo, 2001). Furthermore Cazals et al. claimed that when stimulating with negative current, the AP was present in a wider range of frequencies and lower intensities were required to produce auditory sensation. They placed the electrodes on the promontory or round window, and a reference one on the earlobe (Cazals et al., 1978). The results of our study showed that the direction of current flow (the polarity) is not a deciding factor whether ES evokes AP or not. In the healthy students group, AP appeared to be independent from the current polarity; however, in the tinnitus group, negative stimulations were less likely to evoke AP when compared to positive current. Negative stimulations needed lower intensities to evoke sound perception, which was coherent with Cazals et al.

AP evoked during stimulation of the deaf ears seems to be a confirmation that this perception is generated above the cochlea. Chouard et al. stimulating deaf ears (round window) registered auditory brainstem potentials and noticed sound sensation (AP) in almost all deaf ears (Chouard et al., 1979, 1994). These results may be considered proof for the concept that AP depends on auditory nerve function, not on the cochlea, although the cochlea acts as the first effector of the electric stimulus. Some authors evoke AP during ES on deaf ears (before cochlear implantation) to confirm restored function of the acoustic nerve (Gersuni and

Volokhov, 1936; House and Brackmann, 1974; Bochenek et al., 1977; Portmann et al., 1979; Skarzynski et al., 1999). The use of promontory ES in patients labyrinthectomized due to Meniere's disease, resulted in sound percept despite complete loss of inner ear function (Lambert et al., 1990).

Rattay et al. suggest a correlation between polarity sensitivity and the state of degeneration and demyelination of peripheral neurons. According to them degenerated peripheral neurons would require lower anodic thresholds (Rattay et al., 2001). Our results do not confirm any such effect. In our research, in the tinnitus group, the intensity of current needed to evoke AP was higher than in healthy students group—suggesting the effect of neural degeneration due to natural processes in the tinnitus group, and the levels of current were higher for positive stimulation. Interestingly, in the tinnitus group, AP did not depend on hearing threshold. Since the two subgroups (tinnitus patients with normal hearing and tinnitus patients with hearing loss) were similar in terms of age, we can assume that the condition of the auditory nerve was similar, even if the hearing status differed. Viana et al. demonstrated in five "normal" ears, features of cochlear synaptopathy and the degeneration of cochlear nerve peripheral axons, in the presence of a near-normal hair cell population; they suggest that such changes account for human presbyacusis (Viana et al., 2015). Other animal studies showed that in age-related hearing loss, degeneration of cochlear synapses precedes both hair cell loss and hearing threshold elevation (Kujawa and Liberman, 2015).

In early research by Jellinek and Schreiber (1930) it was reported that ES of the ear with alternating current causes AP, which induces a sound perception at the stimulating frequency. The phenomenon was at first explained by vibratory or mechanical forces arising at the electrode, changes in the fluid volume (filling external ear canal) and mechanical excitation of the tympanum or ossicles. Afterwards Arapova et al. (1937) suggested another two possible mechanisms: the first was that AC evokes mechanical forces in the cochlea, thus stimulating the receptors in the usual way, while the second was that AC directly affects the receptor, omitting the stage of transformation from electrical to mechanical forces. For Gersuni and Volokhov, the proof of cochlea excitation was their production of beats by simultaneous electrical and sound stimulation at approximately similar frequencies (Gersuni and Volokhov, 1936).

Lusted and Simmons give two possible explanations for electrically-evoked AP: the OHC stimulation or afferent auditory fiber excitation (Lusted et al., 1984). Stevens et al. accounts for the "electrophonic effect" by direct stimulation of the auditory nerve, and by electromechanical transduction in the inner ear or tympanum (Stevens and Jones, 1939). Kellaway argues in favor of cochlear structure excitation: sound sensation was always a pure tone, never noise, the change in the polarity of current did not change the character of the sound effect, the absence of tympanic membrane did not influence the perceived sound (Kellaway, 1946). In our research, the AP was a pure tone sound, never noise, suggesting the involvement of sensory elements rather than mechanical. Furthermore, it did not depend on current polarity, since it was present for both.

Many papers have confirmed the value of ES of the ear in tinnitus treatment (Cazals et al., 1978; Portmann et al., 1979; Rubinstein et al., 2003; Arts et al., 2015). Our study proved that extracochlear ES with positive and negative current was efficient to stimulate the auditory system. The auditory nerve appeared to be the most probable place of AP generation. Stimulating tinnitus ears with two polarities, we obtained a higher ratio of improvement (75%) comparing to positive stimulation alone (Mielczarek and Olszewski, 2014). However, since the presence of AP was not correlated with the improvement in tinnitus, some other mechanisms may account for this effect.

AUTHOR CONTRIBUTIONS

MM, AN, WS, and JO: Substantial contribution to the design of the study and the draft of the manuscript; MM and JO: Data acquisition; WS and MM: Performed statistical testing; MM, WS, and AN: Created figures. All authors revised and approved the manuscript.

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REFERENCES

Arapova, A. A., Gersuni, G. V., and Volokhov, A. A. (1937). A further analysis of the action of alternating currents on the auditory apparatus. *J. Physiol.* 89, 122–131. doi: 10.1113/jphysiol.1937.sp003468

Arts, R. A., George, E. L., Chenault, M. N., and Stokroos, R. J. (2015). Optimizing intracochlear electrical stimulation to suppress tinnitus. *Ear Hear.* 36, 125–135. doi: 10.1097/AUD.0000000000000090

Bochenek, W., Chorzempa, A., Hazell, J. W., Kiciak, J., and Kukwa, A. (1989). Non-invasive electrical stimulation of the ear canal as a communication aid in acquired total deafness. *Br. J. Audiol.* 23, 285–291. doi: 10.3109/03005368909076516

Bochenek, Z., Bochenek, W., and Bieniek, J. (1977). Electric stimulation of the human auditory system with transtympanic electrodes. *Otolaryngol. Pol.* 31, 225–228.

Cazals, Y., Negrevergne, M., and Aran, J. M. (1978). Electrical stimulation of the cochlea in man: hearing induction and tinnitus suppression. *J. Am. Aud. Soc.* 3, 209–213.

Chouard, C. H., Koca, E., Meyer, B., and Jacquier, I. (1994). Test of electrical stimulation of the round window. Diagnostic and prognostic value of the rehabilitation of total deafness by cochlear implant. *Ann. Otolaryngol. Chir. Cervicofac.* 111, 75–84.

Chouard, C. H., Meyer, B., and Donadieu, F. (1979). Auditory brainstem potentials in man evoked by electrical stimulation of the round window. *Acta Otolaryngol.* 87, 287–293. doi: 10.3109/00016487909126422

Chouard, C. H., Meyer, B., and Maridat, D. (1981). Transcutaneous electrotherapy for severe tinnitus. *Acta Otolaryngol.* 91, 415–422. doi: 10.3109/00016488109138522

Dehmel, S., Cui, Y. L., and Shore, S. E. (2008). Cross-modal interactions of auditory and somatic inputs in the brainstem and midbrain and

their imbalance in tinnitus and deafness. *Am. J. Audiol.* 17, S193–S209. doi: 10.1044/1059-0889(2008/07-0045)

Gersuni, G. V., and Volokhov, A. A. (1936). On the electrical excitability of the auditory organ: on the effect of alternating current on the normal auditory apparatus. *J. Exp. Phychol.* 16, 370–382. doi: 10.1037/h0060771

Guo, M. (2001). Effects of direct current on vibration of cochlear basilar membrane. *Zhonghua Er Bi Yan Hou Ke Za Zhi* 36, 338–341.

House, W. F. (1976). Cochlear implants. *Ann. Otol. Rhinol. Laryngol.* (Suppl. 27), 50–56. doi: 10.1177/00034894760850S303

House, W. F., and Brackmann, D. E. (1974). Electrical promontory testing in differential diagnosis of sensori-neural hearing impairment. *Laryngoscope* 84, 2163–2171. doi: 10.1288/00005537-197412000-00007

Ito, J., and Sakakihara, J. (1994). Tinnitus suppression by electrical stimulation of the cochlear wall and by cochlear implantation. *Laryngoscope* 104(6 Pt 1), 752–754. doi: 10.1288/00005537-199406000-00017

Jellinek, S., and Schreiber, T. (1930). Eine neue methode des Horens. *Wien klin. Wsche.* 43:417.

Kellaway, P. (1946). The mechanism of the electrophonic effect. *J. Neurophysiol.* 9, 23–31. doi: 10.1152/jn.1946.9.1.23

Konopka, W., Zalewski, P., Olszewski, J., Olszewska-Ziaber, A., and Pietkiewicz, P. (2001). Tinnitus suppresion by electrical promontory stimulation (EPS) in patients with sensorineural hearing loss. *Auris Nasus Larynx* 28, 35–40. doi: 10.1016/S0385-8146(00)00086-9

Kujawa, S. G., and Liberman, M. C. (2015). Synaptopathy in the noise-exposed and aging cochlea: primary neural degeneration in acquired sensorineural hearing loss. *Hear. Res.* 330(Pt B), 191–199. doi: 10.1016/j.heares.2015.02.009

Lambert, P. R., Ruth, R. A., and Halpin, C. F. (1990). Promontory electrical stimulation in labyrinthectomized ears. *Arch. Otolaryngol. Head Neck Surg.* 116, 197–201. doi: 10.1001/archotol.1990.01870020073019

Latkowski, B. (1981). Original improvement of the technique of implantation of microelectrodes in cochlear deafness. *Minerva Otolaringol* 1:69

Lusted, H. S., Shelton, C., and Simmons, F. B. (1984). Comparison of electrode sites in electrical stimulation of the cochlea. *Laryngoscope* 94, 878–882. doi: 10.1288/00005537-198407000-00003

Mielczarek, M., Michalska, J., Polatyńska, K., and Olszewski, J. (2016). An increase in alpha band frequency in resting state EEG after electrical stimulation of the ear in tinnitus patients-a pilot study. *Front. Neurosci.* 10:453. doi: 10.3389/fnins.2016.00453

Mielczarek, M., and Olszewski, J. (2014). Direct current stimulation of the ear in tinnitus treatment: a double-blind placebo-controlled study. *Eur. Arch. Otorhinolaryngol.* 271, 1815–1822. doi: 10.1007/s00405-013-2849-6

Møller, A. R. (2016). Sensorineural tinnitus: its pathology and probable therapies. *Int. J. Otolaryngol.* 2016:2830157. doi: 10.1155/2016/2830157

van de Heyning, P., Muehlmeier, G., Cox, T., Lisowska, G., Maier, H., Morawski, K., and Meyer, T. (2014). Efficacy and safety of AM-101 in the treatment of acute inner ear tinnitus–a double-blind, randomized, placebo-controlled phase II study. *Otol. Neurotol.* 35, 589–597. doi: 10.1097/MAO.0000000000000268

Mulders, W. H., and Robertson, D. (2009). Hyperactivity in the auditory midbrain after acoustic trauma: dependence on cochlear activity. *Neuroscience* 164, 733–746. doi: 10.1016/j.neuroscience.2009.08.036

Noreña, A. J. (2011). An integrative model of tinnitus based on a central gain controlling neural sensitivity. *Neurosci. Biobehav. Rev.* 35, 1089–1109. doi: 10.1016/j.neubiorev.2010.11.003

Noreña, A. J. (2015). Revisiting the cochlear and central mechanisms of tinnitus and therapeutic approaches. *Audiol. Neurootol.* 20(Suppl. 1), 53–59. doi: 10.1159/000380749

Noreña, A. J., and Eggermont, J. J. (2005). Enriched acoustic environment after noise trauma reduces hearing loss and prevents cortical map reorganization. *J. Neurosci.* 25, 699–705. doi: 10.1523/JNEUROSCI.2226-04.2005

Noreña, A. J., Mulders, W. H., and Robertson, D. (2015). Suppression of putative tinnitus-related activity by extra-cochlear electrical stimulation. *J. Neurophysiol.* 113, 132–143. doi: 10.1152/jn.00580.2014

Nuttall, A. L., and Ren, T. (1995). Electromotile hearing: evidence from basilar membrane motion and otoacoustic emissions. *Hear. Res.* 92, 170–177. doi: 10.1016/0378-5955(95)00216-2

Pantev, C., Okamoto, H., and Teismann, H. (2012). Tinnitus: the dark side of the auditory cortex plasticity. *Ann. N.Y. Acad. Sci.* 1252, 253–258. doi: 10.1111/j.1749-6632.2012.06452.x

Portmann, M., Cazals, Y., Negrevergne, M., and Aran, J. M. (1979). Temporary tinnitus suppression in man through electrical stimulation of the cochlea. *Acta Otolaryngol.* 87, 294–299. doi: 10.3109/00016487909126423

Puel, J. L. (2007). Cochlear NMDA receptor blockade prevents salicylate-induced tinnitus. *B-ENT* 3 (Suppl. 7), 19–22.

Quaranta, N., Wagstaff, S., and Baguley, D. M. (2004). Tinnitus and cochlear implantation. *Int. J. Audiol.* 43, 245–251. doi: 10.1080/14992020400050033

Rattay, F., Lutter, P., and Felix, H. (2001). A model of the electrically excited human cochlear neuron. I. Contribution of neural substructures to the generation and propagation of spikes. *Hear Res.* 153, 43–63. doi: 10.1016/S0378-5955(00)00256-2

Ren, T., and Nuttall, A. L. (1995). Extracochlear electrically evoked otoacoustic emissions: a model for *in vivo* assessment of outer hair cell electromotility. *Hear. Res.* 92, 178–183. doi: 10.1016/0378-5955(95)00217-0

Rubinstein, J. T., Tyler, R. S., Johnson, A., and Brown, C. J. (2003). Electrical suppression of tinnitus with high-rate pulse trains. *Otol. Neurotol.* 24, 478–485. doi: 10.1097/00129492-200305000-00021

Ruel, J., Chabbert, C., Nouvian, R., Bendris, R., Eybalin, M., Leger, C. L., et al. (2008). Salicylate enables cochlear arachidonic-acid-sensitive NMDA receptor responses. *J. Neurosci.* 28, 7313–7323. doi: 10.1523/JNEUROSCI.5335-07.2008

Shulman, A. (1987). External electrical tinnitus suppression: a review. *Am. J. Otol.* 8, 479–484.

Skarzynski, H., Czyzewski, A., and Kostek, B. (1999). "Prediction of post-operative profits in cochlear implanted patients using the electrostimulation procedure," in *Proceedings of the 1999 Workshop on Applications of Signal to Audio and Acoustics* (New Palts, NY), 17–20.

Stevens, S. S., and Jones, R. C. (1939). The mechanism of hearing by electrical stimulation. *J. Acoust. Soc. Amer* 10, 261–269. doi: 10.1121/1.1915984

Sun, W., Ding, D., Reyes, S., and Salvi, R. J. (2000). Effects of AC and DC stimulation on chinchilla SOAE amplitude and frequency. *Hear. Res.* 150, 137–148. doi: 10.1016/S0378-5955(00)00195-7

Tass, P. A., and Popovych, O. V. (2012). Unlearning tinnitus-related cerebral synchrony with acoustic coordinated reset stimulation: theoretical concept and modelling. *Biol. Cybern.* 106, 27–36. doi: 10.1007/s00422-012-0479-5

Viana, L. M., O'Malley, J. T., Burgess, B. J., Jones, D. D., Oliveira, C. A., Santos, F., et al. (2015). Cochlear neuropathy in human presbycusis: confocal analysis of hidden hearing loss in post-mortem tissue. *Hear. Res.* 327, 78–88. doi: 10.1016/j.heares.2015.04.014

Watanabe, K., Okawara, D., Baba, S., and Yagi, T. (1997). Electrocochleographic analysis of the suppression of tinnitus by electrical promontory stimulation. *Audiology* 36, 147–154. doi: 10.3109/00206099709071968

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A Pilot Study of Peripheral Muscle Magnetic Stimulation as Add-on Treatment to Repetitive Transcranial Magnetic Stimulation in Chronic Tinnitus

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While brain stimulation techniques have been examined as treatment options for chronic tinnitus for many years, they have recently been extended to multimodal treatment approaches. As chronic tinnitus is often accompanied by comorbid muscular tension in the neck and back, we performed a one-arm pilot study to explore the feasibility of a new multimodal treatment approach. In detail, repetitive peripheral magnetic stimulation (rPMS) of the back was performed before and after each session of repetitive transcranial magnetic stimulation (rTMS) of the brain. Data of 41 patients were analyzed, all of which were treated with ten sessions of rTMS of the left prefrontal and left temporoparietal cortex followed by rPMS of the neck and back muscles. Tinnitus severity was measured using the tinnitus questionnaire (TQ). Neck pain was assessed using the neck pain and disability scale (NPAD). The new treatment approach was feasible and well accepted by the majority of patients. However, the overall patient group did not improve significantly in either of the questionnaires. If patients were divided in different subgroups depending on whether they were suffering from neck pain or somatosensory tinnitus, explorative *post-hoc* tests suggested differential effects: patients with both neck pain and somatosensory tinnitus had better outcomes than patients without those conditions or with neck pain only. This was true for both the TQ and the NPAD. This effect was of transient nature though: the TQ score went back to its baseline level after a follow-up period of 12 weeks. Based on our results we recommend that in studies that investigate tinnitus treatments targeting somatosensory afferents patients should be stratified according to somatic co-morbidities and somatosensory influence on the tinnitus percept.

Clinical trial registration: www.clinicaltrials.gov, NCT02306447.

Keywords: rPMS, rTMS, tinnitus, brain stimulation, muscle magnetic stimulation, chronic tinnitus

INTRODUCTION

Chronic subjective tinnitus is a very heterogeneous condition with respect to its causes, clinical characteristics and the emotional distress perceived by a patient. Therefore, it has been suggested that there exist various subtypes of tinnitus which might respond to different treatment approaches (Landgrebe et al., 2010). Accordingly, there are also multiple models for tinnitus pathophysiology, all of which might be able to explain different aspects of tinnitus generation or maintenance. While cognitive models highlight the importance of top-down mechanisms such as selective attention, interpretation and emotional evaluation of the phantom sound (McKenna et al., 2014; Elgoyhen et al., 2015; Ghodratitoostani et al., 2016), there are also pathophysiological models of tinnitus which emphasize bottom-up influences by suggesting neuroplastic changes in somatosensory afferents (Shore et al., 2016). The current study seeks to target both bottom-up and top-down mechanisms by using a combined treatment of rPMS (hypothesized bottom-up influence) and rTMS (hypothesized top-down influence via cortical stimulation of DLPFC and temporoparietal cortex).

Tinnitus has been shown to be accompanied by altered activity of and connectivity between different cortical networks including temporal, parietal and frontal cortices (Schlee et al., 2009; Schmidt et al., 2013; Elgoyhen et al., 2015). As rTMS is considered to be able to interfere with alterations of cortical activity, it has been examined as a treatment option for patients suffering from tinnitus (Theodoroff and Folmer, 2013; Lefaucheur et al., 2014). The effect sizes for this treatment remain small (Lefaucheur et al., 2014). Therefore, different strategies have been tried to increase treatment effects such as targeting multiple brain areas with rTMS (Kreuzer et al., 2011; Lehner et al., 2016) or varying the frequency by which the rTMS pulses are applied (Schecklmann et al., 2016). Up to now, the stimulation of temporal and frontal cortical areas has been suggested to exert beneficial effects on tinnitus (Kleinjung et al., 2008; Langguth and De Ridder, 2013).

Besides the importance of auditory and non-auditory cortical structures, there is also strong evidence for the somatosensory bottom-up system to be involved in tinnitus pathophysiology. Even if controversial, one tinnitus subtype might be cervicogenic somatic tinnitus (Bhatt et al., 2015; Michiels et al., 2015). It is known that auditory-somatosensory integration takes place in the cochlear nucleus (Dehmel et al., 2008) and auditory brainstem activity was shown to be modulated by trigeminal and also somatosensory stimulation (Dehmel et al., 2012; Markovitz et al., 2015). Somatosensory inputs are thought to be functionally relevant with respect to suppression of body-generated sounds (Shore and Zhou, 2006). Pathological conditions are supposed to spread into to auditory system via the cochlear nucleus. Actually, plastic changes in this bimodal system have already been observed in animal models of tinnitus (Dehmel et al., 2012). Furthermore, many patients suffering from tinnitus are able to modulate their phantom sound by moving face or neck muscles (Levine et al., 2007; Sanchez and Rocha, 2011). This somatosensory tinnitus component has already been targeted by different treatment approaches. For instance, myofascial trigger point deactivation was shown to bring tinnitus relief for patients with tinnitus and comorbid myofascial pain syndrome (Rocha and Sanchez, 2012). There is also some evidence that the reduction of muscle tension of neck and back muscles can bring relief to some tinnitus patients. For example, it was shown that Qigong—a system of movements, body postures and breathing exercises—leads to an improvement of tinnitus severity especially in patients with somatosensory tinnitus (Biesinger et al., 2010). Additionally, a recent case report describes a patient whose tinnitus disappeared after the application of a cervical collar, underscoring the involvement of cervical muscles in tinnitus generation (Bechter et al., 2016). In a very recent study, Marks et al. (2018) found that bimodal auditory-somatosensory treatment was effective in reducing tinnitus loudness and severity in patients suffering from somatic tinnitus. With respect to rTMS, it has been hypothesized that rTMS effects may also be partly mediated by modulation of somatosensory afferents (Vanneste et al., 2011; Lehner et al., 2012). There is some preliminary evidence that magnetic stimulation can also be used for reducing muscle tension in neck muscles and for inducing analgetic effects (Smania et al., 2003, 2005; Zunhammer et al., 2011; Sollmann et al., 2016).

Only recently, brain stimulation techniques have been extended to multimodal treatment approaches by combining them with e.g., acoustic stimulation (Shekhawat et al., 2015) or relaxation techniques (Kreuzer et al., 2016). Integrating the knowledge about the central nervous dysfunction as well as the importance of the somatosensory system for chronic tinnitus, we investigate a new multimodal treatment approach which targets both systems by combining rTMS with repetitive peripheral magnetic stimulation (rPMS) of the neck muscles. For rTMS, a stimulation protocol was chosen which combines low-frequency stimulation of auditory cortical areas with high-frequency stimulation of the prefrontal cortex and which has already shown promising effects in the past (Kleinjung et al., 2008; Langguth et al., 2014). While low-frequency rTMS of the temporoparietal cortex is a standard procedure (Lefaucheur et al., 2014) high frequency rTMS of the prefrontal cortex is supposed to induce activity changes in the anterior cingulate cortex (Speer et al., 2000) which is thought to be involved in tinnitus distress (Vanneste et al., 2010). rPMS treatment is supposed to bring relief to muscle tension (Smania et al., 2003, 2005) which might alter the somatosensory input to the cochlear nucleus. We investigated the feasibility of this bimodal treatment approach in a one-arm pilot study (Dobie, 1999; Landgrebe et al., 2012).

MATERIALS AND METHODS

Subjects

The study was registered at Clinical Trials (NCT02306447). Inclusion criteria for study participation were age between 18 and 80 years and presence of chronic subjective tinnitus for at least 6 months. Exclusion criteria were objective tinnitus, a treatable cause of tinnitus and the involvement in other treatments for tinnitus at the same time. Furthermore, patients with clinically relevant psychiatric comorbidities, alcohol or drug abuse, acute neck or back pain, neck or back pain with unknown etiology as well as unstable internal or neurological comorbidities were

excluded. In addition, general exclusion criteria for rTMS or rPMS stimulation applied (history or evidence of significant brain malformation or neoplasm, head injury, cerebral vascular events, neurodegenerative disorders affecting the brain, prior brain surgery, metal objects in and around the body that cannot be removed, pregnancy). Patients were recruited during routine clinical tinnitus consultations. All data were collected at the Department of Psychiatry and Psychotherapy, University of Regensburg between September 2014 and April 2016 (last follow-up visit). All research participants provided written, informed consent to participate in this research as well as for the data to be used for analysis and publication. Data were gathered and analyzed within the framework of the Tinnitus Research Initiative database (Landgrebe et al., 2010) which was approved by the Ethics Committee of the University Hospital of Regensburg (Germany, reference number 08/046).

Questionnaires and Outcome Measures

Patients completed the below listed questionnaires at four measurement time points: at baseline (treatment day 1), week 2 (treatment day 10, last treatment day), week 4 and week 12 (2 and 10 weeks after the last treatment session, respectively). Tinnitus severity was assessed using the German version of the Tinnitus Questionnaire (TQ, Goebel and Hiller, 1994), the Tinnitus Handicap Inventory (THI, Newman et al., 1996) and five rating scales measuring how loud, uncomfortable, annoying, unpleasant and how easy to ignore the tinnitus was. Those scales ranged from 0 (not at all loud/uncomfortable etc.) to 10 (extremely loud/uncomfortable etc.). In addition, depressive symptoms were assessed by the Major Depression Inventory (MDI) and quality of life was measured by the WHO-QoL BREF (World Health Organization Quality of Life) assessment which is divided into four domains: physical health (domain 1), psychological health (domain 2), social relationships (domain 3), and environment (domain 4). In addition, patients completed the neck pain and disability scale (NPAD, Scherer et al., 2008) at baseline and week 2. The NPAD was only available for a subgroup of 34 patients though. In order to assess demographic and clinical patient characteristics at baseline, patients filled in the Tinnitus Sample Case History Questionnaire (Langguth et al., 2007) and underwent pure-tone audiometry. The mean hearing threshold is reported which represents the average of all thresholds measured bilaterally for frequencies between 125 Hz and 8 kHz.

Primary outcome was defined as the change of tinnitus severity as measured by the TQ from baseline to week 12. Secondary outcomes were changes in TQ, THI, MDI, numeric rating scales, and WHO-QoL over the course of the trial (baseline, week 2, week 4, and week 12). Furthermore the change in the neck pain and disability scale (NPAD) from baseline to week 2 was analyzed.

rTMS and rPMS Treatment

The present clinical trial was designed as a one-arm open-label proof of concept study. Therefore, all patients underwent the same treatment procedures during which they were treated in 10 sessions on 10 consecutive working days with a break over the weekend. Each treatment session consisted of four parts which were applied successively without break in between (apart from the break which was necessary to change coils; see **Figure 1**): (1) rPMS of the neck and back muscles; (2) rTMS stimulation of the left dorso-lateral prefrontal cortex (DLPFC, 2000 stimuli, 20 Hz, which were applied in 20 trains with an intertrain interval of 25 s); (3) rTMS stimulation of the left temporo-parietal cortex (2000 stimuli, 1 Hz). (4) rPMS of the neck and back muscles.

(2) and (3) were done at a stimulation intensity of 110% resting motor threshold using a Medtronic MagPro X100 stimulator (Medtronic, Denmark) and a 70 mm figure-of-eight coil. The temporo-parietal cortex was localized using the 10–20 system: The coil was placed between the temporal (T3) and parietal (P3) EEG electrode sites. The DLFPC was targeted by centring the TMS coil 6 cm anterior from the part of the motor cortex which had been used for defining the motor threshold (Lehner et al., 2013). Combined temporoparietal plus frontal stimulation protocol have been examined before and were shown to be safe (Langguth et al., 2014; Kreuzer et al., 2015; Lehner et al., 2016). The rPMS protocol was based on clinical experience in the use of rPMS in rehabilitative medicine and consisted of four medial-lateral movements starting from the neck (1: left trapezius and deltoid muscle; 2: right trapezius and deltoid muscle; 3: left trapezius and latissimus dorsi muscle; 4: right trapezius and latissimus dorsi muscle) and one cranio-caudal movement over the backbone (see **Figure 2**). The series of those five movements was repeated eight times: the first four repetitions with a stimulation frequency of 5 Hz, the remaining four repetitions with 20 Hz. Each movement consisted of 20 stimulation pulses. As a consequence, the duration of a 20 Hz

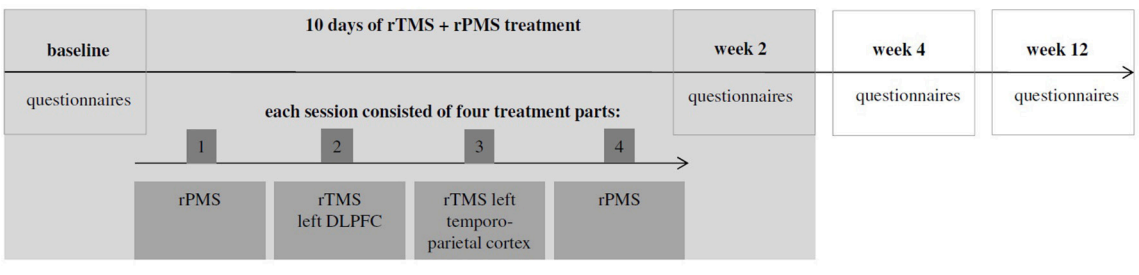

FIGURE 1 | Overall treatment schedule.

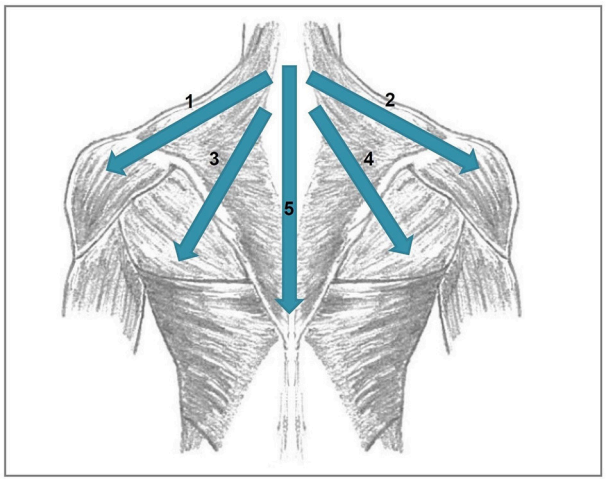

FIGURE 2 | Directions of the movements done during rPMS stimulation. 1, left trapezius and deltoid muscle; 2, right trapezius and deltoid muscle; 3, left trapezius and latissimus dorsi muscle, 4, right trapezius and latissimus dorsi muscle; 5, cranio-caudal movement over the backbone.

movement was 1 s, the duration of a 5 Hz movement was 4 s. Between the movements, there was a 2s interval. In total, one rPMS treatment part had a duration of 100 s. rTMS treatment lasted 2,575 s (2,000 s for the 1 Hz treatment, 575 s for the 20 Hz treatment incl. intertrain intervals). As a consequence, a complete session of two rPMS treatment parts plus rTMS treatment lasted 2,775 s or 46.25 min. rPMS stimulation was done using a round coil with 126 mm outer diameter (MagVenture MMC-140-II) at an intensity that was determined as individually comfortable in a pretest (typically 20–30% of maximal stimulator output). Before the first treatment session, the resting motor threshold was measured. It was defined as the minimal intensity at which at least five of ten motor evoked potentials were 50 μV in amplitude in the right abductor digiti minimi.

Statistical Analysis

For statistical analyses IBM SPSS Statistics for Windows (Version 22.0, Armonk, NY: IBM Corp.) was used. Four missing values were replaced by using a last observation carried forward (LOCF) procedure: The TQ score of one patient was missing for the final visit and the score for the rating scale "annoying" was missing for another patient for week 2. Furthermore, the MDI score for week 2 was missing for one patient and for week 12 for another patient. Recently, we could demonstrate that the LOCF method induces no statistical bias in comparison to linear mixed effects analyses for missing data <10% (Kreuzer et al., 2016). The changes of the TQ score from baseline to week 12 (primary outcome) and of the NPAD score from baseline to week 2 were tested using paired *t*-tests with the within-subjects factor measurement time point. To test for changes in tinnitus severity scores, MDI and WHO-QoL over all four measurement time points an analysis of variance (ANOVA) with the within-subjects factor measurement time point (baseline, week 2, week 4, week 12) was calculated for all questionnaires and rating scales. For the ANOVAs, the sphericity of data was checked with Mauchly Tests (Mauchly, 1940). In case of significant Mauchly-Tests, Greenhouse-Geisser corrections were applied.

In addition to the statistical analyses described above, some exploratory data analyses were conducted in order to understand the results in more detail. To this end, some analyses with a special focus on neck pain and somatosensory tinnitus were done. Patients were divided into different groups depending on whether they were suffering from neck pain ("Do you suffer from neck pain?") and/or from somatosensory tinnitus ("Does any head and neck movement (e.g., moving the jaw forward or clenching the teeth), or having your arms/hands or head touched, affect your tinnitus?"), based on their answers in the Tinnitus Sample Case History Questionnaire (Langguth et al., 2007). A number of 11 patients did not suffer from neck pain or somatosensory tinnitus, 16 patients suffered from neck pain only, and 11 patients reported both neck pain and somatosensory tinnitus. Another 3 patients only reported somatosensory tinnitus. Because of the small sample size, this group was excluded from the following analyses. For the subgroup of 34 patients who filled in the NDPAD, 9 patients did not suffer from neck pain or somatosensory tinnitus, 13 suffered from neck pain only, 9 reported both neck pain and somatosensory tinnitus and 3 reported somatosensory tinnitus only.

Repeated measures ANOVAs were done to compare the resulting three groups with respect to the change of the NPAD score and the TQ from baseline to week 2. The homogeneity of variances between groups was tested with Levene's Tests. In case of significant Levene's Tests, F_{max}-Tests were done. Those tests revealed that an adaptation of the level of significance was not necessary for the ANOVAs with the TQ as dependent variable. For the ANOVA with the NPAD as dependent variable, the significance level had to be adapted to.025.

RESULTS

Dropouts

Forty-nine patients were enrolled in the study. Three patients dropped out of the study during the treatment phase. One of them reported a light subjective cardiac arrhythmia. Although he had had cardiac arrhythmias before and the relation to rPMS seemed to be doubtful, the rPMS treatment was terminated. Another patient dropped out due to an ongoing loudening of the tinnitus percept. The third patient dropped out due to a hypertensive crisis with doubtful relation to rTMS treatment (pre-known hypertension). Five further patients dropped out of the study after the treatment phase during the follow-up phase, all for unknown reasons. One of them had described a transient loudening of the tinnitus before. All in all, data of 41 patients were left to be statistically analyzed (see **Table 1** for demographic and clinical characteristics of this sample at baseline).

Adverse Events

In all treated patients, both the rPMS as well as the rTMS part of the treatment were tolerated without severe side effects. Among the 41 patients who completed the study 5 patients (13%)

TABLE 1 | Demographical data and clinical characteristics at baseline (M ± SD) for the overall patient group and for the three exploratory subgroups.

	Overall patient group ($n = 41$)	Neither neck pain nor somatosensory tinnitus ($n = 11$)	Neck pain ($n = 16$)	Neck pain and somatosensory tinnitus ($n = 11$)
Age (years)	50.70 ± 12.69	48.21 ± 12.67	52.20 ± 11.67	53.62 ± 14.37
Gender	26 m, 15 f	8m, 3f	9m, 7f	6m, 5f
Mean hearing threshold	18.18 ± 11.84	15.01 ± 10.22	21.59 ± 10.89	20.45 ± 13.26
[dB HL]	($n = 40$)		($n = 15$)	
Tinnitus laterality (r/l/l>r/r>l/both/inside head)	6/10/8/6/8/3	0/5/2/0/4/0	2/5/3/1/4/1	3/0/3/4/0/1
Tinnitus duration in years	7.69 ± 7.70	11.07 ± 8.80	4.34 ± 4.93	8.95 ± 9.11
	($n = 38$)	($n = 10$)	($n = 15$)	($n = 10$)
TQ (0–84)	37.83 ± 16.23	25.27 ± 16.62	41.19 ± 16.85	43.27 ± 8.01
THI (0–100)	42.34 ± 21.57	31.73 ± 21.99	44.63 ± 22.71	48.36 ± 16.46
MDI ($N = 40$; 0–50)	7.23 ± 5.43	4.36 ± 4.91	8.44 ± 6.11	8.55 ± 4.59
WHO-QoL Domain 1 (4–20)	15.47 ± 2.57	17.12 ± 2.37	14.52 ± 2.87	15.21 ± 1.77
WHO-QoL Domain 2 (4–20)	14.35 ± 2.66	15.12 ± 3.43	13.98 ± 2.17	14.10 ± 2.90
WHO-QoL Domain 3 (4–20)	15.62 ± 2.85	15.76 ± 3.15	16.10 ± 2.63	15.27 ± 3.00
WHO-QoL Domain 4 (4–20)	16.71 ± 1.62	17.91 ± 1.76	16.16 ± 1.21	16.77 ± 1.49
Rating scales (0–10)				
Strong/loud	6.73 ± 1.88	5.55 ± 2.16	7.13 ± 1.86	7.27 ± 1.49
Uncomfortable	6.76 ± 2.05	5.73 ± 2.01	6.75 ± 2.27	7.55 ± 1.64
Annoying	6.56 ± 2.18	5.27 ± 2.20	7.13 ± 2.28	7.00 ± 1.95
Ignoring	6.20 ± 2.52	4.45 ± 2.30	7.19 ± 2.54	6.55 ± 2.30
Unpleasant	6.68 ± 2.15	5.45 ± 1.92	6.87 ± 2.28	7.45 ± 2.12
Somatosensory tinnitus	14 yes, 27 no			
Suffer from neck pain	27 yes, 14 no			
NPAD score (0–100)	31.47 ± 24.26	4.22 ± 5.97	42.15 ± 19.04	47.33 ± 20.97
	($n = 34$)	($n = 8$)	($n = 13$)	($n = 9$)

Mean hearing threshold (in dB HL): average of all thresholds measured bilaterally ranging from 125 Hz to 8 kHz. Tinnitus laterality is defined in categories: r, right-sided; l, left-sided, l > r, both sides but louder on the left side; r > l, both sides but louder on the right side; both, both sides; inside head, tinnitus is perceived in the middle of/ inside the head. TQ, Tinnitus Questionnaire; THI, Tinnitus Handicap Inventory; MDI, Major Depression Inventory; rating scales ranging from 0 (not at all loud/uncomfortable etc.) to 10 (extremely loud/uncomfortable etc.); WHO-QoL, World Health Organization-Quality of Life; NPAD, neck pain and disability scale.

reported transient headaches and one patient (2.5%) reported headache which was still present at week 12. Furthermore, six patients (14.6%) complained of an increase in tinnitus loudness. In two of them, this increase was still present at week 12. Additionally, one patient reported a transient pain in his fingers.

Statistical Analysis

Concerning the primary outcome (change of the TQ score from baseline to week 12), no significant treatment effect was observed [$t_{(40)} = -0.27$; $p = 0.787$; $d = 0.04$]. The ANOVAs testing for changes in the different questionnaire scores and rating scales over all measurement time points were not significant (see **Figure 3**, **Table 2**). The NPAD score changed marginally from an average total score of 31.47 points at baseline to 28.00 at week 2 [$t_{(33)} = 1.80$; $p = 0.081$; $d = 0.31$).

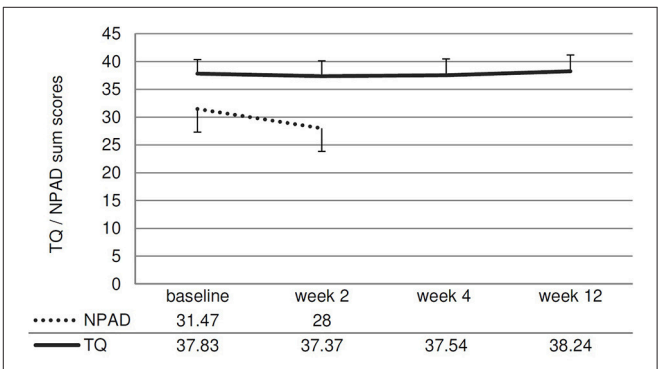

FIGURE 3 | Line chart showing the NPAD and TQ scores over time. Error bars represent standard errors. The change of the NPAD sum score is marginally significant.

Exploratory Data Analysis

If the patients with/without neck pain and/ or somatosensory tinnitus were compared, the interaction effect time*group was significant for the change of the NPAD score from baseline to week 2 [$F_{(2, 28)} = 4.88$; $p = 0.015$; $eta^2 = 0.258$]. For the three *post hoc t*-tests, the Bonferroni-corrected significance level has to be set at 0.016. *Post hoc t*-tests of the mean NPAD differences from baseline to week 2 revealed that patients with both neck pain and somatosensory tinnitus showed more NPAD change ($M = -12.78$; $SD = 10.63$) than patients with neck pain only ($M = 0.85$; $SD = 13.20$). This difference was marginally significant [$t_{(20)} = -2.57$; $p = 0.018$; $d = 1.14$]. Patients with both conditions

TABLE 2 | Results from repeated measures analyses of variance.

	F (*df*)	*p*	Eta^2
TQ	$F_{(3, 120)} = 0.18$	0.912	0.004
THI	$F_{(2.28, 91.23)} = 0.27$	0.792	0.007
MDI	$F_{(1.85, 72.09)} = 0.90$	0.404	0.023
Loudness	$F_{(3, 120)} = 0.49$	0.687	0.012
Uncomfortable	$F_{(3, 120)} = 0.11$	0.954	0.003
Annoyance	$F_{(3, 120)} = 0.36$	0.779	0.009
Ignoring	$F_{(3, 120)} = 0.11$	0.952	0.003
Unpleasant	$F_{(3, 120)} = 1.11$	0.348	0.027
WHO-QoL domain 1	$F_{(2.55, 102)} = 1.40$	0.250	0.034
WHO-QoL domain 2	$F_{(2.1, 83.88)} = 0.22$	0.810	0.006
WHO-QoL domain 3	$F_{(2.57, 102.78)} = 1.20$	0.312	0.029
WHO-QoL domain 4	$F_{(2.52, 100.74)} = 1.90$	0.144	0.045

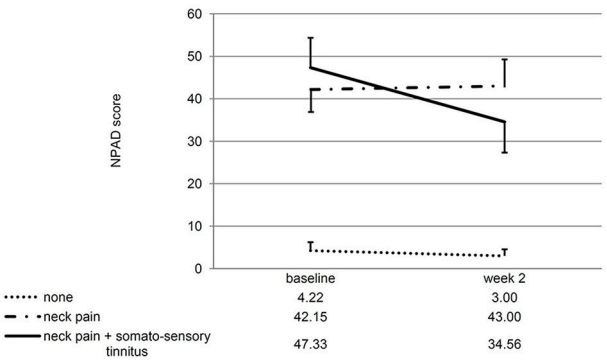

FIGURE 4 | Line chart showing the NPAD score at baseline and week 2 for all three subgroups of patients. Error bars represent standard errors. The NPAD change of patients with both somatosensory tinnitus and neck pain differed significantly from the NPAD change of patients with neither condition. The difference to the change of patients with neck pain only was marginally significant.

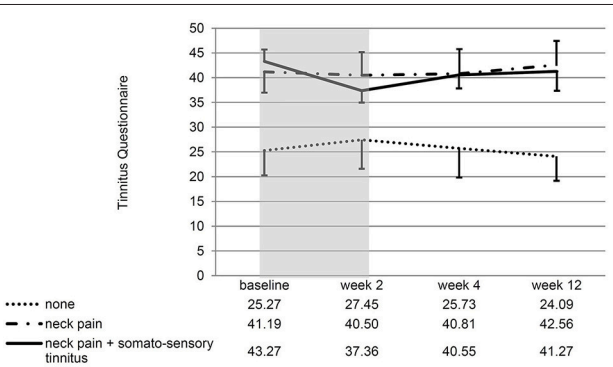

FIGURE 5 | Line chart showing the score at all measurement time points for all three subgroups of patients. Error bars represent standard errors. The 10-day treatment phase is marked in gray color. The TQ change from baseline to week 2 of patients with both somatosensory tinnitus and neck pain differed significantly from the TQ change of patients with neither condition.

also showed significantly more NPAD change than patients with neither condition [$M = -1.22$; $SD = 2.77$; $t_{(9.09)} = -3.16$; $p = 0.011$; $d = 1.49$]. There was no significant difference between the group with neither condition and the group with neck pain only [$t_{(13.50)} = 0.55$; $p = 0.593$; $d = 0.22$]. See **Figure 4** for an illustration of the NPAD changes in all three groups. Also, the overall group effect was significant [$F_{(2, 28)} = 15.73$, $p < 0.001$; $eta^2 = 0.022$]: patients without neck pain or somatosensory tinnitus scored lower on the NPAD than the other two patient groups. If the change of the TQ score from baseline to week 2 was analyzed, there was also a significant time*group interaction effect [$F_{(2, 35)} = 5.47$; $p = 0.009$; $eta^2 = 0.238$]. Again, *post-hoc t*-tests of the mean TQ differences from baseline to week 2 (Bonferroni-corrected alpha = 0.016) revealed that the group with both conditions ($M = -5.91$; $SD = 6.64$) was significantly different from the group suffering from neither condition [$M = 2.18$; $SD = 5.53$; $t_{(20)} = -3.11$; $p = 0.006$; $d = 1.32$] and different by trend from the group suffering from neck pain only [$M = -0.69$; $SD = 5.46$; $t_{(25)} = -2.24$; $p = 0.034$; $d = 0.86$]. There was no significant difference between the group with neither condition and the group with neck pain only [$t_{(25)} = -1.34$; $p = 0.194$; $d = 0.52$]. Again, the main effect "group" was significant [$F_{(2,35)} = 3.35$; $p = 0.047$; $eta^2 = 0.028$]: patients without neck pain or somatosensory tinnitus scored lower on the TQ than the other two patient groups. If the TQ changes of all three subgroups were compared over all four measurement time points, the ANOVA revealed no significant time*group interaction effect [$F_{(4.46, 78)} = 1.40$; $p = 0.238$; $eta^2 = 0.074$]. There was no significant main effect of time [$F_{(2.23, 78)} = 0.44$; $p = 666$; $eta^2 = 0.012$] but a significant main effect of group [$F_{(2, 35)} = 3.75$; $p = 0.033$; $eta^2 = 0.177$]. See **Figure 5** for an illustration of the TQ changes in all three groups.

DISCUSSION

This is the first study to report combined rTMS and rPMS for the treatment of patients suffering from chronic subjective tinnitus. As it was designed as a pilot study, there are some weaknesses which should be kept in mind when interpreting the results. First, in order to examine the feasibility of the combined treatment, a one-arm trial with no control group was chosen. Second, we chose a well-studied standard rTMS protocol in order to combine it with rPMS treatment. This standard rTMS protocol consisted of left-hemispherical treatment and did not account for tinnitus laterality. The question if and how tinnitus laterality should be considered for the choice of the "right" rTMS treatment protocol has partly been examined for unilateral tinnitus (Khedr et al., 2010) but is still an open question for bilateral tinnitus or tinnitus which is perceived inside the head. A recent study indicates that tinnitus laterality has no association with rTMS response (Lehner et al., 2012). Although important, it was not part of the current study's hypothesis to add to this question. Third, the exploratory analyses were done post-hoc, which means that also the subdivision of patients to the different subgroups was done post-hoc. Therefore, the subgroups

were not matched with respect to demographical and clinical characteristics.

It was shown that this treatment approach is feasible in clinical routine. The amount of side effects in the current study is similar to previous results. For example, in Lehner et al. (2016), 12% of the patients treated with triple-site stimulation and 25% of patients treated with standard single-site left temporoparietal stimulation reported transient headaches. In Kreuzer et al. (2015) 16.7% of patients reported headaches after combined left temporoparietal plus left DLPFC stimulation. In the current study, 13% of the patients reported transient, 2.5% ongoing headaches. An increase in tinnitus loudness was reported in 12.5% of the patients treated with single-site stimulation in Lehner et al. (2016), 11.1% in Kreuzer et al. (2015) and in 14.6% of the patients in the current study.

However, there was no significant improvement in tinnitus severity (as measured by the TQ) or neck pain (as measured using the NPAD). This outcome is worse than results of previous studies examining combined frontal plus temporal rTMS stimulation protocols (e.g., Kleinjung et al., 2008; Langguth et al., 2014). One promising approach in increasing treatment effects in chronic tinnitus is the combination of different therapeutic approaches. In tinnitus, several multimodal approaches including rTMS combined with relaxation (Kreuzer et al., 2016), transcranial electric stimulation combined with hearings aids (Shekhawat et al., 2014), tinnitus retraining therapy (Rabau et al., 2015) or tailor-made notched music therapy (Teismann et al., 2014; Lee et al., 2017), vagal nerve stimulation paired with acoustic stimulation (Li et al., 2015) or trigeminal nerve stimulation combined with acoustic stimulation (Hamilton et al., 2016) were introduced. Beside tinnitus other neuropsychiatric disorders were also the focus of combined therapies—for example combined brain stimulation and cognitive training in dementia (Nguyen et al., 2017) or combined brain stimulation and physiotherapy after stroke (Elsner et al., 2017; Salazar et al., 2018). Combination of different neuronal treatments is a challenging task as several open issues have to be resolved. The most important one is the timing or temporal order of both therapies (Bajbouj and Padberg, 2014; Martin et al., 2014; Marks et al., 2018). Combining different therapies might not only result in augmentation of effects. Complex interaction effects might also lead to reduced efficacy. The present trial combined rTMS with preceding and succeeding rPMS. This was a pilot study showing the feasibility and efficacy of the combined approach. In this study we found that for the whole group the combined approach had no beneficial effects, neither on neck muscle pain, nor on tinnitus severity. A possible explanation for this result is that the combination of cortical rTMS with rPMS, as it was investigated in this trial, is not synergistic in the overall patient group. Furthermore this combined protocol may act differentially on different subgroups of patients. This is in line with the findings of the exploratory analyses which suggest additive effects, i.e., linear increase of efficacy from the group without additional conditions over the group with neck pain to the group with neck pain and somatosensory tinnitus. Another explanation of course is that TMS is not effective in tinnitus. A recent review article concluded that it is possibly effective (Lefaucheur et al., 2014).

Nonetheless, a reduction of the TQ score of nearly 6 points from baseline to week 2 is rather large as compared to other rTMS studies. For example, Langguth et al. (2014) reported TQ changes of 2 points from baseline to week 2 for left temporal stimulation and of 3.32 points for a combined left temporal plus frontal stimulation. Lehner et al. (2016) reported a difference of 4.59 points in the TQ score from baseline to week 2 for the overall patient group. Up to now, the most effective treatment option for patients suffering from chronic tinnitus is a specialized care treatment protocol as suggested by Cima et al. (2012) where TQ differences of 7.38 points are seen after 3 months and 15.96 points after 12 months. On an individual patient level a reduction of 5 points in the TQ has been identified a minimal clinically important difference (Adamchic et al., 2012). If related to these results, a mean reduction by 6 points seems to be a rather large change which might be worth future research.

Importantly, the subgroups also differed with respect to the treatment outcome concerning neck pain: patients with both neck pain and somatosensory tinnitus improved with respect to the NPAD score while patients with neck pain only did not. All in all, this suggests that tinnitus patients with both conditions might represent a subgroup of patients for which combined rPMS and rTMS might be a promising treatment approach. There are different possible explanations for these findings. There are studies backing the hypothesis that an improvement of muscle tension leads to an improvement of tinnitus severity (Biesinger et al., 2010; Bechter et al., 2016). A recent systematic review has shown that cervical physical therapy is an effective treatment approach for patients with somatosensory tinnitus (Michiels et al., 2016). Furthermore, Marks et al. (2018) reported that a combined auditory-somatosensory treatment was able to reduce tinnitus loudness and severity in patients suffering from somatosensory tinnitus whereas unimodal auditory treatment was not, emphasizing the importance of the somatosensory system in these patients. This bimodal stimulation examined by Marks et al. has been shown to exert its effects via long term depression in the cochlear nucleus. The changes we observed in patients with neck pain and somatosensory tinnitus in our study might be mediated by similar mechanisms. The fact that we observed improvement only in patients with neck pain and somatosensory tinnitus suggests that both altered neuronal input from the neck area and an interaction between the somatosensory system and the tinnitus percept represent a requirement for a beneficial effect of rPMS.

This result emphasizes the relevance of individualized treatment for tinnitus patients. Tinnitus should be understood as a symptom with diverse causes and variable subgroups all of which might benefit from different treatment approaches (Landgrebe et al., 2010). Besides somatic tinnitus (Ward et al., 2015), typewriter tinnitus was defined as a very specific subtype which is responsive to carbamazepine (Levine, 2006). Further subtypes such as trauma-associated tinnitus (Kreuzer et al., 2012) or tinnitus in combination with specific comorbid symptoms such as temporomandibular joint disorders (Vielsmeier et al.,

2011) have been reported. Therefore, bottom-up oriented treatment strategies might be useful for a different group of tinnitus patients than top-down oriented treatment options.

For electromagnetic stimulation, individual differences in the response to different central and peripheral stimulation techniques have already been demonstrated in the past (Vanneste et al., 2011). As brain stimulation effects depend particularly on the excitability state of the stimulated structure (Rossini et al., 2015), individualized treatment might be particularly relevant for treatment with electrical or magnetic stimulation. Moreover the combination of two techniques—such as rPMS plus rTMS—is challenging to explore, as the complexity is increased by additional aspects such as the temporal relationship between peripheral and central stimulation. Consequently, future studies should try to concentrate on subgroup-specific effects of different treatment strategies or, more generally, on individualized treatment programs considering the very specific combination of possible causes and/or tinnitus-related alterations of a particular patient (Kreuzer et al., 2017).

AUTHOR CONTRIBUTIONS

BL and MS conceived the idea of the study. VV, PK, and TP contributed to data acquisition. AL analyzed the data and drafted the manuscript. All authors contributed to the interpretation of the result and revised the manuscript. All authors approved its final version. The authors declare no competing financial interests with respect to the study.

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REFERENCES

Adamchic, I., Tass, P. A., Langguth, B., Hauptmann, C., Koller, M., Schecklmann, M., et al. (2012). Linking the Tinnitus Questionnaire and the subjective Clinical Global Impression: which differences are clinically important? *Health Qual. Life Outcomes* 10:79. doi: 10.1186/1477-7525-10-79

Bajbouj, M., and Padberg, F. (2014). A perfect match: noninvasive brain stimulation and psychotherapy. *Eur. Arch. Psychiatry Clin. Neurosci.* 264(Suppl. 1), S27–S33. doi: 10.1007/s00406-014-0540-6

Bechter, K., Wieland, M., and Hamann, G. F. (2016). Chronic cervicogenic tinnitus rapidly resolved by intermittent use of cervical collar. *Front. Psychiatry* 7:43. doi: 10.3389/fpsyt.2016.00043

Bhatt, J., Ghavami, Y., Lin, H. W., and Djalilian, H. (2015). Cervical spine dysfunctions in patients with chronic subjective tinnitus. *Otol. Neurotol.* 36, 1459–1460. doi: 10.1097/MAO.0000000000000827

Biesinger, E., Kipman, U., Schätz, S., and Langguth, B. (2010). Qigong for the treatment of tinnitus: a prospective randomized controlled study. *J. Psychosom. Res.* 69, 299–304. doi: 10.1016/j.jpsychores.2010.04.013

Cima, R. F., Maes, I. H., Joore, M. A., Scheyen, D. J., El Refaie, A., Baguley, D. M., et al. (2012). Specialised treatment based on cognitive behaviour therapy versus usual care for tinnitus: a randomised controlled trial. *Lancet* 379, 1951–1959. doi: 10.1016/S0140-6736(12)60469-3

Dehmel, S., Cui, Y. L., and Shore, S. E. (2008). Cross-modal interactions of auditory and somatic inputs in the brainstem and midbrain and their imbalance in tinnitus and deafness. *Am. J. Audiol.* 17, S193–209. doi: 10.1044/1059-0889(2008/07-0045)

Dehmel, S., Pradhan, S., Koehler, S., Bledsoe, S., and Shore, S. (2012). Noise overexposure alters long-term somatosensory-auditory processing in the dorsal cochlear nucleus–possible basis for tinnitus-related hyperactivity? *J. Neurosci.* 32, 1660–1671. doi: 10.1523/JNEUROSCI.4608-11.2012

Dobie, R. A. (1999). A review of randomized clinical trials in tinnitus. *Laryngoscope* 109, 1202–1211. doi: 10.1097/00005537-199908000-00004

Elgoyhen, A. B., Langguth, B., De Ridder, D., and Vanneste, S. (2015). Tinnitus: perspectives from human neuroimaging. *Nat. Rev. Neurosci.* 16, 632–642. doi: 10.1038/nrn4003

Elsner, B., Kwakkel, G., Kugler, J., and Mehrholz, J. (2017). Transcranial direct current stimulation (tDCS) for improving capacity in activities and arm function after stroke: a network meta-analysis of randomised controlled trials. *J. Neuroeng. Rehabil.* 14:95. doi: 10.1186/s12984-017-0301-7

Ghodratitoostani, I., Zana, Y., Delbem, A. C., Sani, S. S., Ekhtiari, H., and Sanchez, T. G. (2016). Theoretical tinnitus framework: a neurofunctional model. *Front. Neurosci.* 10:370. doi: 10.3389/fnins.2016.00370

Goebel, G., and Hiller, W. (1994). [The tinnitus questionnaire. A standard instrument for grading the degree of tinnitus. Results of a multicenter study with the tinnitus questionnaire]. *HNO* 42, 166–172.

Hamilton, C., D'Arcy, S., Pearlmutter, B. A., Crispino, G., Lalor, E. C., and Conlon, B. J. (2016). An investigation of feasibility and safety of bi-modal stimulation for the treatment of tinnitus: an open-label pilot study. *Neuromodulation* 19, 832–837. doi: 10.1111/ner.12452

Khedr, E. M., Abo-Elfetoh, N., Rothwell, J. C., El-Atar, A., Sayed, E., and Khalifa, H. (2010). Contralateral versus ipsilateral rTMS of temporoparietal cortex for the treatment of chronic unilateral tinnitus: comparative study. *Eur. J. Neurol.* 17, 976–983. doi: 10.1111/j.1468-1331.2010.02965.x

Kleinjung, T., Eichhammer, P., Landgrebe, M., Sand, P., Hajak, G., Steffens, T., et al. (2008). Combined temporal and prefrontal transcranial magnetic stimulation for tinnitus treatment: a pilot study. *Otolaryngol. Head Neck Surg.* 138, 497–501. doi: 10.1016/j.otohns.2007.12.022

Kreuzer, P. M., Landgrebe, M., Schecklmann, M., Poeppl, T. B., Vielsmeier, V., Hajak, G., et al. (2011). Can temporal repetitive transcranial magnetic stimulation be enhanced by targeting affective components of tinnitus with frontal rTMS? A randomized controlled pilot trial. *Front. Syst. Neurosci.* 5:88. doi: 10.3389/fnsys.2011.00088

Kreuzer, P. M., Landgrebe, M., Schecklmann, M., Staudinger, S., and Langguth, B. (2012). Trauma-associated tinnitus: audiological, demographic and clinical characteristics. *PLoS ONE* 7:e45599. doi: 10.1371/journal.pone.0045599

Kreuzer, P. M., Lehner, A., Schlee, W., Vielsmeier, V., Schecklmann, M., Poeppl, T. B., et al. (2015). Combined rTMS treatment targeting the anterior cingulate and the temporal cortex for the treatment of chronic tinnitus. *Sci. Rep.* 5:18028. doi: 10.1038/srep18028

Kreuzer, P. M., Poeppl, T. B., Bulla, J., Schlee, W., Lehner, A., Langguth, B., et al. (2016). A proof-of-concept study on the combination of repetitive transcranial magnetic stimulation and relaxation techniques in chronic tinnitus. *J. Neural Transm. (Vienna)*. 123, 1147–1157. doi: 10.1007/s00702-016-1588-4

Kreuzer, P. M., Poeppl, T. B., Rupprecht, R., Vielsmeier, V., Lehner, A., Langguth, B., et al. (2017). Individualized repetitive transcranial magnetic stimulation treatment in chronic tinnitus? *Front. Neurol.* 8:126. doi: 10.3389/fneur.2017.00126

Landgrebe, M., Azevedo, A., Baguley, D., Bauer, C., Cacace, A., Coelho, C., et al. (2012). Methodological aspects of clinical trials in tinnitus: a proposal for an international standard. *J. Psychosom. Res.* 73, 112–121. doi: 10.1016/j.jpsychores.2012.05.002

Landgrebe, M., Zeman, F., Koller, M., Eberl, Y., Mohr, M., Reiter, J., et al. (2010). The Tinnitus Research Initiative (TRI) database: a new approach for delineation of tinnitus subtypes and generation of predictors for treatment outcome. *BMC Med. Inform. Decis. Mak.* 10:42. doi: 10.1186/1472-6947-10-42

Langguth, B., and De Ridder, D. (2013). Tinnitus: therapeutic use of superficial brain stimulation. *Handb. Clin. Neurol.*, 116, 441–467. doi: 10.1016/B978-0-444-53497-2.00036-X

Langguth, B., Goodey, R., Azevedo, A., Bjorne, A., Cacace, A., Crocetti, A., et al. (2007). Consensus for tinnitus patient assessment and treatment outcome

measurement: tinnitus research initiative meeting, Regensburg, July 2006. *Prog. Brain Res.* 166, 525–536. doi: 10.1016/S0079-6123(07)66050-6

Langguth, B., Landgrebe, M., Frank, E., Schecklmann, M., Sand, P. G., Vielsmeier, V., et al. (2014). Efficacy of different protocols of transcranial magnetic stimulation for the treatment of tinnitus: pooled analysis of two randomized controlled studies. *World J. Biol. Psychiatry* 15, 276–285. doi: 10.3109/15622975.2012.708438

Lee, H. Y., Choi, M. S., Chang, D. S., and Cho, C. S. (2017). Combined bifrontal transcranial direct current stimulation and tailor-made notched music training in chronic tinnitus. *J. Audiol. Otol.* 21, 22–27. doi: 10.7874/jao.2017.21.1.22

Lefaucheur, J. P., André-Obadia, N., Antal, A., Ayache, S. S., Baeken, C., Benninger, D. H., et al. (2014). Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). *Clin. Neurophysiol.* 125, 2150–2206. doi: 10.1016/j.clinph.2014.05.021

Lehner, A., Schecklmann, M., Greenlee, M. W., Rupprecht, R., and Langguth, B. (2016). Triple-site rTMS for the treatment of chronic tinnitus: a randomized controlled trial. *Sci. Rep.* 6:22302. doi: 10.1038/srep22302

Lehner, A., Schecklmann, M., Kreuzer, P. M., Poeppl, T. B., Rupprecht, R., and Langguth, B. (2013). Comparing single-site with multisite rTMS for the treatment of chronic tinnitus - clinical effects and neuroscientific insights: study protocol for a randomized controlled trial. *Trials* 14:269. doi: 10.1186/1745-6215-14-269

Lehner, A., Schecklmann, M., Landgrebe, M., Kreuzer, P. M., Poeppl, T. B., Frank, E., et al. (2012). Predictors for rTMS response in chronic tinnitus. *Front. Syst. Neurosci.* 6:11. doi: 10.3389/fnsys.2012.00011

Levine, R. A. (2006). Typewriter tinnitus: a carbamazepine-responsive syndrome related to auditory nerve vascular compression. *ORL J. Otorhinolaryngol. Relat. Spec.* 68, 43–46; discussion: 46–47. doi: 10.1159/000090490

Levine, R. A., Nam, E. C., Oron, Y., and Melcher, J. R. (2007). Evidence for a tinnitus subgroup responsive to somatosensory based treatment modalities. *Prog. Brain Res.* 166, 195–207. doi: 10.1016/S0079-6123(07)66017-8

Li, T. T., Wang, Z. J., Yang, S. B., Zhu, J. H., Zhang, S. Z., Cai, S. J., et al. (2015). Transcutaneous electrical stimulation at auricular acupoints innervated by auricular branch of vagus nerve pairing tone for tinnitus: study protocol for a randomized controlled clinical trial. *Trials* 16:101. doi: 10.1186/s13063-015-0630-4

Markovitz, C. D., Smith, B. T., Gloeckner, C. D., and Lim, H. H. (2015). Investigating a new neuromodulation treatment for brain disorders using synchronized activation of multimodal pathways. *Sci. Rep.* 5:9462. doi: 10.1038/srep09462

Marks, K. L., Martel, D. T., Wu, C., Basura, G. J., Roberts, L. E., Schvartz-Leyzac, K. C., et al. (2018). Auditory-somatosensory bimodal stimulation desynchronizes brain circuitry to reduce tinnitus in guinea pigs and humans. *Sci. Transl. Med.* 10:eaal3175. doi: 10.1126/scitranslmed.aal3175

Martin, D. M., Liu, R., Alonzo, A., Green, M., and Loo, C. K. (2014). Use of transcranial direct current stimulation (tDCS) to enhance cognitive training: effect of timing of stimulation. *Exp. Brain Res.* 232, 3345–3351. doi: 10.1007/s00221-014-4022-x

Mauchly, J. W. (1940). Significance test for sphericity of a normal n-variate distribution. *Ann. Math. Statist.* 11, 204–209. doi: 10.1214/aoms/1177731915

McKenna, L., Handscomb, L., Hoare, D. J., and Hall, D. A. (2014). A scientific cognitive-behavioral model of tinnitus: novel conceptualizations of tinnitus distress. *Front. Neurol.* 5:196. doi: 10.3389/fneur.2014.00196

Michiels, S., De Hertogh, W., Truijen, S., and Van de Heyning, P. (2015). Cervical spine dysfunctions in patients with chronic subjective tinnitus. *Otol. Neurotol.* 36, 741–745. doi: 10.1097/MAO.0000000000000670

Michiels, S., Van de Heyning, P., Truijen, S., Hallemans, A., and De Hertogh, W. (2016). Does multi-modal cervical physical therapy improve tinnitus in patients with cervicogenic somatic tinnitus? *Man. Ther.* 26, 125–131. doi: 10.1016/j.math.2016.08.005

Newman, C. W., Jacobson, G. P., and Spitzer, J. B. (1996). Development of the Tinnitus Handicap Inventory. *Arch. Otolaryngol. Head Neck Surg.* 122, 143–148. doi: 10.1001/archotol.1996.01890140029007

Nguyen, J. P., Suarez, A., Kemoun, G., Meignier, M., Le Saout, E., Damier, P., et al. (2017). Repetitive transcranial magnetic stimulation combined with cognitive training for the treatment of Alzheimer's disease. *Neurophysiol. Clin.* 47, 47–53. doi: 10.1016/j.neucli.2017.01.001

Rabau, S., Van Rompaey, V., and Van de Heyning, P. (2015). The effect of Transcranial Direct Current Stimulation in addition to Tinnitus Retraining Therapy for treatment of chronic tinnitus patients: a study protocol for a double-blind controlled randomised trial. *Trials* 16:514. doi: 10.1186/s13063-015-1041-2

Rocha, C. B., and Sanchez, T. G. (2012). Efficacy of myofascial trigger point deactivation for tinnitus control. *Braz. J. Otorhinolaryngol.* 78, 21–26. doi: 10.5935/1808-8694.20120028

Rossini, P. M., Burke, D., Chen, R., Cohen, L. G., Daskalakis, Z., Di Iorio, R., et al. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin. Neurophysiol.* 126, 1071–1107. doi: 10.1016/j.clinph.2015.02.001

Salazar, A. P. S., Vaz, P. G., Marchese, R. R., Stein, C., Pinto, C., and Pagnussat, A. S. (2018). Noninvasive brain stimulation improves hemispatial neglect after stroke: a systematic review and meta-analysis. *Arch. Phys. Med. Rehabil.* 99, 355–366. doi: 10.1016/j.apmr.2017.07.009

Sanchez, T. G., and Rocha, C. B. (2011). Diagnosis and management of somatosensory tinnitus: review article. *Clinics (Sao Paulo)* 66, 1089–1094. doi: 10.1590/S1807-59322011000600028

Schecklmann, M., Giani, A., Tupak, S., Langguth, B., Raab, V., Polak, T., et al. (2016). Neuronavigated left temporal continuous theta burst stimulation in chronic tinnitus. *Restor. Neurol. Neurosci.* 34, 165–175. doi: 10.3233/RNN-150518

Scherer, M., Blozik, E., Himmel, W., Laptinskaya, D., Kochen, M. M., and Herrmann-Lingen, C. (2008). Psychometric properties of a German version of the neck pain and disability scale. *Eur. Spine J.* 17, 922–929. doi: 10.1007/s00586-008-0677-y

Schlee, W., Hartmann, T., Langguth, B., and Weisz, N. (2009). Abnormal resting-state cortical coupling in chronic tinnitus. *BMC Neurosci.* 10:11. doi: 10.1186/1471-2202-10-11

Schmidt, S. A., Akrofi, K., Carpenter-Thompson, J. R., and Husain, F. T. (2013). Default mode, dorsal attention and auditory resting state networks exhibit differential functional connectivity in tinnitus and hearing loss. *PLoS ONE* 8:e76488. doi: 10.1371/journal.pone.0076488

Shekhawat, G. S., Kobayashi, K., and Searchfield, G. D. (2015). Methodology for studying the transient effects of transcranial direct current stimulation combined with auditory residual inhibition on tinnitus. *J. Neurosci. Methods* 239, 28–33. doi: 10.1016/j.jneumeth.2014.09.025

Shekhawat, G. S., Searchfield, G. D., and Stinear, C. M. (2014). Randomized trial of transcranial direct current stimulation and hearing aids for tinnitus management. *Neurorehabil. Neural Repair* 28, 410–419. doi: 10.1177/1545968313508655

Shore, S. E., Roberts, L. E., and Langguth, B. (2016). Maladaptive plasticity in tinnitus–triggers, mechanisms and treatment. *Nat. Rev. Neurol.* 12, 150–160. doi: 10.1038/nrneurol.2016.12

Shore, S. E., and Zhou, J. (2006). Somatosensory influence on the cochlear nucleus and beyond. *Hear. Res.* 216-217, 90–99. doi: 10.1016/j.heares.2006.01.006

Smania, N., Corato, E., Fiaschi, A., Pietropoli, P., Aglioti, S. M., and Tinazzi, M. (2003). Therapeutic effects of peripheral repetitive magnetic stimulation on myofascial pain syndrome. *Clin. Neurophysiol.* 114, 350–358. doi: 10.1016/S1388-2457(02)00367-X

Smania, N., Corato, E., Fiaschi, A., Pietropoli, P., Aglioti, S. M., and Tinazzi, M. (2005). Repetitive magnetic stimulation: a novel therapeutic approach for myofascial pain syndrome. *J. Neurol.* 252, 307–314. doi: 10.1007/s00415-005-0642-1

Sollmann, N., Trepte-Freisleder, F., Albers, L., Jung, N. H., Mall, V., Meyer, B., et al. (2016). Magnetic stimulation of the upper trapezius muscles in patients with migraine - A pilot study. *Eur. J. Paediatr. Neurol.* 20, 888–897. doi: 10.1016/j.ejpn.2016.07.022

Speer, A. M., Kimbrell, T. A., Wassermann, E. M., D Repella, J., Willis, M. W., Herscovitch, P., et al. (2000). Opposite effects of high and low frequency rTMS on regional brain activity in depressed patients. *Biol. Psychiatry* 48, 1133–1141. doi: 10.1016/S0006-3223(00)01065-9

Teismann, H., Wollbrink, A., Okamoto, H., Schlaug, G., Rudack, C., and Pantev, C. (2014). Combining transcranial direct current stimulation and tailor-made

notched music training to decrease tinnitus-related distress–a pilot study. *PLoS ONE* 9:e89904. doi: 10.1371/journal.pone.0089904

Theodoroff, S. M., and Folmer, R. L. (2013). Repetitive transcranial magnetic stimulation as a treatment for chronic tinnitus: a critical review. *Otol. Neurotol.* 34, 199–208. doi: 10.1097/MAO.0b013e31827b4d46

Vanneste, S., Langguth, B., and De Ridder, D. (2011). Do tDCS and TMS influence tinnitus transiently via a direct cortical and indirect somatosensory modulating effect? A combined TMS-tDCS and TENS study. *Brain Stimul.* 4, 242–252. doi: 10.1016/j.brs.2010.12.001

Vanneste, S., Plazier, M., vander Loo, V., de Heyning, P. V., Congedo, M., and De Ridder, D. (2010). The neural correlates of tinnitus-related distress. *Neuroimage* 52, 470–480. doi: 10.1016/j.neuroimage.2010.04.029

Vielsmeier, V., Kleinjung, T., Strutz, J., Bürgers, R., Kreuzer, P. M., and Langguth, B. (2011). Tinnitus with temporomandibular joint disorders: a specific entity of tinnitus patients? *Otolaryngol. Head Neck Surg.* 145, 748–752. doi: 10.1177/0194599811413376

Ward, J., Vella, C., Hoare, D. J., and Hall, D. A. (2015). Subtyping somatic tinnitus: a cross-sectional UK cohort study of demographic, clinical and audiological characteristics. *PLoS ONE* 10:e0126254. doi: 10.1371/journal.pone.0126254

Zunhammer, M., Busch, V., Griesbach, F., Landgrebe, M., Hajak, G., and Langguth, B. (2011). rTMS over the cerebellum modulates temperature detection and pain thresholds through peripheral mechanisms. *Brain Stimul.* 4, 210–217 e211. doi: 10.1016/j.brs.2010.11.002

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Individualized Repetitive Transcranial Magnetic Stimulation Treatment in Chronic Tinnitus?

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Background: Prefrontal and temporo-parietal repetitive transcranial magnetic stimulation (rTMS) in patients suffering from chronic tinnitus have shown significant but only moderate effectiveness with high interindividual variability in treatment response. This open-label pilot study was designed to examine the general feasibility of an individualized fronto-temporal rTMS protocol and to explore what criteria are needed for a more detailed evaluation in randomized clinical studies.

Methods: During the first session of a 2-week rTMS protocol, we applied different rTMS protocols to the left and right temporo-parietal and dorsolateral prefrontal cortex (DLPFC) in 25 tinnitus patients. Short trains of 1, 5, 10, and 20 Hz and continuous theta burst stimulation were applied, and patients were asked for immediate tinnitus reductions after each train. If a patient reported such improvements, rTMS treatment was applied over nine sessions with a combined protocol consisting of the most effective frontal and the most effective temporo-parietal stimulation protocol. Those patients who did not improve after the test session were treated with a standard prefrontal plus temporo-parietal protocol (20 Hz over left DLPFC + 1 Hz over temporo-parietal cortex).

Results: Almost half of the patients (12 of 25) reported immediate tinnitus reductions during the test session. In this group, the mean pre- to post-treatment amelioration in the tinnitus questionnaire was higher (medium to high effect sizes) in contrast to the patients who did not respond to the test session. Treatment outcome remained stable over a follow-up period of 10 weeks.

Discussion: Individualized rTMS was shown to be feasible and effective in chronic tinnitus. The results obtained from this study provide tentative evidence in support of an individualized rTMS treatment approach and might provide a basis for a "tailored" application of rTMS in tinnitus and other neuropsychiatric disorders.

Keywords: chronic tinnitus, repetitive transcranial magnetic stimulation, individualized repetitive transcranial magnetic stimulation, neuromodulation, neurostimulation

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INTRODUCTION

Chronic Tinnitus

Subjective tinnitus is characterized by the perception of sound in the absence of a corresponding sound source (1). Approximately 1% of the general population report severe tinnitus-related impairment of daily living (2) and seek medical help (3). In contrast to auditory hallucinations that occur in mental disorders and mainly refer to the perception of voices, tinnitus sensations are usually of an unformed acoustic nature such as buzzing, hissing, or ringing (4). Severe tinnitus is frequently associated with depressive symptoms (5), anxiety (6, 7), and insomnia (8), and its socioeconomic relevance is illustrated by the dramatically increased risk for disability pension among tinnitus patients (9). The available evidence-based treatments for tinnitus have only small effect sizes (4, 10, 11), indicating the urgent need for the development and optimization of innovative therapeutic attempts.

Neurobiological Underpinnings of Chronic Tinnitus

Formerly considered as an otological disorder, tinnitus treatment approaches exclusively targeting the cochlea have led to discouraging results in most cases (12). However, during the last years, advances in neuroimaging and the development of animal models have shifted the perspective toward the neuronal pathologies underlying the different forms of tinnitus (13). There is convincing evidence emerging from functional imaging (14) and neurophysiological studies (15, 16) that tinnitus is not only related to abnormal functioning of the central auditory system (1) but also related to abnormal activity of non-auditory brain regions (17, 18) and abnormal functional connectivity between these regions (19–22). Imaging studies have shown that coactivation of prefrontal areas might especially be related to the affective components of tinnitus (5, 22, 23). It has been further proposed that limbic and paralimbic structures may form a fronto-thalamic gating system for tinnitus perception (24). According to this model, increased neuronal activity arises in the auditory pathways as a consequence of deafferentation due to hearing loss. If altered activity in auditory networks is connected to activation of motivational and emotional brain networks, the inhibitory influence from this fronto-thalamic gating system is downregulated, thus allowing the tinnitus signal to propagate to the auditory cortices, which finally leads to conscious perception. This is in line with electrophysiological studies that demonstrated the relevance of dysfunctional top–down inhibitory mechanisms originating in the prefrontal lobe for tinnitus generation (22, 25, 26). Therefore, two potential targets for brain stimulation can be identified: first, the frontal cortex with the aim to enhance the activity of the fronto-striato-thalamic gating mechanism, and second, the temporal cortex with the aim to reduce cortical activity in the auditory cortex. In line with this notion, tinnitus reduction has been demonstrated after repetitive transcranial magnetic stimulation (rTMS), tDCS, and direct cortical stimulation of both frontal and temporal cortices (27, 28).

rTMS in Chronic Tinnitus

On the basis of initial findings of abnormal functioning of central auditory structures (1), rTMS of the temporal and temporo-parietal cortex has been proposed as a potential treatment for chronic tinnitus (29). Several clinical studies showed a sham-controlled reduction of tinnitus severity after repeated 1 Hz rTMS applied to the left temporal cortex (30–34), but some studies were also negative (35, 36). In a meta-analysis, treatment effects were shown to be significant, but effect sizes were moderate at best and interindividual variability in treatment response is high (37).

The *Status Quo*

As mentioned above, rTMS treatment results in chronic tinnitus are currently burdened by only moderate improvement (38) and high interindividual variability indicating the need for optimization strategies. No demographic or clinical criteria could be identified as a predictor for rTMS treatment response in large samples of patients with chronic tinnitus (39). Several studies reported transient tinnitus suppression after a single session of rTMS (40). When the effects of different rTMS protocols over the temporal cortex were compared, it has been shown that the protocol with the best tinnitus suppressing effect differed from patient to patient (41). However, the individually best protocols induced similar changes of oscillatory brain activity (42). Although single rTMS sessions reduced tinnitus only transiently for seconds to minutes, repeated daily rTMS stimulation resulted in longer lasting tinnitus reduction up to several months (30, 43, 44) in some patients. Most studies investigating repeated sessions of rTMS used low-frequency rTMS with 1 Hz, even though two studies comparing the effects of 1 Hz rTMS with those of 10 and 25 Hz revealed similar treatment effects for high-frequency rTMS (45, 46).

In light of these data, the question arises whether a test session, in which the immediate effects of different TMS protocols are evaluated, may be a feasible and useful approach for selecting the rTMS protocol for repeated rTMS sessions. To our knowledge, this question has not yet been addressed by any study, even if the sham-controlled response to single rTMS sessions has been used as a selection criterion for surgical implantation with epidural electrodes (47).

Both auditory and non-auditory brain regions are involved in tinnitus (48, 49), and combined frontal and temporal stimulation protocols exerted best effects in previous studies (22, 50, 51). Thus, the aim of the present study was to investigate the feasibility of an rTMS treatment approach that is (i) individualized based on the effects of single test sessions and that (ii) comprises both prefrontal and temporo-parietal cortices.

MATERIALS AND METHODS

Study Design and Conduct

The study was conducted following a two-armed pilot study design. As a first step, an initial rTMS testing session was conducted in which 1, 5, 10, and 20 Hz; continuous theta burst rTMS; and sham were applied to (i) the left dorsolateral prefrontal cortex (DLPFC), (ii) the right DLPFC, (iii) the left temporo-parietal

junction area, and (iv) the right temporo-parietal junction area. The number of applied stimuli during the rTMS testing session on day 1 was 200 stimuli for each stimulation frequency and location (exception: 50 stimuli at 1 Hz frequency), resulting in a total of up to 4,200 stimuli. After the application of each protocol, patients were asked whether they had experienced a change in their tinnitus and were asked to rate the changes on a percentage level regarding to loudness. Patients wore insert earplugs during the testing session and were instructed to report immediate tinnitus changes in form of percentage values. The most effective protocol of each of the four stimulation locations was (i) repeated to assure retest validity and (ii) controlled by sham stimulation (applying the same paradigm with a tilted coil angle of 90° with one wing of the figure-of-eight coil on the head and magnetic field main direction, thus paralleling the scalp surface). Thus, sham conditions were different for each stimulation site and patient during the testing phase. For patients reporting the same tinnitus reduction for different stimulation protocols for the left and right frontal or for the left and right temporo-parietal stimulation sites, equally effective protocols were repeated and contrasted directly. Patients reporting immediate tinnitus modulation during the testing session on day 1 (Monday) were consecutively treated by application of 9 further daily (only working days) combined stimulation sessions consisting of the most effective prefrontal stimulation protocol (2,000 stimuli) at the left or right hemisphere followed by the most effective temporo-parietal stimulation protocol (left or right, 2,000 stimuli/session). Patients who did not experience immediate tinnitus modulation during the testing session on day 1 were treated by a "standard" combined treatment consisting of 2,000 stimuli of 20 Hz rTMS delivered to the left DLPFC followed by 2,000 stimuli of 1 Hz rTMS of the left temporo-parietal junction area ($n = 3$) or a "triple" paradigm consisting of 2,000 stimuli of 20 Hz rTMS of the left DLPFC followed by rTMS (1,000 stimuli left, 1,000 stimuli right) of the bilateral temporo-parietal junction area ($n = 9$) (52). Due to ethical reasons, we switched treatment for the standard group as it turned out during the course of the present study that the triple stimulation is more effective than the former standard treatment (53). The treatment sessions following the testing session consisted of a total of 4,000 stimuli/day for both the individualized and the standard group and were conducted on nine subsequent working days. Follow-up-visits were conducted 2 and 10 weeks after the end of the stimulation period (=week 4 and week 12 visit, respectively).

Stimulation was performed with a 70-mm figure-of-8 (=butterfly) coil (Cool-B65, Magventure A/S, Denmark) in 40 trains with 50 stimuli and an intertrain interval of 25 s (exception: 1 Hz stimulation was administered in one train). The coil was powered by a MagPro ×100 stimulator (Magventure A/S, Denmark). Stimulation was performed at 110% resting motor threshold (RMT) but not higher than 60% maximum stimulator output. RMT was defined as the lowest intensity sufficient to produce left abductor digiti minimi muscle activation (magnetic evoked potentials >50 μV) with a single pulse delivered to the motor cortex in at least 5 of 10 trials and was determined before the testing session on day 1. For treatment of the left DLPFC, the conventional butterfly coil was positioned 6 cm anterior of the left motor hotspot in a sagittal direction with the handle pointing backward in a 45° angle toward the midline (28). Temporo-parietal cortex localization was conducted following a protocol suggested by Khedr et al. (46) positioning the coil between the temporal (T3/T4-electrode location) and parietal (P3/P4-location) (54) according the 10–20 EEG-positioning system (55). Coil positioning was tangential to the scalp with the handle pointing upward.

All participants gave written informed consent after a comprehensive explanation of the study procedures that data were gathered and analyzed for the Tinnitus Research Initiative database (56), which was approved by the Ethics Committee of the University Hospital of Regensburg (Germany, reference number 08/046). All study procedures were carried out in accordance with the approved guidelines.

Patient Enrollment

Inclusion criterion was subjective tinnitus with duration of more than 6 months. Exclusion criteria comprised objective tinnitus (with a treatable cause), ongoing other tinnitus treatments during or 3 months before study enrollment, the presence of clinically relevant psychiatric comorbidities or unstable medical conditions, history or evidence of significant brain malformation or neoplasm, history of head injuries, cerebral vascular events, the presence of irremovable metal objects in and around the body, pregnancy, alcohol abuse or intake of illicit substances, and history of prior TMS treatment. Patients were recruited for participation in the study after presentation in the outpatient clinic of the Interdisciplinary Tinnitus Centre at the University of Regensburg, Regensburg, Germany. The study was conducted between November 2011 and June 2012.

Outcome Measures and Data Presentation

The outcome parameters were change in tinnitus distress and loudness measured with the tinnitus questionnaire (TQ; range 0–84) (57) and a numeric rating scale (range 0–10), respectively. We present change from baseline to the week 2 visit to measure immediate treatment effects and also from pretreatment to post-treatment defined as a mean value of the screening and baseline visits (pretreatment) and of the week 2, 4, and 12 visits (post-treatment). In addition, we demonstrate the number of treatment responders defined as a minimal reduction of 5 points in the TQ (58). Further objectives were the assessment of adverse events and safety information at all visits. Data were assessed according to international standards (59) and registered in a tinnitus database following ICH-GCP regulations (60, 61). We contrasted the group of patients who reported changes in tinnitus after a single session with the group of patients with no response.

All data are displayed as mean ± SD if not otherwise labeled. In case of missing data, the last observation was carried forward, and participants who did not complete rTMS treatment (dropouts) were excluded from analysis ($n = 1$). Due to the pilot character of the study, we focus on the feasibility and identification of modifications for future studies (62, 63). For this purpose, we report raw data and basic statistical tests for rough estimation of the efficacy of the tested treatments. All statistical tests were conducted two tailed, unadjusted for multiple comparisons, and a value of $p < 0.05$ was assumed as statistically significant. For group comparisons, we calculated Student's *t*-tests for independent

measures. Effect sizes are reported according to G*Power 3.1.2 (64). Statistical data analysis was performed using IBM SPSS Statistics for Windows, version 22.0 (released 2013; IBM Corp., Armonk, NY, USA).

RESULTS

Feasibility

Twenty-five patients were recruited for participation in the study. One participant aborted the treatment after the first day due to headache after finishing the testing session on day 1 (without reporting immediate effects). Exactly 50% of the 24 remaining patients ($n = 12$) reported immediate modulation of their tinnitus percept during day 1 testing procedures and were therefore allocated to the individualized treatment arm receiving combined rTMS of prefrontal and temporo-parietal cortical areas for another 9 consecutive working days. Nine of the 12 patients who perceived a tinnitus change after TMS were able to indicate the change in a percentage value (ranging between 3 and 70%). The other three patients provided only vague estimations like "better" (see **Table 1**). Two patients experiencing the most pronounced improvement after active rTMS reported similar improvement after sham TMS during the testing session. No other patient reported tinnitus improvement after sham TMS. Also, these patients were assigned to the individualized arm (subject number 1 and 7 in **Table 1**). Three patients in each of the study groups were under a psychotropic medication (individualized group: 1× citalopram 40 mg/day, 1× venlafaxine 225 mg/day, 1× opipramol 100 mg/day; control group: 1× citalopram 40 mg/day + trimipramine 40 mg/day, 1× agomelatine 25 mg/day, 1× opipramol 100 mg/day).

Concerning the other 12 patients without immediate tinnitus changes during the testing rTMS session, 3 were treated with the "standard double protocol" consisting of 2,000 stimuli to the left DLPFC at 20 Hz frequency followed by 2,000 stimuli to the left temporo-parietal cortex at 1 Hz and 9 were treated with the "standard triple protocol" consisting of high-frequency rTMS of the left prefrontal cortex (2,000 stimuli, 20 Hz) followed by low-frequency rTMS of the bilateral temporo-parietal junction areas (total of 2,000 stimuli, 1 Hz). For graphical illustration of the study conduct, see **Figure 1**.

The comparison of participants of both treatment groups revealed no differences in the clinical and demographic baseline characteristics. Detailed information is provided in **Table 2**. In three patients (all from the individualized group), screening and baseline visits were performed at the same visit resulting in only one pretreatment assessment. One patient did not provide data for visit week 4 (standard treatment). These missing values were added by LOCF using data from the screening visit. For the other patients, interval between screening and baseline was between

TABLE 1 | Individual data for treatment with individualized repetitive transcranial magnetic stimulation (rTMS) and average group data for individualized and standard rTMS (each subject was stimulated with 4,000 pulses per day).

	Subject order	Kind of prefrontal stimulation	Single-session tinnitus reduction[a]	Kind of temporo-parietal stimulation	Single-session tinnitus reduction[a]	Resting motor threshold (RMT); stimulation intensity (stimulator output)[b]	Change in tinnitus questionnaire (TQ) total score from baseline to week 2 visit[d]	Change in TQ total score from pretreatment to posttreatment[c,d]
Individualized treatment (single subject data)	1	Right continuous theta burst stimulation (cTBS)	70%	Right 5 Hz	50%	42%; 46%	24 (–)	20.33 (–)
	3	Right 10 Hz	"Better"	Left cTBS	"Better"	34%; 37%	7 (–)	2.50 (+)
	5	Left cTBS	40%	Left 10 Hz	40%	43%; 47%	0 (+)	1.17 (+)
	7	Left 20 Hz	50%	Left 5 Hz	40%	46%; 51%	11 (–)	15.17 (–)
	9	Left 5 Hz	20%	Right cTBS	10%	37%; 41%	11 (–)	3.17 (+)
	10	Left 20 Hz	2%	Right 10 Hz	2%	33%; 36%	–3 (+)	–8.33 (+)
	13	Left 20 Hz	"Not sure"	Left 20 Hz	"Good"	50%; 55%	9 (–)	9.33 (–)
	16	Right 20 Hz	30%	Left 5 Hz	100%	57%; 60%	–9 (+)	20.83 (–)
	17	Left 5 Hz	"Shortly better"	Right cTBS	"Shortly off"	30%; 33%	20 (–)	5.67 (–)
	18	Left cTBS	10%	Left cTBS	10%	38%; 42%	3 (+)	9.83 (–)
	23	Left 20 Hz	10%	Right 20 Hz	10%	52%; 57%	10 (–)	8.33 (–)
	24	Left 20 Hz	3%	Left 10 Hz	4%	42%; 46%	3 (+)	5.33 (–)
Individualized treatment (mean ± SD)	n.a.	9/12 left; 5 Hz ($n = 2$), 10 Hz ($n = 1$), 20 Hz ($n = 6$),cTBS ($n = 3$)	n.a.	7/12 left; 5 Hz ($n = 3$), 10 Hz ($n = 3$), 20 Hz ($n = 2$), cTBS ($n = 4$)	n.a.	42 ± 8; 46 ± 9	7.17 ± 9.24 (58% responder)	8.57 ± 8.92 (67% responder)
Standard protocol (mean ± SD)	n.a.	Left 20 Hz	n.a.	Left and/or right 1 Hz	n.a.	43 ± 17; 43 ± 9	3.42 ± 7.04 (42% responder)	1.46 ± 8.48 (42% responder)

[a]*Numbers indicate the percent of tinnitus reduction after a single-session stimulation; verbal statements are indicated by quotation marks.*

[b]*Stimulation intensity was 110% of RMT; for safety reasons, 60% was the upper limit for stimulation intensity; RMT and stimulation intensity were comparable in the standard protocol group as two subjects had thresholds highly above 60%.*

[c]*Visits after treatment (week 2 + week 4 + final visit) minus visits before treatment (screening + baseline).*

[d]*Responder information in brackets (+ = yes, – = no).*

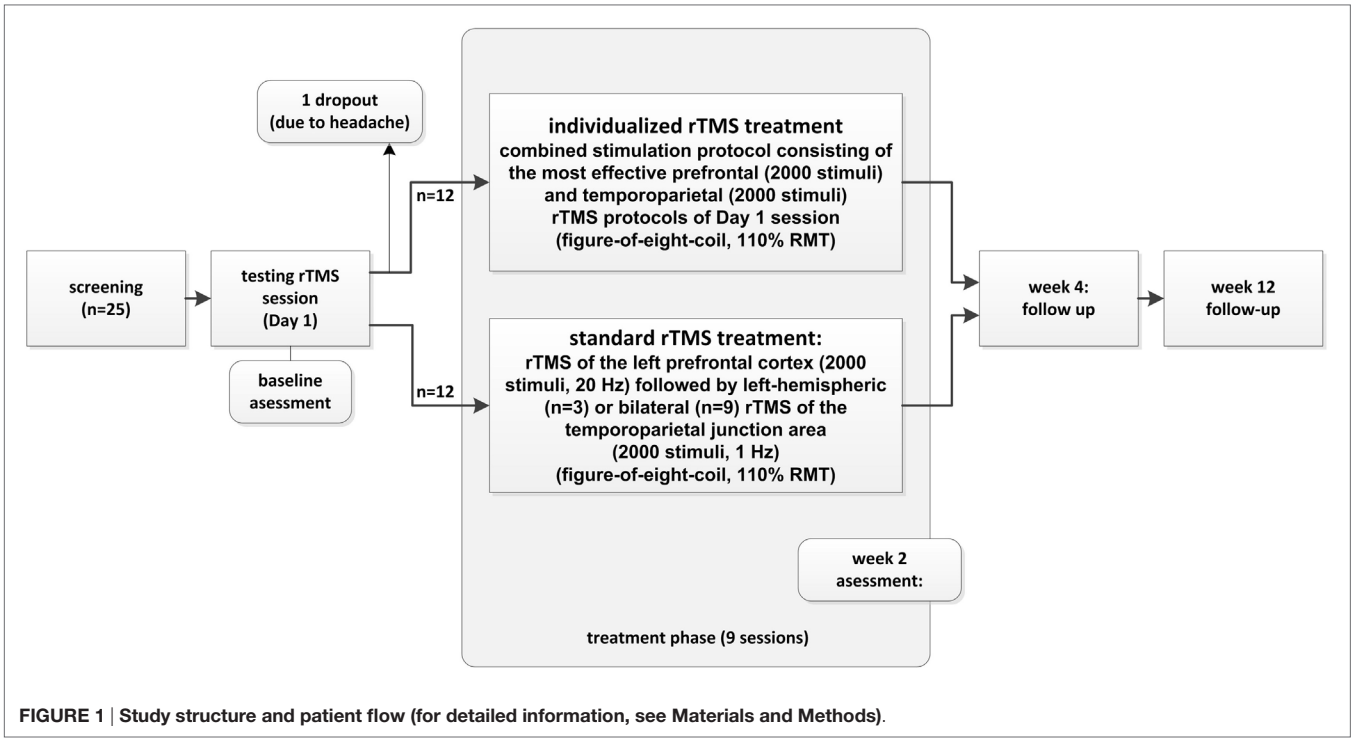

FIGURE 1 | **Study structure and patient flow (for detailed information, see Materials and Methods)**.

TABLE 2 | Group comparisons of the two treatment arms.

	Individualized rTMS (n = 12)	Standard rTMS (n = 12)	Statistics: individualized vs. standard rTMS
Gender (female/male)	2/10	2/10	n.a.
Age (years)	57.1 ± 7.4	50.6 ± 12.1	$t(22) = 1.582$; $p = 0.128$
Mean hearing level (dB HL)	25.7 ± 15.9	22.9 ± 13.4	$t(22) = 0.476$; $p = 0.639$
Tinnitus duration (months)	108.2 ± 98.9	154.3 ± 106.8	$t(21) = 1.072$; $p = 0.296$
Tinnitus laterality (right/left/both)	0/2/10	0/2/10	n.a.
TQ (baseline minus week 2)	7.2 ± 9.2	3.4 ± 7.0	$t(22) = 1.118$; $p = 0.276$; $d = 0.465$
TQ (screening/ baseline minus week 2/4/12)	8.6 ± 8.9	1.5 ± 8.5	$t(22) = 2.002$; $p = 0.058$; $d = 0.816$
NRS loudness (baseline minus week 2)	0.8 ± 1.9	1.4 ± 2.5	$t(22) = 0.649$; $p = 0.289$; $d = 0.270$
NRS loudness (screening/baseline minus week 2/4/12)	0.9 ± 1.9	1.0 ± 1.5	$t(22) = 0.181$; $p = 0.858$; $d = 0.058$

TQ, tinnitus questionnaire (scale: 0–84); NRS, numeric rating scale (scale: 0–10); rTMS, repetitive transcranial magnetic stimulation.

13 and 349 days (or screening and baseline was the same visit) with a mean of 81.4 and a SD of 86.03 (median = 53). In the individualized treatment group, we had 10 right-handed patients and 2 ambidextrous patients; in the standard group, we had 8 right-handed patients, 2 left-handed patients, and 2 ambidextrous patients. A statistical comparison with the Fisher's exact test shows no difference between groups with respect to handedness ($p = 0.329$). In the individualized group, three patients chose right frontal and nine left frontal stimulation as a best stimulation site ($\chi^2 = 2.222$; df = 2; $p = 0.045$) and five right temporal and seven left temporal stimulation as a best stimulation site ($p = 0.470$), indicating an association of right-handedness with left frontal stimulation. However, this association of left frontal stimulation with right-handedness is from correlational nature, but highlights the need for accounting handedness in rTMS trials.

Safety

No severe adverse events occurred during the course of the study. One participant withdrew his consent to participate due to the experience of headache during the testing session on day 1. This patient did not report changes in tinnitus loudness after single rTMS sessions and was determined as dropout for the standard treatment arm (see **Figure 1**). A deterioration of tinnitus was reported by two patients in the testing session, but did not lead to an interruption of the treatment. These patients were treated with the standard protocol. Further adverse events included headache in two patients (one on 1 day of treatment and one in 2 days of treatment) of the standard treatment and in one patient in the individualized group on 1 treatment day. One patient of the standard treatment missed two treatment days due to a common cold.

Results of the Test Session

For the majority of patients, the testing session revealed best results for left-sided stimulation for both frontal and temporal stimulation resulting in predominant left-hemispheric treatment location in the individualized treatment group (**Table 1**).

With respect to tinnitus localization, two patients of both treatment groups had purely left-sided tinnitus, and the other patients had tinnitus either in both ears or within the head. No patient had reported purely right-sided tinnitus (**Table 2**). In the majority of the patients, high-frequency stimulation protocols or TBS revealed best results in the testing session (**Table 1**).

Efficacy

The mean reduction of tinnitus severity measured by the TQ (57) was numerically higher for the individualized treatment group in contrast to the standard group (**Table 2**). Number of responders was also higher in the individualized group (**Table 1**). Graphical information on the TQ score at the different assessment time points is depicted in **Figure 2**. Decrease in tinnitus loudness was in the same range for the individualized and the standard group (**Table 2**). On a statistical level, individualized treatment (vs. standard treatment) showed numerically superior efficacy with medium or high effect size on a non-significant level contrasting week 2 and baseline or postvisits and previsits, respectively (screening and baseline vs. week 2, 4, and 12) as elicited by the TQ. Changes in tinnitus loudness were not significantly different between groups with small or negligible effects sizes.

DISCUSSION

From this pilot study examining the feasibility of an individualized treatment approach in patients suffering from chronic tinnitus, three main conclusions can be drawn:

(i) The application of a testing rTMS session with different frequencies and stimulation sites has proven to be feasible with almost 50% of the patients reporting immediate effects in our study sample.
(ii) An individualized treatment approach following this testing session was well tolerated by the majority of patients. Adverse events and dropout rates were comparable among both study arms and matched prior observations (28).
(iii) The clinical efficacy of an individualized treatment approach exceeded the results of a standard treatment approach on a descriptive level. Effects for TQ were not significant, but showed medium to high effect sizes. Improvement was stable for several weeks after the treatment.

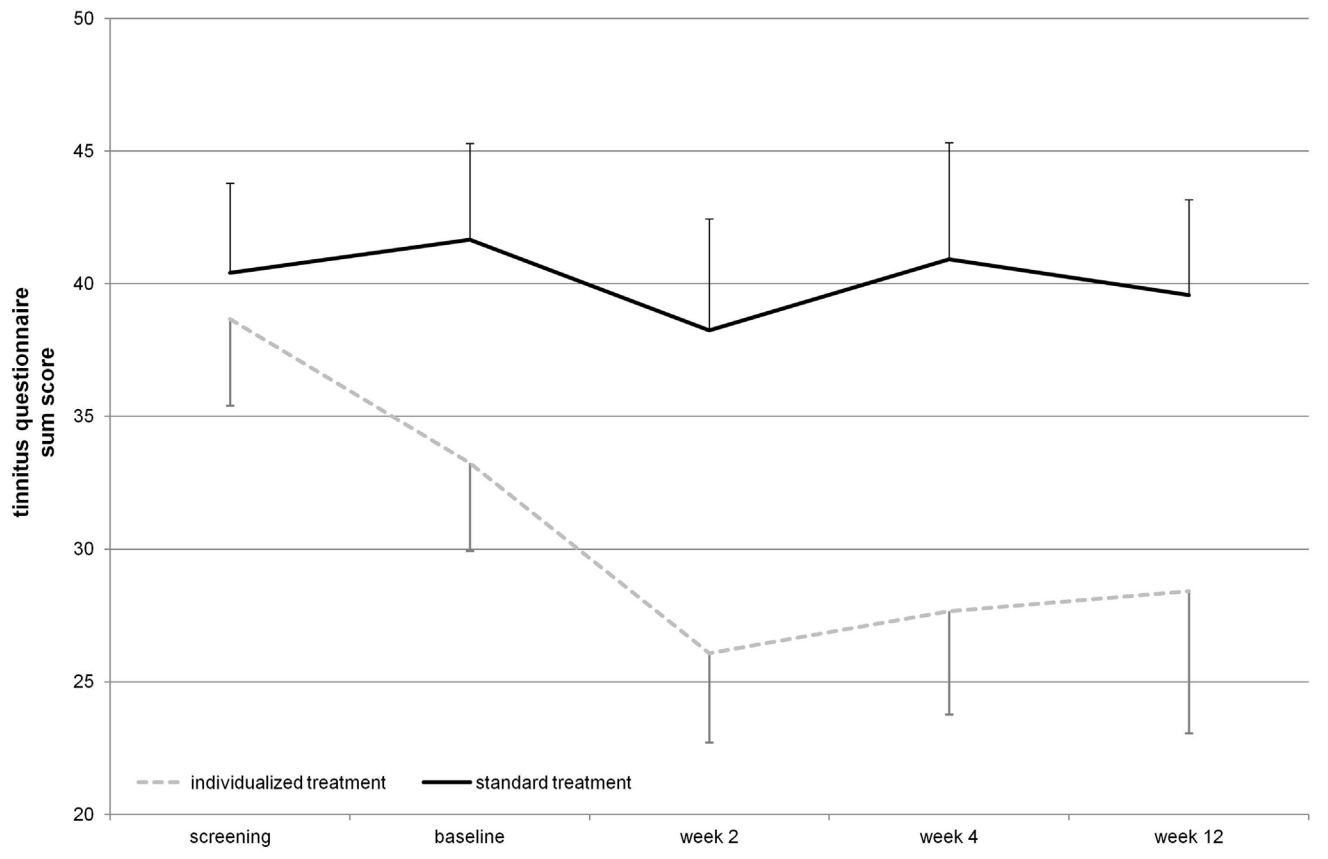

FIGURE 2 | Tinnitus questionnaire total score over the time course of the study (displayed mean ± SEM). Repetitive transcranial magnetic stimulation treatment took place from baseline until week 2 visit. Week 4 and week 12 assessments represent follow-up.

This pilot study addressed the question to what extent clinical effects of a single session may serve as predictors for treatment response of repeated sessions of rTMS. On a descriptive level, the individualized treatment group reported a higher reduction of tinnitus severity than the control group treated with a standard combined rTMS paradigm.

Our finding that response to a single session may predict treatment outcome of prolonged stimulation has similarly been detected in the approach to use a single session of rTMS as a selection criterion for the implantation of epidural electrodes for intractable tinnitus (48, 49). Among patients who responded to a single session of TMS, two-third had also long-term benefit from epidural stimulation (48). Notably, epidural stimulation in chronic tinnitus has been only conducted in few cases of intractable tinnitus, and the results should not carelessly be transferred to our sample and settings. However, this ratio corresponds nicely to our finding that most patients who responded to the testing sessions also responded to repeated rTMS application.

Our finding that the effect of testing sessions predicts the effect of daily treatment is of high clinical relevance as rTMS treatment results are characterized by high interindividual variability and no demographic or clinical tinnitus baseline characteristics could be identified as predictors of rTMS treatment response yet (65). The high interindividual variability in rTMS treatment response in previous studies has been mainly interpreted as an expression of the variability of tinnitus pathophysiology (40). If this was correct, it would be plausible that different patients might respond to different stimulation paradigms, thus profiting from individualized "trial-and-error" treatment attempts (65). Even, if the individualized treatment showed higher efficacy in contrast to standard treatment, the SD of TQ change in the two groups was similar for both treatment arms. This indicates a similar variability in treatment response and suggests that individualized treatment does not increase efficacy by reducing interindividual variability.

One discussable issue is the identification of the most efficient protocol in the testing session. In each patient, 20 different protocols were tested (5 different frequencies at 4 different sites). For practical reasons, the intervals between the different protocols were rather short. Investigators were instructed to wait until the tinnitus returned to baseline level before applying the next protocol. Thus, the interval between different protocols was typically in the range between 1 and 5 min. A testing session, therefore, took approximately 1 h per individual, in some cases even longer. However, we are aware that it cannot be excluded that the effects of the different protocols were influenced by after-effects of the preceding protocols. Thus, the increased responsiveness to high frequency and TBS stimulation may have been partly due to the accumulation of stimulation effects as the order of the different protocols was not randomized during the testing session. It could also be the case that it took some time for the individuals to acclimate to the testing scenario. However, it is unlikely that the effects were only driven by accumulation of stimuli or habituation to the testing situation as TBS was always applied as the last protocol, but not every patient reported best improvement with TBS. Nevertheless, future studies investigating individualized TMS should take a potential order effect into account and follow a randomized protocol regarding the sequence of stimulation frequencies and stimulated hemispheres.

A certain dissociation of the effects of a single session and repeated sessions was observed. Although patients reported loudness reductions of their tinnitus during the testing session, daily treatment had only an effect on tinnitus distress but not on tinnitus loudness. Possible reasons might be related to differences in the applied scales for tinnitus loudness (percentage change after a single session vs. 11 graded numeric rating for tinnitus loudness). Furthermore, ratings during a single session were conducted with inserted earplugs, whereas clinical evaluation over the course of the treatment was done without hearing protection obscuring possible changes in tinnitus loudness.

We are well aware that the comparison between the individualized treatment group and the standard treatment group does not enable us to disentangle whether the response in the testing session serves as a general predictor for response to repeated rTMS sessions or whether the individualized protocol was relevant for the good outcome in the individualized treatment group. In other words, the test session might have only served for the identification of responders at the starting point of the rTMS treatment period. To further address this issue, future studies should be conducted in which patients, who respond to TMS testing sessions, are randomized into standard treatment vs. individualized treatment.

Notably, the present study was designed as a proof-of-concept pilot study, which should primarily evaluate the feasibility and tolerability of an individualized rTMS treatment regime in chronic tinnitus. Thus, we are well aware about the limiting factor of the lack of a sham-controlled study group. It cannot be excluded that the improved outcome in the individualized group was driven by non-specific effects. Patients who experienced tinnitus improvement in the testing session might have developed higher expectations with respect to the daily treatment, which could have contributed to the better outcome. However, active control conditions have especially been recommended in rTMS studies due to the inherent limitations of sham conditions (36, 66, 67). For future studies using this individualized approach, we would suggest the splitting of the patients reporting changes in tinnitus perception into two arms—one arm treated with the individualized protocol and one arm treated with a standard protocol. An optimal design would also include a third sham arm (68).

In summary, our pilot data confirm the potential of individualized rTMS treatment as a non-invasive, safe, and well-tolerated method of brain stimulation in the treatment of chronic tinnitus. Descriptive analyses indicate a remarkable superior effect of the individualized treatment in contrast to standard treatment even if the standard treatment with two or three stimulation sites was shown to be more effective than single-site stimulations (36, 66, 67, 69). Individualized rTMS in chronic tinnitus might provide a basis for an individualized, "tailored" rTMS-based therapeutic approach also in other neuropsychiatric disorders. Combining a single session with electroencephalography during the first treatment day (70) might help to identify neuronal markers, which

might enable reliable predictions regarding treatment response after daily rTMS. This approach could eventually be useful in identifying successful TMS protocols based on EEG markers also in those patients, who were not able to detect perceptual improvements in the test session.

AUTHOR CONTRIBUTIONS

PK, BL, RR, and MS: conception of the study, data interpretation, and manuscript edition. AL and MS: statistics and data management. PK, VV, and TP: patient recruitment and conduction of clinical visits.

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REFERENCES

1. Moller AR. Pathophysiology of tinnitus. *Otolaryngol Clin North Am* (2003) 36:249–266, v–vi. doi:10.1016/S0030-6665(02)00170-6
2. Harter M, Maurischat C, Weske G, Laszig R, Berger M. [Psychological stress and impaired quality of life in patients with tinnitus]. *HNO* (2004) 52(2):125–31. doi:10.1007/s00106-003-0889-8
3. Coles RR. Epidemiology of tinnitus: (1) prevalence. *J Laryngol Otol Suppl* (1984) 9:7–15. doi:10.1017/S1755146300090041
4. Langguth B, Kreuzer PM, Kleinjung T, De Ridder D. Tinnitus: causes and clinical management. *Lancet Neurol* (2013) 12:920–30. doi:10.1016/S1474-4422(13)70160-1
5. Langguth B, Landgrebe M, Kleinjung T, Sand GP, Hajak G. Tinnitus and depression. *World J Biol Psychiatry* (2011) 12(7):489–500. doi:10.3109/15622975.2011.575178
6. Krog NH, Engdahl B, Tambs K. The association between tinnitus and mental health in a general population sample: results from the HUNT Study. *J Psychosom Res* (2010) 69:289–98. doi:10.1016/j.jpsychores.2010.03.008
7. Langguth B. A review of tinnitus symptoms beyond 'ringing in the ears': a call to action. *Curr Med Res Opin* (2011) 27:1635–43. doi:10.1185/03007995.2011.595781
8. Cronlein T, Langguth B, Geisler P, Hajak G. Tinnitus and insomnia. *Prog Brain Res* (2007) 166:227–33. doi:10.1016/S0079-6123(07)66021-X
9. Friberg E, Jansson C, Mittendorfer-Rutz E, Rosenhall U, Alexanderson K. Sickness absence due to otoaudiological diagnoses and risk of disability pension: a nationwide Swedish prospective cohort study. *PLoS One* (2012) 7:e29966. doi:10.1371/journal.pone.0029966
10. Hoare DJ, Kowalkowski VL, Kang S, Hall DA. Systematic review and meta-analyses of randomized controlled trials examining tinnitus management. *Laryngoscope* (2011) 121:1555–64. doi:10.1002/lary.21825
11. Kreuzer PM, Vielsmeier V, Langguth B. Chronic tinnitus: an interdisciplinary challenge. *Dtsch Arztebl Int* (2013) 110:278–84. doi:10.3238/arztebl.2013.0278
12. Dobie RA. A review of randomized clinical trials in tinnitus. *Laryngoscope* (1999) 109:1202–11. doi:10.1097/00005537-199908000-00004
13. Shore SE, Roberts LE, Langguth B. Maladaptive plasticity in tinnitus – triggers, mechanisms and treatment. *Nat Rev Neurol* (2016) 12:150–60. doi:10.1038/nrneurol.2016.12
14. Elgoyhen AB, Langguth B, De Ridder D, Vanneste S. Tinnitus: perspectives from human neuroimaging. *Nat Rev Neurosci* (2015) 16:632–42. doi:10.1038/nrn4003
15. Weisz N, Dohrmann K, Elbert T. The relevance of spontaneous activity for the coding of the tinnitus sensation. *Prog Brain Res* (2007) 166:61–70. doi:10.1016/S0079-6123(07)66006-3
16. Weisz N, Muller S, Schlee W, Dohrmann K, Hartmann T, Elbert T. The neural code of auditory phantom perception. *J Neurosci* (2007) 27:1479–84. doi:10.1523/JNEUROSCI.3711-06.2007
17. Lanting CP, De Kleine E, Van Dijk P. Neural activity underlying tinnitus generation: results from PET and fMRI. *Hear Res* (2009) 255:1–13. doi:10.1016/j.heares.2009.06.009
18. Leaver AM, Renier L, Chevillet MA, Morgan S, Kim HJ, Rauschecker JP. Dysregulation of limbic and auditory networks in tinnitus. *Neuron* (2011) 69:33–43. doi:10.1016/j.neuron.2010.12.002
19. Schlee W, Weisz N, Bertrand O, Hartmann T, Elbert T. Using auditory steady state responses to outline the functional connectivity in the tinnitus brain. *PLoS One* (2008) 3:e3720. doi:10.1371/journal.pone.0003720
20. Schlee W, Hartmann T, Langguth B, Weisz N. Abnormal resting-state cortical coupling in chronic tinnitus. *BMC Neurosci* (2009) 10:11. doi:10.1186/1471-2202-10-11
21. Schlee W, Mueller N, Hartmann T, Keil J, Lorenz I, Weisz N. Mapping cortical hubs in tinnitus. *BMC Biol* (2009) 7:80. doi:10.1186/1741-7007-7-80
22. De Ridder D, Elgoyhen AB, Romo R, Langguth B. Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc Natl Acad Sci U S A* (2011) 108:8075–80. doi:10.1073/pnas.1018466108
23. Vanneste S, Plazier M, Der Loo E, De Heyning PV, Congedo M, De Ridder D. The neural correlates of tinnitus-related distress. *Neuroimage* (2010) 52:470–80. doi:10.1016/j.neuroimage.2010.04.029
24. Rauschecker JP, Leaver AM, Muhlau M. Tuning out the noise: limbic-auditory interactions in tinnitus. *Neuron* (2010) 66:819–26. doi:10.1016/j.neuron.2010.04.032
25. Norena A, Cransac H, Chery-Croze S. Towards an objectification by classification of tinnitus. *Clin Neurophysiol* (1999) 110:666–75. doi:10.1016/S1388-2457(98)00034-0
26. Schecklmann M, Landgrebe M, Poeppl TB, Kreuzer P, Manner P, Marienhagen J, et al. Neural correlates of tinnitus duration and distress: a positron emission tomography study. *Hum Brain Mapp* (2013) 34:233–40. doi:10.1002/hbm.21426
27. Vanneste S, Plazier M, Ost J, Van Der Loo E, Van De Heyning P, De Ridder D. Bilateral dorsolateral prefrontal cortex modulation for tinnitus by transcranial direct current stimulation: a preliminary clinical study. *Exp Brain Res* (2010) 202:779–85. doi:10.1007/s00221-010-2183-9
28. Frank E, Schecklmann M, Landgrebe M, Burger J, Kreuzer P, Poeppl TB, et al. Treatment of chronic tinnitus with repeated sessions of prefrontal transcranial direct current stimulation: outcomes from an open-label pilot study. *J Neurol* (2011) 259(2):327–33. doi:10.1007/s00415-011-6189-4
29. Eichhammer P, Langguth B, Marienhagen J, Kleinjung T, Hajak G. Neuronavigated repetitive transcranial magnetic stimulation in patients with tinnitus: a short case series. *Biol Psychiatry* (2003) 54:862–5. doi:10.1016/S0006-3223(02)01896-6
30. Kleinjung T, Eichhammer P, Langguth B, Jacob P, Marienhagen J, Hajak G, et al. Long-term effects of repetitive transcranial magnetic stimulation (rTMS) in patients with chronic tinnitus. *Otolaryngol Head Neck Surg* (2005) 132:566–9. doi:10.1016/j.otohns.2004.09.134
31. Plewnia C, Reimold M, Najib A, Reischl G, Plontke SK, Gerloff C. Moderate therapeutic efficacy of positron emission tomography-navigated repetitive transcranial magnetic stimulation for chronic tinnitus: a randomised, controlled pilot study. *J Neurol Neurosurg Psychiatry* (2007) 78:152–6. doi:10.1136/jnnp.2006.095612
32. Rossi S, De Capua A, Ulivelli M, Bartalini S, Falzarano V, Filippone G, et al. Effects of repetitive transcranial magnetic stimulation on chronic tinnitus:

a randomised, crossover, double blind, placebo controlled study. *J Neurol Neurosurg Psychiatry* (2007) 78:857–63. doi:10.1136/jnnp.2006.105007
33. Smith JA, Mennemeier M, Bartel T, Chelette KC, Kimbrell T, Triggs W, et al. Repetitive transcranial magnetic stimulation for tinnitus: a pilot study. *Laryngoscope* (2007) 117:529–34. doi:10.1097/MLG.0b013e31802f4154
34. Folmer RL, Theodoroff SM, Casiana L, Shi Y, Griest S, Vachhani J. Repetitive transcranial magnetic stimulation treatment for chronic tinnitus: a randomized clinical trial. *JAMA Otolaryngol Head Neck Surg* (2015) 141:716–22. doi:10.1001/jamaoto.2015.1219
35. Piccirillo JF, Kallogjeri D, Nicklaus J, Wineland A, Spitznagel EL Jr, Vlassenko AG, et al. Low-frequency repetitive transcranial magnetic stimulation to the temporoparietal junction for tinnitus: four-week stimulation trial. *JAMA Otolaryngol Head Neck Surg* (2013) 139:388–95. doi:10.1001/jamaoto.2013.233
36. Langguth B, Landgrebe M, Frank E, Schecklmann M, Sand PG, Vielsmeier V, et al. Efficacy of different protocols of transcranial magnetic stimulation for the treatment of tinnitus: pooled analysis of two randomized controlled studies. *World J Biol Psychiatry* (2014) 15:276–85. doi:10.3109/15622975.2012.708438
37. Soleimani R, Jalali MM, Hasandokht T. Therapeutic impact of repetitive transcranial magnetic stimulation (rTMS) on tinnitus: a systematic review and meta-analysis. *Eur Arch Otorhinolaryngol* (2016) 237:1663–75. doi:10.1007/s00405-015-3642-5
38. Frank E, Eichhammer P, Burger J, Zowe M, Landgrebe M, Hajak G, et al. Transcranial magnetic stimulation for the treatment of depression: feasibility and results under naturalistic conditions: a retrospective analysis. *Eur Arch Psychiatry Clin Neurosci* (2011) 261(4):261–6. doi:10.1007/s00406-010-0137-7
39. Peng Z, Chen XQ, Gong SS. Effectiveness of repetitive transcranial magnetic stimulation for chronic tinnitus: a systematic review. *Otolaryngol Head Neck Surg* (2012) 147(5):817–25. doi:10.1177/0194599812458771
40. Lefaucheur JP, Andre-Obadia N, Antal A, Ayache SS, Baeken C, Benninger DH, et al. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). *Clin Neurophysiol* (2014) 125:2150–206. doi:10.1016/j.clinph.2014.05.021
41. De Ridder D, Verstraeten E, Van Der Kelen K, De Mulder G, Sunaert S, Verlooy J, et al. Transcranial magnetic stimulation for tinnitus: influence of tinnitus duration on stimulation parameter choice and maximal tinnitus suppression. *Otol Neurotol* (2005) 26:616–9. doi:10.1097/01.mao.0000178146.91139.3c
42. Muller N, Keil J, Obleser J, Schulz H, Grunwald T, Bernays RL, et al. You can't stop the music: reduced auditory alpha power and coupling between auditory and memory regions facilitate the illusory perception of music during noise. *Neuroimage* (2013) 79:383–93. doi:10.1016/j.neuroimage.2013.05.001
43. Khedr EM, Rothwell JC, El-Atar A. One-year follow up of patients with chronic tinnitus treated with left temporoparietal rTMS. *Eur J Neurol* (2009) 16:404–8. doi:10.1111/j.1468-1331.2008.02522.x
44. Burger J, Frank E, Kreuzer P, Kleinjung T, Vielsmeier V, Landgrebe M, et al. Transcranial magnetic stimulation for the treatment of tinnitus: 4-year follow-up in treatment responders – a retrospective analysis. *Brain Stimul* (2011) 4:222–7. doi:10.1016/j.brs.2010.11.003
45. Khedr EM, Rothwell JC, Ahmed MA, El-Atar A. Effect of daily repetitive transcranial magnetic stimulation for treatment of tinnitus: comparison of different stimulus frequencies. *J Neurol Neurosurg Psychiatry* (2008) 79:212–5. doi:10.1136/jnnp.2007.127712
46. Khedr EM, Abo-Elfetoh N, Rothwell JC, El-Atar A, Sayed E, Khalifa H. Contralateral versus ipsilateral rTMS of temporoparietal cortex for the treatment of chronic unilateral tinnitus: comparative study. *Eur J Neurol* (2010) 17:976–83. doi:10.1111/j.1468-1331.2010.02965.x
47. Vanneste S, De Ridder D. Differences between a single session and repeated sessions of 1 Hz TMS by double-cone coil prefrontal stimulation for the improvement of tinnitus. *Brain Stimul* (2013) 6:155–9. doi:10.1016/j.brs.2012.03.019
48. De Ridder D, Vanneste S, Kovacs S, Sunaert S, Menovsky T, Van De Heyning P, et al. Transcranial magnetic stimulation and extradural electrodes implanted on secondary auditory cortex for tinnitus suppression. *J Neurosurg* (2011) 114:903–11. doi:10.3171/2010.11.JNS10197
49. De Ridder D, Vanneste S, Plazier M, Menovsky T, Van De Heyning P, Kovacs S, et al. Dorsolateral prefrontal cortex transcranial magnetic stimulation and electrode implant for intractable tinnitus. *World Neurosurg* (2012) 77:778–84. doi:10.1016/j.wneu.2011.09.009
50. Langguth B, Eichhammer P, Wiegand R, Marienhegen J, Maenner P, Jacob P, et al. Neuronavigated rTMS in a patient with chronic tinnitus. Effects of 4 weeks treatment. *Neuroreport* (2003) 14:977–80. doi:10.1097/00001756-200305230-00014
51. Plewnia C, Bartels M, Gerloff C. Transient suppression of tinnitus by transcranial magnetic stimulation. *Ann Neurol* (2003) 53:263–6. doi:10.1002/ana.10468
52. Lehner A, Schecklmann M, Poeppl TB, Kreuzer PM, Vielsmeier V, Rupprecht R, et al. Multisite rTMS for the treatment of chronic tinnitus: stimulation of the cortical tinnitus network – a pilot study. *Brain Topogr* (2013) 26:501–10. doi:10.1007/s10548-012-0268-4
53. Lehner A, Schecklmann M, Greenlee MW, Rupprecht R, Langguth B. Triple-site rTMS for the treatment of chronic tinnitus: a randomized controlled trial. *Sci Rep* (2016) 6:22302. doi:10.1038/srep22302
54. Hoffman RE, Boutros NN, Berman RM, Roessler E, Belger A, Krystal JH, et al. Transcranial magnetic stimulation of left temporoparietal cortex in three patients reporting hallucinated "voices". *Biol Psychiatry* (1999) 46:130–2. doi:10.1016/S0006-3223(98)00358-8
55. Jasper HH. The ten-twenty electrode system of the international federation. *Electroencephalogr Clin Neurophysiol* (1958) 10:371–5.
56. Landgrebe M, Zeman F, Koller M, Eberl Y, Mohr M, Reiter J, et al. The Tinnitus Research Initiative (TRI) database: a new approach for delineation of tinnitus subtypes and generation of predictors for treatment outcome. *BMC Med Inform Decis Mak* (2010) 10:42. doi:10.1186/1472-6947-10-42
57. Goebel G, Hiller W. The tinnitus questionnaire. A standard instrument for grading the degree of tinnitus. Results of a multicenter study with the tinnitus questionnaire. *HNO* (1994) 42:166–72.
58. Adamchic I, Tass PA, Langguth B, Hauptmann C, Koller M, Schecklmann M, et al. Linking the Tinnitus Questionnaire and the subjective clinical global impression: which differences are clinically important? *Health Qual Life Outcomes* (2012) 10:79. doi:10.1186/1477-7525-10-79
59. World's Health Organization. *WHOQOL-BREF: Introduction, Administration, Scoring*. Geneva, Switzerland: WHO (1996).
60. Langguth B, Goodey R, Azevedo A, Bjorne A, Cacace A, Crocetti A, et al. Consensus for tinnitus patient assessment and treatment outcome measurement: Tinnitus Research Initiative meeting, Regensburg, July 2006. *Prog Brain Res* (2007) 166:525–36. doi:10.1016/S0079-6123(07)66050-6
61. Landgrebe M, Azevedo A, Baguley D, Bauer C, Cacace A, Coelho C, et al. Methodological aspects of clinical trials in tinnitus: a proposal for an international standard. *J Psychosom Res* (2012) 73:112–21. doi:10.1016/j.jpsychores.2012.05.002
62. Leon AC. Implications of clinical trial design on sample size requirements. *Schizophr Bull* (2008) 34:664–9. doi:10.1093/schbul/sbn035
63. Leon AC, Davis LL, Kraemer HC. The role and interpretation of pilot studies in clinical research. *J Psychiatr Res* (2011) 45:626–9. doi:10.1016/j.jpsychires.2010.10.008
64. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav Res Methods* (2007) 39:175–91. doi:10.3758/BF03193146
65. Lehner A, Schecklmann M, Landgrebe M, Kreuzer PM, Poeppl TB, Frank E, et al. Predictors for rTMS response in chronic tinnitus. *Front Syst Neurosci* (2012) 6:11. doi:10.3389/fnsys.2012.00011
66. Kleinjung T, Eichhammer P, Landgrebe M, Sand P, Hajak G, Steffens T, et al. Combined temporal and prefrontal transcranial magnetic stimulation for tinnitus treatment: a pilot study. *Otolaryngol Head Neck Surg* (2008) 138:497–501. doi:10.1016/j.otohns.2007.12.022
67. Lehner A, Schecklmann M, Kreuzer PM, Poeppl TB, Rupprecht R, Langguth B. Comparing single-site with multisite rTMS for the treatment of chronic tinnitus – clinical effects and neuroscientific insights: study protocol for a randomized controlled trial. *Trials* (2013) 14:269. doi:10.1186/1745-6215-14-269

68. Duecker F, Sack AT. Rethinking the role of sham TMS. *Front Psychol* (2015) 6:210. doi:10.3389/fpsyg.2015.00210
69. Kreuzer PM, Landgrebe M, Schecklmann M, Poeppl TB, Vielsmeier V, Hajak G, et al. Can temporal repetitive transcranial magnetic stimulation be enhanced by targeting affective components of tinnitus with frontal rTMS? A randomized controlled pilot trial. *Front Syst Neurosci* (2011) 5:88. doi:10.3389/fnsys.2011.00088
70. Schecklmann M, Lehner A, Gollmitzer J, Schmidt E, Schlee W, Langguth B. Repetitive transcranial magnetic stimulation induces oscillatory power changes in chronic tinnitus. *Front Cell Neurosci* (2015) 9:421. doi:10.3389/fncel.2015.00421

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CASE REPORT
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Management of Chronic Tinnitus and Insomnia with Repetitive Transcranial Magnetic Stimulation and Cognitive Behavioral Therapy – a Combined Approach

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It has been estimated that up to 80% of people will experience symptoms of tinnitus over the courses of their lives, with rates of comorbid sleeping problems ranging from 50 to 77%. Because of a potential connection between tinnitus and sleep disorders as well as high rates of comorbid psychiatric disorders, interdisciplinary approaches to treatment seem to be the most efficient option. In this study, we present the case of a 53-year-old male patient, who started to experience symptoms of tinnitus at the age of 49, most likely caused by work-related stress. Over the course of his illness, the patient developed comorbid insomnia. He consulted us for treatment of both conditions and we developed a treatment plan with ten sessions of repetitive transcranial magnetic stimulation (rTMS) followed by 10 sessions of cognitive behavioral therapy (CBT). We used the *Tinnitus Fragebogen* (TF) to assess the severity of the tinnitus, the Beck Depression Inventory (BDI-II) for depressive symptoms, and the WHO Well-being Index (WHO-5) for subjective well-being. Improvements could be achieved with regard to both diagnoses and the patient went from severe (48) to clinically negligible (12) TF scores, from minimal (BDI-II score 10) to no (0) depressive symptoms, and from just above critical (WHO-5 percentile 52) to above average (84) well-being. The combination of technological and psychological approaches to treat tinnitus and insomnia thus proved successful in this case. One may therefore conclude that rTMS may be considered an effective first therapeutic step for tinnitus treatment prior to CBT. To our knowledge this is the first published case in which rTMS and CBT were combined for tinnitus therapy. The approach proved successful since it led to a considerable increase in well-being and everyday functioning. To gauge the effect on a more general level, large-scale studies are still needed to cancel out potential placebo effects. Likewise, the importance of the order of the two treatments, and the possibility of using other therapies in combination with CBT to address certain tinnitus subtypes and different etiologies must be studied in greater detail.

Keywords: tinnitus, insomnia, sleep, rTMS, CBT

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INTRODUCTION

When the patient first consulted us for treatment at the interdisciplinary tinnitus clinic at Klinikum Nürnberg, we performed an ear-nose-throat examination, an assessment of his psychological-psychiatric state and a basic sleep examination. These resulted in the diagnosis of an ear noise that had been chronified for 4 years and not been treated in any way yet. The patient reported that he had noticed the ear noise for the first time in connection with high pressure in his job. The ear noise made him feel helpless and caused severe difficulties to fall and stay asleep at night. He could not relax and his ability to work as well as his general well-being were severely impaired.

To deal with this situation, the patient had initially resorted to alcohol consumption in the evening as a first coping strategy (up to 7 servings of beer at a time, 3–4 days a week, at a bodyweight of 120 kg) but had already returned to abstinence when first consulting our clinic. At the time of this first examination, the patient was a non-smoker, working full time, and married.

As part of the initial assessment, we performed conventional audiometry, which showed a discrete sensorineural hearing loss in frequencies higher than 6 kHz. A cranial MRI did not show any anomalies in the patient's brain structure. Blood work revealed no signs of anemia, decreased kidney function or abnormal levels of glucose. Two markers of liver function were slightly above normal ranges (GPT > 50 U/l at 56 U/l; gamma-GT > 55 U/l at 73 U/l), possibly due to the patient's history of alcohol abuse. Levels of triglycerides (>150 mg/dl at 372 mg/dl) and cholesterol (>200 mg/dl at 238 mg/dl) were also increased, corresponding to the patient's diagnosed adiposis. TSH levels were very low (<0.40 mU/l at 0.03 mU/l), indicating possible hyperthyroidism. The patient adhered to the following medication plan: Allopurinol 300 mg, Olmesartan medoxomil – Amlodipin 40 mg/5 mg combination.

The patient's tinnitus symptoms were diagnosed as decompensated and severe according to the German tinnitus questionnaire TF (raw score 48, corresponds to percentile rank 54 and degree of severity 3; Goebel and Hiller, 1998). For tinnitus characterization, we used the TSCHQ (Landgrebe et al., 2010; Müller et al., 2016) in the German translation. The patient himself described the tinnitus as a permanent bilateral buzzing inside the head at very high frequencies, and a loudness level of 70/100 (based on a numerical rating scale from the TSCHQ). Detailed results of the assessment are presented in **Table 1**. An otoscopic examination showed normal, bilaterally intact tympanic membranes. In the Weber test, there was no lateralization at 440 Hz. A PTA test showed mild bilateral sensorineural hearing loss (right ear with a maximum of 30 dB HL at 6 kHz, left ear with a maximum of 40 dB HL at 4 kHz; assessed range: 125 Hz–8 kHz). TOAEs and DPOAEs could not be determined on a repeated basis (Supplementary Figure 1). We did not perform tone matching procedures to characterize "tinnitus pitch."

In his self-anamnesis, the patient listed tension headaches up to three times a week and arthrosis in the shoulder joints. The

TABLE 1 | Tinnitus anamnesis by TSCHQ (Landgrebe et al., 2010; Müller et al., 2016).

Item	Answer
Age	53
Sex	Male
Handedness	Right
Tinnitus occurrence in family	No
Onset of tinnitus	4 years ago
Perceiving the onset of tinnitus	Gradual
Relation of initial onset of tinnitus	None
Pulsation of tinnitus	No
Location of tinnitus	Inside the head
Tinnitus manifestation over time	Constant
Tinnitus loudness variation from day to day	Yes
Loudness of tinnitus (1–100)	70
Description of tinnitus	Buzzing, similar to a defective fluorescent tube
Sound of tinnitus	Noise
Pitch of tinnitus	Very high
Percentage of total awake time of tinnitus awareness	90
Percentage of total awake time being distressed by tinnitus	30
Number of different tinnitus treatments	0
Reduction of tinnitus by music or environmental sounds	Yes
Worsening of tinnitus by loud noise	Do not know
Tinnitus affected by head movement or touch	No
Tinnitus affected by nap	Yes, worsening of Tinnitus
Tinnitus affected by sleep at night	Do not know
Tinnitus affected by stress	Yes, worsening of Tinnitus
Tinnitus affected by medication	No
Hearing problem	Yes
Hearing aids	No
Problems tolerating sounds	Sometimes
Sounds cause pain or physical discomfort	Yes
Headache	Yes
Vertigo or dizziness	Yes
Temporomandibular disorder	No
Neck pain	Yes
Other pain syndromes	Yes (shoulder)
Currently under treatment for psychiatric problems	Yes

Abbreviations: ADANO, Arbeitsgemeinschaft Deutschsprachiger Audiologen, Neurootologen und Otologen German-speaking Audiologists, Neurootologists and Otologists; AHI, apnea–hypopnea index; BDI-II, Beck Depression Inventory (Hautzinger et al., 2006); CBT, cognitive behavioral therapy; DGHNOKHC, Deutsche Gesellschaft für Hals-Nasen-Ohren-Heilkunde, Kopf- und Hals-Chirurgie German Society of Oto-Rhino-Laryngology, Head and Neck Surgery; ESS, Epworth Sleepiness Scale (Johns, 1991); ICD-10, International Classification of Diseases (Dilling et al., 1994); MRI, magnetic resonance imaging; n-CPAP, nasal continuous positive airway pressure; PTA, pure tone audiometry; RDI, respiratory disturbance index; rTMS, repetitive transcranial magnetic stimulation; TEOAE, transiently evoked otoacoustic emissions; TF, Tinnitus Fragebogen (Goebel and Hiller, 1998); TSCHQ, Tinnitus Sample Case History Questionnaire (Landgrebe et al., 2010); WHO-5, WHO-Five Well-Being Index (Brähler et al., 2007).

pain caused by the arthrosis in the left shoulder was treated with conservative forms of therapy in an outpatient setting.

Regarding his sleep quality and daytime alertness, the patient stated to be experiencing considerable daytime sleepiness (Epworth Sleepiness Scale ESS = 14 – severe sleepiness, pre-therapeutic; Johns, 1991), which was aggravated by difficulties to fall and/or stay asleep at night. Here, a clear worsening could be observed over the course of time: while initially it took the patient approximately 30 min to fall asleep, this period gradually extended to times between 30 and 90 min. The patient also reported severe sleep maintenance problems, combined with early morning awakening. This meant that, in general, the patient's overall sleeping time amounted to approximately 4.5 h. A screening with polygraphy showed habitual and at times obstructive snoring as well as a RDI of 7/h. Due to the pauses in respiration found by third-party anamnesis, a polysomnography was performed, in which an upper airway resistance syndrome was diagnosed.

In the consultation sessions, the patient made the impression to be awake, completely oriented, alert and conscious. However, a further psychological exploration showed that he was clearly suffering from inner tension, irritability and an impaired mood and a considerable decrease in drive, both in his private and professional life. The patient tried to counteract his daytime sleepiness by taking several compensatory rests and sleep breaks during the day. With regard to his cognitive and mnestic functions the psychological assessment did not show any signs of an impairment. No suicidal tendencies were observed. Regarding his general mood and possible depressive symptoms, our assessment using the BDI-II (Hautzinger et al., 2006) resulted in a raw score of 10, which corresponds to minimal depressive symptoms.

As to the patient's ear noise, the following diagnoses were made according to ICD-10 research criteria (Dilling et al., 1994): holocephalic, decompensated and chronified Tinnitus aurium (ICD-10 H93.1) with comorbid decompensated insomnia (ICD-10 F51.0) as well as habitual and obstructive snoring (ICD-10 R06.5) and an upper airway resistance syndrome (ICD-10 G47.31). Moreover, arterial hypertonia (ICD-10 I10.0), adipositas (ICD-10 E66.0) and alcohol abuse with current abstinence (ICD 10 F10.1) were diagnosed.

BACKGROUND

Subjective tinnitus is defined as the perception of a sound in absence of an objective source (Møller, 2003). Describing its prevalence can provide a challenge, as numbers vary with age and gender (Møller, 2011). Studies postulate a lifetime prevalence of about 80% (Møller, 2011) and a point prevalence between 1 and 22.2% (Møller, 2011; Langguth et al., 2013; Kim et al., 2015; Kreuzer et al., 2016). It is assumed that between 50 and 77% of all tinnitus patients develop sleeping problems, especially those suffering from more severe forms (Crönlein et al., 2016).

With regard to effective forms of treatment, tinnitus "remains a clinical enigma" (Müller et al., 2016) due to its high levels of heterogeneity and complexity. Tinnitus may appear in various forms, regarding pattern, site, loudness or pitch of the sound, as well as other perceptual characteristics (Langguth et al., 2013). Furthermore, it can relate to several possible etiologies and comorbidities (Müller et al., 2016). A great majority of tinnitus cases do involve sensorineural hearing loss. Central auditory system reorganization is thought to be a prerequisite for chronic tinnitus.

Recent reviews (Langguth et al., 2013; Minen et al., 2014) concluded, that tinnitus may be caused by disturbances in peripheral auditory, central auditory, or non-auditory systems, by temporomandibular joint disorders, as well as by psychological factors. Regarding this last etiology, which, in our opinion, is central in this case, Mazurek et al. (2015) summarize several studies, that demonstrate associations between stress and tinnitus. According to current research, chronic tinnitus may also be triggered by muscular tension (somatosensory tinnitus), specifically in the neck (cervicogenic tinnitus) and can also appear in connection with a cervical pain syndrome, both with and without vertigo (Bechter et al., 2016). We therefore hypothesized, that a multidisciplinary, combinatory approach to treatment targeting multiple blocks could improve the treatment outcome.

When it comes to comorbid sleeping problems, reports on cases like the one discussed above suggest treating tinnitus and insomnia in conjunction: here, psychotherapy (Crönlein et al., 2007), surgery (Chen et al., 2010), as well as non-traditional approaches (Okamoto et al., 2005) may be appropriate means of therapy.

Cognitive behavioral therapy (CBT), relaxation techniques, and rTMS likewise appear to be safe and effective treatments for chronic tinnitus as they have been shown to increase the general quality of life and lower tinnitus symptoms without adverse events (Cima et al., 2012; Soleimani et al., 2016; Zenner et al., 2016). However, rTMS in particular still seems to lack the necessary methodological fine-tuning and has therfore produced moderate to large, but highly variable effects so far (Hesser et al., 2011; Langguth et al., 2013; Kreuzer et al., 2016; Soleimani et al., 2016). Other current forms of treatment were either not feasible in this case due to a lack of time and resources (e.g., notched music therapy (Okamoto et al., 2010), cochlear implantation (Olze et al., 2012) or because of their reported non to mixed-success [e.g., vagus nerve stimulation (De Ridder et al., 2014), AM-101 (van de Heyning et al., 2014), or other drugs (Kingwell, 2016)].

RESULTS

Tinnitus Therapy

Tinnitus Therapy with rTMS

A first treatment attempt was made in October 2010. This initial treatment consisted of 10 sessions of 1 Hz rTMS over 10 consecutive working days with a stimulation intensity of 110% related to the individual resting motor threshold. 2000 stimuli per session were applied, with coil position 10–20 guided over the left primary auditory cortex.

At the time of therapy, our research group had 2 years of clinical experience in treating patients with a chronified tinnitus (Acker et al., 2010). In choosing the stimulation protocol, we followed the experiences by the interdisciplinary Regensburg working group (Kleinjung et al., 2005), who had proven a metabolic activation of the auditory cortex in a series of tinnitus patients with a similar disease process. A placebo-controlled, multicenter study including eight German participant centers on the efficacy of rTMS likewise used the treatment protocol described above (Landgrebe et al., 2008).

The primary therapeutic aim of the rTMS treatment was a favorable modulation of the patient's tinnitus perception. An improvement in the subjective degree of severity of the symptoms could be achieved without any side effects. The main effect of the therapy was a change in the frequency and the subjective intensity of the tinnitus directly after the treatment. The initially very strong effect of the therapy decreased markedly after 3–4 h after the end of each treatment session, though. Limitations in our evaluation are the missing tone matching of tinnitus frequency and the missing high frequency audiometry (>8 kHz).

Figures 1A–C show the development of the patient's raw scores across three different psychometric questionnaires (TF, BDI-II, and WHO-5) assessed at four points in time (Timespans between measurements: pre rTMS to post rTMS: 3 weeks; post TMS to pre CBT: 2 weeks; pre CBT to post CBT: 1 weeks).

The TF questionnaire is an instrument to measure tinnitus severity ($\alpha = 0.94$; range: 0–84; Goebel and Hiller, 1998). Within the approximately 3 weeks of rTMS treatment, TF scores decreased from 48 (degree 3 of 4, decompensated tinnitus) to 38 (degree 2 of 4, compensated tinnitus). The BDI-II was used to measure depressive symptoms ($\alpha = 0.84$–0.94; range: 0–63; Hautzinger et al., 2006). After rTMS treatment, BDI-II scores only slightly changed from 10 to 9 (minimal depressive symptoms). For the measurement of subjective well-being we used the WHO-5 ($\alpha = 0.92$; range: 0–100, critical cut-off: 52, average score: 70; Brähler et al., 2007). WHO-5 scores increased from 52 (critical cut-off) to 76 (normal well-being).

Tinnitus Therapy with CBT

Due to the persistent professional impairment, we continued our treatment with 10 sessions of CBT as recommended by German guidelines for tinnitus management by the ADANO (Society of German-speaking Audiologists, Neurootologists, and Otologists; Lenarz, 1998) and the DGHNOKHC (German Society of Oto-Rhino-Laryngology, Head and Neck Surgery; AWMF, 2015).

The primary therapeutic aim of the behavioral therapy was to develop coping strategies to reduce the subjective impairment caused by the tinnitus as well as the resulting emotional stress. To do so, the following steps were taken: the subjective illness hypothesis was explored and discussed and medical information and explanatory models were provided. Subsequently, a connection between situative stress and tinnitus perception was worked out and, with the help of cognitive restructuring, helpful forms of behavior were successfully transferred to hitherto discouraging situations.

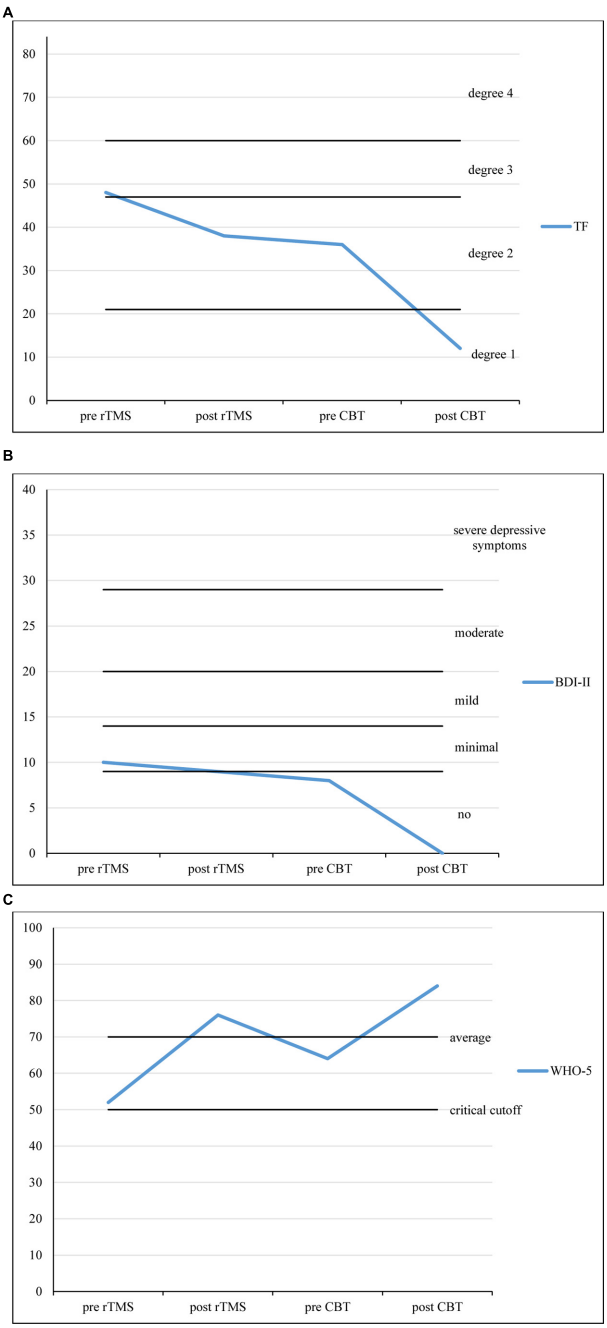

FIGURE 1 | (A) TF scores over the course of treatment. **(B)** BDI-II scores over the course of treatment. **(C)** WHO-5 scores over the course of treatment. Timespans between measurements: pre rTMS to post rTMS: 3 weeks; post TMS to pre CBT: 2 weeks; pre CBT to post CBT: 15 weeks.

The patient particularly profited from practicing progressive muscle relaxation according to Jacobson as well as breathing exercises as a further relaxation method.

Within the approximately 15 weeks of CBT treatment, the patient's TF scores decreased from 36 (degree 2 of 4, compensated tinnitus) to 12 (degree 1 of 4, compensated tinnitus). BDI-II

scores had decreased to 8 (no depressive symptoms) within the 2 weeks between the last session of rTMS and the first session of CBT. They stayed at 0 after CBT treatment. WHO-5 scores increased once more, from 64 (average well-being) to 84 (above average well-being).

Therapy of Insomnia

In view of the high subjective affliction, the patient's sleep problems were likewise addressed with behavioral therapeutic means. Here, the main measures employed were sleep restriction and sleep hygiene (Backhaus and Riemann, 1999), as well as psychoeducation regarding sleep, cognitive restructuring of negative thoughts, stimulus control, and relaxation techniques (Piehl et al., 2010; Richter, 2015).

A polysomnography carried out subsequently found an AHI of 8/h and an increased number of respiratory arousal reactions (arousal index 15/h sleep). On diagnosis of an upper airway resistance syndrome (American Academy of Sleep Medicine, 2005) treatment with n-CPAP therapy was initiated. The snoring could be eliminated completely with average application times of 6 h/night. The patient's compliance with CPAP treatment was very good in general.

Further Development

After both courses of treatment (rTMS and CBT) for the tinnitus symptoms had been completed, the patient reported a notable improvement of his general state. GAF scores (global assessment of functioning; range: 0–90; Hall, 1995) increased from 45 (serious symptoms or impairment) to 68 (some mild symptoms). The ear noise and the resulting emotional distress had been reduced considerably. TF scores decreased from 48 (degree 3 of 4, decompensated tinnitus) to 12 (degree 1 of 4, compensated tinnitus). BDI-II scores decreased from 10 (minimal depressive symptoms) to 0 (no depressive symptoms). This 10-point change may be interpreted as moderate and clinically significant (Hautzinger et al., 2006). However, it must be mentioned that a placebo response to r-TMS treatment may be responsible for this change as well as intraindividual variation of subclinical BDI-scores.

WHO-5 scores increased from 52 (critical cut-off 52) to 84 (above average well-being). Likewise, there were great improvements regarding emotional balance, drive, the level of daytime sleepiness, the patient's ability to concentrate and his general mood. Light improvements were reported concerning the ability to relax and the ability to perform.

The patient profited especially from psychoeducation on tinnitus. He could be discharged from treatment with a clearly increased self-efficacy. Throughout the entire course of treatment, the patient's compliance was very good and co-operative. The patient also did not return to alcohol as a coping strategy but stayed abstinent throughout the entire course of treatment.

Summary

The case of our patient demonstrates the complexity of a chronified Tinnitus aurium modulated by comorbidities. Conventional PTA showed a mild notch like sensorineural hearing loss, while high frequency hearing > 8 kHz was not tested.

At the beginning of the therapy, the patient demanded a technical approach to a somatically fixated disease model. After comprehensive information on the neurological nexuses (Tyler, 2006) we agreed to a treatment attempt using rTMS. The treatment protocol follows the experiences of the Regensburg working group led by Kleinjung et al. (2007) and Langguth et al. (2015).

The psychiatric comorbidity of the present case is low for the characteristic tinnitus patient. Other working groups report a high number of afflictions from the depression – anxiety disorders range in their patient collectives (Schaaf et al., 2003; Zirke et al., 2010).

Tinnitus can have a highly negative impact on a person's professional and private life (Zirke et al., 2013) and cause society considerable follow-up costs. Here, the severity of the tinnitus and psychiatric comorbidities are the main factors increasing costs (Maes et al., 2013). For this reason, multimodal therapy up to the point where a stability of symptoms is reached has considerable significance.

A connection between ear noises and sleeping disorders has been reported since the beginning of the 1990s (Alster et al., 1993). In some current reviews, the frequently comorbid sleep problems are conceptualized as an attendant symptom of psychiatric disorders (Mazurek et al., 2005). In contrast, other studies find insomnia-related problems in older tinnitus patients in over 50% of cases (Lasisi and Gureje, 2011). Likewise, there seems to be an increased prevalence of sleep-related respiratory disorders in tinnitus collectives (Fischer et al., 2003; Herold et al., 2010).

Regarding our patient, we hypothesize a modulation of the tinnitus perception by the insomnia understood as a hyperarousal disorder (Crönlein et al., 2016). In the presence of high subjective stress levels the interaction of auditory and limbic brain areas may be disturbed at the thalamic level, leading to a breakdown of an internal "noise-cancelation" mechanism. In this case, the activation by catastrophizing and fear seem to have played a central role (Cima et al., 2011). Potentially, there is also a bi-directional connection between tinnitus and insomnia (Rauschecker et al., 2010).

Our patient did not suffer from cervical pain or vertigo, and the tinnitus did not change after neck inclination. This lead us to exclude the cervical etiology in this case (Bechter et al., 2016).

Modern therapy concepts recommend a tailored approach in planning the therapy (Seydel et al., 2013). Similarly, for an inpatient-setting, evaluated therapy concepts are available by now (Täuber, 2012).

CONCLUDING REMARKS

If healthcare professionals were to choose a standalone treatment for tinnitus symptoms, current evidence suggests selecting validated tinnitus-specific CBT over alternatives, such as rTMS (Zenner et al., 2016). In our case, however, rTMS therapy made it possible to start therapy at all because of a somatically

fixated disease model. An optimization of the treatment protocol to improve the sustainability of the therapy seems sensible. We noticed a relevant improvement of the patient's everyday functionality could be achieved by a stabilization of his insomnia problems. According to our assessment, a successful therapy could only be established by the combination of rTMS with tinnitus and sleep-related elements from CBT. The efficacy of this personalized combined treatment approach (Golubnitschaja and Costigliola, 2012) in larger samples will be investigated in future research.

ETHICS STATEMENT

As all described interventions were part of clinical everyday practice, we did not consult with an ethics committee for this study. The patient gave written informed consent in accordance with the Declaration of Helsinki. The Patient also gave the consent for the analysis, processing, and publication of his data.

AUTHOR CONTRIBUTIONS

KR and JA contributed equally as first authors and wrote the manuscript. LM and LP contributed to the scientific design. GN contributed to data acquisition.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fpsyg.2017.00575/full#supplementary-material

REFERENCES

Acker, J., Scholz, F., Richter, K., and Niklewski, G. (2010). Behandlungseffekte der repetitiven transkraniellen Magnetstimulation (rTMS) bei Patienten mit chronischem Tinnitus aurium: Erste Ergebnisse der Behandlung mit niederfrequenter rTMS. *Psychiatr. Forsch. Suppl.* 1, 9–12. doi: 10.5283/pf.19

Alster, J., Shemesh, Z., Ornan, M., and Attias, J. (1993). Sleep disturbance associated with chronic tinnitus. *Biol. Psychiatry* 34, 84–90. doi: 10.1016/0006-3223(93)90260-K

American Academy of Sleep Medicine (2005). *International Classification of Sleep Disorders, Revised: Diagnostic and Coding Manual*, 2nd Edn. Chicago, IL: American Sleep Disorders Association.

AWMF (2015). *German S3 Guideline Ol 7/064: Chronic tinnitus. Current revision 02/2015. Leitlinie Chronischer Tinnitus Deutsche Gesellschaft für Hals-Nasen-Ohren-Heilkunde*. Available at: http://www.awmf.org/fileadmin/user_upload/Leitlinien/017_D_G_f_Hals-Nasen-Ohrenheilkunde__Kopf-_und_Halschirurgie/017-064e_S3_guideline_tinnitus_english_2015-08.pdf

Backhaus, J., and Riemann, D. (1999). *Schlafstörungen. Fortschritte der Psychotherapie*, Vol. 7. Göttingen: Hogrefe, Verl. für Psychologie.

Bechter, K., Wieland, M., and Hamann, G. F. (2016). Chronic cervicogenic tinnitus rapidly resolved by intermittent use of cervical collar. *Front. Psychiatry* 7:43. doi: 10.3389/fpsyt.2016.00043

Brähler, E., Mühlan, H., Albani, C., and Schmidt, S. (2007). Teststatistische prüfung und normierung der deutschen versionen des EUROHIS-QOL lebensqualität-Index und des WHO-5 wohlbefindens-index. *Diagnostica* 53, 83–96. doi: 10.1026/0012-1924.53.2.83

Chen, M.-C., Chung, W.-Y., Luo, C.-B., and Wu, H.-M. (2010). Arteriovenous malformation in the parotid region presenting as pulsatile tinnitus: a case report. *Head Neck* 32, 262–267. doi: 10.1002/hed.21063

Cima, R. F. F., Crombez, G., and Vlaeyen, J. W. S. (2011). Catastrophizing and fear of tinnitus predict quality of life in patients with chronic tinnitus. *Ear Hear.* 32, 634–641. doi: 10.1097/AUD.0b013e31821106dd

Cima, R. F. F., Maes, I. H., Joore, M. A., Scheyen, D. J., El Refaie, A., Baguley, D. M., et al. (2012). Specialised treatment based on cognitive behaviour therapy versus usual care for tinnitus: a randomised controlled trial. *Lancet* 379, 1951–1959. doi: 10.1016/S0140-6736(12)60469-3

Crönlein, T., Langguth, B., Geisler, P., and Hajak, G. (2007). Tinnitus and insomnia. *Prog. Brain Res.* 166, 227–233. doi: 10.1016/S0079-6123(07)66021-X

Crönlein, T., Langguth, B., Pregler, M., Kreuzer, P. M., Wetter, T. C., and Schecklmann, M. (2016). Insomnia in patients with chronic tinnitus: cognitive and emotional distress as moderator variables. *J. Psychosom. Res.* 83, 65–68. doi: 10.1016/j.jpsychores.2016.03.001

De Ridder, D., Vanneste, S., Engineer, N. D., and Kilgard, M. P. (2014). Safety and efficacy of vagus nerve stimulation paired with tones for the treatment of tinnitus: a case series. *Neuromodulation* 17, 170–179. doi: 10.1111/ner.12127

Dilling, H., Mombour, W., Schmidt, M. H., and Schulte-Markwort, E. (1994). *Internationale Klassifikation Psychischer Störungen, ICD-10, Forschungskriterien*. Göttingen: Hogrefe.

Fischer, Y., Yakinthou, A., and Mann, W. J. (2003). Zur Prävalenz der obstruktiven Schlafapnoe (OSA) bei Patienten mit Hörsturz. Eine Pilotstudie [Prevalence of obstructive sleep apnea syndrome (OSA) in patients with sudden hearing loss. A pilot study]. *HNO* 51, 462–466. doi: 10.1007/s00106-002-0712-y

Goebel, G., and Hiller, W. (1998). *Tinnitus-Fragebogen:(TF); ein Instrument zur Erfassung von Belastung und Schweregrad bei Tinnitus; Handanweisung*. Göttingen: Hogrefe, Verl. für Psychologie.

Golubnitschaja, O., and Costigliola, V. (2012). General report and recommendations in predictive, preventive and personalized medicine 2012: white paper of the European Association for Predictive, Preventive and Personalized Medicine. *EPMA J.* 3:14. doi: 10.1186/1878-5085-3-14

Hall, R. C. (1995). Global assessment of functioning. A modified scale. *Psychosomatics* 36, 267–275. doi: 10.1016/S0033-3182(95)71666-8

Hautzinger, M., Keller, F., and Kühner, C. (2006). *Beck Depressions-Inventar II (BDI-II)*. Frankfurt: Harcourt Test Service.

Herold, J., Piehl, A., Richter, K., Ficker, J., Niklewski, G., and Acker, J. (2010). Prävalenz einer obstruktiven Schlafapnoe bei Tinnituspatienten mit insomnischen Beschwerden. *Somnologie* 14(Suppl. 1), 71. doi: 10.1007/s11818-010-0489-2

Hesser, H., Weise, C., Westin, V. Z., and Andersson, G. (2011). A systematic review, and meta-analysis of randomized controlled trials of cognitive-behavioral therapy for tinnitus distress. *Clin. Psychol. Rev.* 31, 545–553. doi: 10.1016/j.cpr.2010.12.006

Johns, M. W. (1991). A new method for measuring daytime sleepiness: the Epworth Sleepiness Scale. *Sleep* 14, 540–545. doi: 10.1093/sleep/14.6.540

Kim, H.-J., Lee, H.-J., An, S.-Y., Sim, S., Park, B., Kim, S. W., et al. (2015). Analysis of the prevalence and associated risk factors of tinnitus in adults. *PLoS ONE* 10:e0127578. doi: 10.1371/journal.pone.0127578

Kingwell, K. (2016). First hearing-disorder drugs stumble. *Nat. Rev. Drug Discov.* 15, 733–735. doi: 10.1038/nrd.2016.222

Kleinjung, T., Eichhammer, P., Langguth, B., Jacob, P., Marienhagen, J., Hajak, G., et al. (2005). Long-term effects of repetitive transcranial magnetic stimulation (rTMS) in patients with chronic tinnitus. *Otolaryngol. Head Neck Surg.* 132, 566–569. doi: 10.1016/j.otohns.2004.09.134

Kleinjung, T., Steffens, T., Londero, A., and Langguth, B. (2007). Transcranial magnetic stimulation (TMS) for treatment of chronic tinnitus: clinical effects. *Prog. Brain Res.* 166, 359–367. doi: 10.1016/S0079-6123(07)66034-8

Kreuzer, P. M., Poeppl, T. B., Bulla, J., Schlee, W., Lehner, A., Langguth, B., et al. (2016). A proof-of-concept study on the combination of repetitive transcranial magnetic stimulation and relaxation techniques in chronic tinnitus. *J. Neural Transm.* 123, 1147–1157. doi: 10.1007/s00702-016-1588-4

Landgrebe, M., Binder, H., Koller, M., Eberl, Y., Kleinjung, T., Eichhammer, P., et al. (2008). Design of a placebo-controlled, randomized study of the efficacy

of repetitive transcranial magnetic stimulation for the treatment of chronic tinnitus. *BMC Psychiatry* 8:23. doi: 10.1186/1471-244X-8-23

Landgrebe, M., Zeman, F., Koller, M., Eberl, Y., Mohr, M., Reiter, J., et al. (2010). The Tinnitus Research Initiative (TRI) database: a new approach for delineation of tinnitus subtypes and generation of predictors for treatment outcome. *BMC Med. Inform. Decis. Mak.* 10:42. doi: 10.1186/1472-6947-10-42

Langguth, B., Kreuzer, P. M., Kleinjung, T., and de Ridder, D. (2013). Tinnitus: causes and clinical management. *Lancet Neurol.* 12, 920–930. doi: 10.1016/S1474-4422(13)70160-1

Langguth, B., Landgrebe, M., Schecklmann, M., Schönfeldt-Lecuona, C., Höppner, J., Padberg, F., et al. (2015). Chronischer tinnitus. therapeutischer einsatz von rTMS. *Nervenheilkunde* 34, 987–993.

Lasisi, A. O., and Gureje, O. (2011). Prevalence of insomnia and impact on quality of life among community elderly subjects with tinnitus. *Ann. Otol. Rhinol. Laryngol.* 120, 226–230. doi: 10.1177/000348941112000402

Lenarz, T. (1998). Leitlinie tinnitus der deutschen gesellschaft für hals-nasen-ohren-heilkunde, kopf-und hals-chirurgie. *Laryngorhinootologie* 77, 531–535. doi: 10.1055/s-2007-997020

Maes, I. H. L., Cima, R. F. F., Vlaeyen, J. W., Anteunis, L. J. C., and Joore, M. A. (2013). Tinnitus: a cost study. *Ear Hear.* 34, 508–514. doi: 10.1097/AUD.0b013e31827d113a

Mazurek, B., Georgiewa, P., Seydel, C., Haupt, H., Scherer, H., Klapp, B. F., et al. (2005). Integrierte tinnitusintensivbehandlung: konzept und erste praktische erfahrungen. *Gesundheitswesen* 67, 485–491. doi: 10.1055/s-2005-858379

Mazurek, B., Szczepek, A. J., and Hebert, S. (2015). Stress and tinnitus. *HNO* 63, 258–265. doi: 10.1007/s00106-014-2973-7

Minen, M. T., Camprodon, J., Nehme, R., and Chemali, Z. (2014). The neuropsychiatry of tinnitus: a circuit-based approach to the causes and treatments available. *J. Neurol. Neurosurg. Psychiatr.* 85, 1138–1144. doi: 10.1136/jnnp-2013-307339

Møller, A. R. (2003). Pathophysiology of tinnitus. *Otolaryngol. Clin. North Am.* 36, 249–266. doi: 10.1016/S0030-6665(02)00170-6

Møller, A. R. (2011). *Textbook of Tinnitus*. New York, NY: Springer Publishing Company. doi: 10.1007/978-1-60761-145-5

Müller, K., Edvall, N. K., Idrizbegovic, E., Huhn, R., Cima, R. F., Persson, V., et al. (2016). Validation of online versions of tinnitus questionnaires translated into Swedish. *Front. Aging Neurosci.* 8:272. doi: 10.3389/fnagi.2016.00272

Okamoto, H., Okami, T., Ikeda, M., and Takeuchi, T. (2005). Effects of Yoku-kan-san on undifferentiated somatoform disorder with tinnitus. *Eur. Psychiatry* 20, 74–75. doi: 10.1016/j.eurpsy.2004.09.034

Okamoto, H., Stracke, H., Stoll, W., and Pantev, C. (2010). Listening to tailor-made notched music reduces tinnitus loudness and tinnitus-related auditory cortex activity. *Proc. Natl. Acad. Sci. U.S.A.* 107, 1207–1210. doi: 10.1073/pnas.0911268107

Olze, H., Szczepek, A. J., Haupt, H., Zirke, N., Graebel, S., and Mazurek, B. (2012). The impact of cochlear implantation on tinnitus, stress and quality of life in postlingually deafened patients. *Audiol. Neurootol.* 17, 2–11. doi: 10.1159/000323847

Piehl, A., Richter, K., and Niklewski, G. (2010). Therapieeffekte einer kognitiv-verhaltenstherapeutisch orientierten Insomniebehandlung. *Verhaltenther. Verhaltensmed.* 31, 375–389.

Rauschecker, J. P., Leaver, A. M., and Muhlau, M. (2010). Tuning out the noise: limbic-auditory interactions in tinnitus. *Neuron* 66, 819–826. doi: 10.1016/j.neuron.2010.04.032

Richter, K. (2015). *Schlafen Sie gut!: Ihr Schlafprogramm für Aufgeweckte Tage und Erholsame Nächte,* 1st Edn. Stuttgart: Trias.

Schaaf, H., Dölberg, D., Seling, B., and Märtner, M. (2003). Komorbidität von Tinnituserkrankungen und psychiatrischen Störungen. *Nervenarzt* 74, 72–75. doi: 10.1007/s00115-001-1222-y

Seydel, C., Haupt, H., Olze, H., Szczepek, A. J., and Mazurek, B. (2013). Gender and chronic tinnitus: differences in tinnitus-related distress depend on age and duration of tinnitus. *Ear Hear.* 34, 661–672. doi: 10.1097/AUD.0b013e31828149f2

Soleimani, R., Jalali, M. M., and Hasandokht, T. (2016). Therapeutic impact of repetitive transcranial magnetic stimulation (rTMS) on tinnitus: a systematic review and meta-analysis. *Eur. Arch. Otorhinolaryngol.* 273, 1663–1675. doi: 10.1007/s00405-015-3642-5

Täuber, S. (2012). *Einfluss Einer Stationären Verhaltenstherapeutischen Intervention bei Patienten mit Tinnitus und Insomnie.* Ph.D. dissertation, Universität Regensburg, Regensburg.

Tyler, R. S. (2006). "Neurophysiological models, psychological models, and treatments for tinnitus," in *Tinnitus Treatment. Clinical Protocols*, ed. R. S. Tyler (New York, NY: Thieme), 1–22.

van de Heyning, P., Muehlmeier, G., Cox, T., Lisowska, G., Maier, H., Morawski, K., et al. (2014). Efficacy and safety of AM-101 in the treatment of acute inner ear tinnitus: a double-blind, randomized, placebo-controlled phase II study. *Otol. Neurotol.* 35, 589–597. doi: 10.1097/MAO.0000000000000268

Zenner, H.-P., Delb, W., Kroner-Herwig, B., Jager, B., Peroz, I., Hesse, G., et al. (2016). A multidisciplinary systematic review of the treatment for chronic idiopathic tinnitus. *Eur. Arch. Otorhinolaryngol.* doi: 10.1007/s00405-016-4401-y [Epub ahead of print].

Zirke, N., Goebel, G., and Mazurek, B. (2010). Tinnitus und psychische Komorbiditäten. *HNO* 58, 726–732. doi: 10.1007/s00106-009-2050-9

Zirke, N., Seydel, C., Szczepek, A. J., Olze, H., Haupt, H., and Mazurek, B. (2013). Psychological comorbidity in patients with chronic tinnitus: analysis and comparison with chronic pain, asthma or atopic dermatitis patients. *Qual. Life Res.* 22, 263–272. doi: 10.1007/s11136-012-0156-0

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ORIGINAL RESEARCH
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Results of an Interdisciplinary Day Care Approach for Chronic Tinnitus Treatment: A Prospective Study Introducing the Jena Interdisciplinary Treatment for Tinnitus

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Objective: Considering the heterogeneity of the symptoms shown by patients suffering from chronic tinnitus, there are surprisingly few interdisciplinary treatments available, and mostly available only for inpatients. In order to provide an interdisciplinary treatment, we developed a day care concept in which each patient was treated by an ENT doctor, a cognitive behavioral therapist, a specialist for medical rehabilitation and an audiologist (Jena Interdisciplinary Treatment for Tinnitus, JITT). The aim of this study was to observe the changes of tinnitus related distress due to interdisciplinary day care treatment and to determine which factors mediate this change.

Subjects and Methods: Tinnitus annoyance was measured using the Tinnitus Questionnaire on 308 patients with chronic tinnitus. They were treated in the day care unit over five consecutive days between July 2013 and December 2014. Data were collected before treatment when screened (T0), at the beginning (T1) and at the end of the 5 day treatment (T2), as well as 20 days (T3) and 6 months after treatment (T4).

Results: Overall, tinnitus annoyance improved significantly from the screening day to the beginning of treatment, and to a much larger degree from the beginning to the end of treatment. The treatment outcome remained stable 6 months after treatment. Patients with the following symptoms displayed higher tinnitus annoyance at T0: dizziness at tinnitus onset, tinnitus sound could not be masked with background noise, tinnitus worsening during physical stress, comorbid psychiatric diagnosis, higher age and higher hearing loss. Loudness of tinnitus perceived in the right ear correlated with tinnitus annoyance significantly. Demographic, tinnitus and strain variables could only explain 12.8% of the variance of the change in tinnitus annoyance from T0 to T4. Out of 39 predictors, the only significant ones were "sick leave 6 months before treatment" and "tinnitus annoyance at T0."

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Conclusion: The newly developed JITT represents a valuable treatment for chronic tinnitus patients with improvement remaining stable for at least 6 months after treatment. Using a large number of variables did not allow predicting treatment outcome which underlines the heterogeneity of tinnitus.

Keywords: chronic tinnitus, treatment, interdisciplinary, day care, prediction, tinnitus questionnaire

INTRODUCTION

Tinnitus is widely prevalent and is characterized by experiencing ringing, hissing or similar noises in one or both ears without an external acoustic source. This symptom is in its mild form most often transient, but persists in about 5–10% of the population, leading patients to seek treatment (Henry et al., 2005). As tinnitus is a sensory phenomenon, patients usually consult a physician. Moreover, since tinnitus is a symptom that can arise as a consequence of several disorders, it makes the diagnosis and the resulting treatment rather complex. Some of the possible tinnitus causes are exposure to loud noise, presbyacusis, cardiovascular and cerebrovascular diseases, drugs or medication, ear infections/inflammation, head or neck trauma, hyper- and hypo-thyroidism, Menière's disease, otosclerosis, sudden deafness, or vestibular schwannoma (Hoffman and Reed, 2004). The goal within the first 3 months (acute tinnitus) is to find the cause for the symptomatology and to treat it accordingly. However, in almost 50% of cases no physical origin of the tinnitus can be found (Feldmann, 1992; Lenarz, 1992), leaving the medical practitioner without causal treatment options. To make matters worse, the assumed cause of tinnitus is often successfully treated, but without any influence on the tinnitus itself. This argues in favor of a multifactorial cause, which is supported by the high heterogeneity seen in tinnitus patients.

When lasting more than 3 months and no response to medical treatment can be observed, tinnitus is generally considered as a chronic condition. While most people with chronic tinnitus are able to ignore the sound and do not feel impaired by it, approximately 3–5% of the general adult population perceive tinnitus as extremely bothersome, often to such an extent that it is difficult for them to carry out everyday activities (Davis and El Rafaie, 2000). The most prevalent complaints are concentration problems, mood changes as well as problems with sleep and hearing (Tyler and Baker, 1983; Henry et al., 2005). Additionally, high rates of comorbid psychiatric disorders such as depression, anxiety or somatoform disorders are observed in the group of tinnitus patients with bothersome tinnitus (Sullivan et al., 1988; Zöger et al., 2001). Thus, overall the patients are characterized by a rather large heterogeneity of associated symptoms.

One driving question for the development of therapeutic approaches is why some patients suffer from chronic tinnitus and others do not. Auditory aspects such as pitch, loudness and maskability have been found to be insufficient to explain tinnitus distress (Biesinger and Heiden, 1999; Bleich et al., 2001; Hausotter, 2004; Konzag et al., 2006; Hesse and Schaaf, 2007). One theory that tries to explain the co-occurrence of tinnitus and distress is the neurophysiological model of tinnitus (Jastreboff et al., 1996). According to this model, damage to the auditory pathways plays a crucial role in the development of tinnitus, while other parts of the nervous system (e.g. the limbic system) are responsible for developing tinnitus annoyance. Thus, the dysfunctional interplay between the two systems is responsible for the impact of the impairment on everyday life.

Currently, there is no scientifically proven therapy available that can be considered as a cure for chronic tinnitus. According to the American and German tinnitus guidelines (e.g., American Academy of Otolaryngology-Head Neck Surgery, 2014; German Society of Otorhinolaryngology Head, and Neck Surgery, 2015), the only realistic therapeutic goal is the determination of the tinnitus sensitizing antecedents and their therapeutic manageability, as well as the long term habituation to the phantom noise. Taken together, due to the high heterogeneity of tinnitus and the associated symptoms, an interdisciplinary approach for treatment recommends itself. A recent multidisciplinary systematic review emphasizes the combination of tinnitus specific counseling and cognitive behavior therapy. In the case of hearing loss, additional auditory therapeutic measures (e.g. hearing aids or cochlear implants) should be considered. Comorbidities such as depression should be treated additionally and, if necessary, with drugs (Zenner et al., 2016).

Despite the high prevalence of the impairment, there are only few specialized treatment centers in Germany, and the few existing recommendations for treatment are only rarely fulfilled in clinical practice (Hoare et al., 2012). Very often only inpatients receive interdisciplinary treatments, which is a financially expensive approach. To avoid high expenses, we implemented an interdisciplinary tinnitus treatment in our day care unit. The goal of the treatment was to reduce tinnitus annoyance by addressing the most frequent symptoms that patients with chronic tinnitus complain about: fear of tinnitus, problems with sleep and hearing, inability to relax and concentration problems. The individual treatment was tailored to the specific needs of a patient to account for the individual occurrence and combination of symptoms. Accordingly, we call this approach Jena Interdisciplinary Treatment for Tinnitus (JITT).

The aim of this study was (a) to observe the changes of tinnitus-related distress due to JITT, (b) to investigate in which patients tinnitus annoyance was most strongly expressed at the beginning of the treatment and (c) to explore if treatment success can be predicted.

MATERIALS AND METHODS

Assessment of Patients

The study was conducted in the Tinnitus-Center at the ENT department of Jena University Hospital, including one screening

day, 5 days of day care treatment as well as two follow-up examinations (20 days and 6 months after treatment).

On the screening day all tinnitus patients underwent an examination by an ENT doctor, including ear microscopy, tinnitus case history and history of other ENT symptoms (particularly dizziness). All patients received routine audiometric evaluation including discrete-tone threshold testing and speech audiometry. Audiometry and tinnitus matching were done with calibrated audiometer (MAICO KS5) over Telephonics (TDH 39) headphones. Hearing level (HL in dB) was determined at following frequencies: 125, 250, 500, 750, 1,000, 1,500, 2,000, 3,000, 4,000, 6,000, and 8,000 Hz for the right and left ear for each individual. Pure tone average thresholds (4 PTA) were calculated over the frequencies of 500, 1,000, 2,000, and 4,000 Hz according to the WHO-standard (International Bureau for Audiophonology, 2017). In a second step, the frequency/pitch of the tinnitus was determined. When the patient reported binaural tinnitus, pitch was matched for each ear individually. In case of multiple tinnitus it was suggested that one should concentrate on the most troublesome tinnitus. The patients were asked whether the tinnitus sounds like a pure tone as just perceived during the audiometry, or if it sounds like a broad band or a narrow band noise. Under this directive pure tones or narrow bands of noise or broad band noise were presented to the tinnitus ear. If the pure tone threshold was too high to perceive a test signal at the side of the tinnitus, the contralateral better ear was used to present the sound. When tinnitus pitch was determined, subject's threshold was determined at that frequency. The procedure started at the frequency determined during pitch matching and at a level just below threshold. Then the intensity was increased in 1-dB steps until the patient signaled a match.

In cases of dizziness, vestibular testing was conducted. If indicated (e.g., asymmetric hearing loss, vertigo, headache), other diagnostic procedures (e.g., magnetic resonance imaging) or drug treatment were performed prior to treatment.

Tinnitus-related distress was assessed with the Tinnitus Questionnaire (TQ; Goebel and Hiller, 1998), a standard measure to differentiate patients with mild and severe tinnitus distress based on Hallam's Tinnitus-Questionnaire (Hallam, 1996). Specific fields of distress were assessed by subscales labeled as emotional and cognitive distress, intrusiveness, hearing problems, sleep disturbances, and somatic complaints evoked by the tinnitus. A total sum score ranging 0–30 implies mild tinnitus annoyance (grade 1), 31–46 moderate tinnitus annoyance (grade 2), 47–59 severe tinnitus annoyance (grade 3) and 60–84 very severe tinnitus annoyance (grade 4). The TQ is a standard questionnaire in tinnitus research showing good reliability in terms of retest-reliability ($r = 0.94$) and internal consistency (Cronbachs $\alpha = 0.94$). Validity coefficients are moderate to high for psychological distress ($r = 0.5$ up to >0.7) and high for tinnitus annoyance ($r = 0.69$–0.74). There is no agreement regarding which treatment-related change in the TQ-score is needed in order for a tinnitus condition to be considered as "improved" (Hall, 2016). The given relevant improvement of the score ranges from an absolute reduction of 5 points to a relative 20% reduction in TQ (Hiller and Haerkötter, 2005; Langguth et al., 2014).

Tinnitus is considered to be at a decompensated level (permanent annoyance and psychological strain) with a TQ score of 47 points or higher (grade 3 and 4) and to be at a compensated level (low secondary symptoms) at 46 points or lower (grade 1 and 2) (Lenarz, 1992; Goebel and Hiller, 1998; Stobik et al., 2005; Mazurek et al., 2009). Therefore, we defined a clinically relevant change as a change from a decompensated to a compensated level, i.e., below 47 points.

Screening of psychological symptoms included a semi-structured interview with a clinical psychologist as well as the full German version of the Patient Health Questionnaire (PHQ; Spitzer et al., 1999), consisting of somatic symptom, depressive mood, anxiety and stress scales. Patients with acute suicidal tendencies or severe psychiatric diagnoses, which prevented a benefit from the day care program, were referred to other specialists.

For the treatment to be covered by health insurance, two main inclusion criteria were mandatory: moderate to very severe tinnitus-related distress (measured by TQ) and tinnitus duration of more than 3 months (chronic tinnitus). Tinnitus patients who fulfilled these inclusion criteria and who accepted the treatment goal of tinnitus habituation were included. On average, one out of three tinnitus patients with an appointment in the ENT outpatient department fulfilled the inclusion criteria for the day care treatment. From those not included in the treatment, only 115 patients provided informed consent that the gathered data can be analyzed for scientific purposes. These data are presented in **Table 1**. The most frequent reasons for exclusion in this group were unwillingness to accept habituation as treatment goal (35%), low tinnitus-related distress measured by TQ (23%) or acute tinnitus (10%). If symptoms of anxiety or depression appeared too severe for treatment participation, patients were transferred to the psychiatric or psychotherapeutic department. Patient flow is shown in **Figure 1**.

Patient Sample and Treatment

Participants were 308 patients with chronic tinnitus, who fulfilled the above inclusion criteria and were treated in the day care unit of the ENT department of Jena University Hospital between July 2013 and December 2014 (the Tinnitus Center was founded in July 2013 and December 2014 served as the deadline for patient inclusion in the current study). Fifty-two Percent were male participants. The mean age of the sample was 57.08 years ($\pm$12.05, ranging from 22 to 81 years). Tinnitus onset was approximately 7 years (85.22 $\pm$ 94.88 months, ranging from 3 to 602 months) before treatment. At the first appointment, the tinnitus annoyance indexed by the TQ was 52.39 $\pm$ 11.92 points, which is considered as severe. Baseline characteristics of the patient population can be found in **Table 1**.

The interdisciplinary day care treatment lasted for 5 days (Monday to Friday) with an average of 7 h of therapy per day. Group therapy has been shown to be as effective as individual therapy (universality of the symptom, interpersonal learning, imparting of information, direct advice, imitative behavior, and instillation of hope for more see Yalom, 1995; Andersson and Lyttkens, 1999; Olderog, 1999). For this reason, about 80% (23 h/Week) of our day care treatment was conducted in closed

TABLE 1 | Baseline characteristics of tinnitus sample ($N = 308$) and excluded tinnitus-patients ($N = 115$).

Parameter		Experimental Group ($N = 308$)		Excluded ($N = 115$)		
Gender: male		52%		50%		
Age (M ± sd)		57.08 ± 12.05		58.53 ± 16.55		
Tinnitus duration (M ± sd)		85.22 ± 94.88		104. 95 ± 120.77		
Tinnitus-annoyance (TQ)		52.39 ± 11.92		48.68 ± 14.17		
Tinnitus grade	2 (mild)	32.8%		46.4%		
	3 (severe)	39.9%		28.2%		
	4 (very severe)	27.3%		22.7%		
Tinnitus duration	<1 year	16.6%		20.0%		
	1–5 years	37.3%		27.3%		
	>5 years	46.1%		52.7%		
Number of tinnitus sounds	1	74.7%		–		
	2	21.4%		–		
	3	1.9%		–		
Hyperacusis		57.1%		–		
Comorbid psychiatric disorder		20.5%		10%		
Parameter		**Ear: Left**	**Right**	**Ear: Left**	**Right**	
Hearing loss in dB (4 PTA: M ± sd)		28.58 ± 18.79	29.63 ± 21.22	33.27 ± 19.29	31.28 ± 16.57	
Parameter	**Left**	**Right**	**Both**	**Left**	**Right**	**Both**
Tinnitus localization	20.5%	15.9%	63.6%	15.2%	14.3%	70.5%
Hearing aid at baseline	5.3%	3.9%	41.9%			
Sound generator at baseline	0.3%	0.3%	0.3%			
Cochlear implant at baseline	0	0.3%	0			

4 PTA, pure tone average for 500 Hz, 1, 2, and 4 kHz; dB, decibel; M, arithmetic mean; sd, standard deviation.

groups of four to six tinnitus patients. Apart from the group therapy, every patient received several individual therapy sessions (6–7 h/Week) and was treated by an ENT doctor, a cognitive behavioral therapist, a specialist for medical rehabilitation and an audiologist. Therefore, the treatment was conducted within 4 modules, which will be described below. The selection of the modules is based on the "Algorithm for the Diagnostic & Therapeutic Management of Tinnitus" (Tinnitus Research Initiative: Biesinger et al., 2008) and the German S3 guideline for chronic tinnitus, in accordance with the recommendations from Zenner et al. (2015).

Module 1: ENT Counseling

Tinnitus-specific counseling has been repeatedly proven to be an effective way of diminishing tinnitus-related distress (Coles, 1995; Henry and Wilson, 1996; Hall and Ruth, 1999; Mazurek et al., 2006). For this reason, ENT doctors conducted tinnitus-specific counseling in a group setting according to the neurophysiological model of tinnitus (Jastreboff et al., 1996). The anatomy of the ear and auditory system, hearing processes as well as hearing impairment and possible mechanisms of tinnitus generation were explained. The benefit of sound therapy with sound enrichment, masker and hearing aids were discussed and patients' questions were answered. ENT counseling was performed in 3 h in group settings. In the individual sessions with ENT doctors, the tinnitus case history was taken into account and all diagnostic outcomes were explained in detail, setting a basis for an individual tinnitus model from a somatic point of view. Individual treatment options for the time after tinnitus day care treatment were discussed and planed.

Module 2: Cognitive Behavioral Therapy (CBT)

Considering the problems reported as tinnitus related (concentration loss, sleeping disorder, inability to relax, anxiety, depression, fear of aggravation, etc.), it is no surprise that CBT is one of the most validated treatments that reduces tinnitus-related distress (Frenzel, 1998; Andersson and Lyttkens, 1999; Olderog et al., 2004; Martines-Devesa et al., 2010; Cima et al., 2012; for a systematic review see Hesser et al., 2011b). There is a series of studies evidencing the effectiveness of CBT as an internet-based version (Andersson et al., 2002; Kaldo et al., 2008, 2013; Abbott et al., 2009; Hesser et al., 2011b, 2012; Jasper et al., 2014; Weise et al., 2016).

CBT was based on Delb et al. (2002) and Ivansic-Blau (2012) and was administered in 8 group sessions in closed group over 4 days. At first, the roles of attention and emotion in hearing process were explored. The vicious circle of tinnitus distress was explained and factors increasing/decreasing tinnitus awareness were explored. The habitation model of

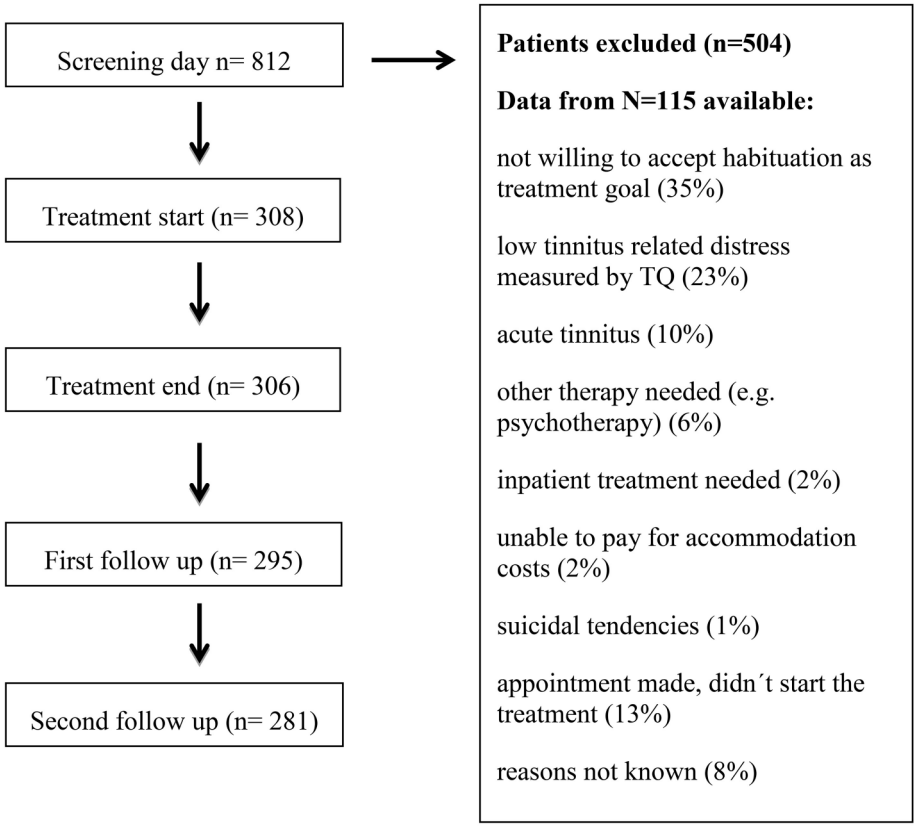

FIGURE 1 | Patient flow chart.

tinnitus (Hallam et al., 1984) was presented, which states that every unknown sound induces an orientation reaction of our body and increases attention to it. If the signal does not change, appears repeatedly and is not considered important, the amount of attention to the signal will reduce as a consequence of habituation. Most tinnitus patients habituate well to their tinnitus sound. Beliefs and emotions hindering habituation were analyzed. The ABC model from Rational-emotive therapy (RET; Ellis, 1993) was introduced and adapted to tinnitus. According to this model, people's beliefs (B) about one activating event (A) strongly affect their emotional and behavioral functioning (C), and not the event itself. Attention switching techniques were learned. Patients were educated about acute/chronic stress responses and about stress reduction techniques. Many tinnitus patients suffering from sleep problems consume alcohol or some medication to improve sleep. We taught our patients about normal sleep patterns and discussed how tinnitus influences sleep. Sleep hygiene recommendations were given and a beneficial sleeping environment (e.g., sound enriched) for tinnitus patients was explored.

In the individual therapy sessions with the CBT therapist an individual tinnitus model was developed, taking into account the following questions: Which individual factors accounted for the tinnitus onset? Which factors helped in dealing with tinnitus? What prevented the implementation of positive management strategies in everyday life? If necessary, patients were encouraged to apply for outpatient psychotherapy subsequent to the treatment in the Tinnitus-Center.

Module 3: Physiotherapy

One subtype of tinnitus is related to a dysfunction of the cervical spine, called cervicogenic somatic tinnitus. Previous research has shown that cervicogenic somatic tinnitus is present in 36–43% of the overall tinnitus population (Abel and Levine, 2004; Fabijanska et al., 2014; Ostermann et al., 2014; Michiels et al., 2015). For that reason, all tinnitus patients underwent physical examination by specialists for medical rehabilitation. In addition to a routine checkup, the influence of head movement, chewing or posture on tinnitus was examined. Individual therapy options for the time after the treatment were discussed and trained. Group physiotherapy was administrated on three consecutive days in 9 sessions, in duration of 50 min each. A physiotherapist conducted one session progressive muscle relaxation (Jacobson, 2006), one session back therapy training and one session physical therapy every day. The goal of the physiotherapy module was to teach patients stress reduction techniques on the one hand, and to expose the patients to ameliorating movement patterns on the other hand. As dysfunctional movements are a common cause for tinnitus aggravation, many tinnitus patients avoid exercise, losing in turn methods for stress reduction.

Module 4: Hearing Therapy

Hearing problems are one of most prevalent complaints of tinnitus patients. Significant hearing loss is found in 70–80% of tinnitus patients (Henry et al., 2005; Hesse and Schaaf, 2015) and the tinnitus frequency is usually within the range of the greatest hearing loss (Noreña et al., 2002). Hearing levels are displayed in **Figure 2**. However, inner ear damage is not necessarily obvious in the pure tone audiogram (Weisz et al., 2006; Schaette and McAlpine, 2011; Epp et al., 2012; Tan et al., 2013). Tinnitus patients with normal hearing thresholds have also more difficulties to understand speech in situations with background noise than persons without tinnitus (Hennig et al., 2011). Increased excitation, plasticity, and connectivity along the entire central auditory path can be compensatory responses to the reduced sensory input (De Ridder et al., 2011; Galazyuk et al., 2012; Stein et al., 2013).

To increase the sensory input, at the beginning of day care treatment, every patient was binaurally fitted with hearing aids and received Terzo® hearing therapy (Funk et al., 2008). Terzo® hearing therapy was originally developed for patients with profound hearing loss and combines hearing aid fitting with auditory speech-in-noise training (Terzo® auditory training). In this 1 h per day training, different stimuli and tasks (e.g., a sentence) were administered from a CD player and the responses (e.g., so-called "key words") were written down in a workbook. Similar to everyday life listening situations, tasks were presented with competing background noise. The Terzo® auditory training was available in three different difficulty levels defined by the initial signal-to-noise ratio (SNR). Depending on listening and comprehension abilities without hearing aids, the suitable training was individually selected. Signal-to-noise-ratio was adaptive and reduced every 2 days in 2 dB SNR. A key section of the workbook contained answers to all exercises, giving the tinnitus patients an important feedback of what was said in the particular sentences. This should increase the awareness for personal hearing (dis)ability. The hearing therapy lasted for 25 days starting at the first day of day care treatment and was continued at home. Terzo® hearing therapy is currently under evaluation; the results will be presented elsewhere.

Follow-up

The first follow-up measurement took part 20 days after the end of the day care treatment. All patients completed the TQ and gave anonymous feedback about the treatment, involving ratings of each treatment module as "very helpful," "helpful," "somewhat helpful," and "not helpful." Hearing aid log data were uploaded and Terzo® hearing therapy workbooks were evaluated. In an individual session with an ENT doctor, it was considered whether patients wanted to continue wearing hearing aids in the future or not. If so, hearing aids were subscribed.

The second follow-up was conducted 6 months after the end of day care treatment. In the individual session with an ENT doctor the treatment was evaluated once more, and patients had a possibility to ask questions or inquire about further help. In a group session with a CBT therapist the personally most useful strategies for reduction of tinnitus-related distress were summarized and problems with implementation of the new, favorable behaviors in everyday life were discussed. All patients completed the TQ once more.

Data were collected before the treatment when each patient was screened (T0), at the beginning (T1) and at the end of the 5 day treatment (T2), as well as 20 days (T3) and 6 months after treatment (T4). In case of missing data (**Figure 1**), we used the last observation carried forward method (Bortz and Döring, 2006). The dependent variable was tinnitus annoyance measured with TQ and its subscales (emotional and cognitive distress, intrusiveness, hearing problems, sleep disturbance, and somatic complains). To identify differences over time, repeated measures analysis of variance (ANOVA) was performed. When significant, *post hoc* testing with Bonferroni correction was performed. To determine the magnitude of change between two points of assessment, effect sizes (ES) using the *d* statistic of Cohen (1992) were calculated; ES between 0.2 and 0.5 are considered as small, from 0.5 to 0.8 as medium and higher than 0.8 as large. The possible impact of patient characteristics on tinnitus annoyance at T0 was tested with Wilcoxon rank sum tests for dichotomized values or with correlations for variables with an interval scale. Regression analysis was used to investigate factors associated with a change in tinnitus annoyance due to treatment. The dependent variable in the regression analysis was change in TQ scores between T0 and T4. The following variables were included in the first regression analysis as independent variables:

- Demographics: age, gender
- Tinnitus-related: duration, localization, frequency, type of onset, subjective loudness, maskability, type of sound, number of sounds, presence during the day, change due to somatic factors like head or jaw movements, change to psychological factors like stress
- Strain variables: baseline TQ score, hearing loss, sound intolerance, sick leave 6 months before treatment, somatic symptoms, depressive mood, anxiety, stress, comorbid psychological disorder
- Otological comorbidity: Menière's disease, dizziness, ear barotrauma, sudden hearing loss, otosclerosis, chronic otitis media, vestibular schwannoma, acoustic trauma

In a second regression analysis, we additionally included "early change" as a predictor. This variable was defined as the TQ change from T0 to T1, i.e., before the actual day care treatment began.

This study was carried out in accordance with the recommendations of ICH harmonized tripartite guideline for Good Clinical Practice, as well as the Declaration of Helsinki. All 308 study participants gave their written informed consent that they were willing to take part in the treatment and that the gathered data can be analyzed for scientific purposes. The protocol was approved by the ethics committee of the Jena University Hospital (4366-03/15).

RESULTS

Treatment Compliance

Overall, treatment compliance was excellent with very low dropout rates (0.6%, $N = 2$) and with 95.2% of patients taking

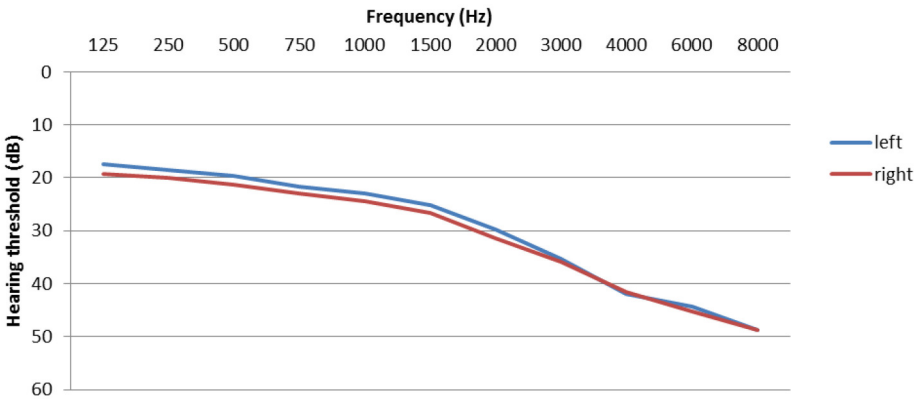

FIGURE 2 | Hearing thresholds.

part in all sessions. According to data logs from the hearing aids at the first follow-up, hearing aids were used for 7.80 ± 4.13 h/day for 21.64 ± 7.79 days (87% of intended 25 days). Almost 90% (89.86%) of the patients completed all tasks of the hearing training.

The anonymous questioner ratings of treatment modules at the end of the treatment showed that all modules were mostly rated as "very helpful" or "helpful": ENT counseling 99%, CBT 99%, physiotherapy 92%, and hearing therapy 92%. As an example, 99% of patients indicated that they would recommend the treatment to family members if they would suffer from chronic tinnitus.

General Treatment Effects

Our treatment goal was to reduce the tinnitus-related annoyance. The repeated measures ANOVA showed that there was a statistical significant difference in tinnitus annoyance measured with TQ over time ($F_{(4, 304)} = 202.201$, $p < 0.001$).

The *post hoc* analysis for tinnitus distress showed a significant reduction from T0 to T1 ($t_{(307)} = 6.737$, $p < 0.001$; from 52.36 ± 11.95 to 48.79 ± 13.74; Cohen's $d = 0.29$, i.e., a small effect), and an even larger reduction from T1 to T2 ($t_{(307)} = 22.710$, $p < 0.001$; from 48.79 ± 13.74 to 34.29 ± 14.98; Cohen's $d = 1.51$, i.e., a large effect). In comparison to T2, significant changes in tinnitus distress were observed neither at T3 ($t_{(307)} = 0.021$, $p = 0.983$), nor at T4 ($t_{(307)} = 0.378$, $p = 0.706$), implying that the outcome remained stable for at least 6 months.

To investigate in more detail which specific tinnitus-related problems did undergo changes over time, we performed repeated measures ANOVA for each TQ subscale. The results are summarized in **Table 2**. The overall changes over time in all TQ subscales were significant ($p < 0.001$). The *post hoc* analyses showed that the results in 4 subscales (emotional and cognitive distress, intrusiveness and sleep disturbance) were significantly reduced from T0 to T1 and from T2 to T3, but remained stable from T2 to both T3 and T4. In two subscales, namely in subscales "hearing problems" and "somatic complains," no change appeared from T0 to T1, but there was a significant reduction from treatment begin (T1) to treatment end (T2), remaining stable at T3 and T4.

Predictors of Tinnitus Annoyance at Baseline

To identify which patient subgroups suffer more from tinnitus, we dichotomized the tinnitus patients in subgroups according to baseline characteristics (e.g., tinnitus onset involving/not involving pressure in ears), and compared differences in tinnitus annoyance between these subgroups at T0. If variables were continuous (e.g., tinnitus duration), we correlated them with TQ sum scores at T0. The results are presented in **Tables 3**, **4**.

Patients with the following symptoms displayed higher tinnitus annoyance at T0: dizziness at tinnitus onset, tinnitus sound could not be masked with background noise, tinnitus worsening during physical stress (e.g., exercise), subjective hearing loss, comorbid psychiatric diagnosis (ICD-10) as well as acute multiple somatic complaints, depressive mood, anxiety and high stress level according to PHQ (Spitzer et al., 1999). Higher tinnitus annoyance at the first appointment was correlated with higher age and higher hearing loss. While tinnitus loudness (tinnitus matching, dB) in the right ear correlated with tinnitus annoyance at T0 significantly, there was no correlation for the left ear.

Predictors of Treatment Success

To learn if all tinnitus annoyance subgroups benefit from treatment, patients were classified into 3 groups (moderate, severe, and very severe tinnitus annoyance) depending on their TQ scores at T0, and repeated measures ANOVAs were performed for each subgroup. As can be seen in **Table 5** as well as in **Figure 3**, all three subgroups had a significant overall change in tinnitus annoyance over time (see Data Sheet 1 in the Supplementary Material for individual change in each subgroup). *Post hoc t*-tests with Bonferroni correction showed the following:

In the subgroup with *moderate tinnitus annoyance* there was no change in tinnitus annoyance from T0 to T1, but from T1 to T2 tinnitus annoyance reduced significantly by 11 points at TQ, changing the grading of tinnitus annoyance from "moderate" to

TABLE 2 | Tinnitus Questionnaire subscale scores over time and results of repeated measures analysis of variance (ANOVA).

TQ subscales (Min-Max)	T0 Baseline M (sd)	T1 Therapy begin M (sd)	T2 Therapy end M (sd)	T3 1st follow up (2.5 weeks) M (sd)	T4 2nd follow up (6 months) M (sd)	F	df	*p**
Hearing problems (0–14)	7.21 (3.59)	7.36 (3.37)	5.57[a,b] (3.22)	5.49[a,b] (3.37)	5.40[a,b] (3.42)	53.18	4/304	**<0.001**
Emotional distress (0–24)	14.77 (4.31)	13.23[a] (4.82)	8.84[a,b] (4.88)	8.74[a,b] (5.26)	8.57[a,b] (5.55)	189.37	4/304	**<0.001**
Cognitive distress (0–16)	10.15 (3.28)	8.94[a] (3.71)	5.41[a,b] (3.82)	5.67[a,b] (4.02)	5.84[a,b] (4.18)	184.20	4/304	**<0.001**
Intrusiveness (0–16)	12.62 (2.22)	12.05[a] (2.61)	9.07[a,b] (3.22)	9.08[a,b] (3.60)	9.18[a,b] (3.79)	133.97	4/304	**<0.001**
Sleep disturbance (0–8)	4.67 (2.36)	4.41[a] (2.42)	3.30[a,b] (2.54)	3.21[a,b] (2.59)	3.11[a,b] (2.56)	68.16	4/304	**<0.001**
Somatic complains (0–6)	2.91 (1.85)	2.81 (1.91)	2.10[a,b] (1.76)	2.09[a,b] (1.88)	1.93[a,b] (1.80)	39.02	4/304	**<0.001**

*TQ, Tinnitus Questionnaire (Goebel and Hiller, 1998); M, arithmetic mean; sd, standard deviation; Min–Max, Range of subscale; [a]Significant change ($p < 0.05$ corrected) to baseline according to paired t-test with Bonferroni correction; [b]Significant change ($p < 0.05$ corrected) to T1 according to paired t-test with Bonferroni correction; *Significant values ($p < 0.05$) in bold.*

TABLE 3 | Baseline difference in tinnitus annoyance measured with Tinnitus Questionnaire between patient-subgroups.

Variable	Tinnitus annoyance M ± sd (N)				*p* (Mann-Whitney *U*-Test)
Gender	**Male**		**Female**		
	52.49 ± 12.12	(156)	52.22 ± 11.81	(152)	0.824
Tinnitus onset	**Subtle**		**Sudden**		
	52.48 ± 11.58	(114)	52.91 ± 12.39	(130)	0.727
Symptom reported by patient	**Yes**		**No**		
Tinnitus onset involving pressure in ears	54.39 ± 11.73	(61)	52.02 ± 11.98	(184)	0.129
Tinnitus onset involving hearing loss	53.43 ± 11.10	(67)	52.52 ± 12.20	(178)	0.433
Tinnitus onset involving dizziness	**56.59 ± 12.53**	**(51)**	**51.70 ± 11.49**	**(196)**	**0.007**
Tinnitus masked trough background noise	**51.59 ± 11.80**	**(208)**	**54.67 ± 12.06**	**(78)**	**0.050**
Tinnitus gets louder in noise	53.83 ± 12.83	(88)	52.01 ± 11.62	(146)	0.233
Noise sensitivity	53.76 ± 12.42	(176)	53.45 ± 11.11	(107)	0.111
Physical stress leads to tinnitus change	**54.54 ± 12.37**	**(109)**	**51.32 ± 11.40**	**(179)**	**0.029**
Emotional stress leads to tinnitus change	52.70 ± 11.97	(211)	52.05 ± 11.93	(77)	0.576
Jaw movement leads to tinnitus change	51.14 ± 12.73	(42)	52.74 ± 11.66	(234)	0.273
Head movement leads to tinnitus change	52.36 ± 11.31	(39)	52.41 ± 11.99	(241)	0.971
Sudden hearing loss in the past	52.57 ± 11.36	(49)	52.32 ± 12.07	(259)	0.739
Current subjective hearing loss	**53.66 ± 11.57**	**(196)**	**49.27 ± 12.52**	**(66)**	**0.015**
Comorbid psychiatric disorder (ICD-10 Checklist)	**Yes**		**No**		
	57.65 ± 12.86	**(63)**	**50.91 ± 11.28**	**(244)**	**<0.001**
Psychological symptoms (PHQ)	**High**		**Low**		
Depressive mood	**49.31 ± 11.28**	**(112)**	**57.96 ± 11.65**	**(173)**	**<0.001**
Anxiety	**50.86 ± 11.50**	**(78)**	**57.62 ± 12.60**	**(207)**	**<0.001**
Somatic complains	**49.64 ± 11.68**	**(138)**	**55.97 ± 11.86**	**(147)**	**<0.001**
Stress	**51.92 ± 12.19**	**(49)**	**56.18 ± 11.64**	**(237)**	**0.029**

PHQ, Patient Health Questionnaire (Spitzer et al., 1999); M, arithmetic mean; sd, standard deviation; N, number of patients in each category. Significant values ($p < 0.05$) in bold.

"mild" and remaining constant at T3 and T4. Cohen's effect size of this change from T0 to T4 is $d = 1.66$.

In the subgroup with *severe tinnitus annoyance* a significant reduction of tinnitus annoyance from T0 to T1 by 4 points in TQ was observed, but grading of the tinnitus annoyance did not change. During treatment the significant change of tinnitus annoyance in TQ (17 points) was observed, shifting tinnitus grading from "severe" to "moderate," achieving a clinical change and remaining stable at T3 and T4. Cohen's effect size of this change from T0 to T4 is $d = 1.91$.

TABLE 4 | Correlation with tinnitus annoyance measured with Tinnitus Questionnaire at baseline.

Variable	Pearson's *r*	*p*
Age	**0.131**	**0.022**
Tinnitus duration	0.109	0.057
Number of tinnitus sounds	**0.152**	**0.008**
Hearing loss in left ear (4 PTA)	**0.247**	**<0.001**
Hearing loss in right ear (4 PTA)	**0.263**	**<0.001**
Tinnitus frequency right	−0.130	0.633
Tinnitus frequency left	−0.101	0.562
Tinnitus loudness dB right	**0.631**	**0.021**
Tinnitus loudness dB left	0.125	0.494

4 PTA, pure tone average for 500 Hz, 1, 2 and 4 kHz. Significant values ($p < 0.05$) in bold.

In the subgroup with *very severe tinnitus annoyance* there was a significant reduction in tinnitus annoyance from T0 to T1 by 7 points in TQ, without the change in grading of the tinnitus annoyance. During treatment a further change of tinnitus annoyance in TQ (14 points) was observed leading to a change in tinnitus grading from "very severe" to "severe" with no further reduction at T3 and T4. Cohen's effect size of this change from T0 to T4 is $d = 1.47$.

To analyze, which factors predict the change of tinnitus annoyance during treatment, we used the difference between T0 and T4 in TQ sum score as dependent variable (Bonate, 2000). After including various personal, tinnitus and strain variables as independent variables in a multiple regression analysis, the model could explain only 12.8% of the variance (R Square = 0.128) if all predictors were used. Because this model explained only so little of the variance despite the large number of predictors used, we refrained from using stepwise procedures. The only significant predictors were "sick leave 6 months before treatment onset" (B = 7.190, SE B = 3.268, ß = 0.148, $p = 0.016$) and "tinnitus annoyance at T0" (B = 0.204, SE B = 0.081, ß = 0.171, $p = 0.012$). Including "early change" as a predictor in second regression analysis allowed to explain 27.4% (R Square = 0.274) of the variance with "early change" being the strongest predictor (B = −0.661, SE B = 0.90, ß = −0.429, $p < 0.001$).

DISCUSSION AND CONCLUSION

For most patients with tinnitus annoyance, the only available therapy option at the moment is a basic ENT examination, because all other guideline recommended therapies are hardly available (at least in Germany). Even though German public health insurance covers the cost for CBT for decompensated chronic tinnitus, the barrier to seek help at a psychotherapist (e.g., public, perceived and self-stigmatizing attitudes to mental illness or difficulty identifying the symptoms of mental illness; for more see Gulliver et al., 2010) is high. This significantly reduces the number of those taking part in CBT. Only patients with very severe tinnitus annoyance and comorbid psychiatric diagnosis receive interdisciplinary stationary treatment covered by the German public health insurance.

A fixed duration for a given intervention and the subsequent decrease in the resources needed for accommodation (overnight rooms, beds, meals, night-shift employees, etc.) would reduce the overall costs and makes them predictable. For this reason we developed a 5-day day care treatment program (JITT), in order to fill the need for a broadly available interdisciplinary treatment for tinnitus. We measured the changes in tinnitus annoyance from an initial consultation (T0) up to 6 months after the end of treatment (T4). To summarize the results briefly, the developed treatment is highly promising in reducing tinnitus annoyance and treatment effects remained stable until at least 6 months after the end of the day care program. This was indexed by the generally high compliance and the overall measure for tinnitus annoyance from the TQ. While inspecting this questionnaire in more detail, it turned out that complaints about emotional and cognitive distress, intrusiveness and disturbed sleep, already improved to some degree after initial consultation (T0), but improved even more in response to the day care treatment (T1–T4). In contrast, complaints about hearing and somatic problems improved only upon treatment. High annoyance was characterized at T0 by several somatic and psychiatric symptoms, but predicting the outcome of treatment proved unsatisfactory. We will discuss each of these aspects below.

General Treatment Effects and Compliance

The general index for tinnitus annoyance demonstrated a considerable reduction from the first consultation (T0) to the final measurement (T4). There was not only a reduction upon the treatment itself, but already earlier, i.e., between the first appointment (T0) and the start of the daycare treatment (T1) corresponding to 4 points in TQ. We assume that this reduction is the outcome of the extensive diagnostic procedures paired with a first, very brief counseling, reassuring the patient that no severe physical abnormality was detected. This information by itself obviously provided some relief. Support for this assumption is provided by a change in the subscales on emotional and cognitive distress as well as intrusiveness and sleep disturbance, but not in the scales addressing hearing and somatic problems. Similarly, waiting for the treatment and certainty of "getting help soon" could have induced this reduction (T0–T1). A meta-analysis of 11 studies included 314 individuals with tinnitus distress that were randomly allocated to a waiting phase lasting 6–12 weeks (Hesser et al., 2011a). The patients revealed a mean decrease in symptom severity between 3 and 8% (Hedges' $g = 0.17$). Thus, already in response to a waiting period tinnitus patients improve slightly on psychometrically robust tinnitus-specific measures.

In our study, the effect size of the overall change before the start of the day care treatment is considered as small. Consequently, at the beginning of treatment the mean tinnitus annoyance was on average still severe. This means that broad and interdisciplinary (ENT doctor, psychologist and audiologist) diagnostics and counseling leads to a significant reduction of tinnitus annoyance, but it does not lead to patients reaching the non-severe range. Perhaps this is why many patients reported during treatment that they were already somewhat relieved after the primary consultation in our clinic or elsewhere, but that they did not know how to cope better with tinnitus in the future.

TABLE 5 | TQ sum scores over time and results of repeated measures analysis of variance (ANOVA) as well as *post hoc t*-tests.

Tinnitus annoyance	T0 Baseline	T1 Therapy begin	T2 Therapy end	T3 1st follow up (2.5 weeks)	T4 2nd follow up (6 months)	*F*	*df*	*p**
	M (sd)	*M* (sd)	*M* (sd)	(M sd)	*M* (sd)			
Moderate ($N = 101$)	38.77 (4.46)	37.24 (9.14)	25.98[a,b] (10.06)	25.98[a,b] (11.84)	24.13[a,b] (11.64)	56.21	4/97	**<0.001**
Severe ($N = 123$)	53.24 (3.83)	49.46[a] (10.09)	32.07[a,b] (12.00)	31.77[a,b] (13.37)	32.49[a,b] (14.91)	118.96	4/119	**<0.001**
Very severe ($N = 84$)	67.39 (5.29)	61.71[a] (10.77)	47.52[a,b] (15.10)	48.32[a,b] (17.05)	48.17[a,b] (17.59)	49.45	4/80	**<0.001**

[a]*Significant change ($p < 0.05$ corrected) to baseline according to paired t-test with Bonferroni correction;* [b]*Significant change ($p < 0.05$ corrected) to T1 according to paired t-test with Bonferroni correction; *significant values ($p < 0.05$) in bold.*

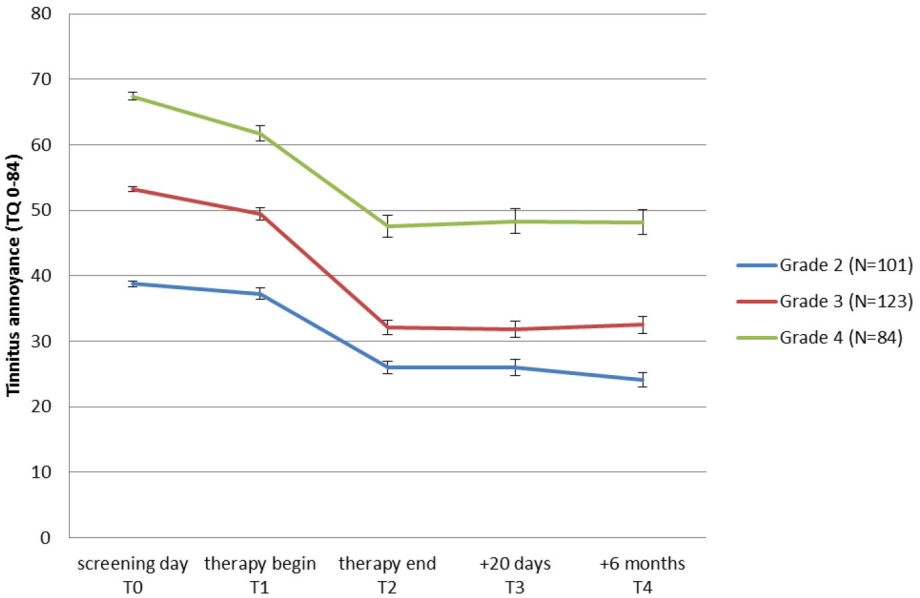

FIGURE 3 | Change of tinnitus annoyance measured with Tinnitus Questionnaire (TQ: Goebel and Hiller, 1998) over time. A higher score represents a higher annoyance. Mean values for patient-groups which started the JITT with moderate (grade 2), severe (grade 3), and very severe (grade 4) tinnitus annoyance are shown. Standard errors of mean are plotted for each point in time and group.

The change in tinnitus annoyance from the beginning (T1) to the end of treatment (T2) is in comparison to the earlier effect considerable, reaching a large effect size with a change in the TQ sum score of further 15 points (in total 18 points); i.e., the mean tinnitus annoyance at treatment end is in the moderate range. This clinically relevant improvement from a decompensated, clinically severe state to a compensated, moderate state remained stable at follow-ups 20 days as well as 6 months later. In contrast to the different responsiveness of subscales for the early change, all TQ subscales reduced significantly upon treatment. Currently, we have no evidence on which of the modules proved more or less successful, but patients considered all modules important.

In general, reported TQ changes in response to different therapeutic approaches differ widely: between 5.2 points (Rief et al., 2005), 7.8 points (Goebel, 1995), 13 points (Haerkötter, 2001), 18.6 points (Weise, 2008), and up to 23.2 points (Weise et al., 2007). Most often the changes at follow-up are smaller compared to the end of treatment, but still significantly larger compared to the onset of treatment (Jakes et al., 1992). As such, JITT seems highly promising, but we also would like to point out several difficulties in directly comparing the different approaches.

The efficacy of most tinnitus management interventions recommended for clinical practice remains to be demonstrated. Currently, only few studies allow making informed conclusions. The efficacy of therapist-delivered CBT appears to be reasonably established (e.g., Hoare et al., 2011). A multidisciplinary CBT-based approach, in which professionals in audiology and psychology share treatment goals aimed at coping with tinnitus through education and counseling, is likely to optimize the benefit for patients (Cima et al., 2012). Thus, multidisciplinary approach was recommended for some time (Henry and Wilson, 1996; El Refaie et al., 2004; Andersson et al., 2005; Henry et al., 2005; Cima et al., 2009; Hoare et al., 2011).

Nevertheless, there are only few researchers reporting the effects of multidisciplinary treatment, often with the limitation that only inpatients of a specialized hospital were examined (Goebel, 1998, 2008; Hiller and Goebel, 1999; Goebel et al., 2006;

Schaaf et al., 2017). These patients are known to be generally more severely impaired and suffer from more psychological complaints than the average patients of ENT practitioners or audiological outpatient departments (Hiller and Goebel, 1999). Therefore, results obtained from inpatient treatment do not seem representative of an outpatient population.

Mazurek et al. (2005) described a 7-day day care interdisciplinary tinnitus-treatment and evaluated it on 46 outpatients. Tinnitus annoyance was reduced significantly from 33.8 points to 27.8 points after 7 days and continued to attenuate to 25.2 points 6 months after treatment. A significant reduction in TQ was observed up to 3 years after treatment (Seydel et al., 2015).

In a large randomized clinical trial, a multidisciplinary stepped care approach involving counseling and elements of CBT and tinnitus retraining therapy (TRT) demonstrated a significant reduction in tinnitus severity (from 49.39 points in TQ to 36.47 points in TQ after 8 months) and tinnitus impairment, as well as improvement of health-related quality of life as compared to usual care (Cima et al., 2012).

Even though the optimal exposure-response relation between number of hours in treatment and outcome remains unknown (Andersson, 2002), the burden for patients and clinicians, as well as the cost-benefit ratio are important (Hoare et al., 2011). JITT lasted 5 days with 2 follow-ups, while other authors report up to 2 years of contact with patients (e.g., Von Wedel and von Wedel, 2000). The very low dropout rate in our study (<1%) suggests that tinnitus patients were generally satisfied with the treatment and that 5 days of treatment seemed to be a reasonable amount of time patients were willing to invest. Such a low dropout rate is remarkable given the much higher rates reported in a review of CBT for tinnitus patients with dropouts ranging between 5 and 22% (Martines-Devesa et al., 2010). These rates were even higher when CBT was delivered via internet (51% in Andersson et al., 2002; 75% in Abbott et al., 2009), even though there was no difference in the reduction of tinnitus annoyance between internet-delivered or therapist-delivered CBT (Kaldo et al., 2008).

Predictors of Tinnitus Annoyance

To describe the heterogeneity of tinnitus patients and in the search for factors related to tinnitus annoyance at T0, we found that patients with the following symptoms displayed higher tinnitus annoyance at the beginning of treatment: dizziness at tinnitus onset, tinnitus sound could not be masked with background noise, tinnitus worsening during physical stress (e.g., exercise), subjective hearing loss, comorbid psychiatric diagnosis (ICD-10) as well as acute multiple somatic complaints, depressive mood, anxiety and high stress level according to PHQ (Spitzer et al., 1999). Additionally, higher tinnitus annoyance at the first appointment was correlated with higher age and greater hearing loss and tinnitus loudness (only for the right ear). This relation is supported by a series of studies that we will briefly review below.

The association between hearing loss and tinnitus corroborates earlier research and is a long standing finding. Prevalence of hearing loss increases with age (Davis, 1995), hearing loss increases the risk for developing tinnitus (Hoffman and Reed, 2004), and on a population level there is a linear increase in tinnitus annoyance with increasing age (Davis and El Rafaie, 2000; Andersson et al., 2005). Studies indicate that 70–80% of tinnitus patients have significant hearing difficulties (Henry et al., 2005; Hesse and Schaaf, 2015). Other studies also reported the positive link between higher hearing loss and higher tinnitus distress (e.g., Goebel and Hiller, 1999; Davis and El Rafaie, 2000; Stobik et al., 2003). For example, Savastano (2008) investigated 520 persons suffering from tinnitus and compared tinnitus patients with and without hearing loss. The author found that subjective discomfort is higher in the presence of hearing loss than in the case of normal hearing (according to Bureau International D'Audiophonologie pure tone average for 500 Hz, 1, 2, and 4 kHz < 20 dB). Among subjects with normal hearing, the level of disturbance was mostly in the moderate range, whereas among subjects with hearing loss, the level of disturbance was mostly elevated. Savastano concluded that the presence of hearing loss increases the complaint of tinnitus considerably, even if the hearing deficit is not severe.

Similarly, the correlation of tinnitus annoyance with otological symptoms reported here corroborates earlier research. Goebel and Hiller (2007) found a strong association between otological conditions and the development of high annoyance: subjects with additional hearing loss and dizziness/vertigo reported both higher loudness and higher annoyance. When subjects with high versus low annoyance were compared, the following odds ratios (OR) were found: hearing loss OR $= 5.64$ and dizziness/vertigo OR $= 3.76$. Hallam and Stephens (1985) found that tinnitus patients who complained of dizziness also suffered from higher emotional distress. Both Erlandsson et al. (1992) and Langenbach et al. (2005) observed a worsening of mood and tinnitus symptoms when tinnitus was accompanied by vertigo. The latter study also confirms our finding that loudness of the tinnitus perceived in the right ear correlated with higher tinnitus annoyance at the first appointment. Thus, the sound perceived in the right ear has a stronger impact on the associated emotional processes. This mechanism is not well understood and should be investigated in future studies.

The association between high tinnitus annoyance and poor maskability was also reported in several studies. Goebel and Hiller (1999) reported higher tinnitus annoyance when maskability was poor. Maskability of tinnitus at admission to CBT was a predictor of tinnitus-related distress at a 5-year follow-up (Andersson et al., 2001). Stobik et al. (2005) compared patients with low/moderate and severe/very severe tinnitus distress and also found that patients with severe/very severe tinnitus reported greater difficulty to mask their tinnitus with background sounds.

The relationship between tinnitus and emotional distress or psychiatric problems has long been recognized and is well documented, at least in the help-seeking group (Harrop-Griffiths et al., 1987; Dobie et al., 1992; Andersson, 2002). Sixty-three to seventy-seven percent of tinnitus inpatients have at least one psychiatric diagnosis (mostly mood or anxiety disorder; Kaldo, 2008). The prevalence of concurrent depression or mood disorders ranges between 39 and 60%, whereas the lifetime prevalence amounts to 62–78% (Andersson, 2002). Other psychological causes of distress associated with tinnitus

include anxiety, depression, irritability, anger, and insomnia (Wilson et al., 1991). Approximately half of the patients with tinnitus without severe hearing impairment also suffer from psychiatric disorders, the most frequent being anxiety disorders and mood disorders (Zöger et al., 2001). Belli et al. (2008) applied the Structured Clinical Interview for DSM-III-R at 90 acute tinnitus patients and found that 24.4% had at least one axis-I psychiatric diagnosis. The most prevalent disorders were anxiety, somatoform and mood disorders. Only 6% of controls without tinnitus had at least one axis-I psychiatric diagnosis. Depression, sleep disorders and difficulties in concentration were significant predictors of tinnitus annoyance (Scott et al., 1990). Erlandsson and Hallberg (2000) investigated in 122 tinnitus inpatients which factors predict quality of life, and found that impaired concentration, feeling depressed and perceived negative attitudes were the most significant predictors and explained most of the variance in quality of life. In the study from Langenbach et al. (2005) on acute tinnitus, insomnia attributed to tinnitus was the best predictor and accounted for 34% of the variance of tinnitus distress 6 months after tinnitus onset. Anxiety in the acute phase accounted for 30% of variance of tinnitus distress, while life satisfaction and somatic complaints accounted together for 41% of the variance.

We conclude that our results of predicting tinnitus annoyance corroborate previous results. Taking Jastreboff's neurophysiological model of tinnitus into account, the negative impact of these symptoms on developing tinnitus annoyance is quite obvious. As the frequency of the individually perceived tinnitus is very likely to be in the range with the highest hearing loss (Henry et al., 1999; Noreña et al., 2002), the masking of tinnitus with background sounds is, in the case of hearing loss, not possible any more. Habituation inhibits tolerance to a stimulus because of its unpredictability. This is possibly why the variability of tinnitus during physical stress attracts attention to the phantom sound, making it difficult to habituate, which in turn leads to higher annoyance. If a person experiences tinnitus onset simultaneously with dizziness, the fear evoked by dizziness will be associated with the noise according to the principles of classical conditioning. Whenever tinnitus is perceived as a danger, no habituation can be achieved. This risk certainly gets higher, due to the belief that hearing loss or dizziness is caused by tinnitus, which is something that many of our patients reported. In the same direction, emotional distress or psychiatric problems are generally regarded as factors hindering habituation.

Predictors of Treatment Outcome

Inspecting changes in tinnitus annoyance in response to treatment, data from patients with moderate, severe and very severe tinnitus annoyance reached high effect sizes. But if we consider only a clinically significant change, JITT displays the strongest effects in patients with severe tinnitus (grade 3). Although tinnitus annoyance is significantly reduced in patients with very severe tinnitus (grade 4), the 5-day treatment is not sufficient to lead to a clinically significant change in this group. Perhaps this group of patients needs an extended duration of JITT or some other outpatient therapeutic approaches. Another possibility would be intensive inpatient care, which however removes patients from their daily routine. Also other researchers found that patients with high tinnitus annoyance at baseline were more often non-responders (Rübler, 1997; Frenzel, 1998) in outpatient setting.

Demographic, tinnitus and strain variables explained only 12.8% of the variance of the change in tinnitus annoyance from T0 to T4. The only predictors for reduction of tinnitus-related distress were "sick leave 6 months before the treatment onset" and "tinnitus annoyance at T0." Patients who were on sick leave before the treatment or with high tinnitus annoyance at T0 showed less improvement in tinnitus annoyance from treatment begin to the final follow-up. We did not inquire further why the patients were on sick leave, but they most likely experienced somatic/psychological symptoms to such an extent that they were unable to continue with their daily activities. The 5-day treatment was perhaps too short for this subgroup. It is yet unclear if they would benefit from a longer treatment duration or a combination of treatments as suggested below. Including "early change" as a predictor allowed to explain 27.4% of the variance, i.e., considerably more. Early change has generally proven to be a strong predictor in psychotherapy and CBT in particular (Schibbye et al., 2014). We did not include this variable in the first regression analysis, because it already requires knowledge about the responsiveness of a patient which was not given at T0. Nevertheless, it constitutes an early and easy calculable indicator who will respond to treatment and who is more resilient. Providing knowledge about early change to therapists could result in more effective treatment (Lambert and Ogles, 2003). Measuring early change seems well constituted for psychiatric disorders (Schibbye et al., 2014; Koffmann, 2017 for recent references), but it is less known in tinnitus research. We propose to integrate such measures in clinical settings for tinnitus treatment. As examples, the Clinical Outcomes in Routine Evaluation questionnaire (CORE, Evans et al., 2000) and the Outcome Questionnaire-45 (OQ-45; Lambert et al., 2004) in combination with the TQ as a disorder specific measure provide validated tools.

Even though the low predictability for treatment success is unsatisfactory, previous studies similarly failed to predict therapy outcome. Rief et al. (2005) found that age and illness duration had only marginal associations with treatment success. Baseline scores of tinnitus annoyance (TQ), gender of the patient or comorbidity with mental disorders were not significant predictors of outpatient psychological treatment. Neither duration of tinnitus nor the level of sleep disturbance, comorbid psychopathology, hearing problems, or experienced stress level affected the outcome of outpatient treatment (Kröner-Herwig et al., 2006). On the other hand, patients with high tinnitus annoyance and comorbid psychopathology at baseline were more often non-responders (Rübler, 1997; Frenzel, 1998). Goebel (2008) conducted a 15-year follow-up after inpatient tinnitus therapy on 271 tinnitus patients. Noise-induced tinnitus, gender and comorbid psychopathology explained 7.6% of the variance in tinnitus annoyance. Male tinnitus sufferers as well as patients with noise-induced tinnitus and high psychopathology reported higher tinnitus annoyance 15 years after the treatment.

Consequently, it appears that there is only little agreement on what can predict treatment outcome, but in no study was predictability good. The complexity of these processes was again stressed by Caffier et al. (2006). In their study, severely affected tinnitus sufferers showed clear improvements in TQ scores without any age-specific differences. In comparison, the groups of younger and older patients with mild tinnitus severity showed higher reductions in TQ scores in comparison to middle-aged patients between 46 and 56 years. Regarding preexisting tinnitus duration, patients with mild tinnitus annoyance demonstrated a particularly strong reduction of annoyance when the tinnitus lasted for less than 1 year. In contrast, in severely affected tinnitus sufferers, preexisting tinnitus duration did not seem to play a role for treatment success.

These results indicate that in order to predict treatment success by patient characteristics, we have to make subgroups and investigate which combinations of subgroup characteristics lead to better/poorer treatment success. Therefore, the next milestone in tinnitus research should be to update large data registries, into which standardized variables can be entered by independent tinnitus researchers. A tinnitus database has already been established by the Tinnitus Research Initiative (http://www.tinnitusresearch.org/index.php/for-researchers/tinnitus-database) and will be improved and enlarged by the TINNET European research network funded by the COST program (http://tinnet.tinnitusresearch.net/). Such a central database will enable the specification of subgroups of tinnitus patients worldwide, making it more possible to develop individually tailored treatments for tinnitus patients.

LIMITATIONS

Due to the lack of a control group receiving a different treatment, we cannot indicate how effective the present approach is compared to other possible treatments or, in the worst case, if it is due to a placebo effect or the mere passing of time. The interdisciplinary treatment comprised several modules, but whether one of them or a specific combination contributed to the reported effects remains to be tested. Future studies might adopt a dismantling approach, leaving out potentially redundant treatment components. Furthermore, cost-effectiveness studies and equivalence trials should be performed.

Even though the results of this study speak in favor of JITT, it must be noted that 35% of the tinnitus patients seen at the ENT department did not accept "habituation to tinnitus" as an objective of the intervention. Therefore, specific treatment approaches adapted to such patients should also be developed. We propose a combination of two therapeutic methods, one addressing tinnitus distress and the other the symptom itself. As an example for the latter, some evidence was presented that tailor-made notched music training reduces tinnitus loudness (Stein et al., 2016). Neurophysiological models (Jastreboff et al., 1996) suggest the proposed combination as a valuable approach that would also satisfy the needs of patients. As an alternative, there are also non-invasive stimulation methods that seem to ameliorate tinnitus symptoms (for a recent case study see Richter et al., 2017).

CONCLUSION

Our interdisciplinary day care tinnitus treatment represents a treatment for patients with chronic tinnitus that reduces tinnitus annoyance. After initial interdisciplinary diagnostic procedures and a first brief tinnitus-specific counseling, a small reduction in tinnitus annoyance was found. A clinically relevant change in tinnitus annoyance was observed between the beginning and the end of treatment and remained stable at least for 6 months.

The best treatment outcome was reached by patients with moderate and severe tinnitus. The improvement in tinnitus annoyance in patients with sick leave within 6 months before treatment onset or with very severe tinnitus annoyance was smaller than for the rest of the investigated population.

Given the high heterogeneity of tinnitus, we predict that the development of adapted JITT to individual needs will be challenging. Additional measurements of neurophysiological correlates might help in understanding which aspects of the symptomatology and the underlying neural network undergo changes in response to treatment and which do not.

AUTHOR CONTRIBUTIONS

DI: Substantial contributions to the conception and design of the work; the acquisition, analysis and interpretation of data for the work; drafting the work and revising it critically for important intellectual content. Wrote the manuscript. CD: Contribution to the analysis and interpretation of data; drafting the work and revising it critically for important intellectual content. Wrote the manuscript. GV, BM and DR: Substantial contributions to the acquisition and interpretation of data for the work; revising the work critically for important intellectual content. US: Substantial contributions to the conception of the work; revising the work critically for important intellectual content. OG: Substantial contributions to the conception and design of the work; the acquisition, analysis and interpretation of data for the work; revising the work critically for important intellectual content. All authors gave their final approval of the version to be published and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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SUPPLEMENTARY MATERIAL

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REFERENCES

Abbott, J. A. M., Kaldo, V., Klein, B., Austin, D., Hamilton, C., Piterman, L., et al. (2009). A cluster randomized trial of an Internet-based intervention program for tinnitus distress in an industrial setting. *Cogn. Behav. Ther.* 38, 162–173. doi: 10.1080/16506070902763174

Abel, M. D., and Levine, R. A. (2004). Muscle contractions and auditory perception in tinnitus patients and nonclinical subjects. *Cranio* 22, 181–191. doi: 10.1179/crn.2004.024

American Academy of Otolaryngology-Head and Neck Surgery (2014). *Clinical Practice Guideline: Tinnitus*. Available online at: https://www.guideline.gov/summaries/summary/48751 (Accessed November 23, 2016).

Andersson, G. (2002). Psychological aspects of tinnitus and the application of cognitive–behavioral therapy. *Clin. Psychol. Rev.* 22, 977–990. doi: 10.1016/S0272-7358(01)00124-6

Andersson, G., Baguley, D. M., McKenna, L., and McFerran, D. J. (2005). *Tinnitus: A Multidisciplinary Approach*. London: Whurr.

Andersson, G., and Lyttkens, L. (1999). A meta-analytic review of psychological treatments for tinnitus. *Br. J. Audiol.* 33, 201–210. doi: 10.3109/03005369909090101

Andersson, G., Strömgren, T., Ström, L., and Lyttkens, L. (2002). Randomized controlled trial of Internet-based cognitive behavior therapy for distress associated with tinnitus. *Psychosom. Med.* 64, 810–816. doi: 10.1097/00006842-200209000-00014

Andersson, G., Vretblad, P., Larsen, H. C., and Lyttkens, L. (2001). Longitudinal follow-up of tinnitus complaints. *Arch. Otolaryngol. Head Neck Surg.* 127, 175–179. doi: 10.1001/archotol.127.2.175

Belli, S., Belli, H., Bahcebasi, T., Ozcetin, A., Alpay, E., and Ertem, U. (2008). Assessment of psychopathological aspects and psychiatric comorbidities in patients affected by tinnitus. *Eur. Arch. Otorhinolaryngol.* 265,279–285. doi: 10.1007/s00405-007-0440-8

Biesinger, E., Del Bo, L., De Ridder, D., Goodey, R., Herraiz, C., Kleinjung, T., et al. (2008). *Algorythm for the Diagnostic & Therapeutic Management of Tinnitus*. TRI Tinnitus Clinic Network. Available online at: http://tinnitusresearch.org/index.php/for-clinicians/diagnostic-flowchart (Accessed March 10, 2017).

Biesinger, E., and Heiden, C. (1999). Die Bedeutung der Retrainingtherapie bei Tinnitus. *Deutsches Ärzteblatt*. 96, 2817–2825.

Bleich, T., Lamprecht, F., Lamm, H., and Jäger, B. (2001). Der Langzeitverlauf des chronischen Tinnitus aurium. *Zeitschrift für Medizinische Psychologie* 2, 79–86.

Bonate, P. L. (2000). *Analysis of Pretest-Posttest Designs*. London: T*aylor & Francis.*

Bortz, J., and Döring, N. (2006). *Forschungsmethoden und evaluation für Human- und Sozialwissenschaftler*. Heidelberg: Springer Medizin Verlag.

Caffier, P. P., Haupt, H., Scherer, H., and Mazurek, B. (2006). Outcomes of long-term outpatient tinnitus-coping therapy: psychometric changes and value of tinnitus-control instruments. *Ear Hear.* 27, 619–627. doi: 10.1097/01.aud.0000240504.77861.1a

Cima, R. F. F., Joore, M., Maes, I., Scheyen, D., El Refaie, A., Baguley, D. M., et al. (2009). Cost-effectiveness of multidisciplinary management of Tinnitus at a specialized Tinnitus centre. *BMC Health Serv. Res.* 9:29. doi: 10.1186/1472-6963-9-29

Cima, R. F. F., Maes, I. H., Joore, M. A., Scheyen, D. J. W. M., El Refaie, A., Baguley, D. M., et al. (2012). Specialised treatment based on cognitive behavior therapy versus usual care for tinnitus: a randomised controlled trial. *Lancet* 379, 1951–1959. doi: 10.1016/S0140-6736(12)60469-3

Cohen, J. (1992). A power primer. *Psychol. Bull.* 112, 155–159. doi: 10.1037/0033-2909.112.1.155

Coles, R. R. A. (1995). "Classification of causes, mechanisms of patient disturbance, and associated counseling," in *Mechanisms of Tinnitus*, eds J. Vernon and A. R. Moller (Boston, MA: Allen & Bacon), 11–19.

Davis, A. (1995). *Hearing in Adults*. London: Whurr.

Davis, A., and El Rafaie, A. (2000). "Epidemiology of tinnitus," in *Tinnitus Handbook*, ed R.S. Tyler (San Diego, CA: Singular), 1–23.

Delb, W., D'Amelio, R., and Schonecke, O. (2002). *Tinnitus: ein Manual zur Tinnitus-Retrainingtherapie*. Göttingen: Hogrefe.

De Ridder, D., Elgoyhen, A. B., Romo, R., and Langguth, B. (2011). Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc. Natl. Acad. Sci. U.S.A.* 108, 8075–8080. doi: 10.1073/pnas.1018466108

Dobie, R. A., Sullivan, M. D., Katon, W. J., Sakai, C. S., and Russo, J. (1992). Antidepressant treatment of tinnitus patients. *Acta Otolaryngol.* 112, 242–247. doi: 10.1080/00016489.1992.11665412

Ellis, A. (1993). *Grundlagen der Rational-Emotiven Verhaltenstherapie*. München: Pfeiffer.

El Refaie, A., Davis, A., Kayan, A., Baskill, J., Lovell, E., and Owen, V. (2004). A questionnaire study of the quality of life and quality of family life of individuals complaining of tinnitus pre- and post-attendance at a tinnitus clinic. *Int. J. Audiol.* 43, 410–416. doi: 10.1080/14992020400050052

Epp, B., Hots, J., Verhey, J. L., and Schaette, R. (2012). Increased intensity discrimination thresholds in tinnitus subjects with a normal audiogram. *J. Acoust. Soc. Am.* 132, EL196-EL201. doi: 10.1121/1.4740462

Erlandsson, S. I., and Hallberg, L. R. M. (2000). Prediction of quality of life in patients with tinnitus. *Br. J. Audiol.* 34, 11–20. doi: 10.3109/03005364000000114

Erlandsson, S. I., Hallberg, L. R. M., and Axelsson, A. (1992). Psychological and audiological correlates of perceived tinnitus severity. *Audiology* 31, 168–179. doi: 10.3109/00206099209072912

Evans, C., Mellor-Clark, J., Margison, F., Barkham, M., Audin, K., Connell, J., et al. (2000). CORE: Clinical Outcomes in Routine Evaluation. *J. Ment. Health* 9, 247–255. doi: 10.1080/713680250

Fabijanska, A. K. K., Raj-Koziak, D., and Skarzynski, H. (2014). *Tinnitus and Normal Hearing: Searching for Underlying Pathology*. Berlin: International tinnitus seminar.

Feldmann, H. (1992). *Tinnitus*. Stuttgart: Georg Thieme Verlag KG.

Frenzel, A. (1998). *Chronischer Tinnitus: Evaluation Eines Kognitiv-Behavioralen Gruppentrainings und Einer Minimalintervention*. Herdecke: GCA-Verl.

Funk, C., Wohlfeil, J., Jauch, E., and Sorg, R. (2008). *Manual der Terzo®Gehörtherapie*. Unpublished manual.

Galazyuk, A. V., Wenstrup, J. J., and Hamid, M. A. (2012). Tinnitus and underlying brain mechanisms. *Curr. Opin. Otolaryngol. Head Neck Surg.* 20, 409–415. doi: 10.1097/MOO.0b013e3283577b81

German Society of Otorhinolaryngology, Head and Neck Surgery (2015). *German S3 guideline Ol 7/064: Chronic tinnitus*. Available online at: http://www.awmf.org/leitlinien/detail/ll/017-064.html (Accessed November 23, 2016).

Goebel, F. (2008). *Langzeitverlauf des chronischen Tinnitus bei Patienten mit Hörsturz, Lärmschädigung und M.* Meniére. Dissertation, Technische Universität, München.

Goebel, G. (1995). Fortschritte bei der verhaltensmedizinischen Diagnostik und Behandlung quälender chronischer Ohrgeräusche. *Oto Rhino Laryngologia Nova* 5, 178–189. doi: 10.1159/000313202

Goebel, G. (1998). *Therapie des chronischen Tinnitus. Evaluation und Prädiktoranalyse einer multimodalen Verhaltenstherapie. Habilitationsschrift*. Postdoctoral thesis, Technische Universität, München.

Goebel, G., and Hiller, W. (1998). *Tinnitus-Fragebogen (TF): ein Instrument zur Erfassung von Belastung und Schweregrad bei Tinnitus*. Göttingen: Hogrefe.

Goebel, G., and Hiller, W. (1999). "Quality management in the therapy of chronic tinnitus" in *Proceedings of the Sixth International Tinnitus Seminar*, ed J.W. Hazell (London: The Tinnitus and Hyperacusis Center Cambridge), 357–363.

Goebel, G., and Hiller, W. (2007). When tinnitus loudness and annoyance are discrepant: audiological characteristics and psychological profile. *Audiol. Neurootol.* 12, 391–400. doi: 10.1159/000106482

Goebel, G., Kahl, M., Arnold, W., and Fichter, M. (2006). 15-year prospective follow-up study of behavioral therapy in a large sample of inpatients with chronic tinnitus. *Acta Otolaryngol. Suppl.* 126, 70–79. doi: 10.1080/03655230600895267

Gulliver, A., Griffiths, K. M., and Christensen, H. (2010). Perceived barriers and facilitators to mental health help-seeking in young people: a systematic review. *BMC Psychiatry* 10:113. doi: 10.1186/1471-244X-10-113

Haerkötter, C. (2001). *Kognitive Verhaltenstherapie bei chronischem Tinnitus: Evaluation neuer Ansätze. Eine Studie zu Potentiellen Therapieeffekten verbesserter Edukation und Apparativer Versorgung Mit therapeutischen Rauschgeneratoren*. Dissertation, Eberhard-Karls-Universität, Tübingen.

Hall, D. (2016). Interpreting Treatment-Related Changes Using the Tinnitus Questionnaire in Argstatter H, Grapp M, Plinkert PK, Bolay HV. Heidelberg neuro-music therapy for chronic-tonal tinnitus - treatment outline and psychometric evaluation. *Int. Tinnitus J.* 20, 73–75. doi: 10.5935/0946-5448.20160014

Hall, J. W., and Ruth, R. A. (1999). "Outcome for tinnitus patients after consultation with an audiologist," in *Proceedings of the Sixth International Tinnitus Seminar*, ed J.W. Hazell (London: The Tinnitus and Hyperacusis Center Cambridge), 378–80.

Hallam, R. S. (1996). *Manual of the Tinnitus Questionnaire (TQ)*. London: Psychological Corporation.

Hallam, R. S., Rachman, S., and Hinchcliffe, R. (1984). "Psychological aspects of tinnitus" in *Contributions to Medical Psychology*, ed S. Rachman (Oxford: Pergamon Press), 31–53.

Hallam, R. S., and Stephens, S. D. (1985). Vestibular disorder and emotional distress. *J. Psychosom. Res.* 29, 407–413. doi: 10.1016/0022-3999(85)90026-1

Harrop-Griffiths, J., Katon, W., Dobie, R., Sakai, C., and Russo, J. (1987). Chronic tinnitus: association with psychiatric diagnosis. *J. Psychosom. Res.* 31, 613–621. doi: 10.1016/0022-3999(87)90040-7

Hausotter, W. (2004). Neurologische und psychosomatische Aspekte bei der Begutachtung des Tinnitus. *Med. Sach.* 100, 5–10.

Hennig, T. A., Costa, M. J., Urnau, D., Becker, K. T., and Schuster, L. C. (2011). Recognition of speech of normal-hearing individuals with Tinnitus and Hyperacusis. *Intl. Arch. Otorhinolaryngol.* 15, 21–28.

Henry, A., Meikle, M., and Gilbert, A. (1999). "Audiometric correlates of tinnitus pitch: insights from the Tinnitus Data Registry," in *Proceedings of the Sixth International Tinnitus Seminar*, ed J.W. Hazell (London: The Tinnitus and Hyperacusis Center Cambridge), 51–57.

Henry, J. A., Dennis, K. C., and Schechter, M. A. (2005). General review of tinnitus: prevalence, mechanisms, effects and management. *J. Speech Lang. Hear. Res.* 48, 1204–1235. doi: 10.1044/1092-4388(2005/084)

Henry, J. L., and Wilson, P. H. (1996). The psychological management of tinnitus: comparison of a combined cognitive educational program, education alone and a waiting-list control. *Int. Tinnitus J.* 2, 9–20.

Hesse, G., and Schaaf, H. (2015). *Manual der Hörtherapie: Schwerhörigkeit, Tinnitus und Hyperakusis*. Göttingen: Hogrefe.

Hesse, G., and Schaaf, H. (2007). Musiktherapie bei Tinnitus. Wirkungsvolle Ergänzung zur Habituations- und Hörtherapie. HNO 55, 328–330. doi: 10.1007/s00106-007-1570-4

Hesser, H., Gustafsson, T., Lundén, C., Henrikson, O., Fattahi, K., Johnsson, E., et al. (2012). A randomized controlled trial of Internet-delivered cognitive behavior therapy and acceptance and commitment therapy in the treatment of tinnitus. *J. Consult. Clin. Psychol.* 80, 649–661. doi: 10.1037/a0027021

Hesser, H., Weise, C., Rief, W., and Andersson, G. (2011a). The effect of waiting: a meta-analysis of wait-list control groups in trials for tinnitus distress. *J. Psychosom. Res.* 70, 378–384. doi: 10.1016/j.jpsychores.2010.12.006

Hesser, H., Weise, C., Westin, V. Z., and Andersson, G. (2011b). A systematic review and meta-analysis of randomized controlled trials of cognitive-behavioral therapy for tinnitus distress. *Clin. Psychol. Rev.* 31, 545–553. doi: 10.1016/j.cpr.2010.12.006

Hiller, W., and Goebel, G. (1999). Assessing audiological, pathophysiological, and psychological variables in chronic tinnitus: a study of reliability and search for prognostic factors. *Int. J. Behav. Med.* 6, 312–330. doi: 10.1207/s15327558ijbm0604_2

Hiller, W., and Haerkötter, C. (2005). Does sound stimulation have additive effects on cognitive-behavioral treatment of chronic tinnitus? *Behav. Res. Ther.* 43, 595–612. doi: 10.1016/j.brat.2004.03.012

Hoare, D. J., Gander, P. E., Collins, L., Smith, S., and Hall, D. A. (2012). Management of tinnitus in English NHS audiology departments: an evaluation of current practice. *J. Eval. Clin. Pract.* 18, 326–334. doi: 10.1111/j.1365-2753.2010.01566.x

Hoare, D. J., Kowalkowski, V. L., Kang, S., and Hall, D. A. (2011). Systematic review and meta-analyses of randomized controlled trials examining tinnitus management. *Laryngoscope* 121, 1555–1564. doi: 10.1002/lary.21825

Hoffman, H. J., and Reed, G. W. (2004). "Epidemiology of tinnitus" in *Tinnitus: Theory and Management*, ed J. B. Snow (Lewiston, NY: BC Decker Inc), 16–41.

International Bureau for Audiophonology (2017). *BIAP Recommendation 02/1: Audiometric Classification of Hearing Impairments*. Available online at: https://www.biap.org/de/empfehlungen/empfehlungen/ct-02-classification-des-deficiences-auditives-1/55-02-1-audiometric-classification-of-hearing-impairments/file (Accessed May 15,2017).

Ivansic-Blau, D. (2012). *Wirksamkeit von ambulanten Gruppentherapien bei chronischem Tinnitus. Eine Psychotherapie-Vergleichsstudie*. Dissertation, Universität Koblenz-Landau, Landau.

Jacobson, E. (2006). *Entspannung als Therapie*. Progressive Relaxation in Theorie und Praxis. Stuttgart: Klett-Cotta.

Jakes, S. C., Hallam, R. S., McKenna, L., and Hinchcliffe, R. (1992). Group cognitive therapy for medical patients: an application to tinnitus. *Cognit. Ther. Res.* 16, 67–82. doi: 10.1007/BF01172957

Jasper, K., Weise, C., Conrad, I., Andersson, G., Hiller, W., and Kleinstauber, M. (2014). Internet-based guided self-help versus groupcognitive behavioral therapy for chronic tinnitus: a randomized controlled trial. *Psychother. Psychosom.* 83, 234–246. doi: 10.1159/000360705

Jastreboff, P. J., Gray, W. C., and Gold, S. L. (1996). Neurophysiological approach to tinnitus patients. *Am. J. Otol.* 17, 236–240.

Kaldo,V. (2008). *Cognitive Behavioural Therapy as Guided Self-Help to Reduce Tinnitus Distress*. Dissertation, Uppsala Universitet, Uppsala.

Kaldo, V., Haak, T., Buhrman, M., Alfonsson, S., Larsen, H. C., and Andersson, G. (2013). Internet-based cognitive behaviour therapy for tinnitus patients delivered in a regular clinical setting: outcome and analysis of treatment dropout. *Cogn. Behav. Ther.* 42, 146–158. doi: 10.1080/16506073.2013.769622

Kaldo, V., Levin, S., Widarsson, J., Buhrman, M., Larsen, H. C., and Andersson, G. (2008). Internet versus group cognitive-behavioral treatment of distress associated with tinnitus: a randomized controlled trial. *Behav. Ther.* 39, 348–359. doi: 10.1016/j.beth.2007.10.003

Koffmann, A. (2017). Has growth mixture modeling improved our understanding of how early change predicts psychotherapy outcome? *Psychother. Res.* 2, 1–13. doi: 10.1080/10503307.2017.1294771

Konzag, T. A., Rübler, D., Bloching, M., Bandemer-Greulich, U., Fikentscher, E., and Frommer, J. (2006). Counselling versus Selbsthilfemanual bei ambulanten Tinnituspatienten. *HNO* 54, 599–604. doi: 10.1007/s00106-005-1350-y

Kröner-Herwig, B., Zachriat, C., and Weigand, D. (2006). Do patient characteristics predict outcome in the outpatient treatment of chronic tinnitus? *Psychosoc. Med.* 3, Doc07.

Lambert, M. J., Morton, J. J., Hatfield, D., Harmon, C., Hamilton, S., Reid, R. C., et al. (2004). *Administration and scoring manual for the Outcome Questionnaire (OQ-45.2)*. Orem, UT: American Professional Credentialing Service.

Lambert, M. J., and Ogles, B. (2003). "The efficacy and effectiveness of psychotherapie," in B*ergin and Garfield's Handbook of Psychotherapy and Behavior Change*, ed M. J. Lambert (New York, NY: John Wiley & Sons), 139–93.

Langenbach, M., Olderog, M., Michel, O., Albus, C., and Köhle, K. (2005). Psychosocial and personality predictors of tinnitus-related distress. *Gen. Hosp. Psychiatry* 27, 73–77. doi: 10.1016/j.genhosppsych.2004.08.008

Langguth, B., Landgrebe, M., Frank, E., Schecklmann, M., Sand, P. G., Vielsmeier, V., et al. (2014). Efficacy of different protocols of transcranial magnetic stimulation for the treatment of tinnitus: pooled analysis of two randomized controlled studies. *World J. Biol. Psychiatry* 15, 276–285. doi: 10.3109/15622975.2012.708438

Lenarz, T. (1992). "Chirurgische Therapie," in *Tinnitus*, ed H. Feldmann (Stuttgart: T*hieme)*, 112–115.

Martines-Devesa, P., Perera, R., Theodoulou, M., and Waddell, A. (2010). Cognitive behavioural therapy for tinnitus (Review): an update. *Cochrane Database Syst. Rev.* 9: CD005233. doi: 10.1002/14651858.CD005233.pub3

Mazurek, B., Fischer, F., Haupt, H., Georgiewa, P., Reisshauer, A., and Klapp, B. F. (2006). A modified version of tinnitus retraining therapy: observing long-term outcome and predictors. *Audiol Neurotol.* 11, 276–286. doi: 10.1159/000093526

Mazurek, B., Georgiewa, P., Seydel, C., Haupt, H., Scherer, H., Klapp, B. F., et al. (2005). Integrierte Tinnitusintensivbehandlung: konzept und erste praktische Erfahrungen. *Gesundheitswesen* 67, 485–491. doi: 10.1055/s-2005-858379

Mazurek, B., Seydel, C., Haupt, H., Szczepek, A., Klapp, B. F., and Schrom, T. (2009). Integrierte Tinnitusintensivbehandlung: verringerung der tinnitusbedingten Belastung während einer 1-Jahres-Katamnese. *Gesundheitswesen* 71, 35–40. doi: 10.1055/s-0028-1082303

Michiels, S., De Hertogh, W., Truijen, S., and Van de Heyning, P. (2015). Cervical spine dysfunctions in patients with chronic subjective tinnitus. *Otol. Neurotol.* 36, 741–745. doi: 10.1097/MAO.0000000000000670

Noreña, A., Micheyl, C., Chery-Croze, S., and Collet, L. (2002). Psychoacoustic characterization of the tinnitus spectrum: implications for the underlying mechanisms of tinnitus. *Audiol. Neurootol.* 7, 358–369. doi: 10.1159/000066156

Olderog, M. (1999). Metaanalyse zur Wirksamkeit psychologisch fundierter Behandlungskonzepte des chronischen dekompensierten Tinnitus. *Zeitschrift für Medizinische Psychologie* 1, 5–18.

Olderog, M., Langenbach, M., Michel, O., Brusis, T., and Köhle, K. (2004). Prädiktoren und Mechanismen der ausblendenden Tinnitus-Toleranzentwicklung - eine Längsschnittstudie. *Laryngo Rhino Otol.* 83, 5–13. doi: 10.1055/s-2004-814235

Ostermann, K. T. M., Vincent, Y., Debaty, M. E., Cotton, P., and Lurquin, P. (2014). *Incidence of Somatic Tinnitus in Current ENT Practice*. Berlin: International tinnitus seminar.

Richter, K., Acker, J., Miloseva, L., Peter, L., and Niklewski, G. (2017). Management of chronic tinnitus and insomnia with repetitive transcranial magnetic stimulation and cognitive behavioral therapy – a combined approach. *Front. Psychol.* 8:575. doi: 10.3389/fpsyg.2017.00575

Rief, W., Weise, C., Kley, N., and Martin, A. (2005). Psychophysiologic treatment of chronic tinnitus: a randomized clinical trial. *Psychosom. Med.* 67, 833–838. doi: 10.1097/01.psy.0000174174.38908.c6

Rübler, D. (1997). *Psychosoziale Belastungsfaktoren und Komorbidität bei Tinnituspatienten - Wie effektiv ist Counselling?* Dissertation, Martin-Luther-Universität, Halle-Wittenberg.

Savastano, M. (2008). Tinnitus with or without hearing loss: are its characteristics different? *Eur. Arch. Otorhinolaryngol.* 265, 1295–1300. doi: 10.1007/s00405-008-0630-z

Schaaf, H., Weiß, S., and Hesse, G. (2017). Catamnesis results of an inpatient neuro-otologic and psychosomatic tinnitus therapy 1-5 years after discharge. *Eur. Arch. Otorhinolaryngol.* 274, 701–710. doi: 10.1007/s00405-016-4316-7

Schaette, R., and McAlpine, D. (2011). Tinnitus with a normal audiogram: physiological evidence for hidden hearing loss and computational model. *J. Neurosci.* 31, 13452–13457. doi: 10.1523/JNEUROSCI.2156-11.2011

Schibbye, P., Ghaderi, A., Ljótsson, B., Hedman, E., Lindefors, N., Rück, C., et al. (2014). Using early change to predict outcome in cognitive behaviour therapy: exploring timeframe, calculation method, and differences of disorder-specific versus general measures. *PLoS ONE* 9:e100614. doi: 10.1371/journal.pone.0100614

Scott, B., Lindberg, P., Melin, L., and Lyttkens, L. (1990). Predictors of tinnitus discomfort, adaptation and subjective loudness. *Br. J. Audiol.* 24, 51–62. doi: 10.3109/03005369009077842

Seydel, C., Haupt, H., Szczepek, A. J., Hartmann, A., Rose, M., and Mazurek, B. (2015). Three years later: report on the state of well-being of patients with chronic tinnitus who underwent modified tinnitus retraining therapy. *Audiol. Neurotol.* 20, 26–38. doi: 10.1159/000363728

Spitzer, R. L., Williams, J., and Kroenke, K. (1999). *Prime MD Today. Evaluation of Mental Disorders. Manual*. New York, NY: Pfizer.

Stein, A., Engell, A., Okamoto, H., Wollbrink, A., Lau, P., Wunderlich, R., et al. (2013). Modulatory effects of spectral energy contrasts on lateral inhibition in the human auditory cortex: an MEG study. *PLoS ONE* 8:80899. doi: 10.1371/journal.pone.0080899

Stein, A., Wunderlich, R., Lau, P., Engell, A., Wollbrink, A., Shaykevich, A., et al. (2016). Clinical trial on tonal tinnitus with tailor-made notched music training. *BMC Neurol.* 16:38. doi: 10.1186/s12883-016-0558-7

Stobik, C., Weber, R. K., Münte, T. F., and Frommer, J. (2003). Psychosomatic stress factors in compensated and decompensated tinnitus. *Psychother. Psychosom. Med. Psychol.* 53, 344–352. doi: 10.1055/s-2003-40947

Stobik, C., Weber, R. K., Münte, T. F., Walter, M., and Frommer, J. (2005). Evidence of psychosomatic influences in compensated and decompensated tinnitus. *Int. J. Audiol.* 44, 370–378. doi: 10.1080/14992020500147557

Sullivan, M. D., Katon, W., Dobie, R. A., Saskai, C., Russo, J., and Harrop-Griffiths, J. (1988). Disabling tinnitus: association with affective disorder. *Gen. Hosp. Psychiatry* 10, 285–291. doi: 10.1016/0163-8343(88)90037-0

Tan, C. M., Lecluyse, W., McFerran, D., and Meddis, R. (2013). Tinnitus and patterns of hearing loss. *J. Assoc. Res. Otolaryngol.* 14, 275–282. doi: 10.1007/s10162-013-0371-6

Tyler, R. S., and Baker, L. J. (1983). Difficulties experienced by tinnitus suffers. *J. Speech Hear. Disord.* 48, 150–154. doi: 10.1044/jshd.4802.150

Von Wedel, H., and von Wedel, U. (2000). Eine Bestandsaufnahme zur Tinnitus-Retraining-Therapie. *HNO* 48, 887–901. doi: 10.1007/s001060050685

Weise, C. (2008). *Biofeedback als Chance für die Tinnitusbehandlung*. Dissertation, Philipps-Universität, Marburg.

Weise, C., Heinecke, K., and Rief, W. (2007). Biofeedback bei chronischem Tinnitus – Behandlungsleitfaden und vorläufige ergebnisse zu wirksamkeit und Akzeptanz. *Verhaltenstherapie* 17, 220–230. doi: 10.1159/000111462

Weise, C., Kleinstäuber, M., and Andersson, G. (2016). Internet-Delivered cognitive-behavior therapy for tinnitus: a randomized controlled trial. *Psychosom. Med.* 78, 501–510. doi: 10.1097/PSY.0000000000000310

Weisz, N., Hartmann, T., Dohrmann, K., Schlee, W., and Norena, A. (2006). High-frequency tinnitus without hearing loss does not mean absence of deafferentation. *Hear. Res.* 222, 108–114. doi: 10.1016/j.heares.2006.09.003

Wilson, P. H., Henry, J., Bowen, M., and Haralambous, G. (1991). Tinnitus reaction questionnaire: psychometric properties of a measure of distress associated with tinnitus. *J. Speech Hear. Res.* 34, 197–201. doi: 10.1044/jshr.3401.197

Yalom, I. D. (1995). *The Theory and Practice of Group Psychotherapy*. New York, NY: Basic Books.

Zenner, H. P., Delb, W., Kröner-Herwig, B., Jäger, B., Peroz, I., Hesse, G., et al. (2015). On the interdisciplinary S3 guidelines for the treatment of chronic idiopathic tinnitus. *HNO* 63, 419. doi: 10.1007/s00106-015-0011-z

Zenner, H. P., Delb, W., Kröner-Herwig, B., Jäger, B., Peroz, I., Hesse, G., et al. (2016). A multidisciplinary systematic review of the treatment for chronic idiopathic tinnitus. *Eur. Arch. Otorhinolaryngol.* 274, 2079–2091. doi: 10.1007/s00405-016-4401-y

Zöger, S., Svedlund, J., and Holgers, K. M. (2001). Psychiatric disorders in tinnitus patients without severe hearing impairment: 24 months follow- up of patients at an audiological clinic. *Audiology* 40, 133–140. doi: 10.3109/00206090109073108

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frontiers in Aging Neuroscience

CLINICAL TRIAL
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Comparison of the Long-Term Effect of Positioning the Cathode in tDCS in Tinnitus Patients

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Objective: Transcranial direct current stimulation (tDCS) is one of the methods described in the literature to decrease the perceived loudness and distress caused by tinnitus. However, the main effect is not clear and the number of responders to the treatment is variable. The objective of the present study was to investigate the effect of the placement of the cathode on the outcome measurements.

Methods: Patients considered for the trial were chronic non-pulsatile tinnitus patients with complaints for more than 3 months and a Tinnitus Functional Index (TFI) score that exceeded 25. The anode was placed on the right dorsolateral prefrontal cortex (DLPFC). In the first group—"bifrontal"—the cathode was placed on the left DLPFC, while in the second group—"shoulder"—the cathode was placed on the shoulder. Each patient received two sessions of tDCS weekly and eight sessions in total. Evaluations took place on the first visit for an ENT consultation, at the start of therapy, after eight sessions of tDCS and at the follow-up visit, which took place 84 days after the start of the therapy. Subjective outcome measures such as TFI, Visual Analog Scales (VAS) for loudness and percentage of consciousness of tinnitus were administered in every patient.

Results: There was no difference in the results for tinnitus loudness and the distress experienced between the placement of the cathode on the left DLPFC or on the shoulder. In addition, no statistically significant overall effect was found between the four test points. However, up to 39.1% of the patients experienced a decrease in loudness, measured by the VAS for loudness. Moreover, 72% of those in the bifrontal group, but only 46.2% of those in the shoulder group reported some improvement in distress.

Conclusion: While some improvement was noted, this was not statistically significant. Both electrode placements stimulated the right side of the hippocampus, which could be responsible for the effect found in both groups. Further research should rule out the placebo effect and investigate alternative electrode positions.

Keywords: neuromodulation, tDCS, electrode placement

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INTRODUCTION

Tinnitus is the perception of sound in the absence of a corresponding external sound source (Eggermont and Roberts, 2004) and is a very common problem. Approximately 25.3% of the US population reports having tinnitus, while 7.9% experience tinnitus frequently (Shargorodsky et al., 2010). Tinnitus affects daily activities and can lead to a high level of distress. The literature describes several mechanisms and structures that could be involved in tinnitus. However, the findings reported cannot always be repeated and the proposed brain structures do not always match. However, it is certain that it is not only the auditory system which is responsible for inducing tinnitus (Jastreboff, 1990). Multiple non-auditory systems involved in cognition, emotion and memory play an important role (De Ridder et al., 2014).

Currently, no treatment is available that eliminates tinnitus completely. Most researchers and clinicians focus on diminishing the level of disturbance experienced and/or the loudness of the tinnitus. One method that can be used is neuromodulation, namely transcranial Direct Current Stimulation (tDCS). In the case of tDCS, the current is applied to the brain by means of two electrodes. The goal of tDCS is to influence those regions involved in tinnitus and consequently lessen the distress caused by, or the loudness of, tinnitus. Previous research has shown that bifrontal tDCS strengthens deficient inhibitory top-down mechanisms in tinnitus and interferes with the emotional processing of tinnitus (Tanaka et al., 2008; Vanneste et al., 2010). However, in the literature tDCS is reported as having a variable effect, with the percentage of responders ranging from 0% to almost 47% (Fregni et al., 2006; Vanneste et al., 2011; Song et al., 2012). The difference in outcomes might be due to different factors such as orientation of the current field, electrode positions, electrode size, stimulation duration and current intensity (Nitsche et al., 2008). Depending on the orientation of the current field, tDCS can either increase or decrease the excitability. Under the cathode, excitability is decreased due to neural hyperpolarization. However, under the anode, excitability is enhanced due to neural depolarization (Nitsche and Paulus, 2000).

The literature also reports the use of different electrode positions to treat tinnitus, with bifrontal tDCS and left temporoparietal area (LTA) tDCS most frequently applied in tinnitus patients. In bifrontal tDCS, the anode and cathode are placed on the right and left dorsolateral prefrontal cortex (DLPFC), respectively. In LTA tDCS, the anode is placed on the left temporal area. Song et al. (2012) found that LTA and bifrontal positioning produced similar results with respect to the percentage of responders and a reduction in tinnitus intensity (Song et al., 2012). Furthermore, the current intensity and duration can also play an important role. Shekhawat et al. (2013) concluded that 2 mA anodal tDCS at LTA for 20 min was the most effective setting for transient tinnitus suppression (Shekhawat et al., 2013).

Concluding, more research is needed towards parameters of tDCS to optimize the effect of it. In that interest, a computational model was calculated to predict differences in the current pathway of two placement montages, namely the "bifrontal" montage and the "shoulder" montage. The "bifrontal" group had the anode and cathode positioned over the F4 (right DLPFC) and F3 (left DLPFC) positions respectively; in the "shoulder" group, the anode electrode was placed over F4 and the cathode on the left shoulder. Considering these differences, interest was raised towards the difference in the perception outcome of the tinnitus patients undergoing tDCS using these two different montages. The objective of the present study was to compare the outcomes of the placement of the cathode on the left DLPFC vs. the shoulder.

MATERIALS AND METHODS

Subjects

Patients considered for the trial were chronic non-pulsatile tinnitus patients who had complaints for more than 3 months and a Tinnitus Functional Index (TFI) score that exceeded 25. Written informed consent was obtained from every patient (n = 65). The study protocol was approved by the ethical committee of Antwerp University Hospital. Of the 65 subjects, six were lost to follow-up for various reasons. The median age of the subjects was 52 years, within a range of 23 years to 70 years. The median duration of tinnitus was 28 months and fluctuated between 3 months and 264 months. In total 59 subjects—41 men and 18 women were included and randomized based on the parameters of age, TFI score, etiology, gender and degree of hearing loss. In order to create two equal groups, the MS-DOS program MINIM (by S. Evans, P. Royston and S. Day) was used to allocate the subjects by minimization. A flow diagram is presented in **Figure 1**.

Transcranial Direct Current Stimulation

In the first group—"bifrontal"—the anode and the cathode were placed on the right and left DLPFC, respectively (F4 and F3), while in the second group—"shoulder"—the cathode was placed on the left upper arm, according to the 10–20 international system for EEG electrode placement. Each patient received two sessions of tDCS weekly and a total of eight sessions. The direct current was transferred by means of two saline-soaked pairs of surface sponge electrodes (35 cm^3) and delivered by a specially developed, battery-driven constant current Neuroconn stimulator (neuroConn, Ilmenau, Germany), with a maximum output of 10 mA. A constant current of 2 mA was applied for 20 min with a fade-in and fade-out of 10 s. **Figure 2** shows the current pattern in both cathode placements. In the bifrontal group, the DLPFC and the hippocampus are stimulated, but not the cingulate cortex. In contrast, in the shoulder placement, the temporal lobe is stimulated, in addition to the cingulate cortex and the right side of the hippocampus.

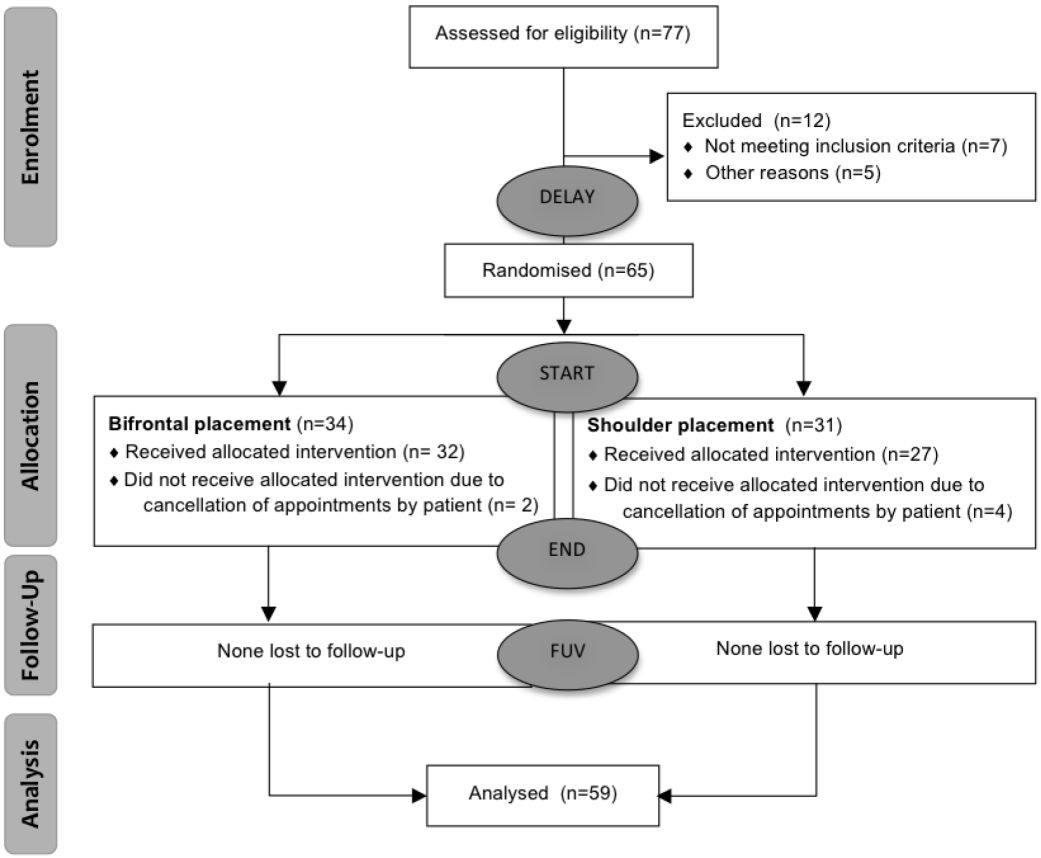

FIGURE 1 | Flow chart.

Outcome Measures

Evaluations took place on the first visit for an ENT consultation (Delay), at the start of therapy (Start), after eight sessions of tDCS (End) and 84 days after the start of the therapy (FUV). The delay was added to test spontaneous recovery after visiting an ENT-specialist. The FUV was added to measure the long-term effect of tDCS. The flow diagram is presented in **Figure 1**.

The subjective outcome measures of TFI (Meikle et al., 2012; Rabau et al., 2014), Visual Analog Scales (VAS) for loudness and the Hyperacusis Questionnaire (HQ; Khalfa et al., 2002) were completed for every patient. The Dutch version of the TFI, a self-report questionnaire consisting of 25 questions, was chosen as the primary outcome measurement. A reduction of 13 points on the TFI is considered a meaningful reduction in annoyance experienced by the patient. In addition to a total score, scores of the subscales of intrusiveness, reduced sense of control, cognitive interference, sleep disturbance, auditory difficulties attributed to tinnitus, interference with relaxation, reduced quality of life and emotional distress can also be calculated (Meikle et al., 2012).

The secondary outcome measures were the VAS for loudness and the HQ. On the VAS the patient had to rate the maximum and mean loudness of their tinnitus on a scale of 0–10, with 0 indicating a very soft sound that is not audible and 10 meaning as loud as possible—the tinnitus could not be any louder. The HQ surveys the over-sensitivity to sounds, and consists of 14 items with four answer possibilities: no (0 points); yes, a little (1 point); yes, quite a lot (2 points) and yes, a lot (3 points). The maximum score is 42, with Khalfa et al. (2002) suggesting a cut-off score of 28. Patients scoring more than 28 are considered to have hyperacusis (Khalfa et al., 2002).

Statistical Analysis

Statistical analyses were performed on the data to determine whether one placement was preferable to the other. The data were analyzed using SPSS statistical software version 22 MAC OS X (IBM; Armonk, NY, USA). Normal distribution of the data was checked using the Shapiro-Wilk test and Q-Q plots. For each of the outcome measurements, a repeated measures ANOVA was performed with test moment as within subject and group (bifrontal vs. shoulder) as between subject variable. The interaction between test moment and group were added to the model. To assess the impact of the demographic details, covariants (hearing loss and age) were added into the model. The significance level was set at $p < 0.05$. Descriptive statistics was used to compare the characteristics of the subjects.

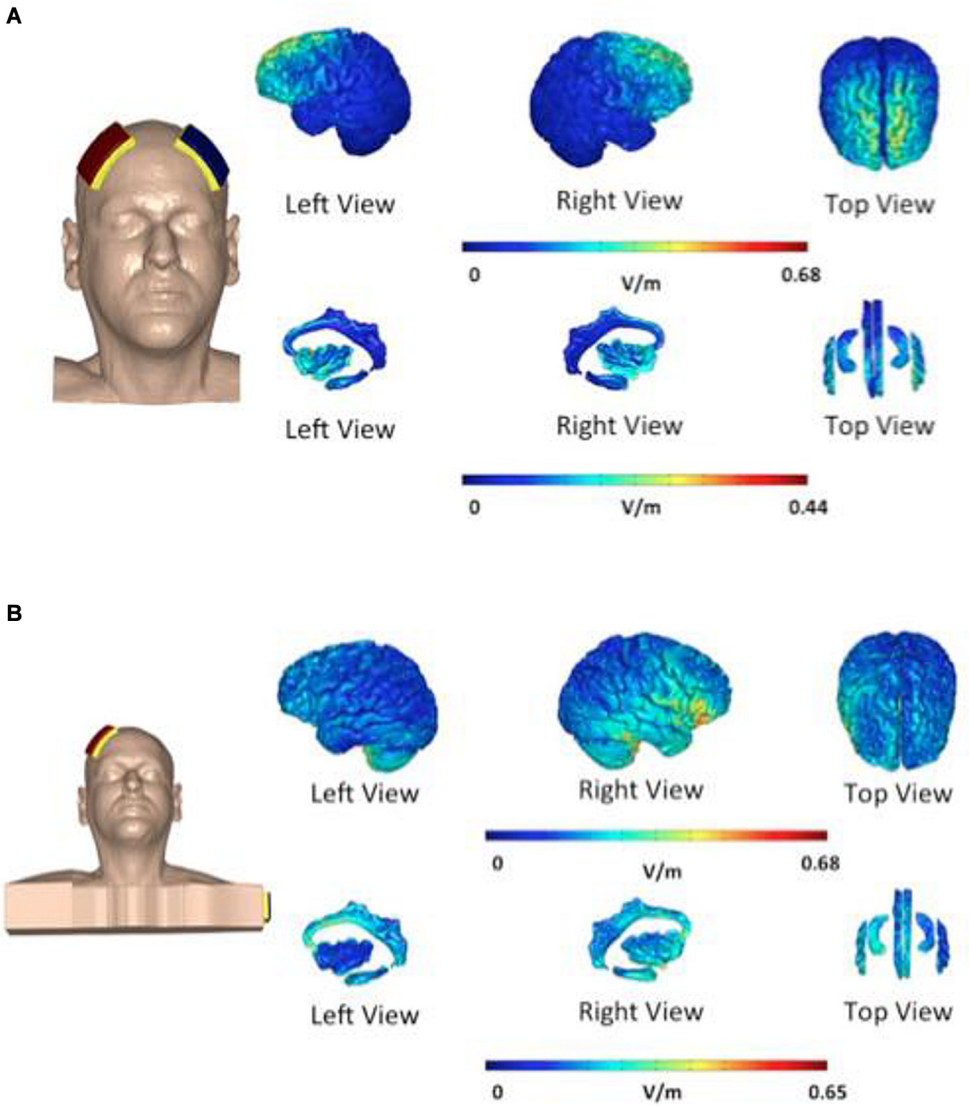

FIGURE 2 | Distribution of electric field during transcranial direct current stimulation (tDCS). The electric field is shown as electric field/current density magnitude (V/m). The cathode placed on the left dorsolateral prefrontal cortex (DLPFC) **(A)** vs. the cathode placed on the shoulder **(B)**. In the bifrontal group **(A)** the DLPFC and hippocampus are stimulated. In the shoulder placement group **(B)**, the temporal lobe is stimulated in addition to the cingulate cortex and the right side of the hippocampus.

Computational Model

A finite element montages were generated in COMSOL multi physics 4.2 for analysis based on previous procedures (Truong et al., 2012a,b). Briefly, initially, a 3-D 1 mm*1 mm*1 mm T1 MRI of an adult male was segmented into different head regions using both automated segmentation algorithms and manual segmentation techniques available in the ScanIP software (Simpleware, Ltd., Exeter, UK) to correct for segmentation errors from the automated algorithms, add fat, segment a number of brain deep structures. 5 × 7 cm sponge pads were then placed on F4 and F3 for the "bifrontal" montage, and F4 and the left shoulder for the "shoulder" montage using ScanCAD (Simpleware, Ltd., Exeter, UK). The mesh was then generated and imported into COMSOL multi physics 4.2 (COMSOL, Inc., Burlington, MA, USA). In COMSOL, the segmented regions were assigned a conductivity (S/m): air, 10^{-15}; skin, 0.465; fat, 0.025; skull, 0.01; CSF, 1.65; gray matter, 0.276; or white matter, 0.126. The electrodes were assigned a conductivity of 5.99×10^7 S/m and the sponges were modeled using the conductivity of saline which is 1.4 S/m. Boundary conditions were set such that the cathode (F3 for "bifrontal"; left shoulder electrode for "shoulder") was the ground and a total of 2 mA of current was applied from the F4 anode. The model was solved and cortical electric field magnitude was plotted for

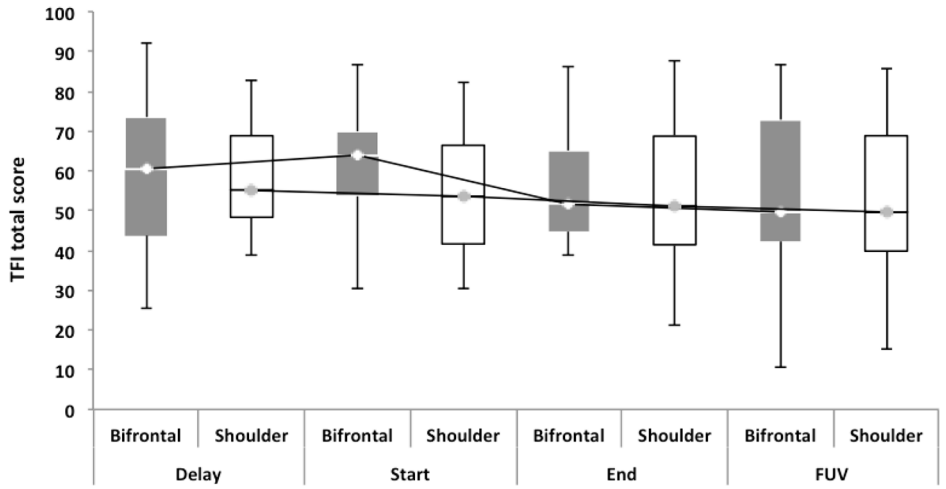

FIGURE 3 | Tinnitus functional index (TFI) total score. No significant effect was found between either cathode placement and the test moments for the TFI total score.

each montage to analyze the differences between the two montages.

RESULTS

Repeated measures showed no statistically significant difference between both groups with regard to the TFI, the subscales of the TFI, the VAS for maximum and mean loudness, the percentage of consciousness, or problems experienced falling asleep and waking up during the night ($p > 0.05$). Looking at the overall effect, no significant effect was found between the test moments for the TFI total score (**Figure 3**), the TFI subscales of intrusiveness, cognitive interference, sleep disturbance, auditory difficulties, quality of life and emotional distress, or with the maximum (**Figure 4**) and mean VAS for loudness and percentage of consciousness ($p > 0.05$). For the subscale of sleep disturbance ($p = 0.048$), a statistically significant effect was found between the Start (mean = 64.03) and the FUV (mean = 52.50) test moments in the bifrontal group (**Figure 5**). Concerning the shoulder group, a statistically significant effect was found for the subscale of intrusiveness ($p = 0.040$) between the Delay (mean = 72.50) and the FUV (mean = 62.50) test moments. The results are shown in **Figure 6**. No statistically significant effect of the demographic details age and hearing loss was found on the outcome measurements ($p > 0.05$).

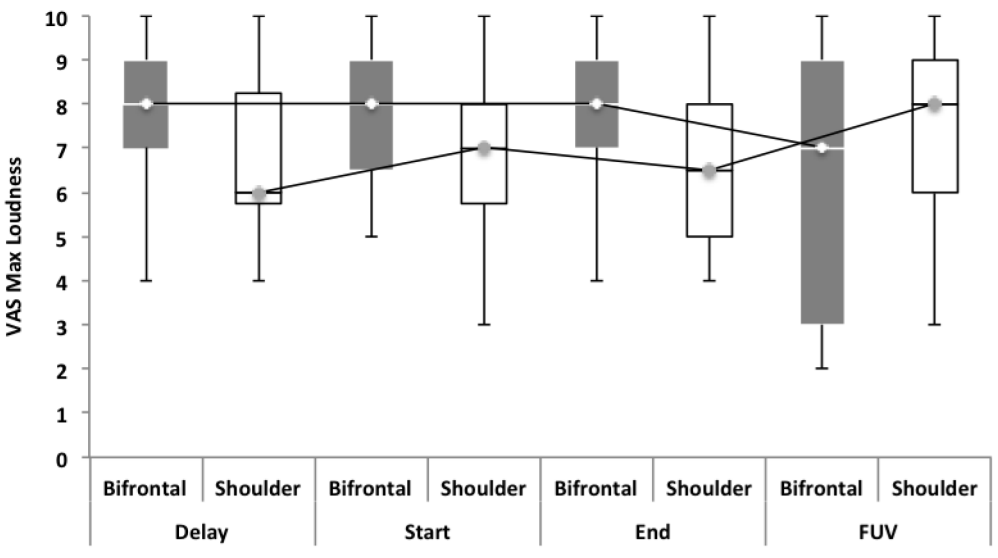

FIGURE 4 | Visual analog scales (VAS) for maximum loudness. No significant effect was found between groups and the test moments for the VAS for maximum loudness.

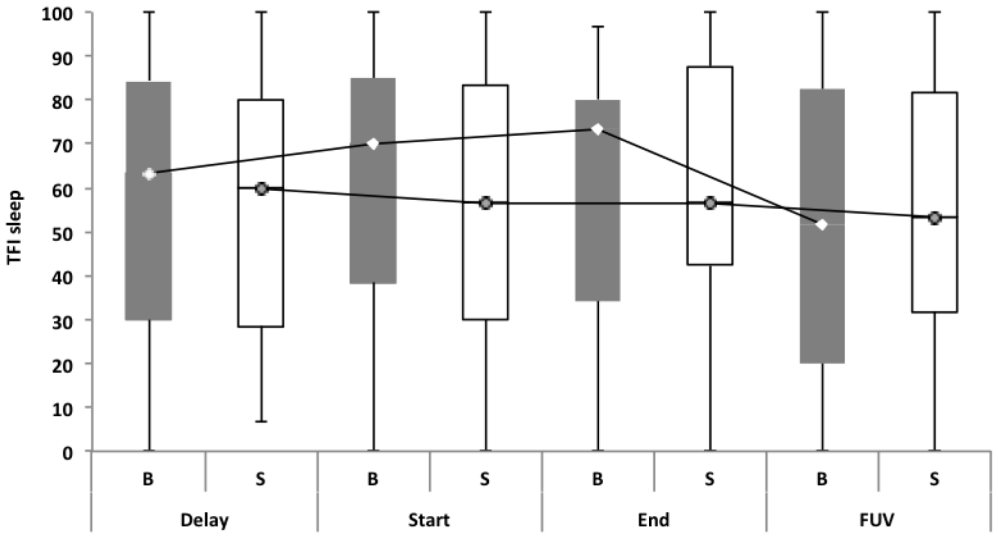

FIGURE 5 | TFI subscale of sleep disturbance. In the bifrontal group, a statistically significant effect (p = 0.048) was found for the subscale of sleep disturbance between the Start (mean = 64.03) and the FUV (mean = 52.50) test moments.

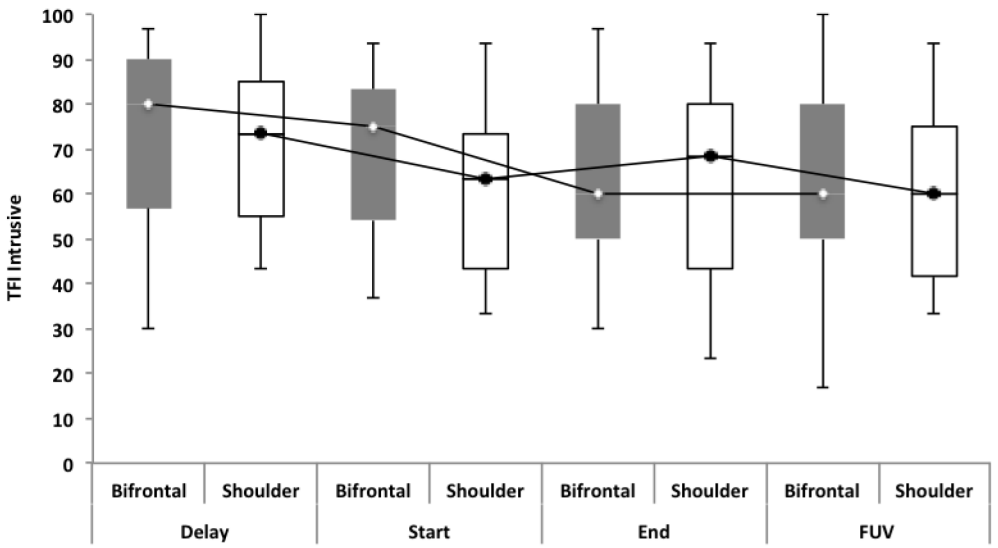

FIGURE 6 | TFI subscale of intrusiveness. Concerning the shoulder group, a statistically significant effect was found for the subscale of intrusiveness (p = 0.040) between the Delay (mean = 72.50) and the FUV (mean = 62.50) test moments. The effect found cannot be attributed to the tDCS therapy, but to the spontaneous recovery or placebo effect found between the Delay (median = 73.30) and Start (median = 63.33) test moments.

Focusing on the measurements before and after the eight sessions of tDCS, we found that 26.8% (n = 7) of the bifrontal group and 37.5% (n = 9) of the shoulder group experienced an improvement of 1 or more on the VAS for maximum loudness, while 30.6% (n = 8) of the subjects in the bifrontal group and 39.1% (n = 9) of the subjects in the shoulder group showed an improvement on the VAS for mean loudness. With respect to the change in TFI scores, 16% (n = 4) of the bifrontal group and 8.4% (n = 2) of the shoulder group showed a clinically significant improvement of more than 13 points, while 56% (n = 14) of the bifrontal group and 37.8% (n = 9) of the shoulder group showed an improvement of less than 13 points on the TFI.

DISCUSSION

The present study failed to find any difference between the electrode placements with respect to their effects on tinnitus distress and tinnitus loudness. One possible explanation for this may be the limited focus of the tDCS, due to

the large electrodes used (Nitsche et al., 2008). The lack of focus prevents the stimulation of a specific area of the brain. Moliadze et al. (2010) have suggested that an extracephalic reference electrode has the potential to better focus stimulation (Moliadze et al., 2010), but direct proof is lacking. In contrast, Parazzini et al. (2012) argued that if the anode and cathode are placed on the far sides of the brain this results in a more widely spread current/electric field (Parazzini et al., 2012). Bikson has studied the role of the position of the return electrode, finding that the repositioning of the return electrode from the contralateral forehead to the upper arm caused the relocation of the current from the frontal regions to the more posterior regions of the brain (Bikson et al., 2010). This finding is consistent with our current pattern, as presented in **Figure 2**. Tissue resistance and altered current flow can also play an important role (Nitsche and Paulus, 2011). Moliadze et al. (2010) claimed that higher current intensity is required to induce identical aftereffects in comparison to bicephalic electrode placement (Moliadze et al., 2010). In relation to bicephalic electrode placement, Shekhawat et al. (2013) concluded that 2 mA anodal tDCS at the LTA for 20 min was sufficient (Shekhawat et al., 2013). Further research should determine whether 2 mA is sufficient to induce aftereffects in the case of extracephalic reference electrode placement and the best dose-response ratio with this electrode position.

No statistically significant overall effect was found between the four test moments. Only for the TFI subscale of sleep disturbance in the bifrontal group was a significant effect found between the Start (median = 70.00) and FUV (median = 51.67) test moments. The statistically significant difference found for the subscale of intrusiveness in the shoulder group cannot be attributed to the tDCS therapy, but to spontaneous recovery or a placebo effect between the Delay (median = 73.30) and Start (median = 63.33) test moments. To rule out a placebo effect, we recommend including a control group in the study protocol for further research. The percentage of responders varied from 26.8% to 39.1% for tinnitus loudness. For the outcome measurement of distress, a clinically significant effect was only found in 16% and 8.4% of the subjects in the bifrontal and shoulder groups respectively. None of these results were statistically significant. The results confirm previous findings reported in the literature, which suggest no long-term effects on distress. However, a positive transient effect of bifrontal tDCS has been reported for tinnitus intensity (Garin et al., 2011; Frank et al., 2012). The present study failed to repeat these results. Most studies have reported a positive effect of tDCS on tinnitus loudness and distress immediately after a tDCS session (Vanneste et al., 2010; Vanneste and De Ridder, 2011; Joos et al., 2014); however, in this study, we focused on the long-term effect.

Another explanation for the lack of statistically significant results may be related to the factors that cause the variance of responders. For tDCS to be considered useful in a clinical setting, it would be interesting to know what causes this variance in the percentage of responders. Further research should also determine what might improve the administration of tDCS to attain clinically significant outcomes. High definition tDCS seems to be a promising alternative in this respect (Shekhawat et al., 2016).

While some improvement was found in both groups, the difference was not statistically significant. This finding would suggest that the area that was stimulated in both groups can be hold responsible for this improvement, namely the right side of the hippocampus, which is the part of the brain that is involved in learning and memory processes. Jastreboff (1990) has pointed out that in addition to the auditory system, the limbic system, including the hippocampus, is responsible for the preseverance of tinnitus. The gating model proposed by Rauschecker et al. (2010) also makes predictions about the structures involved in tinnitus. Although the hippocampus is not a part of the model presented, this structure may be involved in tinnitus but not sufficient to cause tinnitus on its own (Rauschecker et al., 2010; Adjamian et al., 2014). The positioning of an electrode such that it influences the structures involved in the proposed gating model would be an interesting topic for further research. In this study we did not investigate the underlying structures activated during the tDCS stimulation during the two montages (cathode on L DLPFC and shoulder). However, in future it would be insightful to incorporate MRI scans to investigate the neurophysiological structures involved during the tDCS. Another possible explanation is that the effect found can be attributed to a placebo effect. Further research should include a control group to rule out the placebo effect and reveal the real effect of tDCS.

CONCLUSION

There was no significant difference in outcomes between the electrode placements. Both placements stimulated the right side of the hippocampus, which could be responsible for the effect found in both groups. Further research should rule out a placebo effect, while alternative electrode positions, as well as high definition tDCS, could reveal the effects of stimulating brain structures that are involved in the gating model proposed by Rauschecker et al. (2010).

AUTHOR CONTRIBUTIONS

SR, VVR and PV designed and set up the study protocol. SR collected and analyzed the data. SR and MB wrote the manuscript. MA and DG designed the model of the distribution of the electric field of tDCS. GSS, VVR, DG, MA and MB reviewed the manuscript.

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REFERENCES

Adjamian, P., Hall, D. A., Palmer, A. R., Allan, T. W., and Langers, D. R. (2014). Neuroanatomical abnormalities in chronic tinnitus in the human brain. *Neurosci. Biobehav. Rev.* 45, 119–133. doi: 10.1016/j.neubiorev.2014.05.013

Bikson, M., Datta, A., Rahman, A., and Scaturro, J. (2010). Electrode montages for tDCS and weak transcranial electrical stimulation: role of "return" electrode's position and size. *Clin. Neurophysiol.* 121, 1976–1978. doi: 10.1016/j.clinph.2010.05.020

De Ridder, D., Vanneste, S., Weisz, N., Londero, A., Schlee, W., Elgoyhen, A. B., et al. (2014). An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci. Biobehav. Rev.* 44, 16–32. doi: 10.1016/j.neubiorev.2013.03.021

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Frank, E., Schecklmann, M., Landgrebe, M., Burger, J., Kreuzer, P., Poeppl, T. B., et al. (2012). Treatment of chronic tinnitus with repeated sessions of prefrontal transcranial direct current stimulation: outcomes from an open-label pilot study. *J. Neurol.* 259, 327–333. doi: 10.1007/s00415-011-6189-4

Fregni, F., Marcondes, R., Boggio, P. S., Marcolin, M. A., Rigonatti, S. P., Sanchez, T. G., et al. (2006). Transient tinnitus suppression induced by repetitive transcranial magnetic stimulation and transcranial direct current stimulation. *Eur. J. Neurol.* 13, 996–1001. doi: 10.1111/j.1468-1331.2006.01414.x

Garin, P., Gilain, C., Van Damme, J. P., De Fays, K., Jamart, J., Ossemann, M., et al. (2011). Short- and long-lasting tinnitus relief induced by transcranial direct current stimulation. *J. Neurol.* 258, 1940–1948. doi: 10.1007/s00415-011-6037-6

Jastreboff, P. J. (1990). Phantom auditory perception (tinnitus): mechanisms of generation and perception. *Neurosci. Res.* 8, 221–254. doi: 10.1016/0168-0102(90)90031-9

Joos, K., De Ridder, D., Van de Heyning, P., and Vanneste, S. (2014). Polarity specific suppression effects of transcranial direct current stimulation for tinnitus. *Neural Plast.* 2014:930860. doi: 10.1155/2014/930860

Khalfa, S., Dubal, S., Veuillet, E., Perez-Diaz, F., Jouvent, R., and Collet, L. (2002). Psychometric normalization of a hyperacusis questionnaire. *ORL J. Otorhinolaryngol. Relat. Spec.* 64, 436–442. doi: 10.1159/000067570

Meikle, M. B., Henry, J. A., Griest, S. E., Stewart, B. J., Abrams, H. B., Mcardle, R., et al. (2012). The tinnitus functional index: development of a new clinical measure for chronic, intrusive tinnitus. *Ear Hear.* 33, 153–176. doi: 10.1097/AUD.0b013e3182597b3e

Moliadze, V., Antal, A., and Paulus, W. (2010). Electrode-distance dependent after-effects of transcranial direct and random noise stimulation with extracephalic reference electrodes. *Clin. Neurophysiol.* 121, 2165–2171. doi: 10.1016/j.clinph.2010.04.033

Nitsche, M. A., and Paulus, W. (2000). Excitability changes induced in the human motor cortex by weak transcranial direct current stimulation. *J. Physiol.* 527, 633–639. doi: 10.1111/j.1469-7793.2000.t01-1-00633.x

Nitsche, M. A., and Paulus, W. (2011). Transcranial direct current stimulation—update 2011. *Restor. Neurol. Neurosci.* 29, 463–492. doi: 10.3233/RNN-2011-0618

Nitsche, M. A., Cohen, L. G., Wassermann, E. M., Priori, A., Lang, N., Antal, A., et al. (2008). Transcranial direct current stimulation: state of the art 2008. *Brain Stimul.* 1, 206–223. doi: 10.1016/j.brs.2008.06.004

Parazzini, M., Fiocchi, S., and Ravazzani, P. (2012). Electric field and current density distribution in an anatomical head model during transcranial direct current stimulation for tinnitus treatment. *Bioelectromagnetics* 33, 476–487. doi: 10.1002/bem.21708

Rabau, S., Wouters, K., and Van de Heyning, P. (2014). Validation and translation of the dutch version of the tinnitus functional index. *B-ENT* 10, 251–258.

Rauschecker, J. P., Leaver, A. M., and Mühlau, M. (2010). Tuning out the noise: limbic-auditory interactions in tinnitus. *Neuron* 66, 819–826. doi: 10.1016/j.neuron.2010.04.032

Shargorodsky, J., Curhan, G. C., and Farwell, W. R. (2010). Prevalence and characteristics of tinnitus among US adults. *Am. J. Med.* 123, 711–718. doi: 10.1016/j.amjmed.2010.02.015

Shekhawat, G. S., Stinear, C. M., and Searchfield, G. D. (2013). Transcranial direct current stimulation intensity and duration effects on tinnitus suppression. *Neurorehabil. Neural Repair* 27, 164–172. doi: 10.1177/1545968312459908

Shekhawat, G. S., Sundram, F., Bikson, M., Truong, D., De Ridder, D., Stinear, C. M., et al. (2016). Intensity, duration and location of high-definition transcranial direct current stimulation for tinnitus relief. *Neurorehabil. Neural Repair* 30, 349–359. doi: 10.1177/1545968315595286

Song, J. J., Vanneste, S., Van de Heyning, P., and De Ridder, D. (2012). Transcranial direct current stimulation in tinnitus patients: a systemic review and meta-analysis. *ScientificWorldJournal* 2012:427941. doi: 10.1100/2012/427941

Tanaka, E., Inui, K., Kida, T., Miyazaki, T., Takeshima, Y., and Kakigi, R. (2008). A transition from unimodal to multimodal activations in four sensory modalities in humans: an electrophysiological study. *BMC Neurosci.* 9:116. doi: 10.1186/1471-2202-9-116

Truong, D. Q., Datta, A., Xu, J., Fregni, F., and Bikson, M. (2012a). "Prefrontal cortex transcranial direct current stimulation via a combined high definition and conventional electrode montage: a FEM modeling studying," in *34th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBS),* (San Diego, CA), 6608-6611.

Truong, D. Q., Magerowski, G., Pascual-Leone, A., Alonso-Alonso, M., and Bikson, M. (2012b). "Finite element study of skin and fat delineation in an obese subject for transcranial direct current stimulation," in *34th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBS)*, (San Diego, CA), 6587-6590.

Vanneste, S., and De Ridder, D. (2011). Bifrontal transcranial direct current stimulation modulates tinnitus intensity and tinnitus-distress-related brain activity. *Eur. J. Neurosci.* 34, 605–614. doi: 10.1111/j.1460-9568.2011.07778.x

Vanneste, S., Focquaert, F., Van de Heyning, P., and De Ridder, D. (2011). Different resting state brain activity and functional connectivity in patients who respond and not respond to bifrontal tDCS for tinnitus suppression. *Exp. Brain Res.* 210, 217–227. doi: 10.1007/s00221-011-2617-z

Vanneste, S., Plazier, M., Ost, J., van der Loo, E., Van de Heyning, P., and De Ridder, D. (2010). Bilateral dorsolateral prefrontal cortex modulation for tinnitus by transcranial direct current stimulation: a preliminary clinical study. *Exp. Brain Res.* 202, 779–785. doi: 10.1007/s00221-010-2183-9

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Tinnitus Treatment with Oxytocin: A Pilot Study

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Introduction: Tinnitus is the perception of sound in the absence of an external stimulus. It is a frequent condition for which there is as yet no pharmacological treatment approved. Auditory and non-auditory pathways are involved in tinnitus' pathophysiology. Oxytocin is a neurohormone and eventual neurotransmitter that plays a complex role in social cognition and behavior.

Objective: To evaluate the potential of oxytocin as a tinnitus treatment.

Study design: Two studies were performed. Study 1 was a long-term open pilot study, while study 2 investigated short-term effects with a double-blinded placebo-controlled cross-over study.

Setting: Ambulatory ENT care.

Subjects and method: In study 1, 15 patients were investigated over a 10-week period in an open pilot study. In study 2, 16 patients were included in a placebo-controlled crossover trial to investigate short-term effects following a single dose.

Results: For the long-term study (study 1), analysis of variance revealed a significant decrease in tinnitus sensation, both for the Tinnitus Handicap Inventory and Clinical Global Impression (CGI). Also, the short-term effects in study 2 revealed a significant reduction of tinnitus because of the oxytocin nasal spray as measured with the Visual Analog Scale and the CGI Scale.

Conclusion: These preliminary studies demonstrated that oxytocin may represent a helpful tool for treating tinnitus and further larger controlled studies are warranted.

Keywords: tinnitus, oxytocin, hearing disorders, pharmacotherapy, nasal sprays

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INTRODUCTION

Tinnitus is a phantom auditory sensation that is not generated by an external stimulus. It is a symptom that affects about 25% of American adults, in a frequent basis for around 8% of them (1). According to current trends of thoughts, tinnitus is a central nervous system phenomenon that follows an initial peripheral damage and may be modulated by many concurring neuronal circuits (2). Neuroimaging studies suggest that tinnitus results from the dynamic interaction between auditory

and non-auditory pathways, and the result of this interaction, especially when the limbic and autonomous systems are involved, is the trigger of negative emotional associations and appearance of uncomfortable reactions (3). As yet, there is no Food and Drug Administration (FDA)-approved pharmacological treatment for tinnitus. However, various factors structures such as ion channels, neurotransmitters, and receptors have been proposed to be involved in tinnitus pathophysiology (4). Thus, there is no reasonable argument to believe that tinnitus could not be pharmacologically treated (5).

Oxytocin is a neurohormone that may also act as a neurotransmitter, produced by magnocellular neurons in the ventricular nuclei of the hypothalamus (6). Oxytocin's production is stimulated by high estrogen doses and inhibited by high doses of catecholamines (dopamine, epinephrine, and norepinephrine) (7). It is released to the bloodstream by the posterior pituitary. Oxytocin receptors (OTR) are widespread throughout the human body and belong to the family of heterotrimeric G-protein-coupled receptors, which are found in many cell types (8). OTR promote the inhibition of adenilate-cyclase, thus reducing intracellular levels of cyclic-AMP and subsequently opening potassium channels and closing calcium channels, having, thereby, an inhibitory signature (9).

Oxytocin is responsible for important physiological functions, such as uterine contraction, lactation stimulation, sperm transport, and ejaculation (10). In addition, oxytocin plays a complex role in social cognition and behavior. Important aspects of human social interaction such as empathy, trust, and social learning are influenced by oxytocin (11). Among other mechanisms the prosocial effects of oxytocin are mediated by reduction of amygdale activation (12). Recently, it has been shown, that oxytocin also increases the salience of acoustic social stimuli by modulating the inhibitory function in the auditory cortex (13).

Imaging studies have demonstrated that tinnitus loudness and tinnitus distress are reflected by increased activation in networks involving the auditory cortex and the amygdala, respectively (3, 14). Therefore, we hypothesized that oxytocin may influence tinnitus perception and tested in two studies whether its intranasal application reduces tinnitus loudness and distress.

MATERIALS AND METHODS

The trial comprised 2 studies, as follows.

Study 1

Study Design

Study 1 was designed as an open pilot study.

Subjects

Fifteen patients who presented with the primary complaint of tinnitus in a local ENT clinic in São Paulo and Valença were included. Only patients older than 18 years with a continuous perception of tinnitus of at least 6 months duration were included. Patients were excluded if their Tinnitus Handicap Inventory (THI) (15) score in the Brazilian Portuguese validated version (16) was below 16 points. All patients underwent a complete otolaryngological evaluation and were excluded from the study in case of external or media otitis or in the presence of A-s, B, and C tympanogram curves. Also, women in fertile age [up to 49 years old (17)] were excluded from the study, to avoid oxytocin-induced uterine contractions. Informed consent was obtained prior to the study, and the patients were informed about the clinical procedures. Clinical and demographic characteristics of participants (mean age, percentage of females, mean duration of tinnitus in months, laterality and scores on the THI in both studies) are described in **Table 1**.

Experimental Procedure

Oxytocin (Syntocinon) was administered daily for a duration of 10 weeks. Patients were instructed to apply one puff [each puff corresponds to 4 oxytocin IU] of oxytocin in each nostril two times a day, which sums up to a dosage of 16 IU per day. The patients were asked to store the oxytocin in the refrigerator throughout the study period. Subsequent visits were made in weeks 1, 2, 3, 4, 6, 8, and 10 to monitor study progress and screen for side effects. Treatment effects were assessed with the THI (primary outcome measurement) and the Clinical Global Impression (CGI) scale improvement [secondary outcome measurement; CGI-I (18)]. The CGI scale is a well-accepted instrument in clinical research for evaluating the clinical improvement retrospectively. The instruction in the CGI is: "Please rate the total improvement of your tinnitus complaints compared to before beginning of treatment."

Statistical Analysis

For the THI, a mixed model analysis of variance (ANOVA) was calculated with the factor time and a random intercept per patient. A mixed model ANOVA was also calculated for the CGI with the factor time (weeks 1–10) and a random intercept per patient. In case of positive results of the ANOVA further comparisons were calculated.

Study 2

During study 1, five subjects reported an immediate effect (5–10 min after drug administration) on tinnitus sensation (around 50% reduction of tinnitus volume). Considering this unexpected finding, we decided to investigate this tinnitus reduction following one single dose in more detail. Therefore, we performed a second study with a single dose of oxytocin treatment in a double-blind placebo-controlled study using a crossover design. As study 1 turned out with positive results, study 2 was performed with a similar number of patients.

TABLE 1 | Patient groups, clinical and demographic data (mean ± SD).

n	Age	Female (%)	Duration of tinnitus in months	Laterality	THI at baseline
Study 1 (*n* = 15)	60.6 ± 9.6	40	107.8 ± 118.7	5 unilateral 7 bilateral 3 in head	56.8 ± 28.2
Study 2 (*n* = 16)	62.8 ± 10.6	31.3	78.8 ± 147.9	11 unilateral 5 bilateral	52.8 ± 30.5

THI, Tinnitus Handicap Inventory.

Subjects

Study 2 was performed in the same ENT clinic as study 1. Seventeen patients with the primary complaint of tinnitus and who had not participated in study 1 were included.

As in the first study, all patients were older than 18 years and reported chronic tinnitus of more than 6 months duration. Prior to the study, all patients were seen by an experienced ENT doctor. Patients with vascular or muscular origin of their tinnitus, patients with conductive hearing loss and patients with regular intake of other medications during the study period were not included. Patients reporting previous experience with oxytocin nasal spray were not included as well. A further inclusion criterion was a tinnitus loudness rating of at least 4, measured on a scale between 0 and 10.

One patient dropped out of the study because she could not return for the second visit. Clinical and demographic characteristics of the 16 patients, which were included in the analysis, are displayed in **Table 1**. Informed consent was obtained prior to study participation.

Experimental Procedure

In study 2, the effects of nasal oxytocin administration were compared with placebo treatment. The order of drug treatment was counterbalanced. Patients were randomized to receive either a single dosage administration of 16 IU of oxytocin (Syntocinon) or a placebo treatment with a nasal spray containing distilled water, which is indistinguishable to oxytocin nasal spray considering smell and taste. A randomization table was created prior to study start by Winfried Schlee who is not in contact with the study participants. After an interval of 1 week, the patients received a second administration with either placebo (the group who received oxytocin first) or with oxytocin (the group who received placebo first). The patients, as well as the medical doctor who evaluated the results (author 1), were blinded to the treatment assignment. Author 2, who applied oxytocin and placebo, was not blinded. In the oxytocin condition, patients received two puffs of oxytocin in each nostril, which corresponded to 16 IU. Likewise, in the placebo treatment, the patients received two puffs distilled water in each nostril, which is supposed to have no clinical effect.

The primary outcome measurement was a visual analog scale (VAS) asking for the tinnitus loudness (19). The VAS score for tinnitus loudness was assessed directly before administering the nasal spray as well as 30 min and 24 h after the intervention. Additionally, the CGI-I was applied 30 min and 24 h after the intervention as secondary outcome measurement.

Statistical Analysis

For the VAS scores a two-way mixed model ANOVA was calculated with the factors *time* and *treatment*. A random intercept was modeled for each participant. Mixed model ANOVA were also used to analyze the results of the CGI scale. For the CGI scores, again a two-way mixed model ANOVA was calculated with the factors *time* and *treatment*.

The statistical analysis was performed with the statistical software package R, version 3.3.3 [R Core Team (2017). R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/]. For the mixed models analysis, the "nlme" library (version 3.1-131) was used. A p-value ≤ 0.05 was considered as significant. Correction for multiple comparisons was applied using the stepwise Holm–Bonferroni method (20).

Study Registration

The study was approved by the ethics committee and registered at http://clinicaltrials.gov.

RESULTS

Study 1

Mean scores for the THI and the CGI-I are presented in **Figures 1** and **2**, respectively. Both measures show continuous and significant decreases of tinnitus-related symptoms measured with the THI [$F(7,98) = 7.45$, $p < 0.0001$] as well as global clinical improvement (CGI-I) [$F(6,84) = 5.22$, $p < 0.0001$] over the study period.

For the THI scores, further comparisons using paired t-tests between baseline and all other time points reveal significant differences for the THI measurements at week 1, 2, 3, 4, 6, 8, and 10 (p-values of all comparisons survived the Holm–Bonferroni method for multiple comparisons).

For the CGI-I additional tests were calculated to test if scores were different from the value of 4, which indicates "no change." No significant difference was found for week 1, week 2, and week 3. The comparisons for week 4, 6, 8, and 10; however, survived the Holm–Bonferroni correction for multiple comparisons. For week 4 and 6, the CGI scores indicated a change with high significance ($p < 0.01$), for week 8 and 10, the CGI scores indicated a change with very high significance ($p < 0.001$).

Paired t-tests were calculated to compare the maximum hearing loss of the participants before and after the treatment. The hearing function did not change significantly, neither for the right ear ($p = 0.2$) nor for the left ear ($p = 0.36$).

Study 2

Mean scores of the oxytocin treatment and the placebo intervention are presented in **Figures 3** and **4** for the VAS scores and the CGI-I, respectively.

In the ANOVA for the VAS scores, the main effect of *treatment* was highly significant [$F(1,77) = 9.54$, $p = 0.003$] and the main effect of *time* was significant [$F(2,77) = 4.47$, $p = 0.015$]. The interaction effect *time* × *treatment*, however, failed to reach the level of significance [$F(2,77) = 1.13$, $p = 0.33$]. Cohen's d effect sizes for the VAS ratings were 0.55 (30 min) and 0.56 (24 h) for oxytocin treatment and 0.18 (30 min) and 0.19 (24 h) for placebo treatment, respectively.

Furthermore, the ANOVA using the CGI scores as dependent variable, the main effect *treatment* was highly significant [$F(1,46) = 8.72$, $p = 0.005$], while the main effect *time* did not reach statistical significance [$F(1,46) = 0.60$, $p = 0.44$]. There was no statistically significant interaction effect [$F(1,46) = 0.001$, $p > 0.9$]. Additional t-tests were calculated to confirm the results and showed that there was no statistical significant difference between the two time points (30 m and 24 h after treatment) for the oxytocin ($p > 0.63$) nor for the placebo condition ($p > 0.58$). A t-test testing

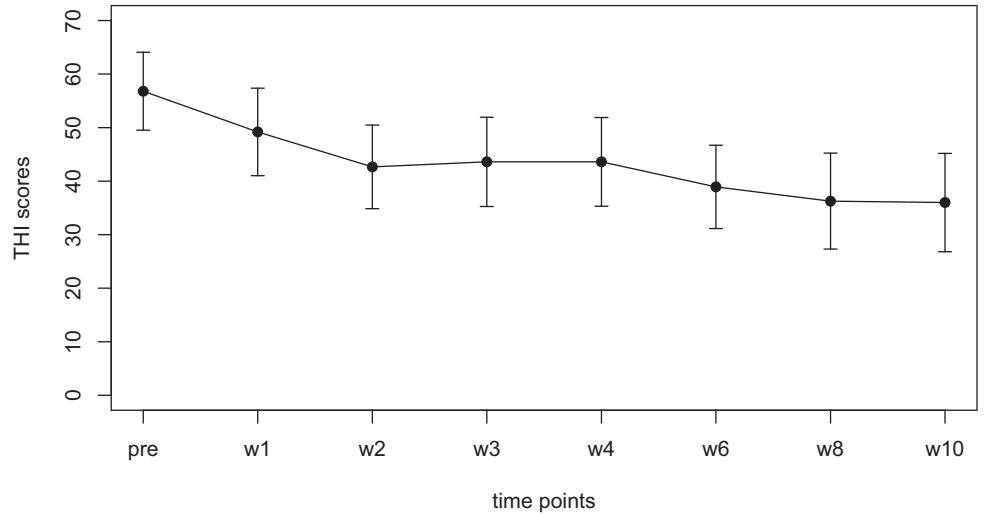

FIGURE 1 | Average scores of the Tinnitus Handicap Inventory (THI) (0–100) before the treatment and at all visits during the study (weeks 1–8). The final assessment was done at the end of treatment in week 10.

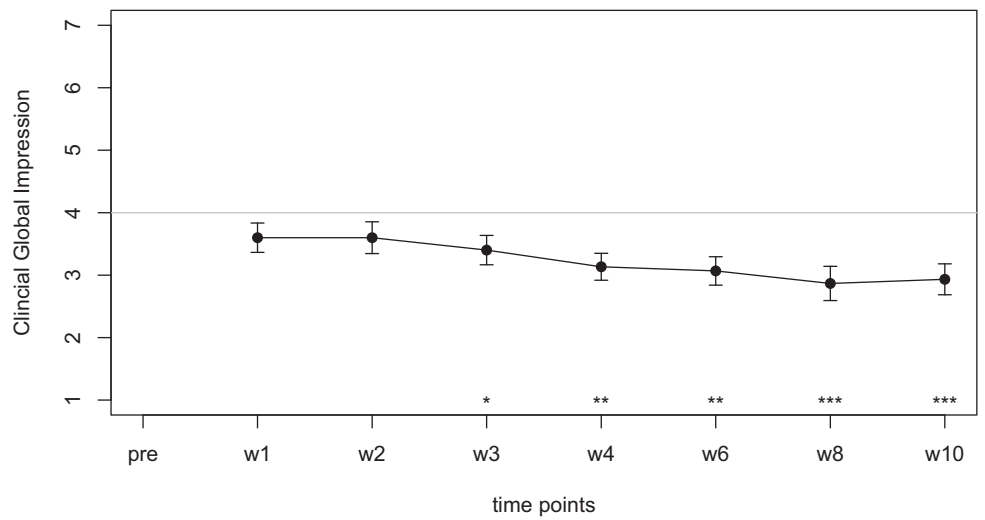

FIGURE 2 | Average scores of the Clinical Global Impression scale (1–7). A rating of 4 on this scale indicates "no change." Smaller numbers indicate an improvement of clinical symptoms (1 = "very much better," 2 = "much better," 3 = "minimally better").

for the difference between the oxytocin and placebo intervention, irrespective of the time point, revealed a statistically significant improvement for the oxytocin treatment ($t = -2.9$, $p = 0.005$).

DISCUSSION

The present results show that in an exploratory non-controlled study, a 10-week treatment with daily intranasal puffs of oxytocin produced a significant reduction in the THI and CGI in a group of tinnitus patients. Moreover, a double-blind controlled study with a single dose of an intranasal oxytocin, although not significant, exhibited a tendency in a reduction in tinnitus distress, as measured by the CGI.

The presented pilot studies were designed to explore possible effects of oxytocin on tinnitus. The open 10 week treatment study (study 1) suggests that regular intake of oxytocin can reduce tinnitus-related handicap. Open pilot studies have been suggested as a useful screening tool to identify potentially promising pharmacological compounds (21–23). Motivated by the promising results of study 1, we performed also a placebo-controlled crossover trial to investigate the short-term effects of a single dose of oxytocin. Whereas questionnaires represent the gold standard for assessing longer lasting effects of therapeutic interventions (24), short-term effects can be best detected with VAS. While the clinical effects of the short-term oxytocin treatment are much smaller than the long-term effects in study 1, they still suggest

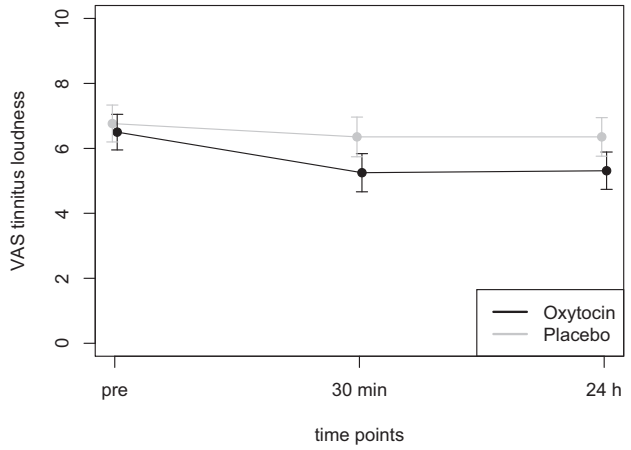

FIGURE 3 | Average scores of the Visual Analogue Scale (0–10) before the treatment and at two time points after the intervention (30 min and 24 h after).

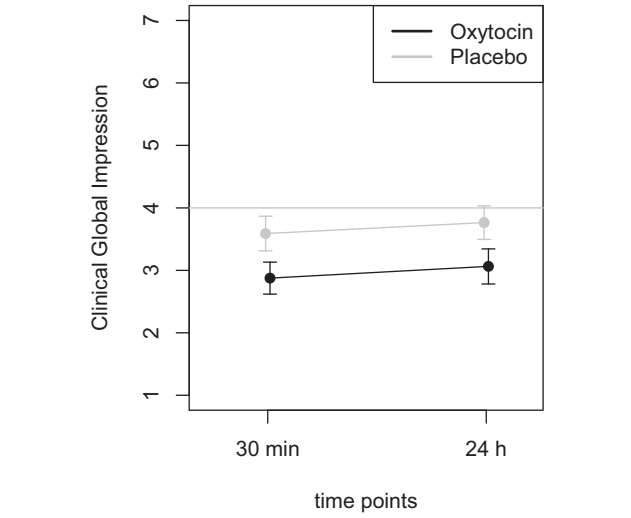

FIGURE 4 | Average scores of the Clinical Global Impression scale (1–7). A rating of 4 on this scale indicates "no change." Smaller numbers indicate an improvement of clinical symptoms (1 = "very much better," 2 = "much better," 3 = "minimally better").

a potential therapeutic role of oxytocin for tinnitus. These preliminary results warrant further controlled studies, which should include more patients and investigate chronic treatments with oxytocin. The results of the presented pilot studies provide an estimation of the effect sizes for both acute and long-term effects of oxytocin to inform the study design of future randomized controlled studies. While the open study with the long-term treatment (study 1) revealed a strong reduction of tinnitus-related distress over time, the short-term treatment (study 2) revealed only small differences between placebo and oxytocin. Further studies on oxytocin treatment for tinnitus should invest in long-term treatment.

A wide variety of compounds are used off-label to treat tinnitus patients (5, 25). However, there is still no US FDA- or European Medicines Agency-approved drug on the market for this clinical unmet use (5). The comprehensive list of compounds includes almost the entire pharmacopeia arsenal, such as anxiolytics, anticonvulsants, antidepressants, *N*-methyl D-aspartate (NMDA) antagonists, cholinergic antagonists, antihistamines, vasodilators, antipsychotics, sodium and calcium channel antagonists, antidiuretics, and herbal medicines, among others (5, 25). In most cases, the pharmacological treatment is used to treat comorbidities which accompany tinnitus, such as frustration, annoyance, anxiety, depression, irritation, concentration difficulties, and sleep disturbances which are most relevant for the perceived tinnitus severity (5, 19). In this context, the exploratory use of oxytocin on tinnitus patients is well justified, since it is a compound with a different mechanism of action from those previously tested.

In particular, Kirsch et al. have shown that when compared with placebo, oxytocin potently reduces activation of the amygdala and coupling of the amygdala to brainstem regions implicated in autonomic and behavioral manifestations of fear (26). In this regard, tinnitus neuroimaging techniques have identified brain networks related to tinnitus associated distress which include the amygdala and the autonomic nervous system (3). Recently, it has been shown that tinnitus distress correlates with enhanced effective connectivity from the amygdala to the auditory cortex (27). An EEG analysis revealed that tinnitus distress is correlated with more synchronized alpha activity in various emotion-related areas, including the subcallosal anterior cingulate cortex, the insula, the parahippocampal area and the amygdale (28). In addition, an MEG functional connectivity analysis has shown long-range coupling between frontal, parietal and cingulate brain areas in alpha and gamma phase synchronization related to tinnitus distress (29). In summary, tinnitus distress has been related to the co-activation of a network which includes amygdala, anterior cingulate cortex, insula, parahippocampal area and which is under direct influence of the posterior cingulate and prefrontal cortex (30, 31). This network partially overlaps with brain areas implicated in distress in patients suffering from pain (32), dyspnea in asthma (33), somatoform disorders (34) and might, therefore, represent a non-specific distress network.

Through source localized resting-state EEG and electrocardiogram recordings, the participation of the autonomic nervous system to tinnitus distress has been investigated, showing that the dorsal and subgenual anterior cingulate, as well as the left and right insula are important in the central control of the autonomic system in tinnitus patients (35, 36). The perceived distress in tinnitus patients seems to be sympathetically mediated. It is of interest that the same areas that are involved in the control of the autonomic system are also involved in salience processing and distress. This suggests that the autonomic nervous system critically influences salience perception and distress associated with tinnitus.

Thus, the observed reduction of tinnitus handicap after long-term oxytocin application could be explained by a modulatory influence of oxytocin on amygdala activity and on a decoupling

between amygdala activation and autonomous nervous system regulation. Further studies investigating the effect of oxytocin on perception and simultaneously on brain activity and connectivity could shed further light on the mechanisms by which oxytocin interacts with tinnitus perception and distress.

ETHICS STATEMENT

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AUTHOR CONTRIBUTIONS

Study design: AA, RF, WS, BL, and AE. Data collection: AA and RF. Statistics: WS. Writing: AA, RF, AE, BL, and NP. Manuscript revision: AA, RF, AE, BL, and PS. All contributed for writing and revision: AA, RF, BL, AE, NP, and WS.

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REFERENCES

1. Shargorodsky J, Curhan JC, Farwell WR. Prevalence and characteristics of tinnitus among US adults. *Am J Med* (2010) 123(8):711–8. doi:10.1016/j.amjmed.2010.02.015
2. Langguth B, Kreuzer PM, Kleinjung T, De Ridder D. Tinnitus: causes and clinical management. *Lancet Neurol* (2013) 12(9):920–30. doi:10.1016/S1474-4422(13)70160-1
3. Elgoyhen AB, Langguth B, De Ridder D, Vanneste S. Tinnitus: perspectives from human neuroimaging. *Nat Rev Neurosci* (2015) 16(10):632–42. doi:10.1038/nrn4003
4. Elgoyhen AB, Langguth B, Nowak W, Schecklmann M, De Ridder D, Vanneste S. Identifying tinnitus-related genes based on a side-effect network analysis. *CPT Pharmacometrics Syst Pharmacol* (2014) 3:e97. doi:10.1038/psp.2013.75
5. Langguth B, Salvi R, Elgoyhen AB. Emerging pharmacotherapy of tinnitus. *Expert Opin Emerg Drugs* (2009) 14(4):687–702. doi:10.1517/14728210903206975
6. Richard P, Moos F, Freund-Mercier MJ. Central effects of oxytocin. *Physiol Rev* (1991) 71(2):331–70.
7. Wang H, Ward AR, Morris JF. Oestradiol acutely stimulates exocytosis of oxytocin and vasopressin from dendrites and somata of hypothalamic magnocellular neurons. *Neuroscience* (1995) 68(4):1179–88. doi:10.1016/0306-4522(95)00186-M
8. Wettschureck N, Moers A, Hamalainen T, Lemberger T, Schütz G, Offermanns S. Heterotrimeric G proteins of the Gq/11 family are crucial for the induction of maternal behavior in mice. *Mol Cell Biol* (2004) 24(18):8048–54. doi:10.1128/MCB.24.18.8048-8054.2004
9. Gimpl G, Fahrenholz F. The oxytocin receptor system: structure, function, and regulation. *Physiol Rev* (2001) 81:629–83.
10. Muin DA, Wolzt M, Marculescu R, Sheikh Rezaei S, Salama M, Fuchs C, et al. Effect of long-term intranasal oxytocin on sexual dysfunction in premenopausal and postmenopausal women: a randomized trial. *Fertil Steril* (2015) 104(3):715–23.e4. doi:10.1016/j.fertnstert.2015.06.010
11. Meyer-Lindenberg A, Domes G, Kirsch P, Heinrichs M. Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. *Nat Rev Neurosci* (2011) 12(9):524–38. doi:10.1038/nrn3044
12. Kirsch P. Oxytocin in the socioemotional brain: implications for psychiatric disorders. *Dialogues Clin Neurosci* (2015) 17(4):463–76.
13. Marlin BJ, Mitre M, D'amour JA, Chao MV, Froemke RC. Oxytocin enables maternal behaviour by balancing cortical inhibition. *Nature* (2015) 520(7548):499–504. doi:10.1038/nature14402
14. De Ridder D, Elgoyhen AB, Romo R, Langguth B. Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc Natl Acad Sci U S A* (2011) 108(20):8075–80. doi:10.1073/pnas.1018466108
15. Newman CW, Jacobson GP, Spitzer JB. Development of the Tinnitus Handicap Inventory. *Arch Otolaryngol Head Neck Surg* (1996) 122(2):143–8. doi:10.1001/archotol.1996.01890140029007
16. Paula Erika Alves F, Cunha F, Onishi ET, Branco-Barreiro FC, Ganança FF. Tinnitus Handicap Inventory: cross-cultural adaptation to Brazilian Portuguese. *Pro Fono* (2005) 17(3):303–10. doi:10.1590/S0104-56872005000300004
17. Wallace WHB, Kelsey TW. Human ovarian reserve from conception to the menopause. *PLoS One* (2010) 5(1):e8772. doi:10.1371/journal.ponee.0008772
18. Guy W, editor. *ECDEU Assessment Manual for Psychopharmacology*. Rockville, MD: US Department of Health, Education and Welfare Public Health Service Alcohol, Drug Abuse, and Mental Health Administration (1976).
19. Adamchic I, Langguth B, Hauptmann C, Tass PA. Psychometric evaluation of visual analog scale for the assessment of chronic tinnitus. *Am J Audiol* (2012) 21(2):215–25. doi:10.1044/1059-0889(2012/12-0010)
20. Holm S. A simple sequentially rejective multiple test procedure. *Scand J Stat* (1979) 6:65–70.
21. Dobie RA. A review of randomized clinical trials in tinnitus. *Laryngoscope* (1999) 109(8):1202–11. doi:10.1097/00005537-199908000-00004
22. Coelho C, Figueiredo R, Frank E, Burger J, Schecklmann M, Landgrebe M, et al. Reduction of tinnitus severity by the centrally acting muscle relaxant cyclobenzaprine: an open-label pilot study. *Audiol Neurootol* (2012) 17(3):179–88. doi:10.1159/000335657
23. Landgrebe M, Azevedo A, Baguley D, Bauer C, Cacace A, Coelho C, et al. Methodological aspects of clinical trials in tinnitus: a proposal for an international standard. *J Psychosom Res* (2012) 73(2):112–21. doi:10.1016/j.jpsychores.2012.05.002
24. Langguth B, Goodey R, Azevedo A, Bjorne A, Cacace A, Crocetti A, et al. Consensus for tinnitus patient assessment and treatment outcome measurement: Tinnitus Research Initiative meeting, Regensburg, July 2006. *Prog Brain Res* (2007) 166:525–36. doi:10.1016/S0079-6123(07)66050-6
25. Figueiredo RR, Azevedo AA, Penido NO. Pharmacological treatment of tinnitus. In: Watson J, editor. *Tinnitus*. New York: Nova Science Publishers (2016). p. 25–42.
26. Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S, et al. Oxytocin modulates neural circuitry for social cognition and fear in humans. *J Neurosci* (2005) 25(49):11489–93. doi:10.1523/JNEUROSCI.3984-05.2005
27. Chen YC, Xia W, Chen H, Feng Y, Xu JJ, Gu JP, et al. Tinnitus distress is linked to enhanced resting-state functional connectivity from the limbic system to the auditory cortex. *Hum Brain Mapp* (2017) 38(5):2384–97. doi:10.1002/hbm.23525
28. Vanneste S, Plazier M, der Loo EV, de Heyning PV, Congedo M, De Ridder D. The neural correlates of tinnitus-related distress. *Neuroimage* (2010) 52(2):470–80. doi:10.1016/j.neuroimage.2010.04.029
29. Schlee W, Weisz N, Bertrand O, Hartmann T, Elbert T. Using auditory steady state responses to outline the functional connectivity in the tinnitus brain. *PLoS One* (2008) 3(11):e3720. doi:10.1371/journal.pone.0003720
30. De Ridder D, Vanneste S, Weisz N, Londero A, Schlee W, Elgoyhen AB, et al. An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci Biobehav Rev* (2014) 44:16–32. doi:10.1016/j.neubiorev.2013.03.021
31. Husain FT. Neural networks of tinnitus in humans: elucidating severity and habituation. *Hear Res* (2016) 334:37–48. doi:10.1016/j.heares.2015.09.010
32. Price DD. Psychological and neural mechanisms of the affective dimension of pain. *Science* (2000) 288(5472):1769–72. doi:10.1126/science.288.5472.1769
33. von Leupoldt A, Sommer T, Kegat S, Baumann HJ, Klose H, Dahme B, et al. Dyspnea and pain share emotion-related brain network. *Neuroimage* (2009) 48(1):200–6. doi:10.1016/j.neuroimage.2009.06.015

34. Landgrebe M, Barta W, Rosengarth K, Frick U, Hauser S, Langguth B, et al. Neuronal correlates of symptom formation in functional somatic syndromes: a fMRI study. *Neuroimage* (2008) 41(4):1336–44. doi:10.1016/j.neuroimage.2008.04.171
35. van der Loo E, Congedo M, Vanneste S, Van De Heyning P, De Ridder D. Insular lateralization in tinnitus distress. *Auton Neurosci* (2011) 165(2):191–4. doi:10.1016/j.autneu.2011.06.007
36. Vanneste S, De Ridder D. Brain areas controlling heart rate variability in tinnitus and tinnitus-related distress. *PLoS One* (2013) 8(3):e59728. doi:10.1371/journal.pone.0059728

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ORIGINAL RESEARCH
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Identification of Candidate Allosteric Modulators of the M1 Muscarinic Acetylcholine Receptor Which May Improve Vagus Nerve Stimulation in Chronic Tinnitus

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Chronic tinnitus is characterized by neuroplastic changes of the auditory cortex. A promising method for therapy of chronic tinnitus is vagus nerve stimulation (VNS) combined with auditory stimulation. The principle of VNS is reversal of pathological neuroplastic changes of the auditory cortex toward physiological neural activity and synchronicity. The VNS mechanism of action in chronic tinnitus patients is prevailingly through the muscarinic neuromodulation of the auditory cortex by the activation of nc. basalis Meynerti. The aim of this study is to propose potential pharmaceutics which may improve the neuromodulatory effects of VNS. The working hypothesis is that M1 receptors have a dominant role in the neural plasticity of the auditory cortex. We propose that allosteric agonists of the muscarinic receptor type 1 (M1) receptor could improve specificity and selectivity of the neuromodulatory effect of VNS on the auditory cortex of chronic tinnitus patients even in the circumstances of lower acetylcholine brain concentration. This intervention would also reinforce the re-learning process of tinnitus (sub)networks by acting on cholinergic memory and learning mechanisms. We performed *in silico* screening of drug space using the EIIP/AQVN filter and selected 50 drugs as candidates for allosteric modulators of muscarinic receptors. Further filtering of these compounds by means of 3D QSAR and docking revealed 3 approved drugs—bromazepam, estazolam and flumazenil as the most promising candidates for combined chronic tinnitus therapy. These drugs should be further evaluated by biological tests and clinical trials.

Keywords: tinnitus, muscarinic allosteric agonists, M1 receptor, vagus nerve stimulation, *in silico* analysis, information spectrum method

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INTRODUCTION

Tinnitus, the perception of phantom sound, is a debilitating condition with notable prevalence: approximately 10–15% of the general population experience tinnitus and for 7 million this is a debilitating condition (Geven et al., 2014). The Royal National Institute for Deaf People estimates that 13 million people in Western Europe and USA seek medical assistance for their tinnitus

symptoms (Vio and Holme, 2005). Demographic development and increase of occupational and ambient noise support the view that this problem will be even more prevalent in the future. In spite of the fact that more than 4 million prescriptions are written for the treatment of tinnitus, there is not a single drug approved by the FDA specific for the treatment of tinnitus. The estimates are that a potential tinnitus drug could have a product value of more than $600 million in the first year after its release (https://www.ata.org/understanding-facts).

The problem of chronic tinnitus is mainly of central origin. The pathophysiological substrate of chronic tinnitus is still a matter of intense debate (De Ridder et al., 2014b; Norena, 2015). The majority of chronic states of tinnitus begin with the functional damage of the cochlea (i.e., noise or hearing loss) and consequent abnormal input to the higher level neural structures of the auditory pathway (Eggermont and Roberts, 2004; Guitton, 2012; Chen et al., 2013; Wu et al., 2016). When tinnitus stabilizes as a chronic condition (not less than 1 year, Malinvaud et al., 2016), then numerous functional disarrangements occur, like the hyperpolarization of thalamic relay cells (Llinas et al., 2005), the changes in the central neural sensitivity or gain (Norena, 2011) and the functional coupling of different parallel brain networks (Kalauzi et al., 2012, 2014) into the "tinnitus (sub)network" (De Ridder et al., 2014b). This finally results also with the functional and anatomical disarrangement of the auditory cortex tonotopic map (Norena and Eggermont, 2005; Guitton, 2012).

The central (Zoccoli et al., 2005; Bojić et al., 2016) and peripheral components of the autonomic nervous system (Bojić, 2003; Silvani et al., 2003; Platiša et al., 2016) play crucial roles in the stabilization and manifestations of chronic tinnitus (Jastreboff, 2011). Cholinergic innervation plays a major role in the development and plasticity of the auditory cortex (Shideler and Yan, 2010). The growth and functional coupling of cholinergic innervations with auditory cortical cells goes in parallel with the forming of thalamocortical connections during embryological development and in the early stage after the birth. The presence of muscarinic acetylcholine receptors plays a crucial role in this process: muscarinic receptor type 1 (M1) regulates the expression of different neurotrophins (brain-derived neurotrophic factor and nerve growth factor; Da Penha Berzaghi et al., 1993; Betancourt et al., 2006), determines the structure of neurons by promoting cell survival (Tobin and Budd, 2003) and stimulates the neural (VanDeMark et al., 2009) and dendritic outgrowth (Zhang et al., 2005). Finally, muscarinic antagonists decrease the frequency specific plasticity of the auditory cortex in the paradigm of auditory fear conditioning (Ji et al., 2005; Ji and Suga, 2009) and, importantly for this concept, the stimulation of nc. basalis Meynerti (Bakin and Weinberger, 1996; Weinberger, 2003). Nc. basalis Meynerti electrical stimulation paired with tones acutely enhances cortical neural plasticity and reverses the neurological and perceptual correlates of tinnitus in adult animals (Nichols et al., 2011). M1 impacts also the processes of learning and memory (Zhang et al., 2006; Butcher et al., 2016) which are crucially important for the genesis and maintenance of tinnitus (De Ridder et al., 2014b; Eggermont and Kral, 2016; Vanneste and De Ridder, 2016).

Different techniques, both noninvasive and invasive, were tested in order to reverse the pathological neuroplastic changes of the auditory cortex toward the physiological state (Vanneste and De Ridder, 2012). Vagus nerve stimulation paired with tone stimulation is among the most promising tools for treatment of chronic tinnitus (Engineer et al., 2011; Shetake et al., 2012; De Ridder et al., 2014a). It is based on the principle of provoking the central neuromodulatory responses by stimulation of vagal afferent fibers and nc. basalis Meynerti which abundantly innervate the auditory cortex. Vagus stimulation paired with tone stimulation has an effect of targeted plasticity, the phenomenon of reversing the map changes in individuals with tinnitus. The area of tinnitus specific cortical neurons is consequently diminished (Engineer et al., 2011). In human studies the efficiency of this method to reduce tinnitus severity was around 40%, and interestingly, all the patients who did not experience an improvement were on drug therapy that included, among others, muscarinic antagonists (De Ridder et al., 2014a). More, it is well-documented in the literature that M1 receptors have a crucial role in the experience-dependent plasticity of the auditory cortex (Shideler and Yan, 2010). Our hypothesis is that *in silico* identification of M1 receptor allosteric agonists would propose a new line for the clinical research for modulated vagus nerve stimulation (VNS) paired with tones, potentially more targeted and more efficient. In addition, by agonizing the cholinergic mechanisms of learning and memory, attention, stress response, wakefulness and sleep and sensory information processing (Ferreira-Vieira et al., 2016), M1 allosteric agonists would stabilize the process of re-learning of neural networks involved in tinnitus perception. In order to choose the best candidates for these drugs we applied *in silico* strategies that resulted with three candidates for M1 allosteric modulators.

METHODS

In this paper, we implemented a virtual screening protocol that includes both short and long-range interactions between interacting molecules. The long-range interactions are denoted by the parameters—the average quasi valence number (AQVN) and the electron-ion interaction potential (EIIP).

First, the EIIP/AQVN filter was applied for *in silico* screening of the DrugBank (http://www.drugbank.ca) (Wishart et al., 2006) and then followed by 3D QSAR and molecular docking for identification of candidate allosteric modulators of M1.

EIIP/AQVN

The EIIP for organic molecules can be determined by the following simple equation derived from the "general model pseudopotential" (Veljkovic et al., 2011)

$$\mathrm{EIIP} = 0.25\, Z^* \sin(1.04\, \pi\, Z^*)/2\pi \quad (1)$$

where Z^* is the average quasi valence number (AQVN) determined by

$$Z^* = \sum^{m} (\mathrm{niZi/N}) \quad (2)$$

where *Zi* is the valence number of the *i*th atomic component, *ni* is the number of atoms of the *i*th component, *m* is the number of atomic components in the molecule, and *N* is the total number of atoms. EIIP values calculated according to Equations (1, 2) are expressed in Rydberg units (Ry).

Further filtering of these compounds was performed by means of 3D QSAR(quantitative structure–activity relationships) in Pentacle software and followed by docking.

3D QSAR

In order to build a pharmacophoric model and select compounds based on their pharmacophoric similarity, 12 literature M1 modulators (Target ID CHEMBL216) were downloaded from the ChEMBL database. All compounds were converted into the SDF format and then imported, along with candidate compounds into the Pentacle QSAR software, protonated at pH 7.4, and oriented according to the principal moments of inertia. Standard GRIND descriptors were calculated and the PCA model was built. From PCA scores that included the first two major components, PC1 and PC2, the most similar drug molecules to literature allosteric modulators were selected for further filtering.

Molecular Docking

Molecular docking of selected candidates was performed into the built homology model of the M1 receptor. The binding site of allosteric modulators was identified from the 4MQT structure and was placed in between extracellular loops of the 2 and 3 region. The grid box for docking, with dimensions 20 × 20 × 20 Å was placed to occupy this space. The receptor and ligands were prepared in Autodock Tools 1.5.6. Docking was carried out in AutodockVina (Trott and Olson, 2010). Exhaustiveness was set to 250.

RESULTS

EIIP/AQVN Filter

The virtual screening (VS) protocol in this study was based on the application of consecutive filters to select candidate allosteric modulators of M1. Previously it was shown for molecular targets in diverse pathological states that small molecules with similar AQVN and EIIP values interact with the common therapeutic agent (Veljkovic et al., 2011, 2013). This resulted in determining criteria for virtual screening of molecular libraries for compounds with similar therapeutic properties (Veljkovic et al., 2013). The selected learning set consisted of allosteric agonists and positive allosteric modulators of M1, reported in literature (Conn et al., 2009). The compounds from the learning set were inside the active domain with AQVN and EIIP values within the intervals of (3–3.08) and (0.004–0.073), respectively, and this domain was selected as a criterion for the selection of compounds representing candidate allosteric modulators of M1 (**Figure 1**). By applying the EIIP/AQVN-based virtual screening criterion, 50 drugs were chosen out of 1463 approved drugs from the DrugBank (http://www.drugbank.ca) (Wishart et al., 2006).

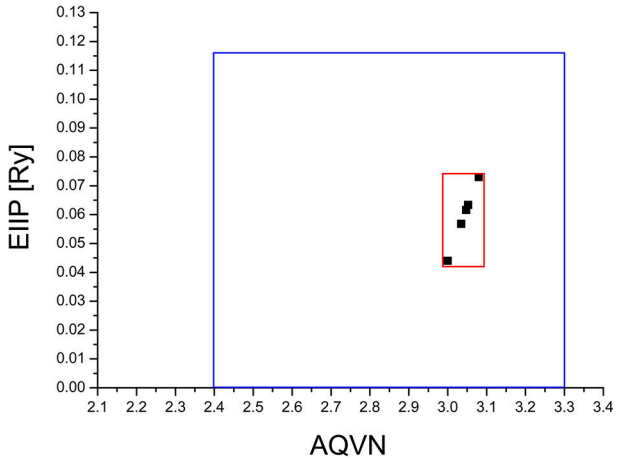

FIGURE 1 | Schematic presentation of the EIIP/AQVN criterion for selection of candidate allosteric modulators of muscarinic receptor M1. Active domain (red): AQVN (3–3.08), EIIP (0.004–0.073). Chemical space (blue) AQVN (2.40–3.30) EIIP (0.000–0.116) EIIP/AQVN domain of homologous distribution of >90% compounds from PubChem Compound Database.

QSAR Selection

The 103 molecules after EIIP filtering were subjected to pharmacophoring modeling and selection. Twelve literature M1 modulators were converted into 3D structures, along with drug candidates and GRIND descriptors for all compounds were calculated, from which a PCA model was built (**Table 1**).

From PCA scores that included the first two major components, PC1 and PC2, based on their vicinity in the PC1-PC2 space to literature M1 modulators, the most pharmacologically similar drug molecules were selected. The QSAR filtering was carried in Pentacle software (Duran et al., 2009). Finally, 10 compounds were selected for molecular docking (**Table 2**).

M1 Muscarinic Receptor Homology Modeling

In order to obtain the relevant structure of the M1 receptor active state, the crystal structure of the M2 muscarinic receptor in the active state with the agonist and allosteric modulator (PDB ID 4MQT) was used as a template for homology modeling. The P11229 sequence of the Homo sapiens muscarinic acetylcholine receptor M1 was used. The modeling was carried out on the Protein Homology/analogY Recognition Engine V 2.0 (Phyre) server (Kelley et al., 2015).

Molecular Docking

Ten selected compounds after 3D QSAR were further subjected to molecular docking into the M1 receptor model. Three compounds with the lowest binding energy values (**Table 2**) and identified hydrophilic and hydrophobic interactions with allosteric binding site amino acid residues (Tyr 82, Tyr 85, Tyr 106, Tyr 179, Trp 400, Glu 401) were selected to be the best candidates, along with considerations of drug side effects and purpose. Finally, BROMAZEPAM, ESTAZOLAM,

TABLE 1 | PCA models for M1 alosteric modulators and candidates.

Component	SSX	SSXacc	VarX	VarXacc
1	44.48	44.48	43.92	43.92
2	11.32	55.8	10.98	54.9
3	5.48	61.29	5.19	60.09
4	4.97	66.26	4.77	64.86
5	4.09	70.35	3.94	68.8

SSX, Percentage of the X sum of squares; SSXacc, accumulative percentage of the X sum of squares; VarX, percentage of the X variance; VarXaac, accumulative percentage of the X variance.

TABLE 2 | QSAR selection of 10 compounds for molecular docking, with docking energies.

Compound	VINA docking energy, kcal mol-1
Estazolam	−9.9
Diloxanide	−9
Flumazenil	−9.2
Rosoxacin	−9.6
Bromazepam	−9.3
Penicilin V	−9
Sulfamethazine	−8.4
Fluconasole	−8.7
Dapsone	−8
Bromfenac	−9.4
Raltegravir	−10.3

and FLUMAZENIL were found to be the best promising candidates (**Figures 2–4**, respectively).

DISCUSSION

We hypothesized that allosteric activation of M1 receptors in the circumstances of increased cholinergic neurotransmission to the auditory cortex by targeted VNS, will provoke an increase of M1 mediated effects:

(1) Neuromodulatory action of BDGF and NGF (Da Penha Berzaghi et al., 1993) in neuronal zones adjacent to the tinnitus;
(2) Neuromodulatory action on frequency specific neuronal zones enabling the recruitment of pathologically active neurons back to the physiological pattern of activity;
(3) Regaining the physiological tonotopy (Zhang et al., 2006) of the auditory cortex;
(4) Recruitment of novel neurons from undifferentiated neuronal pools in the hippocampus (VanDeMark et al., 2009) and
(5) The dendritic and axonal "rewiring" of the tinnitus "subnetwork" (De Ridder et al., 2014b).

The AQVN/EIIP approach was applied previously for the selection of new candidates in pharmacotherapy of the vasovagal syncope (VVS) revealing that the majority of antimuscarinic drugs might have a therapeutical potential for VVS. In this study sequential virtual screening criteria were applied, first the AQVN/EIIP based filter for the selection of candidate allosteric agonists of the M1 receptor selecting 50 drugs out of 1463 approved drugs from the DrugBank (http://www.drugbank.ca; Wishart et al., 2006). This step was followed by 3DQSAR analysis and finally docking. The screening identified bromazepam, estazolam, and flumazenil as the most promising drugs which could be repurposed as allosteric agonists of the M1 receptor.

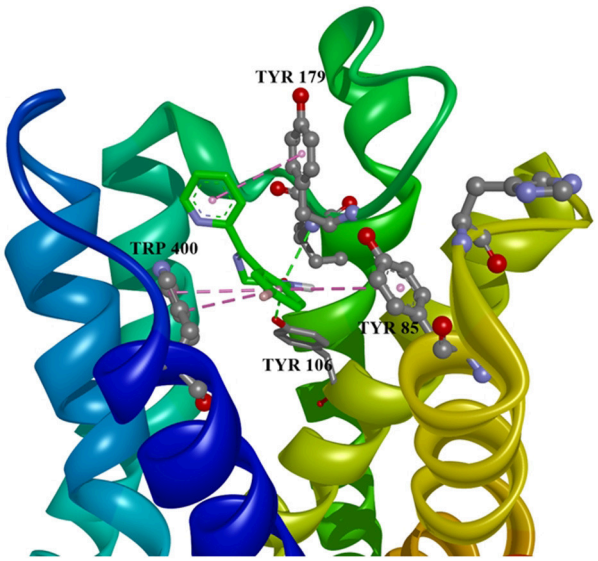

FIGURE 2 | Bromazepam in allosteric modulator binding region of M1 acetylcholine receptor, with marked aminoacids, and intermolecular interactions. Green: hydrogen bonds, purple: hydrophobic interactions.

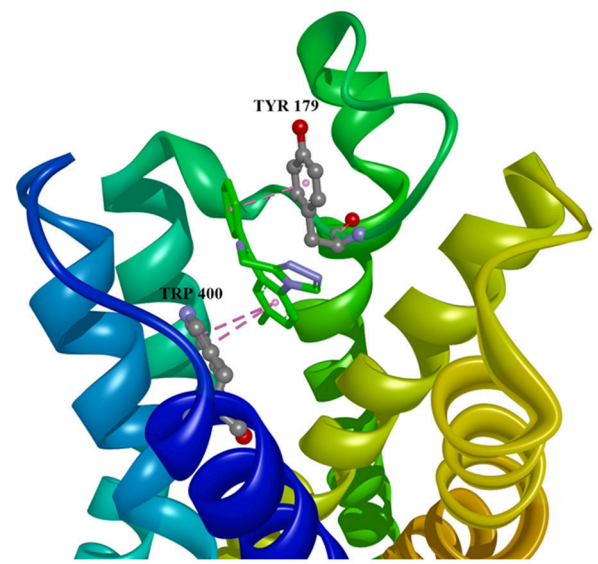

FIGURE 3 | Estazolam in allosteric modulator binding region of M1 acetylcholine receptor, with marked aminoacids, and intermolecular interactions. Green: hydrogen bonds, purple: hydrophobic interactions.

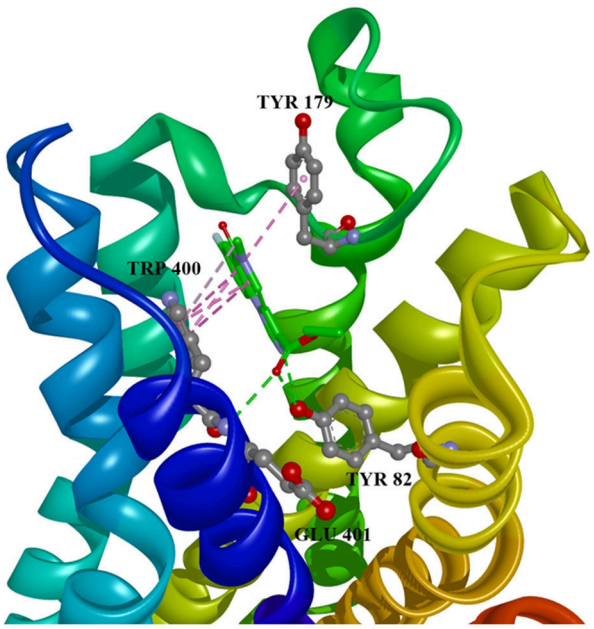

FIGURE 4 | Flumazenil in allosteric modulator binding region of M1 acetylcholine receptor, with marked aminoacids, and intermolecular interactions. Green: hydrogen bonds, purple: hydrophobic interactions.

Bromazepam and estazolam are known anti-anxiety agents with a hypnotic effect. Flumazenil has specific antibenzodiazepine action and is used as an antihypnotic agent in circumstances where the benzodiazepine and nonbenzodizepine hypnotic effect has to be reduced.

If we expand the concept to other methods of treatment of tinnitus (invasive or noninvasive; Vanneste and De Ridder, 2012), it is reasonable to propose that pharmacological facilitation of neuromodulatory changes by the *in silico* identified drugs in our investigation in tinnitus network structures would be beneficial even in these treatments. Future clinical studies of the combined M1 allosteric agonist therapy with VNS and potentially other invasive and noninvasive methods of chronic tinnitus treatment will reveal the final answer regarding their synergistic action.

Learning during wakefulness induces neural plasticity changes. Their stabilization occurs during sleep (Tononi and Cirelli, 2005). The process of sleep selects the synaptic weights of the neural synapses on the basis of their engagement during wakefulness: if the synapses are more used, the sleep process will make them more stabile while the less used synapses during wakefulness will not be strengthened. This is at least partially due to the intensity of cholinergic neurotransmission that increases from wakefulness toward NREM and REM sleep (Hobson and Friston, 2012). The last phase, REM, also known as a dream state, is the state of consciousness where tinnitus is not perceived (De Ridder et al., 2014b). The sleep process in this way supports the mechanisms of learning and memory acquired during wakefulness. If we presume that VNS paired with tones is also the process of re-learning of the cortical networks pathologically changed in tinnitus, then the quality of sleep in chronic tinnitus patients is essential for stabilization of daily acquired neuroplastic changes by the VNS therapy. In line with that, the cholinergic effect together with the hypnotic effect of the proposed drugs could be beneficial and their application justified before bedtime. The caveat that has to be taken into consideration is the feature of benzodiazepines to promote or to reinforce already existing sleep apnea by increased miorelaxation. This effect could diminish the amount of both NREM and REM sleep and potentially prevent the beneficial effect of the sleep on the process of the recovery in tinnitus patients. Biological experiments will address the question of concentration applied to the treatment of tinnitus and the question if sleep apnea as the contraindication for application of the proposed M1 allosteric agonists.

In conclusion, the results presented here suggest a potential novel approach in treatment of chronic tinnitus by VNS paired with tones with *in silico* repurposed drugs as therapeutic candidates that may target M1 and prospectively improve the treatment.

AUTHOR CONTRIBUTIONS

TB, VP, MS, and SG contributed equally to the conception of the work, acquisition, analysis, and interpretation of data. TB, VP, and SG participated in drafting the manuscript, revisiting it critically and gave final approval of the version to be published. The authors reached the agreement to be accountable for all the aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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REFERENCES

Bakin, J. S., and Weinberger, N. M. (1996). Induction of a physiological memory in the cerebral cortex by stimulation of the nucleus basalis. *Proc. Natl. Acad. Sci. U.S.A.* 93, 11219–11224. doi: 10.1073/pnas.93.20.11219

Betancourt, A. M., Burgess, S. C., and Carr, R. L. (2006). Effect of developmental exposure to chlorpyrifos on the expression of neurotrophin growth factors and cell-specific markers in neonatal rat brain. *Toxicol. Sci.* 92, 500–506. doi: 10.1093/toxsci/kfl004

Bojić, T. (2003). *Mechanisms Of Cardiovascular Control And Effects Of Acoustic Stimulation On Cardiovascular System During The Wake-Sleep Cycle*. Ph.D. Dissertation, Alma Mater Università di Bologna.

Bojić, T., Perović, V., and Glišić, S. (2016). *In silico* therapeutics for neurogenic hypertension and vasovagal syncope. *Front. Neurosci.* 9:520. doi: 10.3389/fnins.2015.00520

Butcher, A. J., Bradley, S. J., Prihandoko, R., Brooke, S. M., Mogg, A., Bourgognon, J. M., et al. (2016). An antibody biosensor establishes the activation of the M1 muscarinic acetylcholine receptor during learning and memory. *J. Biol. Chem.* 291, 8862–8875. doi: 10.1074/jbc.M115.681726

Chen, G. D., Stolzberg, D., Lobarinas, E., Sun, W., Ding, D., and Salvi, R. (2013). Salicylate-induced cochlear impairments, cortical hyperactivity and re-tuning, and tinnitus. *Hear. Res.* 295, 100–113. doi: 10.1016/j.heares.2012.11.016

Conn, P. J., Jones, C. K., and Lindsley, C. W. (2009). Subtype-selective allosteric modulators of muscarinic receptors for the treatment of CNS disorders. *Trends Pharmacol. Sci.* 30, 148–155. doi: 10.1016/j.tips.2008.12.002

Da Penha Berzaghi, M., Cooper, J., Castren, E., Zafra, F., Sofroniew, M., Thoenen, H., et al. (1993). Cholinergic regulation of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) but not neurotrophin-3 (NT-3) mRNA levels in the developing rat hippocampus. *J. Neurosci.* 13, 3818–3826.

De Ridder, D., Vanneste, S., Engineer, N. D., and Kilgard, M. P. (2014a). Safety and efficacy of vagus nerve stimulation paired with tones for the treatment of tinnitus: a case series. *Neuromodulation* 17, 170–179. doi: 10.1111/ner.12127

De Ridder, D., Vanneste, S., Weisz, N., Londero, A., Schlee, W., Elgoyhen, A. B., et al. (2014b). An integrative model of auditory phantom perception: tinnitus as a unified percept of interacting separable subnetworks. *Neurosci. Biobihav. Rev.* 44, 16–32. doi: 10.1016/j.neubiorev.2013.03.021

Duran, A., Zamora, I., and Pastor, M. (2009). Suitability of GRIND-based principal properties for the description of molecular similarity and ligand-based virtual screening. *J. Chem. Inf. Model.* 49, 2129–2138. doi: 10.1021/ci900228x

Eggermont, J. J., and Kral, A. (2016). Somatic memory and gain increase as preconditions for tinnitus: insights from congenital deafness. *Hear. Res.* 333, 37–48. doi: 10.1016/j.heares.2015.12.018

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682. doi: 10.1016/j.tins.2004.08.010

Engineer, N. D., Riley, J. R., Seale, J. D., Vrana, W. A., Shetake, J. A., Sudanagunta, S. P., et al. (2011). Reversing pathological neural activity using targeted plasticity. *Nature* 470, 101–104. doi: 10.1038/nature09656

Ferreira-Vieira, T. H., Guimaraes, I. M., Silva, F. R., and Ribeiro, F. M. (2016). Alzheimer's disease: targeting the cholinergic system. *Curr. Neuropharmacol.* 14, 101–115. doi: 10.2174/1570159X13666150716165726

Geven, L. I., De Kleine, E., Willemsen, A. T., and Van Dijk, P. (2014). Asymmetry in primary auditory cortex activity in tinnitus patients and controls. *Neuroscience* 256, 117–125. doi: 10.1016/j.neuroscience.2013.10.015

Guitton, M. J. (2012). Tinnitus: pathology of synaptic plasticity at the cellular and system levels. *Front. Syst. Neurosci.* 6:12. doi: 10.3389/fnsys.2012.00012

Hobson, J. A., and Friston, K. J. (2012). Waking and dreaming consciousness: neurobiological and functional considerations. *Prog. Neurobiol.* 98, 82–98. doi: 10.1016/j.pneurobio.2012.05.003

Jastreboff, P. (2011). "Tinnitus retraining therapy," in *Textbook of Tinnitus,* eds A. Moller, B. Langguth, D. De Ridder, and T. Kleinjung (New York, NY: Springer), 575–596.

Ji, W., and Suga, N. (2009). Tone-specific and nonspecific plasticity of inferior colliculus elicited by pseudo-conditioning: role of acetylcholine and auditory and somatosensory cortices. *J. Neurophysiol.* 102, 941–952. doi: 10.1152/jn.00222.2009

Ji, W., Suga, N., and Gao, E. (2005). Effects of agonists and antagonists of NMDA and ACh receptors on plasticity of bat auditory system elicited by fear conditioning. *J. Neurophysiol.* 94, 1199–1211. doi: 10.1152/jn.00112.2005

Kalauzi, A., Vuckovic, A., and Bojic, T. (2012). EEG alpha phase shifts during transition from wakefulness to drowsiness. *Int. J. Psychophysiol.* 86, 195–205. doi: 10.1016/j.ijpsycho.2012.04.012

Kalauzi, A., Vuckovic, A., and Bojic, T. (2014). Topographic distribution of EEG alpha attractor correlation dimension values in wake and drowsy states in humans. *Int. J. Psychophysiol.* 95, 278–291. doi: 10.1016/j.ijpsycho.2014.11.008

Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N., and Sternberg, M. J. (2015). The phyre2 web portal for protein modeling, prediction and analysis. *Nat. Protoc.* 10, 845–858. doi: 10.1038/nprot.2015.053

Llinas, R., Urbano, F. J., Leznik, E., Ramirez, R. R., and Van Marle, H. J. (2005). Rhythmic and dysrhythmic thalamocortical dynamics: GABA systems and the edge effect. *Trends Neurosci.* 28, 325–333. doi: 10.1016/j.tins.2005.04.006

Malinvaud, D., Londero, A., Niarra, R., Peignard, P., Warusfel, O., Viaud-Delmon, I., et al. (2016). Auditory and visual 3D virtual reality therapy as a new treatment for chronic subjective tinnitus: results of a randomized controlled trial. *Hear. Res.* 333, 127–135. doi: 10.1016/j.heares.2015.12.023

Nichols, J. A., Nichols, A. R., Smirnakis, S. M., Engineer, N. D., Kilgard, M. P., and Atzori, M. (2011). Vagus nerve stimulation modulates cortical synchrony and excitability through the activation of muscarinic receptors. *Neuroscience* 189, 207–214. doi: 10.1016/j.neuroscience.2011.05.024

Norena, A. (2011). An integrative model of tinnitus based on a central gain controlling neural sensitivity. *Neurosci. Biobehav. Rev.* 35, 1089–1109. doi: 10.1016/j.neubiorev.2010.11.003

Norena, A. (2015). Revisiting the cochlear and central mechanisms of tinnitus and therapeutic approaches. *Audiol. Neurootol.* 20, 53–59. doi: 10.1159/000380749

Norena, A. J., and Eggermont, J. J. (2005). Enriched acoustic environment after noise trauma reduces hearing loss and prevents cortical map reorganization. *J. Neurosci.* 25, 699–705. doi: 10.1523/JNEUROSCI.2226-04.2005

Platiša, M. M., Bojić, T., Pavlović, S. U., Radovanović, N. N., and Kalauzi, A. (2016). Generalized poincare plots-a new method for evaluation of regimes in cardiac neural control in atrial fibrillation and healthy subjects. *Front. Neurosci.* 10:38. doi: 10.3389/fnins.2016.00038

Shetake, J. A., Engineer, N. D., Vrana, W. A., Wolf, J. T., and Kilgard, M. P. (2012). Pairing tone trains with vagus nerve stimulation induces temporal plasticity in auditory cortex. *Exp. Neurol.* 233, 342–349. doi: 10.1016/j.expneurol.2011.10.026

Shideler, K. K., and Yan, J. (2010). M1 muscarinic receptor for the development of auditory cortical function. *Mol. Brain* 3:29. doi: 10.1186/1756-6606-3-29

Silvani, A., Bojic, T., Cianci, T., Franzini, C., Lodi, C. A., Predieri, S., et al. (2003). Effects of acoustic stimulation on cardiovascular regulation during sleep. *Sleep* 26, 201–205. doi: 10.1093/sleep/26.2.201

Tobin, A. B., and Budd, D. C. (2003). The anti-apoptotic response of the Gq/11-coupled muscarinic receptor family. *Biochem. Soc. Trans.* 31, 1182–1185. doi: 10.1042/bst0311182

Tononi, G., and Cirelli, C. (2005). "A possible role of sleep in synaptic homeostasis," in *The Physiological Nature of Sleep*, eds P. L. Parmeggiani and R. Velluti (London: Imperial College Press), 77–103.

Trott, O., and Olson, A. J. (2010). AutoDock vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J. Comput. Chem.* 31, 455–461. doi: 10.1002/jcc.21334

VanDeMark, K. L., Guizzetti, M., Giordano, G., and Costa, L. G. (2009). The activation of M1 muscarinic receptor signaling induces neuronal differentiation in pyramidal hippocampal neurons. *J. Pharmacol. Exp. Ther.* 329, 532–542. doi: 10.1124/jpet.108.150128

Vanneste, S., and De Ridder, D. (2012). Noninvasive and invasive neuromodulation for the treatment of tinnitus: an overview. *Neuromodulation* 15, 350–360. doi: 10.1111/j.1525-1403.2012.00447.x

Vanneste, S., and De Ridder, D. (2016). Deafferentation-based pathophysiological differences in phantom sound: tinnitus with and without hearing loss. *Neuroimage* 129, 80–94. doi: 10.1016/j.neuroimage.2015.12.002

Veljkovic, N., Glisic, S., Perovic, V., and Veljkovic, V. (2011). The role of long-range intermolecular interactions in discovery of new drugs. *Expert Opin. Drug Discov.* 6, 1263–1270. doi: 10.1517/17460441.2012.638280

Veljkovic, N., Glisic, S., Prljic, J., Perovic, V., and Veljkovic, V. (2013). Simple and general criterion for "*in silico*" screening of candidate HIV drugs. *Curr. Pharm. Biotechnol.* 14, 561–569. doi: 10.2174/1389201014051311111105301

Vio, M. M., and Holme, R. H. (2005). Hearing loss and tinnitus: 250 million people and a US$10 billion potential market. *Drug Discov. Tod.* 10, 1263–1265. doi: 10.1016/S1359-6446(05)03594-4

Weinberger, N. M. (2003). The nucleus basalis and memory codes: auditory cortical plasticity and the induction of specific, associative behavioral memory. *Neurobiol. Learn. Mem.* 80, 268–284. doi: 10.1016/S1074-7427(03)00072-8

Wishart, D. S., Knox, C., Guo, A. C., Shrivastava, S., Hassanali, M., Stothard, P., et al. (2006). DrugBank: a comprehensive resource for *in silico* drug discovery and exploration. *Nucleic Acids Res.* 34, D668–D672. doi: 10.1093/nar/gkj067

Wu, C., Stefanescu, R. A., Martel, D. T., and Shore, S. E. (2016). Tinnitus: maladaptive auditory-somatosensory plasticity. *Hear. Res.* 334, 20–29. doi: 10.1016/j.heares.2015.06.005

Zhang, Y., Dyck, R. H., Hamilton, S. E., Nathanson, N. M., and Yan, J. (2005). Disrupted tonotopy of the auditory cortex in mice lacking M1 muscarinic acetylcholine receptor. *Hear. Res.* 201, 145–155. doi: 10.1016/j.heares.2004.10.003

Zhang, Y., Hamilton, S. E., Nathanson, N. M., and Yan, J. (2006). Decreased input-specific plasticity of the auditory cortex in mice lacking M1 muscarinic acetylcholine receptors. *Cereb. Cortex* 16, 1258–1265. doi: 10.1093/cercor/bhj067

Zoccoli, G., Bojić, T., and Franzini, C. (2005). "Regulation of cerebral circulation during sleep," in *The Physiological Nature of Sleep, 1st Edn.*, eds P. L. Parmeggiani and R. Velluti (London: Imperial College Press), 351–369.

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Positive Association between Tinnitus and Arterial Hypertension

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Introduction: Tinnitus is the perception of noise in the absence of an external source and is considered by most authors as a multifactorial symptom. A systematic review concerning the association of tinnitus and systemic arterial hypertension retrieved suggestions of a positive association, but the articles included failed to perform a detailed analysis on the theme.

Purpose: To analyze the presence of arterial hypertension in tinnitus and non-tinnitus patients, to analyze differences between tinnitus impact and psychoacoustic measurements in hypertensive and normotensive patients, and to evaluate the association between the presence of tinnitus and the diverse antihypertensive drugs employed.

Materials and methods: This includes cross-sectional transversal study, comparing two groups of subjects (144 in the study group with tinnitus and 140 in the control group without tinnitus). Clinical, demographical, audiometrical, and psychoacoustics characteristics of the subjects were compared.

Results: Hypertension prevalence in tinnitus subjects was 44.4% against 31.4% in subjects without tinnitus ($p = 0.024$). Positive associations with tinnitus were found with hypertension treatment with angiotensin-converting enzyme (ACE) inhibitors ($p = 0.006$), tiazidic diuretics ($p < 0.0001$), potassium-sparing diuretics ($p = 0.016$), and calcium channels blockers ($p = 0.004$).

Conclusion: There is an association between tinnitus and arterial hypertension. This association is particularly strong in older patients. Hypertension treatment with diuretics, ACE inhibitors, and calcium channels blockers were more prevalent in tinnitus patients, suggesting that an eventual ototoxicity of these drugs may be involved in tinnitus pathophysiology, a hypothesis that should be evaluated in further studies.

Keywords: tinnitus, arterial hypertension, hearing loss, cardiovascular diseases, hearing disorders

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INTRODUCTION

Tinnitus is the perception of noise, which is not generated by external stimulus (1). It affects approximately 25% of the general population: one third on a frequent basis (2). Tinnitus may be classified as auditory and para-auditory tinnitus with the former representing the majority of cases, and the latter being subdivided into muscular and vascular tinnitus, sometimes referred as somatosounds (3).

According to the most recent trends of thought, tinnitus is considered a symptom which may have multiple causes, sometimes even in a single patient (4, 5). Noise exposure, metabolic and cardiovascular disease, presbycusis, ototoxicity, and cranial and cervical trauma are the most frequently

considered causes of tinnitus (5, 6). Caffeine abuse, dietary factors, temporomandibular joint, and cervical disease have also been described as contributing factors (7–9).

Systemic arterial hypertension is a multifactorial clinical condition characterized by raised and sustained arterial pressure levels (10). It is defined as systolic levels equal or greater than 140 mmHg and diastolic levels equal or greater than 90 mmHg (10). The prevalence in Brazil, similar to that in other countries, is estimated to be between 22.3 and 43.9% (32.5% average), raising to 50% between 60 and 69 years old and 75% for 70 years and older (10–12). The presence of comorbidities, such as diabetes mellitus and dyslipidemias, and habits, such as smoking, was demonstrated to increase complications risks (13). Arterial hypertension has been described as a possible cause of tinnitus since 1940s (14). Three principle mechanisms suspected of being involved are: damage to inner ear microcirculation (15), ototoxicity by antihypertension drugs (16), and perception of noise generated by blood vessels (3).

As related to inner ear microcirculation, the *stria vascularis* was demonstrated to be the main cochlear site damaged by arterial hypertension (17). Sodium retention could also lead to an increase of extracellular fluid volume, including the perilymph (18), and the endocochlear potential being reduced in hypertensive rats (19). Moreover, hypertension has been associated with a higher risk of hearing loss in brain ischemia (15) and also with a slower recovery in sudden hearing loss (20).

In considering ototoxicity, an extensive review cited diuretics, beta-blockers, angiotensin-conversing enzyme (ACE) inhibitors, angiotensin II receptors blockers, and calcium channels blockers as possible ototoxic medications (21). Furosemide's ototoxicity is the most studied form, producing a quick and reversible decrease of the endocochlear potential (22).

As for vascular tinnitus, some studies cite hypertension as a causal factor, mainly when vascular abnormalities have been ruled out (3). An anatomopathological study demonstrated a high incidence of bony dehiscence of the carotid canal in the middle ear, which may affect the inner ear microcirculation and also generate vascular noises (23).

According to a systematic review, there is evidence of an association between tinnitus and arterial hypertension, but there is a lack of more comprehensive studies (24). The association is stronger in studies that analyzed the presence of arterial hypertension in patients with tinnitus than in those which analyzed the presence of tinnitus in patients with arterial hypertension.

The main purpose of this study is to analyze the presence of arterial hypertension in tinnitus and non-tinnitus patients. Secondary purposes are to analyze differences between tinnitus impact and psychoacoustic measurements in hypertensive and normotensive patients and to evaluate the association between the presence of tinnitus and the diverse antihypertensive drugs employed.

MATERIALS AND METHODS

This is a transversal case–control study in which individuals of 18 years of age or older with and without tinnitus were selected at the author's ENT clinic from 2011 to 2014. The trial was approved by the Institutional Review Board (number 010/CEP-FMV/2011). This study was carried out in accordance with the recommendations of the aforementioned Institutional Review Board with written informed consent from all subjects. All subjects gave written informed consent in accordance with the Declaration of Helsinki.

Two groups were created: the first included patients with tinnitus of at least 3 months duration and the second included patients without tinnitus (control). The control group was paired with the tinnitus group for gender, age, and race. The time of tinnitus onset as related to arterial hypertension onset was not an exclusion criteria. Patients from both groups were submitted to anamnesis (including demographics, comorbidities, and habits), otorhinolaryngological physical examination, and arterial pressure measurements with a calibrated sphygmomanometer (Erka Perfekt Aneroid, Germany), in order to exclude possible undiagnosed arterial hypertension. The criteria for blood pressure evaluation were those from the VII Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, U. S. Department of Health and Human Services, as previously described.

Patients allegedly normotensive with high blood pressure detected at the physical examination were excluded. Patients from both groups also underwent conventional pure tone and speech audiometry.

Tinnitus patients were questioned regarding their tinnitus characteristics (duration, type of sound, laterality, and periodicity) and also classified their tinnitus according to a Visual Analog Scale (VAS), from 1 to 10 (for intensity and distress) and to the Brazilian Portuguese validated version of the Tinnitus Handicap Inventory (THI) (25). They also underwent psychoacoustic measurements of their tinnitus – Pitch Matching (PM) and Minimum Masking Level (MML).

The sample size was determined after the analysis of the arterial hypertension prevalence in a preliminary sample of tinnitus patients ($n = 46$) that was compared to the prevalence of arterial hypertension in the Brazilian general population, obtained from previous studies (10). The minimum number of individuals for each group was determined to be 140.

For whole sample analysis, the comparison of the variables between the tinnitus group and the control group was performed using the Mann–Whitney test for the numerical data and the chi-square or the Fisher test for the categorical data. The Spearman coefficient was performed to analyze the association between the duration of arterial hypertension and the numerical audiometry measurements. The Cochran–Mantel–Haenszel was used to analyze the association between tinnitus and arterial hypertension adjusting for the presence of hearing loss. The significance level was established at 5%, and the statistical analysis was performed with the SAS statistical software, 6.11 version (SAS Institute, Inc., Cary, NC, USA).

RESULTS

The final sample was composed of 144 patients in the tinnitus group and 140 in the control group. The tinnitus group was then divided into two subgroups: one for patients with arterial hypertension and the other without arterial hypertension.

The average age was 57.8 years of age for the tinnitus group and 58.6 for the control group. The averages of tinnitus duration,

VAS for intensity, VAS for distress, and THI scores for the tinnitus group were, respectively, 54.5 months–4.5 years, 5.45, 5.83, and 41.7 points.

Although the duration of arterial hypertension was longer (average of 128.7 months–10.7 years) than the duration of tinnitus (average of 54.5 months), no statistically significant correlation was found, according to the Spearman coefficient. Considering the patients that could ascertain the duration of both arterial hypertension and tinnitus ($n = 56$), for 42 of them (75%) arterial hypertension preceded tinnitus.

Table 1 shows the comparison of numerical variables and **Table 2** shows the categorical variables, the latter confirming the pairing by gender, age, and race. As shown in **Table 2**, arterial hypertension was more prevalent in tinnitus patients ($p = 0.024$), demonstrating an association of tinnitus and arterial hypertension. This association was not found when data were adjusted for the presence of hearing loss ($p = 0.27$, according to the Cochran–Mantel–Haenszel test). Hearing loss was more frequent in the tinnitus group, as shown in **Table 3**, but there was no difference between the two groups as to the type of hearing loss or the shape of the audiogram curve. Sensorineural descendant was the most frequent curve in both groups (75% in the tinnitus patients group and 58% in the control group). Also, there was no difference in the speech recognition index of both groups (100% median in both).

TABLE 1 | Analysis of the numerical variables (age, duration of arterial hypertension, and daily consumption of caffeine) according to the group.

Variable	Tinnitus			No tinnitus			*p*-Value
	n	Median	IQA	*n*	Median	IQA	
Age (years)	144	59	49–69	140	58	50–67	0.97
Arterial hypertension duration (months)	60	**120**	39–216	43	**180**	84–240	**0.019**
Caffeine (ml/day)	144	**100**	50–200	140	**300**	200–400	**0.0001**

Significant values in bold.
IQA, Interquartilic Amplitude: Q1–Q3.

TABLE 2 | Analysis of categorical variables (gender, age, race, and presence of arterial hypertension) according to the groups.

Variable	Category	Tinnitus		No Tinnitus		*p*-Value[a]
		n	%	*n*	%	
Gender	Male	62	43.1	65	46.4	0.57
	Female	82	56.9	75	53.6	
Age (years)	≤40	18	12.5	14	10.0	0.82
	41–59	55	38.2	60	42.9	
	60–69	40	27.8	36	25.7	
	≥70	31	21.5	30	21.4	
Race	White	93	71.5	98	70.0	0.94
	Brown	22	16.9	24	17.1	
	Black	15	11.5	18	12.9	
Arterial Hypertension	Yes	64	**44.4**	44	**31.4**	**0.024**
	No	80	55.6	96	68.6	

Significant values in bold.
[a]χ^2 *or Fisher test.*

The analysis of the antihypertensive drugs used in both groups is shown in **Table 4**.

The analysis of comorbidities and habits demonstrated that dyslipidemia was more frequent in the tinnitus group (p-value of 0.003), while the presence of diabetes mellitus, hypothyroidism, noise exposure, caffeine consumption, and smoking was similar in both groups. When considering the coexistence of arterial hypertension and comorbidities and habits, the association of arterial hypertension and caffeine consumption greater than 150 milliliters per day was more frequent in the tinnitus group ($p < 0.0001$).

Table 4 shows data about the antihypertensive drugs used in both groups.

Table 5 shows data from the numerical variables for the tinnitus' patients subgroups, those with and without arterial hypertension.

There was no statistical difference between the two subgroups concerning gender and race. Diabetes mellitus and dyslipidemia were more frequent in the subgroup with tinnitus and arterial hypertension ($p = 0.017$ and 0.02, respectively), while there were no differences concerning hypothyroidism, noise exposure, caffeine consumption, or smoking. Hearing loss was considered to be more prevalent in the subgroup with arterial hypertension (89.1%), although it was also frequent in the normotensive subgroup (75%, $p = 0.032$). No differences concerning the type or curve were found.

TABLE 3 | Prevalence of hearing loss among tinnitus and non-tinnitus patients (χ^2 test).

Variable	Category	Tinnitus		No Tinnitus		*p*-Value
		N (ears)	%	*N* (ears)	%	
Hearing loss	Yes	111	81.3	75	53.6	<0.0001
	No	32	18.7	65	46.4	

TABLE 4 | Analysis of the categorical variable – antihypertensive drugs used according to the groups.

Variable	Category	Tinnitus		No tinnitus		*p*-Value
		n	%	*n*	%	
B-blocker	Yes	19	13.2	21	15.0	0.66
	No	125	86.8	119	85.0	
ACEI	Yes	23	16.0	8	5.7	0.006
	No	121	84.0	132	94.3	
ARB	Yes	34	23.6	24	17.1	0.18
	No	110	76.4	116	82.9	
Loop diuretic	Yes	0	0.0	4	2.9	0.057
	No	144	100.0	136	97.1	
Thiazidic diuretic	Yes	29	20.1	8	5.7	<0.0001
	No	115	79.9	132	94.3	
K sparing diuretic	Yes	6	4.2	0	0.0	0.016
	No	138	95.8	140	100.0	
CCA	Yes	13	9.0	2	1.4	0.004
	No	131	91.0	138	98.6	

χ-squared or Fisher tests.
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin II receptor blocker; CCA, calcium channel antagonist.

TABLE 5 | Analysis of the numerical variables (age, caffeine consumption, tinnitus duration, Visual Analog Scale, Tinnitus Handicap Inventory, Pitch Masking, and Minimum Masking Level) according to the subgroups of tinnitus patients (with and without arterial hypertension).

Variable	Arterial hypertension subgroup			No arterial hypertension subgroup			*p*-Value[a]
	n	Median	IQA	*n*	Median	IQA	
Age (years)	64	**66**	57–72	80	**52.5**	43–65	**0.0001**
Caffeine (ml/day)	58	100	50–200	67	100	100–200	0.78
Tinnitus duration (months)	59	24	9–60	76	18	5.3–60	0.22
VAS volume (points)	63	5	3–7	80	6	4–8	0.37
VAS distress (points)	63	5	3–8	80	6	4–9	0.37
THI score (points)	63	40	18–70	80	38	16.5–61.5	0.75
PM RE (Hz)	40	4000	1000–8000	64	6000	2000–8000	0.58
PM LE (Hz)	45	4000	1500–8000	56	6000	3250–8000	0.27
MML RE (dB SL)	40	15	5–25	63	15	10–30	0.36
MML LE (dB SL)	44	15	5–29	55	15	5–25	0.48

Nineteen patients reported no caffeine consumption, nine patients could not estimate the duration of their tinnitus, and one patient could not report VAS and THI scores.
IQA, interquartilic amplitude: Q1–Q3.
Bold is to highlight the significant data.
[a]Mann–Whitney test.
VAS, Visual Analog Scale; THI, Tinnitus Handicap Inventory; PM, Pitch Masking; MML, Minimum Masking Level; RE, right ear; LE, left ear; dB, decibel; dB SL, decibel Sensation Level.

TABLE 6 | Analysis of the categorical variables (tinnitus clinical characteristics) according to the tinnitus' patients' subgroups (with and without arterial hypertension).

Variable	Category	Arterial hypertension subgroup		No arterial hypertension subgroup		*p*-Value[a]
		n	%	*n*	%	
Laterality	RE	13	20.3	22	27.5	0.60
	LE	15	23.4	14	17.5	
	Bilateral	32	50.0	41	51.3	
	Head	4	6.3	3	3.8	
Instalation	Sudden	33	55.0	33	42.9	0.0.16
	Gradual	27	45.0	44	57.1	
Periodicity	Constant	45	70.3	55	69.6	0.93
	Intermittent	19	29.7	24	30.4	

[a]χ^2 or Fisher test.
RE, right ear; LE, left ear.

Table 6 shows the tinnitus characteristics in both subgroups.

The most frequently described types of tinnitus were wheezing, whistle, and insect with no differences between the subgroups. The presence of multiple types of sound in the same patient was more frequently found in the hypertensive subgroup ($p = 0.014$). The prevalence of vascular tinnitus was 6.3% in the hypertensive subgroup and 1.3% in the normotensive one ($p = 0.12$) with muscular tinnitus being found in only two cases, both being in normotensive patients.

No significant differences were found between the subgroups concerning the otolaryngologic exam, including evaluation of the temporomandibular joint. Finally, there was no correlation discovered between the duration of arterial hypertension and tinnitus according to the Spearman coefficient ($p = 0.77$).

DISCUSSION

Association studies are key sources of information for the comprehension of how one disorder may affect another. A study on attempt to establish a correlation between arterial hypertension and tinnitus studies will help to elucidate characteristic in common that could be addressed in the diagnosis and therapeutic interventions. However, causal correlate studies would demand a plethora of different cases, including patients without tinnitus or arterial hypertension who were also free from other infirmities or habits which could be linked to tinnitus generation. Oftentimes, this elaborate type of study requires an extensive time period and patients with incredibly varied conditions.

A systematic review on this subject (24) argued that studies which have evaluated the prevalence of tinnitus among hypertensive patients failed to demonstrate an association (26–29). However, data from this study, along with other studies (2, 30, 31), demonstrated an association between tinnitus and arterial hypertension (hypertension prevalence in tinnitus patients = 44.4% against 31.4% in patients without tinnitus, $p = 0.024$).

The information above may lead one to infer that tinnitus could be a causal factor for hypertension, albeit it seems more reasonable to believe arterial hypertension is more a cofactor than a main cause of tinnitus.

Regarding hearing loss, the results are in conformity with prior studies, which found a high prevalence among tinnitus patients, as well as the higher prevalence of sensorineural pattern with descending configuration curves (2, 5).

Once hearing loss was added to (adjusted for) the statistical analysis, the association between tinnitus and arterial hypertension was no longer positive, suggesting that arterial hypertension may be a cause of hearing loss, which is related to most cases of tinnitus, as previously reported (1, 6). Having said so, if we think about tinnitus prevention, arterial hypertension may still be regarded as a possible important causal factor.

Arterial hypertension may affect the inner ear microcirculation, and it is known that comorbidities, such as diabetes mellitus and dyslipidemia, may enhance vascular impairment due to hypertension (10). Although dyslipidemia was more prevalent in the tinnitus group, there was no difference concerning the concomitancy of arterial hypertension and dyslipidemia. Diabetes mellitus also affects the inner ear microcirculation, but it may also have direct metabolic effects on cochlea (4, 5, 20). There

was a statistical tendency in favor of a higher prevalence of the concomitancy of diabetes and hypertension in the tinnitus group. As for hypothyroidism, another metabolic disease frequently cited as related to tinnitus (6), no differences between the two groups were found, either as an isolated factor or in association with arterial hypertension. The comparison between the tinnitus subgroups (with and without hypertension) demonstrated that diabetes mellitus and dyslipidemia were more prevalent in hypertensive tinnitus patients, suggesting that these diseases may act synergically upon the generation, maintenance and/or aggravation of tinnitus.

The excessive consumption of caffeine is believed to have a negative impact on hypertension control (32), but its association with tinnitus has been doubted by recent studies (9, 33, 34). Data from this study failed to demonstrate any association between tinnitus and caffeine consumption. In fact, the concomitancy of hypertension and caffeine intake greater than 150 ml per day was more prevalent in the control group, which may reflect the widespread patients' concept that caffeine worsens tinnitus. Smoking, which is believed to worsen both tinnitus and hypertension, probably by impairing macro and microcirculation (10, 35), was more prevalent in the tinnitus group, either as an isolated factor or concomitant to hypertension.

Considering these findings, one might speculate that vascular changes, which may affect cochlear microcirculation, leading to hair cells damage and, consequently, tinnitus is probably a common pathophysiological scenario for many conditions, such as arterial hypertension, dyslipidemia, diabetes mellitus, smoking, and caffeine abuse. These conditions are not infrequently found in a single patient and may act sinergically, multiplying the damage to the auditory system.

Although noise exposure is considered as one of the main causes of tinnitus (4, 6), data from this study failed to establish an association between noise exposure and tinnitus, either isolated or concomitant to hypertension. This finding should be considered with caution, being that the study was performed in an industrial city where many workers are exposed to industrial or recreational noise.

The average age of patients with tinnitus and arterial hypertension was significantly higher than the age of those in the group with tinnitus and no arterial hypertension. In the hypertension tinnitus subgroup most of the patients were 60 years or older, while the opposite was verified in the subgroup without hypertension. Both tinnitus and arterial hypertension are more prevalent in the elderly (2, 10), but these findings may also be due to some synergistic action of presbycusis and arterial hypertension contributing to tinnitus generation and, eventually, aggravation.

The difference concerning arterial hypertension duration, which was significantly lower in the tinnitus group, may be due to a lack of proper control of blood pressure in the first years, which may lead to perfusion and reperfusion vascular events in the cochlea. More studies are needed to clarify these findings.

The median of the pitch masking was higher (6 kHz) in the subgroup without hypertension than when compared to the hypertension subgroup (4 kHz), although this difference was not statistically significant. Both measures are in agreement with most of the references, tinnitus ranging from 3 to 8 kHz, which corresponds to the usually most affected frequencies in hearing loss (36, 37). As for the MML, the averages of both subgroups were the same (15 dB SL), which is somewhat higher than the usually reported sensation level of 5–10 dB SL (37). No differences were demonstrated concerning the degree of intensity and distress due to tinnitus in both subgroups, considering the VAS and the THI.

The tinnitus' characteristics were similar in both groups, including the type of sound perceived by the patients. The fact that multiple sounds' tinnitus was more prevalent in the hypertensive patients may reflect the multicausality of tinnitus, hypertension being one of the possible factors involved.

Although the average duration of arterial hypertension was longer than the duration of tinnitus and for most of the patients the onset of hypertension preceded the onset of tinnitus, the methodology of this case–control does not allow the conclusion that hypertension is a causal factor for tinnitus.

The ototoxicity of many antihypertension medications has been well established, especially with diuretics (21, 22). Data from this study demonstrated that the use of ACE inhibitors, thiazidic diuretics, potassium-sparing diuretics, and calcium channels blockers was more prevalent in the tinnitus hypertensive patients than in the control group. These findings have a partial correspondence with prior studies (16) and, although they are not strong enough to justify a correlation between an eventual ototoxicity of these drugs and the presence of tinnitus (for example, multidrug therapy for hypertension is very frequent), it appears that further, more detailed studies on this subject should be performed.

CONCLUSION

There is an association between tinnitus and arterial hypertension. This association is particularly strong in older patients and cannot be dissociated from the hearing loss, which was also more prevalent among tinnitus patients. The use of thiazidic and potassium-sparing diuretics, ACE inhibitors, and calcium channels blockers was more prevalent in tinnitus patients.

The clinical and psychoacoustic characteristics of tinnitus in hypertensive and normotensive patients were similar, as well as tinnitus-related distress.

AUTHOR CONTRIBUTIONS

RF – study design, data collection, and writing. AA – data collection. NP – study design and writing.

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REFERENCES

1. Heller AJ. Classification and epidemiology of tinnitus. *Otolaryngol Clin North Am* (2003) 36(2):239–48. doi:10.1016/S0030-6665(02)00160-3
2. Shargorodsky J, Curhan GC, Farwell WR. Prevalence and characteristics of tinnitus among US adults. *Am J Med* (2010) 123(8):711–8. doi:10.1016/j.amjmed.2010.02.015
3. Herraiz C, Aparicio JM. Diagnostic clues in pulsatile tinnitus (somatosounds). *Acta Otorrinolaringol Esp* (2007) 58(9):426–33. doi:10.1016/S0001-6519(07)74960-9
4. Langguth B, Kreuzer PM, Kleinjung T, De Ridder D. Tinnitus: causes and clinical management. *Lancet Neurol* (2013) 12(9):920–30. doi:10.1016/S1474-4422(13)70160-1
5. Tunkel DE, Bauer CA, Sun GH, Rosenfeld RM, Chandrasekhar SS, Cunningham ER Jr, et al. Clinical practice guideline: tinnitus. *Otolaryngol Head Neck Surg* (2014) 151(2 Suppl):S1–40. doi:10.1177/0194599814545325
6. Henry JA, Dennis KC, Schechter MA. General review of tinnitus: prevalence, mechanisms, effects and management. *J Speech Lang Hear Res* (2005) 48(5):49–70. doi:10.1044/1092-4388(2005/084)
7. Rocha CB, Sanchez TG. Efficacy of myofascial trigger point deactivation for tinnitus control. *Braz J Otorhinolaringol* (2012) 78(6):21–6. doi:10.5935/1808-8694.20120028
8. Ferendiuk E, Zajdel K, Pihut M. Incidence of otolaryngological symptoms in patients with temporomandibular joint dysfunctions. *Biomed Res Int* (2014) 2014:824684. doi:10.1155/2014/824684
9. Figueiredo RR, Rates MJ, Azevedo AA, Moreira RK, Penido NO. Effects of the reduction of caffeine consumption on tinnitus perception. *Braz J Otorhinolaryngol* (2014) 80(5):416–21. doi:10.1016/j.bjorl.2014.05.033
10. Nobre F, Amodeo C, Consolim-Colombo FA. VI Diretrizes Brasileiras de Hipertensão. *Rev Bras Hipert* (2010) 17(1):7–60.
11. Barreto SM, Passos VMA, Firmo JOA, Guerra HL, Vidigal PG, Lima-Costa MFF. Hypertension and clustering of cardiovascular risk factors in a community in Southest Brazil – The Bambuí Health and Ageing Study. *Arq Bras Cardiol* (2001) 77(6):576–81. doi:10.1590/S0066-782X2001001200008
12. Cesarino CB, Cipullo JP, Martin JFV, Ciorlia LA, de Godoy MRP, Cordeiro JA, et al. Prevalência e fatores sociodemográficos em hipertensos de São José do Rio Preto – SP. *Arq Bras Cardiol* (2008) 91(1):31–5. doi:10.1590/S0066-782X2008001300005
13. Zanchetti A, Hanson L, Dahlöf B, Elmfeldt D, Kjeldsen S, Kolloch R, et al. Effects of individual risk factors on the incidence of cardiovascular events in the treated hypertensive patients of the Hypertension Optimal Treatment Study. HOT Study Group. *J Hypertens* (2001) 19(6):1149–59. doi:10.1097/00004872-200106000-00021
14. Johnson LF, Zonderman B. The hearing acuity, tinnitus and vertigo in essential hypertension. *Laryngoscope* (1948) 58(5):374–9. doi:10.1288/00005537-194805000-00002
15. Przewoźny T, Gasecki D, Narozny W, Nyka W. Risk factors of sensorineural hearing loss in patients with ischemic stroke. *Otol Neurotol* (2008) 29(6):745–50. doi:10.1097/MAO.0b013e318181336c
16. Borghi C, Brandolini C, Prandin MG, Dormi A, Modugno GC, Pirodda A. Prevalence of tinnitus in patients with hypertension and the impact of different antihypertensive drugs on the incidence of tinnitus: a prospective, single-blind, observational study. *Curr Ther Res Clin Exp* (2005) 66(5):420–32. doi:10.1016/j.curtheres.2005.10.001
17. Tachibana M, Yamamichi I, Nakae S. The site of involvement in hypertension within the cochlea. *Acta Otolaryngol* (1984) 97(3):257–65. doi:10.3109/00016488409130987
18. Marková M. The cochleovestibular syndrome in hypertension. *Cesk Otolaryngol* (1990) 39(2):89–97.
19. Mosnier I, Teixeira M, Loiseau A, Fernandes I, Sterkes O, Amiel C, et al. Effects of acute and chronic hypertension on the labyrinthine barriers in rat. *Hear Res* (2001) 151(1–2):227–36. doi:10.1016/S0378-5955(00)00229-X
20. Nagaoka J, Anjos MF, Takata TT, Chaim RM, Barros F, Penido NO. Idiopathic sudden sensorineural hearing loss: evolution in the presence of hypertension, diabetes mellitus and dyslipidemias. *Braz J Otorhinolaryngol* (2010) 76(3):363–9. doi:10.1590/S1808-86942010000300015
21. Cianfrone G, Pentangelo D, Cianfrone F, Mazzei F, Turchetta R, Orlando MP, et al. Pharmacological drugs inducing ototoxicity, vestibular symptoms and tinnitus: a reasoned and updated guide. *Eur Rev Med Pharmacol Sci* (2011) 15(6):601–36.
22. Rybak LP. Furosemide ototoxicity: clinical and experimental aspects. *Laryngoscope* (1985) 95(9 Pt 2 Suppl 38):1–14. doi:10.1288/00005537-198509010-00001
23. Penido NO, Borin A, Fukuda Y, Lion CN. Microscopic anatomy of the carotid canal and its relation with cochlea and middle ear. *Braz J Otorhinolaryngol* (2005) 71(4):410–4. doi:10.1016/S1808-8694(15)31191-5
24. Figueiredo RR, Azevedo AA, Penido NO. Tinnitus and arterial hypertension: a systematic review. *Eur Arch Otorhinolaryngol* (2015) 272(11):3089–94. doi:10.1007/s00405-014-3277-y
25. Ferreira PEA, Cunha F, Onichi ET, Branco-Barreiro FCA, Ganança FF. Tinnitus Handicap Inventory: adaptação cultural para o português brasileiro. *Pro Fono* (2005) 17(3):303–10. doi:10.1590/S0104-56872005000300004
26. Fasce E, Flores M, Fasce F. Prevalence of symptoms associated with blood pressure in normal and hypertensive population. *Rev Med Chil* (2002) 130(2):160–6. doi:10.1016/j.curtheres.2005.10.001
27. Baraldi GS, Almeida LC, Borges ACLC. Hearing loss and hypertension findings in an older by group. *Braz J Otorhinolaryngol* (2004) 70(5):640–4. doi:10.1590/S0034-72992006000400016
28. Marchiori LLM. Zumbido e hipertensão no processo de envelhecimento. *Rev Bras Hipert* (2009) 16(1):5–8.
29. Mondelli MFCG, Lopes AC. Relação entre a hipertensão arterial e a deficiência auditiva. *Arq Int Otorrinolaringol* (2009) 13(1):63–8.
30. Lasisi AO, Abiona T, Gureje O. Tinnitus in the elderly: profile, correlates and impact on the Nigerian Study of Ageing. *Otolaryngol Head Neck Surg* (2010) 143(4):510–5. doi:10.1016/j.otohns.2010.06.817
31. Negrila-Mezei A, Enache R, Sarafoleanu C. Tinnitus in elderly population: clinic correlations and impact upon QoL. *J Med Life* (2011) 4(4):412–6.
32. Kalyoncu ZB, Pars H, Bora-Günes N, Karabulut E, Aslan D. A systematic review of nutrition-based practices in prevention of hypertension among healthy young. *Turk J Pediatr* (2014) 56(4):335–46.
33. Claire LS, Stothart G, McKenna L, Rogers PJ. Caffeine abstinence: an ineffective and potentially distressing tinnitus therapy. *Int J Audiol* (2010) 49(1):24–9. doi:10.3109/14992020903160884
34. Glicksman JT, Curhan SG, Curhan GC. A prospective study of caffeine intake and risk of incident tinnitus. *Am J Med* (2014) 127(8):739–43. doi:10.1016/j.amjmed.2014.02.033
35. Martines F, Sireci F, Cannizzaro E, Constanzo R, Martines E, Mucia M, et al. Clinical observations and risk factors for tinnitus in a Sicilian cohort. *Eur Arch Otorhinolaryngol* (2014) 272(10):2719–29. doi:10.1007/s00405-014-3275-0
36. Penner MJ. Two-tone forward masking patterns for tinnitus. *J Speech Hear Res* (1980) 23(4):779–86. doi:10.1044/jshr.2304.779
37. Meikle MB. The interaction of central and peripheral mechanisms in tinnitus. In: Vernon JA, Møller A, editors. *Mechanisms of Tinnitus*. Needham Heights, MA: Allyn & Bacon (1995). p. 181–206.

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Innovations in Doctoral Training and Research on Tinnitus: The European School on Interdisciplinary Tinnitus Research (ESIT) Perspective

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Tinnitus is a common medical condition which interfaces many different disciplines, yet it is not a priority for any individual discipline. A change in its scientific understanding and clinical management requires a shift toward multidisciplinary cooperation, not only in research but also in training. The European School for Interdisciplinary Tinnitus research (ESIT) brings together a unique multidisciplinary consortium of clinical practitioners, academic researchers, commercial partners, patient organizations, and public health experts to conduct innovative research and train the next generation of tinnitus researchers. ESIT supports fundamental science and clinical research projects in order to: (1) advancing new treatment solutions for tinnitus, (2) improving existing treatment paradigms, (3) developing innovative research methods, (4) performing genetic studies on, (5) collecting epidemiological data to create new knowledge about prevalence and risk factors, (6) establishing a pan-European data resource. All research projects involve inter-sectoral partnerships through practical training, quite unlike anything that can be offered by any single university alone. Likewise, the postgraduate training curriculum

fosters a deep knowledge about tinnitus whilst nurturing transferable competencies in personal qualities and approaches needed to be an effective researcher, knowledge of the standards, requirements and professionalism to do research, and skills to work with others and to ensure the wider impact of research. ESIT is the seed for future generations of creative, entrepreneurial, and innovative researchers, trained to master the upcoming challenges in the tinnitus field, to implement sustained changes in prevention and clinical management of tinnitus, and to shape doctoral education in tinnitus for the future.

Keywords: tinnitus, education, medical, hearing, PhD studentship, heterogeneity of tinnitus

BACKGROUND

Tinnitus is a condition associated with a continuous auditory percept in the ears or head and can arise as a symptom of many different medical disorders. Assuming a conservative tinnitus prevalence of 10% (1% of severe tinnitus; McCormack et al., 2016) for the 425 million adults living within the European Union (EU), tinnitus affects more than 42 million citizens and is experienced as a severe problem by more than 4 million. Moreover, incidence of new cases is expected to grow over the next few decades (Nondahl et al., 2002, 2010). Although much progress has been made in understanding the pathophysiology (Langguth et al., 2013; Elgoyhen et al., 2015), tinnitus remains a scientific and clinical enigma (Baguley et al., 2013). Unfortunately, tinnitus remains an unmet clinical need and complaining patients are often told "to live with it" (Cederroth et al., 2013). The condition is very common and of varying severity, but the fundamental mechanisms of tinnitus are still incompletely understood. Although not all individuals are unduly troubled, many find the disorder life-changing. In cases with severe tinnitus, mental disorders, and symptoms such as anxiety, depression, insomnia, and concentration problems can impair quality of life often to a level that leads to sick leave and disability pension (Friberg et al., 2012). Therefore, severe tinnitus contributes to a substantial cost to health care and to society at large. In the Netherlands alone, the economic burden of tinnitus is estimated at up to €10.8 billion, with the greater impact being related to socio-economic factors (Maes et al., 2013). The health care costs are enormous. In England there are 750,000 medical consultations yearly with the primary complaint of tinnitus (El-Shunnar et al., 2011).

There is no licensed pharmacological therapy and there is little high quality evidence for the success of palliative management strategies. The Cochrane Library currently lists 9 completed systematic reviews on different tinnitus treatments; namely Tinnitus Retraining Therapy (TRT), Cognitive Behavioral Therapy (CBT), hyperbaric oxygen therapy, sound therapy (masking), hearing aids, repetitive transcranial magnetic stimulation (rTMS), ginkgo biloba, anticonvulsants, and antidepressants (Phillips and McFerran, 2010; Hoekstra et al., 2011; Meng et al., 2011; Baldo et al., 2012; Bennett et al., 2012; Hobson et al., 2012; Hilton et al., 2013; Espinosa-Sánchez et al., 2014; Hoare et al., 2014; Person et al., 2016). However, no uniformly effective treatment for tinnitus has yet been identified. Two main reasons have been discussed: (1) methodological limitations in study design with a paucity of Randomized Controlled Trials (RCTs) and no consensus about what and how to measure therapeutic outcome (Hall et al., 2016), and (2) a large heterogeneity in the patient population with respect to etiology, genetic, and clinical phenotype (Elgoyhen et al., 2015).

Tinnitus is a symptom rather than a distinct disease, and its multivariate manifestations can be subtyped according to various dimensions such as its etiology, time since onset, perceptual characteristics (i.e., pitch, loudness, location, and temporal dynamics), perceived emotional distress, and comorbidities. Progress in scientific understanding and clinical management needs to first address this heterogeneity by identifying scientifically and clinically meaningful subtypes of the condition. Subtyping can then guide the definition of relevant inclusion/exclusion criteria in clinical research and of stratification variables for allocating patients to different intervention groups in RCTS. Subtyping can also guide more sophisticated methods of multivariate data analysis than have been applied hitherto. Current knowledge about etiology, perceptual characteristics, and the neurobiological correlates of tinnitus is not sufficient to enable effective subtyping (Lopez-Escamez et al., 2016). These problems are indicating the need for new approaches.

AN INTERDISCIPLINARY APPROACH IS CRITICAL

Across the EU, no single healthcare system, research organization, or commercial enterprise has an adequate coverage of all relevant issues related to tinnitus and this has resulted in a patchwork of approaches without any coherent framework. Clinically speaking, tinnitus is managed by a variety of different practitioners including general practitioners, otologists, audiologists, psychologists, psychiatrists, neurologists, physical therapists, and dentists (Hall et al., 2011). Academically speaking, tinnitus is of interest to animal neurophysiologists, neuroscientists, epidemiologists, geneticists, trialists, biostatisticians, biomedical engineers, software engineers, and data mining experts. The European School on Interdisciplinary Tinnitus Research (ESIT) uniquely fosters an environment where knowledge and ideas are shared beyond current sectoral borders. The ESIT project provides an international interdisciplinary network of experts from these relevant disciplines working toward a coordinated approach

to tinnitus research. Such a partnership is an essential part of the project. It will support data sharing and meta-analyses to gain new insights about tinnitus, to develop an evidence-based treatment protocol for effective personalized medicine and to identify new innovative treatment approaches.

ESIT MANAGEMENT AND PROJECT DETAILS

The ESIT project started on April 1, 2017 with an overall duration of 4 years. The 15 PhD start their work in October 2017. Each student receives funding for 3 years to undertake their PhD. In **Figure 1** we outline the work packages of the ESIT project. The first three work packages focus on the scientific goals, while work packages 4–7 concentrate on the effective management of the project (i.e., governance, recruitment and training, communications, and longer-term sustainability). The ESIT office is led by Schlee and based at the department of psychiatry and psychotherapy at the University Clinic Regensburg. More details about the ESIT project and the most recent achievements of the project can be found on the project website (www.esit.tinnitusresearch.net).

ESIT's INNOVATIVE PERSPECTIVE ON TINNITUS RESEARCH

ESIT is an EU-funded Marie Skłodowska-Curie Innovative Training Network with 12 top-level research institutions in 10 European countries, supporting 15 PhD projects. The research performed under ESIT will be geared toward a more personalized medicine approach, with improved diagnosis and selection of the best-suited therapy based on the individual patient profile. The 15 PhD projects will collectively address three major objectives that are managed under three research-specific work packages. In total, 2 clinics, 8 commercial enterprises, 2 patient organizations, 5 partner academic institutions, and 2 non-profit organizations are actively partnering in the projects and will support 2–3 month research secondments. Secondments are research visits or practical trainings with other academic institutions or industry partners to promote inter-sectoral exchange and embed a broader perspective into the research collaboration from the outset. Commercial partners will also share technological innovations for research purposes.

Research-specific work package 1. "meaningful individual differences" Co-ordinated by Lopez-Escamez, work package 1 seeks to determine meaningful individual differences in tinnitus by integrating knowledge and experience from all relevant clinical and scientific disciplines, and combining it with patient-centered and commercial perspectives. This will be achieved through interdisciplinary ESIT partnerships with the Meniere's Disease Society (patient organization), Tinnitus Research Initiative Foundation (non-profit research organization), Sensorion Pharmaceuticals (commercial sector), Julius Maximilian's University Würzburg, and the Knowledge Management and Discovery Laboratory at the Otto von Guericke University. Through this work package:

1a) ESIT will create a conceptual framework, as an end goal, that describes an individual's tinnitus profile. This framework will be based on multi-disciplinary data deposited in a centralized ESIT database that then enables the integration of common data variables gathered from all ESIT projects and data analyses targeted toward informing the tinnitus profile framework.
1b) ESIT will assess the genetic contribution to the development and maintenance of specific subtypes of tinnitus. This work will focus on whole-exome sequencing of people with tinnitus who do not have any detectable otological comorbidities, and of patients with Meniere's Disease and tinnitus.
1c) ESIT will identify factors of the individual tinnitus profile, which affect the general responsiveness to tinnitus treatment.

Research-specific work package 2. "novel personalized treatment solutions" Co-ordinated by Weisz, work package 2 seeks to develop novel personalized treatment solutions that respect each patient profile and integrate them with state-of-the-art technological innovations. This will be achieved through interdisciplinary ESIT partnerships with Sivantos, Cochlear Europe, Bee Group AG and Soterix Medical Inc. (commercial sector), and Del Bo Technologia per l'ascolto (independent clinic). Through this work package:

2a) ESIT takes advantage of recent technical developments to develop new, innovative treatment strategies by assessing the effectiveness of enhanced amplification or notched amplification around the frequency corresponding to the dominant tinnitus pitch, individual tomographic neurofeedback, and pseudo-monophasic extra-cochlear stimulation, and by identifying the best placement for an intracochlear microphone.
2b) ESIT seeks to improve existing, clinically well-established treatments by assessing Cognitive Behavior Therapy modified by classical learning, individualized transcranial electric stimulation, repetitive transcranial stimulation combined with auditory stimulation, and an optimized protocol for extra-cochlear electric stimulation.
2c) Using meta-analysis techniques, ESIT will identify the parameters of the individual tinnitus profile which optimize responsiveness to a particular treatment and will use these findings to develop a treatment guide.

Research-specific work package 3. "comparability of scientific and clinical results" Co-ordinated by Gallus, work package 3 seeks to promote international comparability of findings across all major relevant disciplines. This will be achieved through interdisciplinary ESIT partnerships with the British Tinnitus Association (patient organization), Brain Products (commercial sector), Nottingham University Hospitals NHS Trust (clinic), and the Institute of Computer Science, University of Tartu. Through this work package:

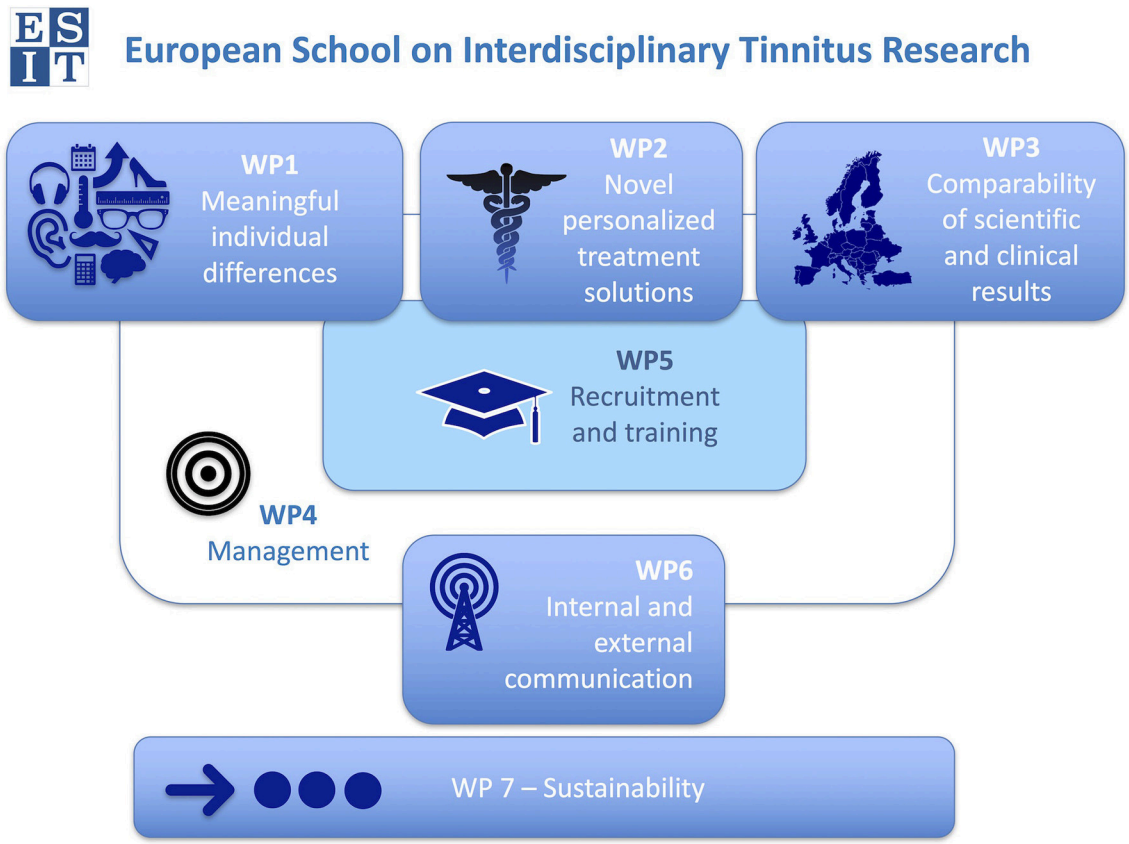

FIGURE 1 | The ESIT structure. Work packages 1–3 deliver the research projects, Work package 4 is to ensure the fluent and effective management of ESIT, Work package 5 is to coordinate the training and recruitment of the students, Work package 6 manages the internal and external communication, and Work package 7 ensures the sustainability of the ESIT achievements. Work packages 4–7 are led by the University of Regensburg, University of Nottingham, Karolinska Institute in Stockholm, and University of Regensburg, respectively.

3a) ESIT will standardize measurements used for diagnostic assessment and inclusion/exclusion criteria for research by developing an evidence-based protocol that will be thoroughly tested using analysis of empirical data, and collecting expert opinions, and patient experiences.
3b) ESIT will integrate large-scale patient data in a central database to support descriptive and inferential analyses.
3c) ESIT will create a standardized framework for collecting longitudinal tinnitus data by implementing novel methods for mobile data collection together with standard assessment tools for validating the crowd-sensing data.
3d) ESIT will collect population-based epidemiological data across many EU countries using the same standardized questions and response options.

This brief overview of the research projects illustrates how the ESIT consortium is characterized by interdisciplinary partnerships both within and across research-specific work packages 1–3. Many projects benefit from the exchange of measurement tools, data sharing, intersectoral knowledge transfer, and dissemination. The overall research program is designed to promote synergies between projects.

ESIT's INNOVATIVE PERSPECTIVE ON TINNITUS TRAINING

ESIT provides the first specialized doctoral curriculum on the topic of tinnitus. Acknowledging that "twenty-first century skills" require more than just knowledge building, ESIT PhD students will develop a balance of transferable competencies, as well as tinnitus-specific knowledge and skills (news feature 2015). In this sense it is unique. Each ESIT PhD student will actively manage his/her own dynamic Personal Career Development Plan. To do this we will promote the Vitae Researcher Development Framework Planner, which is a web-based application for mapping professional development (Vitae, 2017). The planner can be used to keep a unified record of all professional development activities, identify their current expertise and capabilities, record learning and development goals and monitor progress, and upload files such as CVs, conference details, and testimonials to record their personal achievements for lifelong learning. The planner uses the Vitae Researcher Development Framework, which considers four essential domains of learning and development and subdomains which inform the learning objectives of the ESIT training modules (**Figure 2**).

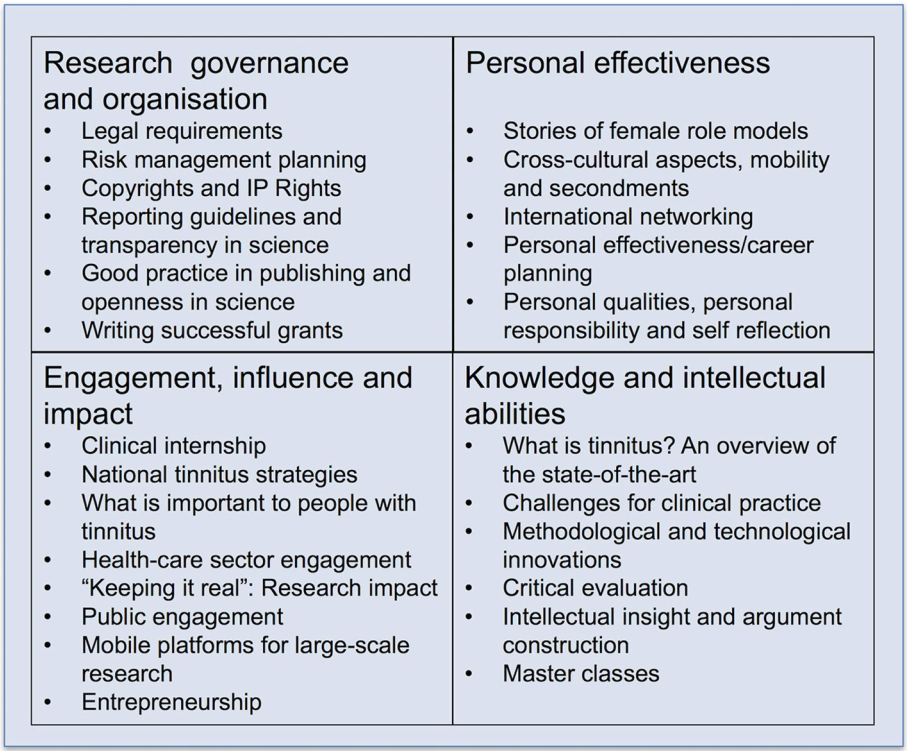

FIGURE 2 | Overview of the ESIT training curriculum structured according to the Vitae Researcher Development Framework. The four essential domains of learning and development are given in the four squares, with individual training modules arranged below.

1. **Knowledge and intellectual abilities** To be able to work at the highest level, ESIT PhD students will gain a foundation-level, tinnitus-specific knowledge base, as well as exposure to critical evaluation, intellectual insight, and argument construction**.** All ESIT PhD students will receive scientific training in the structural and functional components of tinnitus, common diagnostic procedures, and the latest developments in technological innovations for tinnitus care.
2. **Personal effectiveness** Action learning opportunities will foster the personal qualities and approach necessary to become an effective researcher.
3. **Engagement, influence, and impact** Interactions with partners from across the clinical, charity, and commercial sectors will enhance the knowledge and skills that are important for working with others and ensure the wider impact of research.
4. **Research governance and organization** A series of lectures and workshops led by experts in the field will inform students about the standards, requirements, and professionalism to do research.

ESIT offers an innovative doctoral training program (see **Figure 2**) for ambitious young researchers and provides those PhD students with a set of unique academic, clinical, charity, and commercial sector experiences as well as learning opportunities that extend far beyond any typical academic training program. In the same way that all beneficiaries and most partner organizations are contributing to the research projects in work packages 1–3, work package 5 co-ordinated by Hall describes the training curriculum and the input from many of the ESIT consortium members. In total, 10 commercial enterprises, 6 patient and non-profit organizations, 6 partner academic institutions, 1 health authority and 11 independent clinics will provide practical training for the 15 ESIT students. Most training will be provided by ESIT training schools, satellite events at annual conferences and internet-based courses. Self-study will be guided by supervisors and coordinated and monitored by a Supervisory Board.

A prerequisite for impactful research is an awareness of real-world challenges in the tinnitus clinic. At the start of the program, all ESIT PhD students will complete a clinical internship whereby they will experience for 1 week the activities within a tinnitus clinic in a European country. They will meet with many tinnitus patients and tinnitus care providers, develop an appreciation of the symptoms and impact of tinnitus on the lives of real people, learn to understand the care system in the respective country, and critically evaluate the challenges in that system.

PLANNED MAJOR DISSEMINATION ACTIVITIES

Real-world development opportunities are afforded through several major dissemination activities. First, ESIT PhD students

will write a special commissioned feature for publication in "ENT & Audiology News" magazine aimed at hearing healthcare professionals. Second, the ESIT network aims for a completely revised and updated 2nd edition of the "Textbook of Tinnitus" (Møller et al., 2011). The revised edition will be tailored toward becoming the number one choice as a textbook for doctoral and medical training in tinnitus. Other dissemination activities include an updated version of the Wikipedia page for "Tinnitus" (Wikipedia, 2017Q12), an educational video on tinnitus prepared in the major European languages, and dissemination of activities through social media channels. Dissemination will be coordinated by Cederroth, leader of the work package 6.

IMPACT ON CAREER PROSPECTS

To enable clinical relevance of academic research and its rapid translation into improved tinnitus healthcare, there is an urgent need for closer intersectoral collaboration. In conclusion, ESIT will achieve this by providing high-level training with a multidisciplinary supervisory team. The program will equip ESIT PhD students not only with a comprehensive understanding of the challenges faced by healthcare providers in treating tinnitus and by industry in providing technological healthcare solutions, but also to lay the foundations for leadership positions in the relevant academic and non-academic sectors. The network-integrated training program will ensure that the ESIT PhD student can effectively communicate between disciplines and will have the skills to identify synergistic opportunities, to build bridges between disciplines to exploit these connections, and to interact successfully with the private sector. Exposure across disciplines and sectors is unique and is essential to advance ESIT student personal career development and lead the students into European leadership positions in the future. In this way, ESIT will create a new generation of tinnitus experts who are sensitive to the issues of heterogeneity, have a broad knowledge base and have first-hand experience of the possibilities for inter-sectoral disciplines.

AUTHOR CONTRIBUTIONS

WS, DH, and CC drafted the initial version of the manuscript and created the figures. All authors contributed to the development of the ESIT project and contributed equally to all other stages of the manuscript development. All authors approved the manuscript.

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[1] www.cost.eu/COST_Actions/bmbs/BM1306

REFERENCES

Baguley, D., McFerran, D., and Hall, D. (2013). Tinnitus. *Lancet* 382, 1600–1607. doi: 10.1016/S0140-6736(13)60142-7

Baldo, P., Doree, C., Molin, P., McFerran, D., and Cecco, S. (2012). Antidepressants for patients with tinnitus. *Cochrane Database Syst. Rev.* 12:CD003853. doi: 10.1002/14651858.CD003853.pub3

Bennett, M. H., Kertesz, T., Perleth, M., Yeung, P., and Lehm, J. P. (2012). Hyperbaric oxygen for idiopathic sudden sensorineural hearing loss and tinnitus. *Cochrane Database Syst. Rev.* 10:CD004739. doi: 10.1002/14651858.CD004739.pub4

Cederroth, C. R., Canlon, B., and Langguth, B. (2013). Hearing loss and tinnitus–are funders and industry listening? *Nat. Biotechnol.* 31, 972–974. doi: 10.1038/nbt.2736

Elgoyhen, A. B., Langguth, B., De Ridder, D., and Vanneste, S. (2015). Tinnitus: perspectives from human neuroimaging. *Nat. Rev. Neurosci.* 16, 632–642. doi: 10.1038/nrn4003

El-Shunnar, S. K., Hoare, D. J., Smith, S., Gander, P. E., Kang, S., Fackrell, K., et al. (2011). Primary care for tinnitus: practice and opinion among GPs in England. *J. Eval. Clin. Pract.* 17, 684–692. doi: 10.1111/j.1365-2753.2011.01696.x

Espinosa-Sánchez, J. M., Heitzmann-Hernández, T., and López-Escámez, J. A. (2014). [Pharmacotherapy for tinnitus: much ado about nothing]. *Rev. Neurol.* 59, 164–174.

Friberg, E., Jansson, C., Mittendorfer-Rutz, E., Rosenhall, U., and Alexanderson, K. (2012). Sickness absence due to otoaudiological diagnoses and risk of disability pension: a nationwide swedish prospective cohort study. *PLoS ONE* 7:e29966. doi: 10.1371/journal.pone.0029966

Hall, D. A., Haider, H., Szczepek, A. J., Lau, P., Rabau, S., Jones-Diette, J., et al. (2016). Systematic review of outcome domains and instruments used in clinical trials of tinnitus treatments in adults. *Trials* 17:270. doi: 10.1186/s13063-016-1399-9

Hall, D. A., Láinez, M. J., Newman, C. W., Sanchez, T. G., Egler, M., Tennigkeit, F., et al. (2011). Treatment options for subjective tinnitus: self reports from a sample of general practitioners and ENT physicians within Europe and the USA. *BMC Health Serv. Res.* 11:302. doi: 10.1186/1472-6963-11-302

Hilton, M. P., Zimmermann, E. F., and Hunt, W. T. (2013). Ginkgo biloba for tinnitus. *Cochrane Database Syst. Rev.* 28:CD003852. doi: 10.1002/14651858.CD003852.pub3

Hoare, D. J., Edmondson-Jones, M., Sereda, M., Akeroyd, M. A., and Hall, D. (2014). Amplification with hearing aids for patients with tinnitus and co-existing hearing loss. *Cochrane Database Syst. Rev.* 31:CD010151. doi: 10.1002/14651858.CD010151.pub2

Hobson, J., Chisholm, E., and El Refaie, A. (2012). Sound therapy (masking) in the management of tinnitus in adults. *Cochrane Database Syst. Rev.* 11:CD006371. doi: 10.1002/14651858.CD006371.pub3

Hoekstra, C. E., Rynja, S. P., van Zanten, G. A., and Rovers, M. M. (2011). Anticonvulsants for tinnitus. *Cochrane Database Syst. Rev.* 6:CD007960. doi: 10.1002/14651858.CD007960.pub2

Langguth, B., Kreuzer, P. M., Kleinjung, T., and De Ridder, D. (2013). Tinnitus: causes and clinical management. *Lancet Neurol.* 12, 920–930. doi: 10.1016/S1474-4422(13)70160-1

Lopez-Escamez, J. A., Bibas, T., Cima, R. F., Van de Heyning, P., Knipper, M., Mazurek, B., et al. (2016). Genetics of tinnitus: an emerging area

for molecular diagnosis and drug development. *Front. Neurosci.* 10:377. doi: 10.3389/fnins.2016.00377

Maes, I. H., Cima, R. F., Vlaeyen, J. W., Anteunis, L. J., and Joore, M. A. (2013). Tinnitus: a cost study. *Ear. Hear.* 34, 508–514. doi: 10.1097/AUD.0b013e31827d113a

McCormack, A., Edmondson-Jones, M., Somerset, S., and Hall, D. (2016). A systematic review of the reporting of tinnitus prevalence and severity. *Hear. Res.* 337, 70–79. doi: 10.1016/j.heares.2016.05.009

Meng, Z., Liu, S., Zheng, Y., and Phillips, J. S. (2011). Repetitive transcranial magnetic stimulation for tinnitus. *Cochrane Database Syst. Rev.* 5:CD007946. doi: 10.1002/14651858.CD007946.pub2

Møller, A. R., Langguth, B., De Ridder, D., and Kleinjung, T. (2011). *Textbook of Tinnitus.* New York, NY: Springer.

Nondahl, D. M., Cruickshanks, K. J., Wiley, T. L., Klein, B. E., Klein, R., Chappell, R., et al. (2010). The ten-year incidence of tinnitus among older adults. *Int. J. Audiol.* 49, 580–585. doi: 10.3109/14992021003753508

Nondahl, D. M., Cruickshanks, K. J., Wiley, T. L., Klein, R., Klein, B. E., and Tweed, T. S. (2002). Prevalence and 5-year incidence of tinnitus among older adults: the epidemiology of hearing loss study. *J. Am. Acad. Audiol.* 13, 323–331.

Person, O. C., Puga, M. E., da Silva, E. M., and Torloni, M. R. (2016). Zinc supplementation for tinnitus. *Cochrane Database Syst. Rev.* 11:CD009832. doi: 10.1002/14651858.CD009832.pub2

Phillips, J. S., and McFerran, D. (2010). Tinnitus retraining therapy (TRT) for tinnitus. *Cochrane Database Syst. Rev.* 17:CD007330. doi: 10.1002/14651858.CD007330.pub2

Vitae (2017). *About the Vitae Researcher Development Framework Planner - Vitae Website.* Available online at: https://www.vitae.ac.uk/researchers-professional-development/about-the-vitae-researcher-development-framework-planner (Accessed December 31, 2017).

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WHAT DOES TINNITUS HAVE TO DO WITH HEARING LOSS?

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PARTS AND CRAFTS
10–12 YEARS OLD

Our sense organs, along with our brains, give us a detailed understanding of the world around us. If something goes wrong with any of the sense organs, it will affect our everyday functioning. An example of this is hearing loss and tinnitus. Hearing loss is defined as the loss of auditory (sound) information due to damage to the hearing system. Tinnitus is a sound that people can hear, but there is nothing around them that is generating this sound. It can occur as a result of hearing loss. People describe tinnitus as a ringing, buzzing, or hissing sound, but there is no object around that is creating this sound. In this article, we describe some strategies that can be used to protect our hearing, such as moving away from the sound source, protecting the ears, and reducing the volume levels of the sound-producing devices. There is no cure for tinnitus yet. We also discuss ways to manage tinnitus, such as educating yourself about tinnitus, relaxation, focusing your attention away from tinnitus, and seeking the help of a doctor.

HOW DO WE EXPERIENCE THE WORLD AROUND US?

Our sense organs (eyes, ears, tongue, nose, skin) are extremely valuable in giving us a detailed picture of the world around us. To give an example,

imagine you have an apple in your hand. You can touch it and feel its shape, you can see it with your eyes and tell its color, you can bite it and hear the crunch, enjoy its sweet flavor with your tongue, and smell it. Our sense organs send all this information to the brain, which then gives us a complete experience of enjoying the apple.

Each of these sensory organs is valuable to our everyday living and our mighty brain is the center of it all. The brain combines all this sensory information so that we can have a complete experience of the world.

WHAT IF ONE OF THE SENSE ORGANS IS DAMAGED?

Imagine what will happen if one of these sense organs gets damaged. Would we be able to enjoy the full experience of the world around us? Probably not, because now our brain would be missing the important information from this sense organ. We will be explaining this in detail by using the ear as an example. If our ears are damaged and we are not hearing well, this will have two consequences. First of all, the brain will be missing the information from the ears. This is called "**hearing loss**." Second, the brain tries to fill the gap created by this hearing loss. As a result of this, a person with hearing loss might start hearing a sound that others can't hear. This sound is called "**tinnitus**." We will explain both "hearing loss" and "tinnitus" in detail in this article.

HEARING LOSS

Damage to the ear which results in reduced hearing ability.

TINNITUS

Hearing a sound that nobody else can here.

HEARING LOSS

Our hearing is valuable for getting information about our environment and for communicating well with the people around us. We can hear our loved ones' voices or music, which makes us feel happy and creates positive emotions. Hearing can also play an important role in protecting us from danger. For example, look at the boy playing with a ball in the top panel of Figure 1. A car is approaching toward him from the back. Even without seeing it, he instantly recognizes the sound of the car, knows the direction it is coming from, and has a chance to move out of the way.

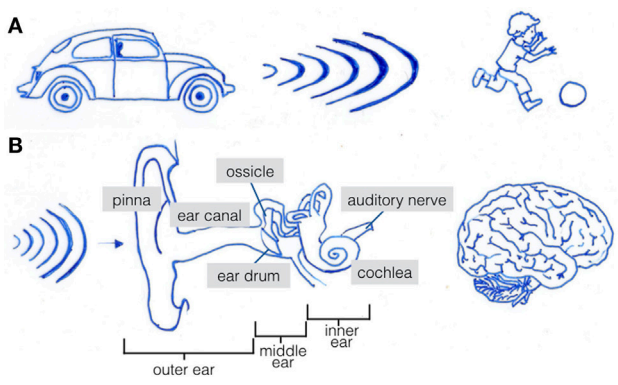

FIGURE 1

FIGURE 1

A. An example how our hearing can protect us while we are playing. **B.** You should read this figure from left to right. On the left side, you see how the sound enters the outer ear. It travels through the middle to the inner ear where it is transformed into electrical signals. The auditory nerve transmits the electrical impulses to the brain (right side).

COCHLEA

The organ which lies in the inner ear and converts the sound into electrical signals.

AUDITORY NERVE

The nerve which sends auditory signals from our ears to the brain.

The bottom panel of Figure 1 explains how our hearing works. The sound is funneled through the outside of the ear, which is called the pinna, into the ear canal. These two parts are called the outer ear. The sound then vibrates the ear drum, which in turn sets the ossicles (a set of three tiny bones in the middle ear) in motion. This motion of the ossicles creates waves in the fluid of the snail-shaped **cochlea**. The cochlea is located deep inside, in an area called the inner ear. The cochlea is the place where the sound energy is converted into electrical impulses by thousands of tiny hair cells. The **auditory nerve** passes this information to the brain, where the details of the sound such as its characteristics, pitch, loudness, and direction, are then understood, so that the boy recognizes the sound of the engine as a car approaching from behind him. This is a rapid process that happens in less than a second. The speed of the hearing process allows the boy to quickly react.

Our hearing system, especially the hair cells in the inner ear, is very sensitive and can easily be damaged by loud sounds. Once these hair cells are damaged, the damage is permanent. Examples of these loud noises could be construction work, traffic noise, airplane engine noise, and others—but also could be pleasurable sounds such a TV at a very loud volume, loud music at a concert, or sound from i-pods, mp3 players, radios, or other music devices. It is extremely important to protect our ears from these loud noises, as they can permanently damage our hearing.

HOW CAN YOU PROTECT YOUR EARS?

You can use the following three strategies to protect your ears [1]. These strategies are also shown in Figure 2.

STRATEGY 1: MOVE AWAY FROM THE SOUND SOURCE

If possible, you should try to move away from loud sounds. For example, if there is construction work happening and it produces very loud noises, you can quickly walk away from it. To give you another example, imagine that you are in an auditorium standing next to a loudspeaker and you feel that it is too loud. You can choose to move away from the speaker. Moving away from the source of the sound reduces the impact of these loud sounds on your ears and minimizes the chances of damaging your hearing.

STRATEGY 2: PROTECT YOUR EARS

In some situations, it is not possible to move away from the sound source, but you can still protect your ears. If you have ear muffs or ear plugs, you should use them to protect your hearing. This is what many people do when they have to work in loud environments—they carry a professional ear protection device and use it when there are loud sounds. Or, if you don't have such an ear protection device, you can simply cover your ears with your hands. Even better, you can put your index fingers in your ear canals to block the sound from getting into the ear.

FIGURE 2

Three strategies to protect our ears from the effects of very loud sounds.

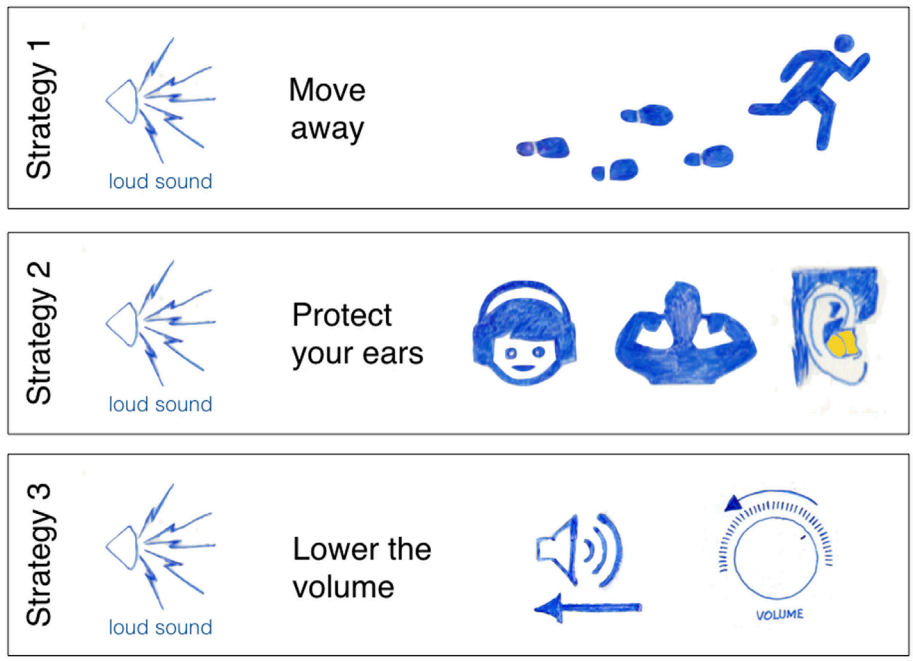

FIGURE 2

STRATEGY 3: LOWER THE VOLUME

When you are listening to music or watching TV, you should keep an eye on the sound level. If you are listening to loud music or TV for a long time, this can lead to permanent damage to your hearing. Sometimes, video games and electronic toys come with high sound levels and don't have a switch to turn the sound down. In this case, you can cover the loudspeaker with several layers of tissue to absorb the sound and reduce the volume.

All these strategies can help you to prevent damage to the ear and preserve your hearing. If you pay attention to professional musicians, many of them wear ear plugs while they are performing on stage because they want to protect their precious hearing, which is extremely important for a good musician.

TINNITUS

Tinnitus is a symptom that can develop as a result of hearing loss. If you have tinnitus you will be hearing a sound but there will be no object around you which is creating that sound. This tinnitus happens because the brain is trying to make up for the lack of input from the ear due to the hearing loss.

Tinnitus is a very common condition affecting millions of people worldwide [2]. Most often, people with tinnitus describe it as a ringing sound or a noise like buzzing, whistling, or hissing. It is very likely that you have heard a tinnitus sound yourself for a very short period of time, for example, after

attending a loud concert or after exposure to a sudden loud noise such as a loud hammer sound. If you have this brief tinnitus, don't be afraid—for most people it will disappear on its own. However, for some people it does not go away and they hear this tinnitus sound constantly, which is irritating to them. Some of these people are bothered by their tinnitus so much that they find it hard to fall asleep, they find it difficult to concentrate, or they have problems following a conversation because they hear the tinnitus all the time. Some of them even feel anxious or depressed and it can affect their quality of life.

WHAT DO RESEARCHERS THINK ABOUT TINNITUS?

Tinnitus means hearing an extra sound, so it's easy to make an assumption that it is a problem of the ear. This is what the researchers thought many years ago. However, in the past few decades, many researchers have studied brain scans that record activity in different parts of the brain. These researchers have realized that tinnitus is not only the problem of the ear, but there are different areas in the brain that are involved in tinnitus as well [3]. Scientists all over the world are working every day toward gaining a better understanding of tinnitus and our knowledge about this condition has been improving every year. These scientists meet every year at conferences and meetings, for example, the "Tinnitus Research Initiative," "International Tinnitus Seminar," and "TINNET," to discuss the latest findings in the area of tinnitus. There are several specialized clinics dedicated to helping people with tinnitus and several ways to manage tinnitus, which have been developed for people who suffer from this condition.

WHAT CAN BE DONE FOR TINNITUS?

There is currently no cure for tinnitus, but there are several ways to manage it or partly reduce it [4, 5]. Here are some useful tips for managing tinnitus. Feel free to share some of these with people you know who have tinnitus.

1. *Know your tinnitus*—it is important to understand tinnitus, its causes, and what can be done to manage it. This information can be valuable in dealing with tinnitus and correcting the wrong beliefs associated with it. It is important to understand that tinnitus is not a sign of danger, it is a non-threatening sound and there is no need to be afraid of it.
2. *Relaxation and distraction*—some people with tinnitus experience that it is reduced when they are relaxed, so it is good to reduce stress. This can be achieved by using relaxation techniques such as deep breathing, walking, sport activities, doing enjoyable things, or any other activities which can be relaxing. Some people also benefit from focusing their attention away from tinnitus by doing things like listening to soothing music or doing something interesting. When they aren't focusing on the tinnitus, it can make it less bothersome.

3. *Seek professional help*—besides the hearing loss described in this article, there can be several other causes of tinnitus. Some medical professionals are trained to help people with tinnitus. Those experts include ear/nose/throat doctors, **audiologists** (hearing experts), neurologists (brain experts), psychologists, and physical therapists. These professionals can offer several management options based on the details of the tinnitus and the other symptoms the patient might be having. Examples of these management options are hearing aids, devices called maskers that "cover up" the sound of the tinnitus, counseling to learn how to deal with the tinnitus, and brain stimulation—to name just a few. It is also important to remember that every person with tinnitus is unique and everyone responds differently to the types of treatment offered. Therefore, it is extremely important to get professional help. The tinnitus experts can help find the best treatments to reduce the tinnitus.

AUDIOLOGISTS

Hearing expert, who deals with people with hearing loss, does hearing aid fitting, and management of hearing loss.

REFERENCES

1. Martin, W. H. 2008. Dangerous decibels: partnership for preventing noise-induced hearing loss and tinnitus in children. *Seminars in Hearing*. Stuttgart: Thieme Medical Publishers.
2. Shargorodsky, J., Curhan, G. C., and Farwell, W. R. 2010. Prevalence and characteristics of tinnitus among US adults. *Am. J. Med.* 123:711–718. doi:10.1016/j.amjmed.2010.02.015
3. De Ridder, D., Elgoyhen, A. B., Romo, R., and Langguth, B. 2011. Phantom percepts: tinnitus and pain as persisting aversive memory networks. *Proc. Natl. Acad. Sci. U.S.A.* 108:8075–8080. doi:10.1073/pnas.1018466108
4. Langguth, B., Kreuzer, P. M., Kleinjung, T., and De Ridder, D. 2013. Tinnitus: causes and clinical management. *Lancet Neurol.* 12:920–930. doi:10.1016/S1474-4422(13)70160-1
5. Baracca, G., Del Bo, L., and Ambrosetti, U. 2011. Tinnitus and hearing loss. In: Møller AR, Langguth B, De Ridder D, Kleinjung T, editors. *Textbook of Tinnitus.* New York: Springer. p. 285–291.

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I am a tinnitus researcher at the University Hospital of Regensburg in Germany. I love to do research. It is great to try out new things that nobody else has done before, and thereby learn a lot of things about the brain and about tinnitus. I am the scientific coordinator of the Tinnitus Research Initiative (TRI), which means that I can work in a great team that is doing research projects on tinnitus all over the world. My big goal is that we find a cure for tinnitus so that nobody in the world needs to suffer from it anymore. *winfried.schlee@gmail.com

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