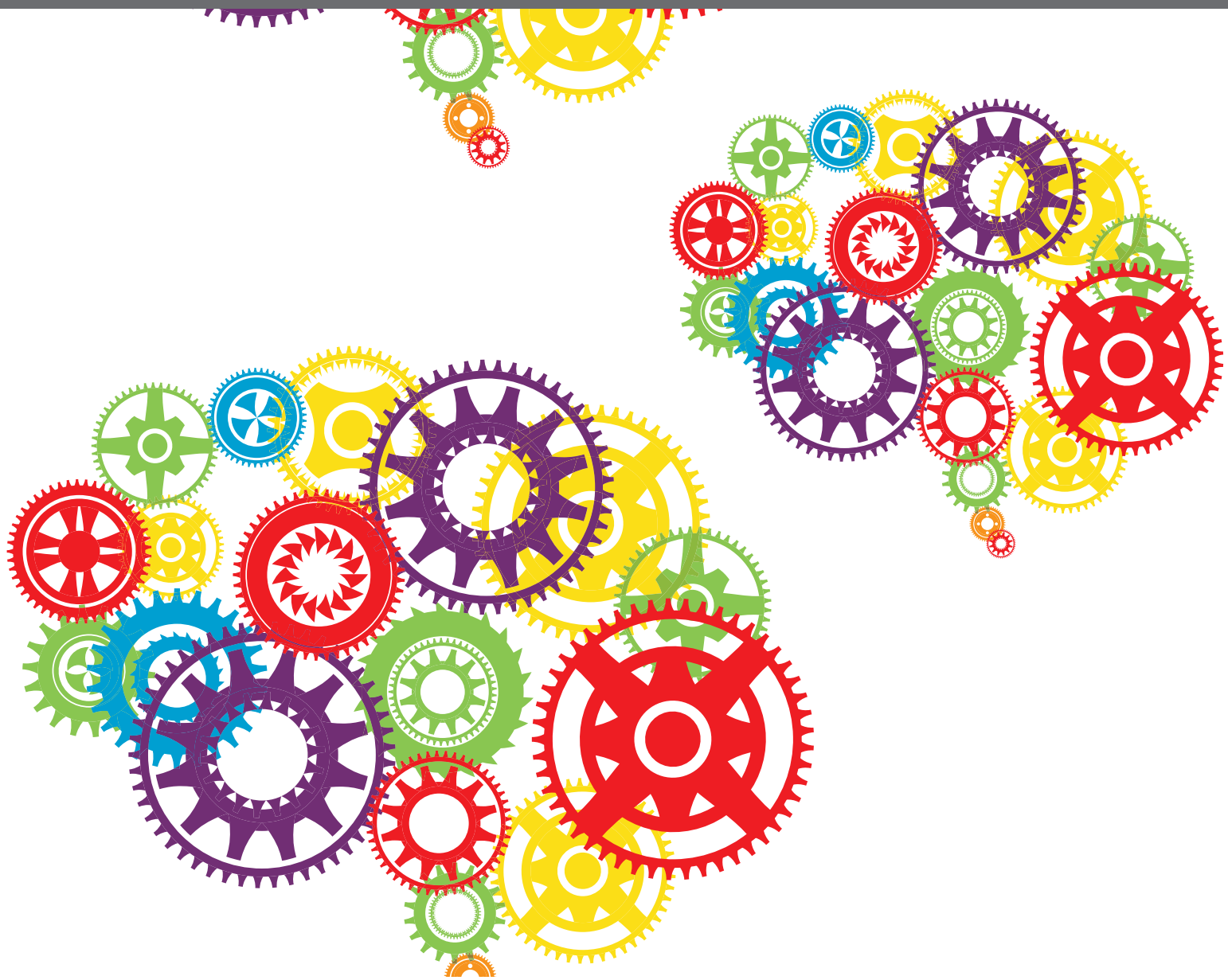
NEW FRONTIERS IN NONINVASIVE BRAIN STIMULATION: COGNITIVE, AFFECTIVE AND NEUROBIOLOGICAL EFFECTS OF TRANSCUTANEOUS VAGUS NERVE STIMULATION

EDITED BY: Mathias Weymar and Tino Zaehle

PUBLISHED IN: Frontiers in Psychology and Frontiers in Human Neuroscience



frontiers Research Topics

ISSN 1664-8714
ISBN 978-2-88971-083-6
DOI 10.3389/978-2-88971-083-6

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: frontiersin.org/about/contact

NEW FRONTIERS IN NONINVASIVE BRAIN STIMULATION: COGNITIVE, AFFECTIVE AND NEUROBIOLOGICAL EFFECTS OF TRANSCUTANEOUS VAGUS NERVE STIMULATION

Topic Editors:
Mathias Weymar, University of Potsdam, Germany
Tino Zaehle, Otto von Guericke University Magdeburg, Germany

Citation: Weymar, M., Zaehle, T., eds. (2021). New Frontiers in Noninvasive Brain Stimulation: Cognitive, Affective and Neurobiological Effects of Transcutaneous Vagus Nerve Stimulation. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88971-083-6

Table of Contents

frontiers in Psychology

EDITORIAL
published: 24 May 2021
doi: 10.3389/fpsyg.2021.694723

Editorial: New Frontiers in Noninvasive Brain Stimulation: Cognitive, Affective and Neurobiological Effects of Transcutaneous Vagus Nerve Stimulation

Mathias Weymar[1,2]* *and Tino Zaehle*[3,4]*

[1] Department of Biological Psychology and Affective Science, Faculty of Human Sciences, University of Potsdam, Potsdam, Germany, [2] Faculty of Health Sciences Brandenburg, University of Potsdam, Potsdam, Germany, [3] Department of Neurology, Otto-von-Guericke University, Magdeburg, Germany, [4] Center for Behavioral Brain Sciences, Magdeburg, Germany

Keywords: vagus nerve stimulation, tVNS, neuromodulation, cognition, affective, neurobiological

Editorial on the Research Topic

New Frontiers in Noninvasive Brain Stimulation: Cognitive, Affective and Neurobiological Effects of Transcutaneous Vagus Nerve Stimulation

OPEN ACCESS

Edited and reviewed by:
Bernhard Hommel, University Hospital Carl Gustav Carus, Germany

***Correspondence:**
Mathias Weymar
mathias.weymar@uni-potsdam.de
Tino Zaehle
tino.zaehle@ovgu.de

Specialty section:
This article was submitted to Cognition, a section of the journal Frontiers in Psychology

Received: *13 April 2021*
Accepted: *15 April 2021*
Published: *24 May 2021*

Citation:
Weymar M and Zaehle T (2021) Editorial: New Frontiers in Noninvasive Brain Stimulation: Cognitive, Affective and Neurobiological Effects of Transcutaneous Vagus Nerve Stimulation. Front. Psychol. 12:694723. doi: 10.3389/fpsyg.2021.694723

Since its first introduction two decades ago, transcutaneous vagus nerve stimulation (tVNS) has drawn tremendous attention as a promising non-invasive tool to stimulate the vagus nerve in the brain. Through the putative activation of afferent vagal projections to distinct brainstem, subcortical and cortical regions, and associated neurotransmitter systems (e.g., noradrenaline, GABA), tVNS was originally used as an alternative treatment option for epilepsy, depression, and other clinical conditions. More recently, it has been used in non-clinical populations to modulate affective and cognitive functions. However, despite the undisputed potential and accumulating evidence for tVNS-related improvements in corresponding domains, such as emotion recognition, fear extinction, cognitive control, and attention, there is also uncertainty in the field with respect to tVNS research settings, e.g. stimulation parameters, duration of stimulation (also concurrent to a certain task or not), mechanisms of action, and related biomarkers for effective stimulation, which is reflected at some point by an even number of non-replicable or mere subtle findings.

The aim of this Frontiers Research Topic on "Non-invasive Brain Stimulation: Cognitive, Affective and Neurobiological Effects of Transcutaneous Vagus Nerve Stimulation" is to present recent advances in the application of tVNS as a useful neuromodulation tool for the systematic study of cognition and emotion as well as its underlying neurobiological processes, to address new trends and emerging neuromodulation technologies, and ultimately foster fruitful discussion and interaction among researchers.

The articles in this Frontiers Research Topic perfectly reflect the current status of the field by encompassing articles that span from the novel but also mixed findings in the newly emerging fields of tVNS (mainly referring to auricular stimulation) application in basic and clinical research to reviews that highlight important anatomical considerations, strategies, and recommendations to overcome current challenges when applying tVNS in future research.

The submissions from basic research include behavioral findings on the impact of tVNS on episodic memory. Mertens et al. showed that auricular tVNS, compared to sham and control

stimulation, had no improving effect on immediate free recall and recognition memory for attended verbal material when applied directly after encoding. Giraudier et al. partly replicated this finding; however, they demonstrated that tVNS during a lexical decision task can improve delayed recognition memory performance for emotional and neutral words when memory quality was taken into account (i.e., tVNS effects on high confidence memory reflecting recollection). Contrary to other studies, in this article, tVNS did not affect a putative indirect marker for central noradrenergic activity (salivary alpha amylase). Two other studies emphasized the role of tVNS in food processing, hedonic assessment, and ingestive behavior (Obst et al., Öztürk et al.). Pre-task tVNS in food deprived individuals showed no effect on the processing of food as revealed by event-related brain potentials (ERP), compared to sham, but found overall ERP differences in components related to visual attention and conflict processing (Obst et al.). Preliminary evidence by Öztürk et al. however, emphasized that concurrent tVNS stimulation increased food related hedonic responses, such as liking of low-fat food during a food sample task, also pointing toward a plausible triggering of dopaminergic reward circuits in the brain by tVNS (although spontaneous eye blink rate as a potential biomarker of dopamine functioning was not modulated by tVNS). Food consumption, however, was not influenced by vagal stimulation in both studies (Obst et al.; Öztürk et al.). Submissions from application in clinical settings further suggest that motivated behavior (such as oral intake) can be improved by tVNS by facilitating oromotor learning over a period of 2 weeks in premature or brain injured infants with oral feeding difficulties when vagal stimulation was paired with bottle-feeding (Badran et al.). Further evidence suggests that tVNS may also be effective as adjunct treatment of symptoms of acute opioid withdrawal, as shown in a study by Jenkins et al., in which auricular nerve stimulation (which also includes vagal branch) resulted in alleviation of withdrawal symptoms and duration of opioid replacement therapy in infants suffering from opioid toxification, which may be explained by the involvement of dopaminergic circuits as an extended vagal system (c.f., Öztürk et al.; Badran et al.). Finally, Ylikoski et al. present outcome data from a treatment program showing that tVNS may be a useful long-term therapeutic adjunct reducing distress in patients suffering from tinnitus. It is also assumed by the authors that tVNS may exert its anti-stress effects via a (parasympathetic) efferent pathway based on treatment baseline data showing short-term improvements in heart rate variability by tVNS (see also Badran et al. for reporting increased heart rate deceleration). It should be noted though that all clinical studies in this Research Topic did not include a control or sham condition, which needs to be considered when interpreting the potential impact of tVNS in these studies.

The current Research Topic on tVNS also includes critical reviews and a perspective paper. Paciorek and Skora emphasize an important link between vagus nerve stimulation and interoception, which also guides various cognitive and affective functions at different levels of processing, encouraging elaborate tVNS research in the future. Cakmak points to important anatomical pathways that modulate central vagal and non-vagal structures depending on the activation of specific auricular zones, helping researchers in the field to improve study designs (in terms of optimal electrode location) and to sharpen the interpretability of results (with respect to the mechanism of action). Finally, as an effort to increase knowledge and reproducibility in the field this Research Topic includes a much needed tVNS consensus paper (Farmer et al.) by Julian Koenig—with the cooperation of a large group of tVNS researchers—on guidelines and recommendations for future tVNS studies in basic research and clinical practice based on the existing tVNS literature.

In conclusion, the Research Topic shows new exciting fields of application for tVNS in basic and clinical settings. However, as reflected by the original research papers and reviews, it is clear that more research is needed to identify the exact mechanism of action including reliable biomarkers, and optimal stimulation parameters and contexts for tVNS to meet the high expectations as effective neuromodulation and treatment tool.

AUTHOR CONTRIBUTIONS

MW and TZ drafted and revised the manuscript. Both authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We thank all authors for their contribution to our Research Topic.

Conflict of Interest: 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.

frontiers in Human Neuroscience

REVIEW
published: 12 December 2019
doi: 10.3389/fnhum.2019.00421

Concerning Auricular Vagal Nerve Stimulation: Occult Neural Networks

Yusuf Ozgur Cakmak [1,2,3,4]*

[1]Department of Anatomy, School of Biomedical Sciences, University of Otago, Dunedin, New Zealand, [2]Brain Health Research Centre, University of Otago, Dunedin, New Zealand, [3]Centre for Health Systems and Technologies, University of Otago, Dunedin, New Zealand, [4]Medical Technologies Centre of Research Excellence, Auckland, New Zealand

Auricular vagal nerve stimulation (AVNS) is an evolving neuromodulation technology that has a wide range of therapeutic applications across multiple disciplines of medical science. To date, AVNS results had been interpreted in the context of a monolog concept of the auricular branch of the vagus nerve (ABVN): that this is the sole network of the mechanism of action and/or structure in the auricular area of the stimulation in the context of activations in the brainstem nuclei, including the nucleus tractus solitarius (NTS), locus coeruleus (LC), trigeminal brainstem nuclei, and the nucleus cuneatus. This review considers the overlooked aspects of neural networks, connections, hijacking axons from cranial nerves and cervical sympathetic ganglions, the inhomogeneous distribution of perivascular sympathetic nerves, and intrinsic/extrinsic auricular muscles in the auricular zone that can explain the vagal and non-vagal nucleus activations in AVNS. In addition, the unique cortical representation of the human ear and interspecies differences in the auricula are discussed. The detailed auricular anatomy of the AVNS zone explored in the present study references structural and functional neural network information to overcome default designs and misinterpretations of existing research on AVNS to provide a better foundation for future investigations that use this modality.

Keywords: auricular, vagus, electrostimulation, nVNS, facial nerve, neuromodulation, network, neuroanatomy

OPEN ACCESS

Edited by:
Tino Zaehle,
Otto von Guericke University Magdeburg, Germany

Reviewed by:
Bashar W. Badran,
Medical University of South Carolina, United States
Andrey Gourine,
Karolinska Institutet (KI), Sweden

***Correspondence:**
Yusuf Ozgur Cakmak
yusuf.cakmak@otago.ac.nz

***Specialty section**:*
This article was submitted to Brain Imaging and Stimulation, a section of the journal Frontiers in Human Neuroscience

Received: *31 July 2019*
Accepted: *13 November 2019*
Published: *12 December 2019*

Citation:
Cakmak YO (2019) Concerning Auricular Vagal Nerve Stimulation: Occult Neural Networks. Front. Hum. Neurosci. 13:421. doi: 10.3389/fnhum.2019.00421

INTRODUCTION

The number of auricular vagal nerve stimulation (AVNS) studies in the literature has increased logarithmically over the last two decades (Cakmak, 2006; Badran et al., 2018a,b; Burger et al., 2019; Hong et al., 2019; Zhao et al., 2019). A broad range of clinical conditions across multiple disciplines—from neurology to immunology—have been investigated for a potential clinical therapeutic response to AVNS. However, most of these studies are designed and interpreted according to the same neuroanatomical model defined by retrograde neuronal tract tracing research, which is limited in terms of denervation levels as well as in the selection of the anatomical structures to be examined. In addition, functional magnetic resonance imaging (fMRI) studies designed to demonstrate the AVNS activations of the influenced networks and structures in response to non-invasive vagal nerve stimulation are also based on the same concept of AVNS neural connectivity and might therefore have overlooked other

neural connections and structures in the zone of the AVNS. Multiple lines of evidence in the literature indicate that anatomical structures in the anatomical zone of AVNS can modulate the same neural vagal and non-vagal structures in the brainstem *via* alternative pathways. The inhomogeneous distribution of the nerves, including the perivascular sympathetic nerves, were not considered by these studies. It is also worth noting that the field electrical stimulation used in studies of the auricular branch of the vagus nerve (ABVN) cannot target a specific axon or nerve bundle in the stimulation zone and instead may modulate all the nerves and structures beneath and between the electrodes. In addition, the potential neural networks that can be manipulated as a result of such stimulation are beyond the monosynaptic anatomical connectivity that is described by neural tract tracing studies; multi-synaptic influences should always be considered to gain a more complete understanding of the potential of AVNS.

The aim of this article is to identify and highlight the anatomical aspects of auricular vagal stimulation zones overlooked by prior studies to yield an improved proxy to inform future research design and the interpretation of AVNS studies.

THE PATH: CONNECTIVITY AND HIJACKER (HITCHHIKER) AXONS

Jugular Foramen to Mastoid

The ABVN pathway is quite complex on account the contribution of multiple axons to this branch along its path (**Figure 1**). The ABVN is known to originate in the superior (also referred to as the jugular) ganglion of the vagus nerve. However, from a functional anatomical perspective, it is more correct to take the sensory content of the axons into consideration and state vice versa. In this context, it is worth noting that only a portion of ABVN axons join with the superior vagal ganglion at the level of the jugular foramen; there are other non-vagal axons that leave the main branch of the ABVN at this same level. In other words, the ABVN axons split into three main bundles at the level of the jugular foramen (after it leaves the mastoid foramen-temporal bone and before terminating in the superior ganglion of the vagus nerve): (1) one bundle joins the glossopharyngeal nerve (Ozveren et al., 2003; Tubbs et al., 2005; Watanabe et al., 2016); (2) another axonal bundle (sympathetic nerves) joins the cervical sympathetic ganglions (that project through the internal carotid nerve, Matthews and Robinson, 2014); and (3) other axons terminate in the superior vagal ganglion. It is worth noting that the non-vagal bundles (1 and 2) branch off after the ABVN leaves the mastoid canaliculus and before the ABVN terminates in the superior vagal ganglion (**Figure 1**).

The most proximal ABVN injections in neural tract-tracing studies of the ABVN have been performed with injections of horseradish peroxidase (HRV) into the cut end (proximal to the facial nerve connection) of the ABVN before it splits into the non-vagal branches of the glossopharyngeal nerve and cervical sympathetic ganglions. Neural labeling has been demonstrated in the superior vagal ganglion, but not in the other cranial nerve ganglia, including the superior/inferior ganglion of the glossopharyngeal nerve, geniculate ganglion of the facial nerve, inferior (nodose) ganglion of the vagus nerve, and trigeminal ganglion (Nomura and Mizuno, 1984). In this context, the superior vagal ganglion has been proposed as the terminal for ABVN axons. On the other hand, the same study labeled fibers in the spinal trigeminal tract and the principal trigeminal nucleus, nucleus tractus solitarius (NTS), cuneate nucleus, and C1–3 dorsal horn segments after the same HRV injection (Nomura and Mizuno, 1984). Considering that most of these anatomical regions are non-vagal centers, a significant implication of this 1984 report has been overlooked: the authors failed to investigate the sympathetic cervical ganglions for potential labeling, and the cervical sympathetic ganglions are likely to be labeled with retrograde tracer injections into the same ABVN segment. In addition, all the reported labeling achieved by this same study, including the spinal trigeminal tract, principal trigeminal nucleus, NTS, cuneate nucleus, and C1–3 dorsal horns of the cervical spinal nerves after HRP injection into the ABVN segment situated distal to the non-vagal contributions to ABVN, cannot be explained by vagal axons. These are likely to be the axons that do not synapse in the superior vagal ganglia but exist in or hitchhike the ABVN. The auricular muscles are modulated along with the neck and shoulder muscles by neurons in the cuneate nucleus (Maslany et al., 1991); hence, the cuneate nucleus is likely to be excited with intrinsic auricular muscle stimulation in the ABVN stimulation zone. The labeled neurons of the cuneate nucleus in a 1984 report are potentially related to the facial nerve fiber contributions that hitchhike the vagus nerve; this will be considered in the following section. The sympathetic axons of the C1–3 spinal nerves also contribute to the superior cervical ganglion since they do not receive direct sympathetic contributions from the spinal cord like the other spinal nerves at T1 and below (Netter, 1999; von Lanz and Wachsmuth, 2003). In addition, the ABVN does not have its own somatosensory sensory nucleus nor any somatosensory fibers. Regardless of whether these somatosensory axons of the ABVN join to the glossopharyngeus or the vagus nerves, they terminate in the trigeminal nucleus in the brainstem (Rhoton et al., 1966; Heimer, 1995) and so should actually be considered trigeminal nerve axons; this will be discussed in the following sections.

In the Mastoid (Temporal Bone)

Before splitting into the three main bundles described in the previous section, the ABVN passes through the mastoid (Tekdemir et al., 1998). In the human mastoid, the ABVN crosses the fallopian canal, where it forms two main branches (Gasser, 1957; Schuknecht, 1974; Lang, 1983; May, 2000; Nageris et al., 2000; Diamond et al., 2011; Watanabe et al., 2016; Mulazimoglu et al., 2017).

The first branch of the ABVN reportedly connects to the *chorda tympani* branch of *the facial nerve* and also receives innervation from the posterior dura mater of the cranial fossa. The other division of the first branch of the ABVN contributes to the *external auditory meatus* and *concha zone of the auricular skin*, where AVNS is performed (Gasser, 1957; Schuknecht, 1974; Lang, 1983; May, 2000; Nageris et al., 2000; Diamond et al., 2011; Watanabe et al., 2016; Mulazimoglu et al., 2017; **Figure 1**).

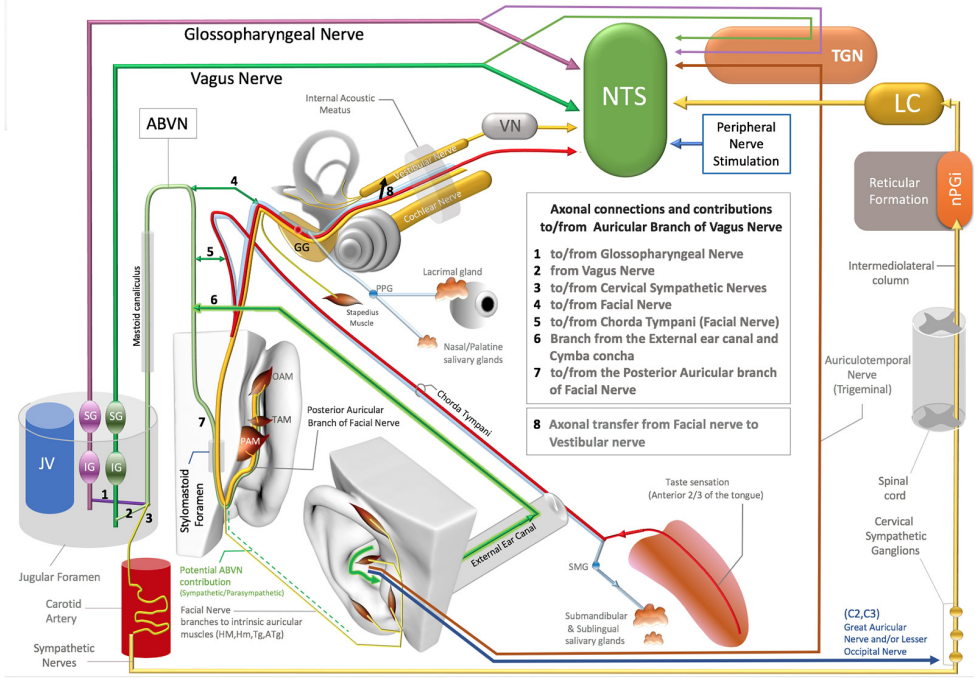


FIGURE 1 | Axonal connections and contibutions to/from the auricular branch of the vagus nerve (ABVN), Non-vagal pathways to nucleus tractus solitarius (NTS), locus coeruleus (LC). TGN, Trigeminal sensory nuclei; nPGI, NucleusParagigantocellularis; VN, Vestibular Nuclei; GG, Geniculate ganglion of Facial Nerve; PPG, Pterygopalatine ganglion of the Facial Nerve; SMG, Submandibular ganglion of the Facial Nerve; OAM, Oblique auricular muscle; TAM, Transverse Auricular Muscle; PAM, Posterior Auricular Muscle; JV, Jugular Vein; IG (magenta), Inferior ganglion of the Glossopharyngeus nerve; SG (magenta), Superior ganglion of the glossopharyngeal nerve; SG (green), Superior Ganglion of the Vagus nerve; IG (Green), Inferior ganglion of the Vagus Nerve.

The second branch of the ABVN also joins to *the facial nerve via the posterior auricular nerve of the facial nerve* before leaving the skull *via* the stylomastoid foramen and contributes to the posterior aspect of the auricula and the adjacent skull region (Gasser, 1957; Schuknecht, 1974; Lang, 1983; May, 2000; Nageris et al., 2000; Diamond et al., 2011; Watanabe et al., 2016; Mulazimoglu et al., 2017; **Figure 1**).

The type of axons that project from the facial nerve to the ABVN and vice versa remain obscure. The contributing axons from the facial nerve may have a *motor, general sensory, special sensory*, or even a *parasympathetic origin*, as the parasympathetic component of the facial nerve (the intermediate nerve, nervus intermedius) also enters the facial canal. Additionally, the sympathetic axons that join to the ABVN at the level of the jugular foramen can distribute to the external ear canal, the concha zone of the auricular skin, and the posterior aspect of the ear *via* the ABVN branch that contributes to the posterior auricular branch of the facial nerve. If we consider the retrograde pathway of the ABVN branch that contributes to the posterior branch of the facial nerve, a particular bundle of the posterior auricular branch of facial nerve axons joins the ABVN after it enters the skull *via* the stylomastoid foramen. In this scenario, which cannot be excluded in the context of the existing literature, these axons may also elongate up to the nucleus cuneatus, which corresponds to the center of the neck and potentially the auricular muscles, labeled and activated after ABVN HRV injections or stimulations in fMRI studies (Tekdemir et al., 1998; Frangos et al., 2015).

Finally, *facial and vestibulocochlear nerves also exchange axons* with the facial canal: another factor that confounds the elucidation of the origins and content of the axons that join to the ABVN in the facial canal (Nageris et al., 2000; Özdoğmuş et al., 2004; Diamond et al., 2011).

In the context of these extensive connections, before exiting the stylomastoid foramen or distributing to the external ear canal and auricula, the complexity of the ABVN is already established on account of its numerous axonal origins. No data in the literature has hitherto revealed the extent to which axons are distributed to each of the ABVN branches. Hence, AVNS studies cannot exclude these neural networks and should consider these potential neural contributions in their designs and interpretations of outcomes.

Out of the Stylomastoid Foramen and the External Ear

The detailed territory of the ABVN in the posterior aspect of the auricula has not been mapped in the literature. The potential territory of ABVN axons in the posterior auricular branch of the facial nerve might distribute to the auricular muscle zones stimulated by the posterior auricular branch of the facial nerve. If we consider retrograde transmission in the ABVN, the posterior auricular nerve of the facial nerve that innervates the intrinsic and extrinsic auricular muscles would be hijacking the ABVN

after it enters the skull *via* the stylomastoid foramen. As stated in the previous section, this model can account for the previously described HRP of the cuneate nucleus following HRP injections into the ABVN.

The existence of sympathetic axons that contribute to both the ABVN at the level of the jugular foramen and the posterior auricular branch of the facial nerve cannot be dismissed by current data. In this case, these sympathetic axons can distribute to the posterior aspect of the auricular skin *via* the posterior ABVN. In contrast, this distribution complicates interpretations of the AVNS because the sympathetic nerves also contribute to the perivascular innervation of the auricular arteries (Cakmak et al., 2018), and these auricular arteries have perforating branches that pierce the posterior aspect of the auricular cartilage (**Figures 2C,D**). These pass through to the internal aspect of the auricula and auricular skin, including the concha area where AVNS is performed (Park and Roh, 2002; Zilinsky et al., 2017; Cakmak et al., 2018). This perforating pathway provides not only a route for the sympathetic nerves to pass from the posterior surface of the ear to its anterior/interior surface but also a path for the parasympathetic axons from which the ABVN may originate. Our group recently demonstrated that the perivascular auricular innervation comprises sympathetic and cholinergic components in the human ear (Cakmak et al., 2018). The significance of this contribution and the inhomogeneity of these axons will be discussed in relation to the design and interpretations of ABVN studies in the following sections.

VAGAL BRAINSTEM NUCLEI ACTIVATION BY NON-VAGAL AURICULAR STRUCTURES

In addition to the complexity of the axonal origins and contributions of ABVN, which must be considered in any analysis of ABVN stimulation, the interpretations of ABVN results in electrostimulation studies, including those that employ fMRI and neural tracing, feature unresolved ambiguities concerning auricular anatomy. This section will focus on the overlooked anatomical structures and non-vagal neural networks that can modulate vagal-related brainstem nuclei.

Facial Nerve, Intrinsic Auricular Muscles, and Nucleus Cuneatus

The intrinsic auricular muscles of the human auricula and their innervation by the facial nerve axons are major anatomical and neural confounding factors of ABVN stimulation zones (**Figure 2D**). The helicis minor muscle, which is innervated by the facial nerve, is localized at the center of the ABVN zone used in numerous AVNS studies (Frangos et al., 2015). Similarly, the tragicus muscle is situated in the zone of the tragus, which is also used for AVNS investigations (Badran et al., 2018a,b). The other intrinsic muscles also constitute conflicting factors for the sham zones chosen in multiple studies. To date, none of the AVNS studies have considered the potential contribution of the intrinsic auricular muscles in the ABVN stimulation zones of the auricular muscles. Furthermore, the sham or control stimulation sites are also determined without this information (Frangos et al., 2015); consequently, while the active stimulation sites are muscle-free zones, the sham/control zones contain intrinsic auricular muscles and vice versa, preventing the proper interpretation of study results. Our group recently demonstrated that the stimulation of intrinsic auricular muscle zones improved the motor functions of patients with Parkinson's disease (Cakmak et al., 2017). A recent study that placed active electrodes on the tragicus muscle zone claimed that the improvement of oromotor function occurs *via* the auricular vagus nerve (Badran et al., 2018b). Unfortunately, this unquestioned, biased approach is widespread among studies of the AVNS; indeed, investigations commonly reapply previous conceptions of the AVNS neural pathways that lack a basis in concise, accurate auricular anatomical knowledge.

Additionally, transcutaneous electrical stimulation features a parabolic electrical field that can induce the deeper stimulation of areas between electrodes. Median nerve stimulation of the wrist is a clear example of such a parabolic field effect: the median nerve situated at the core of the carpal tunnel zone of the wrist can be stimulated by transcutaneous electrical nerve stimulation electrodes placed 3 cm apart on the wrist (Urasaki et al., 1998; Ferretti et al., 2007; Maharjan et al., 2019). The structures, including intrinsic muscles and nerves located in the posterior aspect of the auricular muscles, can also potentially be stimulated by electrodes placed on the anterior surface of the auricula. To date, no study has investigated such a potential contribution, and it therefore cannot be ruled out.

The stimulation of the intrinsic muscles may influence multiple zones in the brainstem, including the nucleus cuneatus, cerebellum, and cortex. Selective muscle afferent nerve stimulation reportedly causes significant activation in motor-related areas relative to that induced by cutaneous stimuli (Wardman et al., 2014). Muscle afferent stimulation evokes more widespread cortical, subcortical, and cerebellar activations than do cutaneous afferents (Wardman et al., 2014). Separate pre- and post-central excitation foci were observed with muscle afferent stimulation, underscoring the importance of muscle afferent nerve stimulation in the modulation of cortical motor areas (Wardman et al., 2014).

Any ABVN stimulation should thus consider muscle-free zones in the ear to interpret the results related to the ABVN if the electrodes are placed on intrinsic auricular muscle zones. The nucleus cuneatus is a hub for neck muscles. As aforementioned, the posterior auricular branch of the facial nerve likely functions as the pathway to the nucleus cuneatus. A recent fMRI study also supported such a connection: stimulation of the antitragicus muscle zone (without considering the fact that this zone contains intrinsic auricular muscles) activated the nucleus cuneatus, whereas no activation in the nucleus cuneatus was observed when a non-muscular area of the ear was stimulated (Frangos et al., 2015). The nucleus cuneatus is also labeled when neurotracers are injected into the ABVN (Maslany et al., 1991). In another fMRI study (Badran et al., 2018a), the researchers also used the tragicus muscle zone as the AVNS zone, and the control zone was a muscle-free zone in the ear lobe. It was found that the supplementary motor area was only activated in the tragicus muscle zone stimulation, and this was postulated to be an outcome of AVNS. Any theories that explain the activation of the

cortical and subcortical motor areas including nucleus cuneatus with auricular stimulation cannot exclude the intrinsic auricular muscle; moreover, these muscles can account for the activation of the nucleus cuneatus rather than that of the vagus nerve.

Trigeminal Brainstem Nuclei

The nucleus ambiguus and the dorsal motor nucleus of the vagus are the two motor brainstem nuclei of the vagus nerve that contribute to striated and cardiac muscle rhythm and smooth and cardiac muscle contractibility, respectively (Geis and Wurster, 1980; Wang et al., 2001; Farmer et al., 2016), while the NTS is the viscerosensory nucleus. As stated in the Introduction, the vagus itself does not receive projections from or terminate in the somatosensory brainstem nuclei and instead conveys its somatosensory information to the brainstem nuclei of the trigeminal nerve (Rhoton et al., 1966; Heimer, 1995). The classification of the nuclei of the brainstem cranial nerve and the axonal bundles that compose cranial nerves is complex and likely inaccurate in many respects. The vagus conveys somatosensory information to the trigeminal nuclei *via* the ABVN, and this bundle is still considered the vagus nerve. In contrast, numerous hijacking axonal bundles in the cranial nerve system have not been classified in the same manner. The parasympathetic axons of the glossopharyngeal nerve hijack the auriculotemporal branch of the trigeminal nerve, and the parasympathetic fibers of the facial nerve also hijack the lingual, zygomatic, and lacrimal branches of the trigeminal nerve; however, these are still classified as being components of the glossopharyngeus and the facial nerves, respectively, because these axons terminate at, or their cell bodies are located in, the parasympathetic brainstem nuclei of the facial and glossopharyngeus nerves but not in the trigeminal brainstem nuclei. Similarly in terms of organization, the facial, glossopharyngeus, and vagus nerves do not have their own sensory brainstem nuclei but convey their somatosensory information to the brainstem nuclei of the trigeminal nerve—i.e., their somatosensory axons terminate in the trigeminal brainstem nuclei. However, these sensory axons are not classified under the trigeminal nerve system but are instead attributed to the ABVN. It can therefore be argued that the facial, glossopharygeus, and vagus nerves are actually hijacked by trigeminal nerve sensory fibers, which actually belong to the trigeminal nerve and not to the facial, glossopharyngeus, or vagus nerves. This line of reasoning indicates that they have traditionally been misclassified. Hence, ABVN stimulation can activate sensory nuclei of the trigeminal nerve not because of the axons of the ABVN itself, but rather because the trigeminal nerve axons hijack the ABVN.

Even in the case of a conventional, conservative argument that insists on the traditional but conflicting classification of these sensory fibers of the ABVN without considering their axonal origins, there is also another distinct neural route of trigeminal nerve sensory axons that project to the ABVN zone in the auricula that is independent of the ABVN route. The auriculotemporal nerve of the mandibular division of the trigeminal nerve contributes to the anterosuperior aspect of the auricula and supplies the skin over the tragus and helical crus regions of the auricula. This area also comprises the non-invasive auricular stimulation zone in numerous studies (Badran et al., 2018a,b; Niamtu, 2018). Further, the auriculotemporal nerve was reportedly found in 80% of the crus helicis. Interestingly, a gross auricular surface dissection study of 14 cadavers observed that 20% of the ABVN branches contribute to this zone (Peuker and Filler, 2002). Stimulation of these zones can also stimulate the trigeminal nuclei and the NTS *via* the auriculotemporal nerve as a distinct pathway independent from the ABVN (**Figure 1**).

It is also worth noting the limitations of Peuker and Filler (2002) in the context of overlooked neural networks because the study has been used as an auricular vagal nerve territory guide in numerous AVNS studies. In this gross dissection of 14 cadavers, the authors did not consider the ABVN division that contributes to the posterior auricular branch of the facial nerve. Moreover, no immunohistochemical mapping of the distal nerve territories was performed, but dissection-based recognition of the nerve bundles was considered for the proposed territories (Peuker and Filler, 2002). In addition, their investigation did not consider the perivascular cholinergic innervation of the human ear (Cakmak et al., 2018). In summary, the results concerning the vagus nerve territory are relatively incomplete and should not therefore be used as an absolute proxy for AVNS study design or interpretations.

Problems With Taking NTS Activation as Proof of ABVN Stimulation

Labeling or activation in the viscerosensory nucleus of the NTS was advanced as a proof-of-concept for previous ABVN stimulation studies (Frangos et al., 2015). However, there are numerous issues and controversies regarding this monolog perspective. Numerous studies have clearly documented that the NTS can be directly and indirectly activated by multiple neural areas, most of which are present in the ABVN zone; this indicates that it is part of a brainstem reflex arc independent of the vagus nerve. In this section, we will focus on non-vagal pathways that can stimulate the NTS *via* AVNS zones.

Trigeminal nerve stimulation is one of the alternative pathways (DeGiorgio et al., 2003; Jean, 2008; Schrader et al., 2011) that can stimulate the NTS *via* its sensory nuclei. Stimulation of the ABVN zone, which overlaps with the trigeminal nerve, can also excite the trigeminal nerve (sensory axons within the ABVN or independent pathways). This may result in the activation of the trigeminal sensory nuclei of the brainstem, and then, as a reflex arc, the NTS can be stimulated (DeGiorgio et al., 2003; Jean, 2008; Schrader et al., 2011; **Figure 1**).

The facial nerve innervates the submandibular and sublingual glands with its parasympathetic component, which is carried by the chorda tympani branch of the facial nerve (Segal et al., 1996; **Figure 1**). The chorda tympani not only carries the parasympathetic axons of the facial nerve but also special sensory fibers with taste information from the anterior third aspect of the tongue. This special taste sensation relays to the rostral pole of the NTS, which also includes viscerotopography that represents visceral organs at its middle and lower poles

(Altschuler et al., 1989, 1991; Broussard and Altschuler, 2000; Lemon and Di Lorenzo, 2002; Reddaway et al., 2012). While the axonal connections between the chorda tympani and the ABVN have also been demonstrated (Gasser, 1957; Schuknecht, 1974; Lang, 1983; May, 2000; Nageris et al., 2000; Diamond et al., 2011; Watanabe et al., 2016; Mulazimoglu et al., 2017), the origin and end terminals of these axons in the brainstem nuclei have yet to be investigated. As the facial nerve axons contribute to the ABVN in the facial canal, a portion of the ABVN axons might relay to the NTS *via* the facial nerve or axons of the facial nerve that hijack the ABVN in the temporal bone. In this context, the elucidation of the viscerotopical representation of the activated zones in the NTS is warranted to clarify the neural route to NTS and identify the axons that play a role in NTS stimulation in AVNS studies.

As emphasized previously, *the facial and vestibular nerves exchange axons* in the facial canal, and considering the axon exchange between the ABVN and the facial nerve, the exchanged axons between the facial and vestibular nerves might originate in the ABVN. Interestingly, it has been shown that the vestibular nerve can also stimulate the NTS (Yates et al., 1994; **Figure 1**).

Finally, several independent studies have shown that the stimulation of the peripheral spinal nerves in the hindlimb and forelimb modulates NTS activity (Noguchi and Hayashi, 1996; Tada et al., 2003; Imai et al., 2008; Wang et al., 2015). The underlying neural networks for this activation have yet to be elucidated, but the two spinal nerves (C2–C3) in the ABVN zone that innervate auricular skin feature well-known neural networks that can interact with the NTS (**Figure 1**). This neural network will be discussed together with sympathetic pathways in the next section.

In conclusion, NTS activation itself cannot be used as absolute proof of ABVN stimulation, and studies considering the clinical or physiological outputs of auricular stimulation should consider the trigeminal, facial, and other spinal nerves (including C2/C3 and their sympathetic nerves) in this zone to ascertain their sole or combined mechanisms of action in the auricular stimulation zone known as the ABVN.

As a final consideration in this discussion regarding the facial nerve and ABVN axonal exchanges, the motor nuclei of the cranial nerves have also been shown to contain cholinergic neurons, and the facial nerve motor nucleus has been reported to be one of the densest concentrations of cholinergic neurons of all the cranial nerve motor nuclei (Li et al., 2018).

Locus Coeruleus (LC) Activation and Sympathetic Nerves in the Auricula

In addition to the aforementioned neural pathways that may be implicated in the mechanism of action of AVNS, the sympathetic nerves in the ABVN zone can also stimulate the NTS. Activity in the bilateral locus coeruleus (LC) observed with c-fos labeling or fMRI is also attributed to the stimulation of the ABVN and to NTS projections to the LC (Frangos et al., 2015). However, the LC also projects to the NTS, indicating an alternative or opposing mechanism of action. A major input to the LC originates from the nucleus paragigantocellularis (nPGi) in the reticular formation (Ennis and Aston-Jones, 1988; Kessel et al., 2008; **Figure 1**), which receives direct inputs from sympathetic neurons (Ennis and Aston-Jones, 1988; Berntson et al., 1998; Aston-Jones et al., 1996). This pathway is involved in transmitting sympathetic control and status information between the nPGi and the LC (Garcia-Rill, 1991; Limousin et al., 1997; **Figure 1**).

The sympathetic nerves distribute to the AVNS zone of the ABVN from at least three different neural routes: (1) the perivascular sympathetic nerves situated in the human ear (Cakmak et al., 2018); (2) the C2–3 spinal nerves that receive sympathetic axons from the superior cervical ganglion (Netter, 1999; von Lanz and Wachsmuth, 2003; Lingford-Hughes and Kalk, 2012); and (3) sympathetic axons that hijack the ABVN in the jugular foramen (Matthews and Robinson, 2014). All these sympathetic pathways can be stimulated within the zones of ABVN stimulation and may potentially stimulate the NTS *via* the LC. In the context of the described neural networks, the ABVN-NTS-LC axis cannot be postulated as the only explanation for the bilateral LC c-fos labeling after the stimulation of the cavum concha *via* AVNS. The cervical sympathetic ganglia should always be considered in the design and interpretation of AVNS studies as a potential labeling and/or a potential contributor of LC and NTS activation.

Therefore, the possible role of auricular sympathetic nerves and, consequently, their possible effects on AVNS study results cannot be excluded without the denervation of sympathetic nerves. It is worthwhile to note that the study by Shu et al. (2005) demonstrated that the antiepileptic effects of the auricular electrostimulation disappeared if the great auricular nerve (C2–C3) of a rat model of seizure was severed before electrical stimulation of the auricula.

A contrary argument is that activations of neither the LC or the NTS have been supported by control/sham region stimulations in AVNS studies (including human), and, hence, only stimulations in the active ABVN zone that includes the cymba concha may be postulated. This argument is addressed in the following section through discussion of the inhomogeneous distribution of the sympathetic nerves in the auricula.

AURICULAR ANATOMY: INHOMOGENEITY, UNIQUE CORTICAL REPRESENTATION, AND INTERSPECIES DIFFERENCES

Significant Confounding Effects of Inhomogeneity Problems

To date, the homogeneous distribution of sympathetic axons on the auricular skin has not been comprehensively investigated. Only a recent study on human ears has considered this topic. In a detailed histological and immunohistochemical labeling study, we demonstrated that perivascular sympathetic neurotransmitters are denser in the upper auricular zones adjacent to the cymba concha (Cakmak et al., 2018). This dense sympathetic labeling was not observed in the lower sections of the human auricula (Cakmak et al., 2018), and such an inhomogeneity should be considered in the design and interpretation of AVNS studies because most control/sham electrodes are placed in the lower aspects of the auricula. Therefore, if the control/sham stimulation is applied to the

lower aspects of the auricula distant from the cymba concha, the potential confounding or otherwise significant role of auricular sympathetic nerves on AVNS study results cannot be excluded.

This problem may be overcome by preferring the upper levels of the auricula when applying electrodes in the sham/control groups and keeping the electrodes in the same auricular density zone of the sympathetic fibers, as in the case of vagal stimulation electrodes.

In addition to the inhomogeneous distribution of the sympathetic nerves in the human ear (Cakmak et al., 2018), perforating auricular arteries are also worth considering when placing electrodes in sham/control groups. Two independent group studies demonstrated that the posterior auricular artery—i.e., the periarterial sympathetic and cholinergic nerves—perforates the auricular cartilage in up to five locations (**Figure 2C**) to emerge from the posterior auricular surface at the anterior auricular surface, where they anastomose with branches of the superior temporal artery (Park and Roh, 2002; Zilinsky et al., 2017); four of the five perforated zones are reported in both of these studies (the perforating artery location in the triangular fossa was documented in only of the studies; Park and Roh, 2002; Zilinsky et al., 2017).

The auricular skin zones, which house these perforating arteries, likely contain a higher density of perivascular sympathetic axon bundles. The perforating arteries also distribute to the auricular skin areas that include the AVNS zones (**Figure 2C**), including the zone in the cymba concha where most AVNS studies place their active electrodes. It has also been reported that the perforator at the root of the helix consistently supplies the cymba and cavum of the concha as well as the posterior wall of the external auditory meatus, which fits perfectly with the AVNS zone (Park and Roh, 2002; Zilinsky et al., 2017). Hence, arteries—as well as perivascular sympathetic and cholinergic innervation—should be considered when determining the sham/placebo zones in the human ear. The concha zone, which corresponds with the perforating auricular artery (**Figure 2C**), as been demonstrated in two independent anatomical studies (Park and Roh, 2002; Zilinsky et al., 2017), is also postulated as the most effective zone in the context of fMRI activations. The confounding factor of the perforating artery in this zone of the ear is also underestimated and is not mentioned in the study report (Yakunina et al., 2017). In addition, all of the control stimulation zones in the same fMRI study were located lower to the concha stimulation zone, which cannot exclude the fact that a higher density of perivascular autonomic innervation exists in the upper sections of the human auricula (Cakmak et al., 2018; **Figure 2D**). To the best of our knowledge, our recent auricular stimulation study in patients with Parkinson's disease is the only human study to uses a sham zone located in the high-density perivascular sympathetic auricular skin area, outside the perforation zone of the arteries and a zone without auricular muscles (Cakmak et al., 2017).

In addition to the documented inhomogeneous distribution of sympathetic fibers in the human ear, the territories of the great auricular nerve, which for the most part originates in C3 with contributions from C2 (Becelli et al., 2014), and of the lesser occipital nerve, which mainly originates in C2 with contributions from C3 (Waxenbaum and Bordoni, 2019), reportedly distributes differentially across the cymba concha area (the AVNS zone) among humans. It has further been demonstrated that the

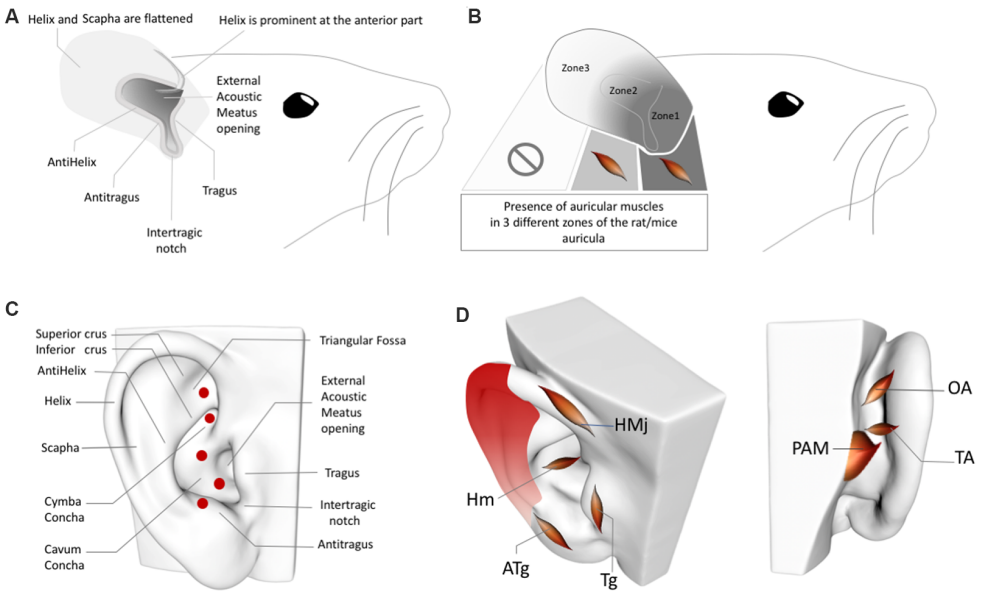


FIGURE 2 | (A) Rat/Mouse Auricular Anatomy. **(B)** Rat/Mouse Auricular Muscle Zones. **(C)** Perforator artery zones of the human ear. **(D)** Inhomogeneous distribution of the perivascular sympathetic nerves on the human ear. Red gradient represents the density of perivascular innervation: Dark red: High density, Light red: Low density. OAM, Oblique auricular muscle; TAM, Transverse Auricular Muscle; PAM, Posterior Auricular Muscle; HMj, Helicis major muscle; Tg, Tragicus muscle; Hm, Helicis minor muscle; ATg, Anti-tragicus muscle. Adapted from Kiernan and Mitchell (1974), Chiu et al. (1979), Park and Roh (2002), Liugan et al. (2018), Cakmak et al. (2018) and Hong et al. (2019).

cymba concha is innervated by the lesser occipital nerve in a quarter (26%) of participants, which indicates that the same proportion of the experimental groups of AVNS studies may exhibit inhomogeneity (Pantaloni and Sullivan, 2000).

In summary, the active/sham/control AVNS zones should be carefully chosen according to documented sympathetic inhomogeneity, which is denser in upper zones, and perforating arteries in the human ear to overcome the potential influence of sympathetic nerves on study findings. In addition, within-study designs should be preferred to overcome documented anatomical variations of C2/C3 in the cymba concha area, which can influence a quarter of the results.

The Unique Cortical Representation of the Auricula (External Ear)

In the final section, I would like to underscore the unique representation of the human ear in the cortex, which should be considered when interpreting cortical activations induced by auricular stimulation.

The representation of the human skin in the somatosensorial cortex is organized such that each region corresponds to one specific part of the human body (e.g., one zone of the somatosensorial cortex represents the hand only, while another zone represents the arm, and so on). In contrast, imaging studies have revealed that the cortical representation of the auricula is not as aligned as for the other parts of the human body. The auricula is represented by numerous different cortical zones that are distributed over the face, head, and neck representation areas (Nihashi et al., 2001) in the somatosensory cortex. Each of these areas belonging to auricular representation is segregated from the others, which means that they are not continuous across the somatosensorial cortex. It has been demonstrated that peripheral nerve stimulation can normalize the distortion of somatotopical representation in the cortex induced by pain conditions, including carpal tunnel and osteoarthritis, when it is applied through the nerves around the pathological condition zone (e.g., the forearm and wrist in carpal tunnel; Chen et al., 2003; Dhond et al., 2008). In this context, any study that performs AVNS for head and neck pain should also consider the unique cortical representation of the ear and other contributions of the sensory pathways that indicated in previous sections to explain the mechanism of action as well as the potential plasticity (normalization) of the somatosensorial representation of the head and neck *via* somatosensory stimulation.

Interspecies Differences of the Auricula: Rat vs. Human

Furthermore, rat and human AVNS studies cannot be comparable in the context of the auricular muscle anatomy. While the rat auricula exhibits skin, cartilage, and auricular muscles, the muscle tissue is present only in the proximal segments, not in the distal third (**Figures 2A,B**). In the proximal two-thirds of the pinna, the dorsal auricular surface overlies muscular tissue, and in the proximal one-third, the external auricular muscles contribute to the dorsal aspect of the auricula, while the intrinsic muscles distribute at both surfaces of the auricular cartilage. In addition, the intrinsic muscles of the rat ear do not correspond with the intrinsic auricular muscles in humans (**Figures 2B,D**; Kiernan and Mitchell, 1974). In this context, any AVNS study of humans or rats should consider interspecies differences in the locations of intrinsic and extrinsic auricular muscles before choosing sham and control zones of the AVNS as well as when interpreting the results. Moreover, the localization of the perforating branches and inhomogeneity of the sympathetic distribution in the rat ear also require clarification in future research.

Some researchers use heart rate variability (HRV) to obtain evidence and/or monitor the AVNS (Badran et al., 2018b). The HRV can be altered by the modulation of the NTS and LC (Ellis and Thayer, 2010), and these brainstem nuclei are easily stimulated by other non-vagal neural networks—as explained in detail in the previous sections and **Figure 1**—in the AVNS zone. In addition, HRV alterations with auricular stimulation cannot be postulated as the mechanism of action of improved functioning/symptoms without excluding the contribution of other neural pathways. The auricula has extensive neural contributions, and it is not unusual for AVNS studies to stimulate multiple neural networks that can all function as key players in the mechanism of action.

In conclusion, AVNS studies (or better to refer to it as auricular electrical stimulation) are a promising avenue by which to explore numerous medical conditions; however, the lack of anatomical knowledge and inadequate study design in the context of a monolog anatomical concept impose clear limitations that may result in the misinterpretation of the mechanism of action. The present article helps to elucidate obscure neuroanatomical aspects of the ABVN and provide a deeper understanding of the auricular anatomy and the multiple neural networks that may influence its mechanism of action, thus helping to inform improved design of AVNS studies and the interpretations of their findings by considering all aspects of the auricular anatomy.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and has approved it for publication.

ACKNOWLEDGMENTS

We would like to thank 3dscanstore/Ten24 Media Limited for the 3D scans of the external ear in **Figure 2**.

REFERENCES

Altschuler, S. M., Bao, X., Bieger, D., Hopkins, D. A., and Miselis, R. R. (1989). Viscerotopic representation of the upper alimentary tract in the rat: sensory ganglia and nuclei of the solitary and spinal trigeminal tracts. *J. Comp. Neurol.* 283, 248–268. doi: 10.1002/cne.902830207

Altschuler, S. M., Ferenci, D. A., Lynn, R. B., and Miselis, R. R. (1991). Representation of the cecum in the lateral dorsal motor nucleus of the vagus

nerve and commissural subnucleus of the nucleus tractus solitarii in rat. *J. Comp. Neurol.* 304, 261–274. doi: 10.1002/cne.903040209

Aston-Jones, G., Rajkowski, J., Kubiak, P., Valentino, R. J., and Shipley, M. T. (1996). Role of the locus coeruleus in emotional activation. *Prog. Brain Res.* 107, 379–402. doi: 10.1016/s0079-6123(08)61877-4

Badran, B. W., Dowdle, L. T., Mithoefer, O. J., LaBate, N. T., Coatsworth, J., Brown, J. C., et al. (2018a). Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: a concurrent taVNS/fMRI study and review. *Brain Stimul.* 11, 492–500. doi: 10.1016/j.brs.2017.12.009

Badran, B. W., Jenkins, D. D., DeVries, W. H., Dancy, M., Summers, P. M., Mappin, G. M., et al. (2018b). Transcutaneous auricular vagus nerve stimulation (taVNS) for improving oromotor function in newborns. *Brain Stimul.* 11, 1198–1200. doi: 10.1016/j.brs.2018.06.009

Becelli, R., Morello, R., Renzi, G., and Matarazzo, G. (2014). Great auricular nerve preservation during parotidectomy for benign tumors. *J. Craniofac. Surg.* 25, 422–424. doi: 10.1097/SCS.0b013e3182a28c50

Berntson, G. G., Sarter, M., and Cacioppo, J. T. (1998). Anxiety and cardiovascular reactivity: the basal forebrain cholinergic link. *Behav. Brain Res.* 94, 225–248. doi: 10.1016/s0166-4328(98)00041-2

Broussard, D. L., and Altschuler, S. M. (2000). Brainstem viscerotopic organization of afferents and efferents involved in the control of swallowing *Am. J. Med.* 108, 79–86. doi: 10.1016/S0002-9343(99)00343-5

Burger, A. M., Van Diest, I., Van der Does, W., Korbee, J. N., Waziri, N., Brosschot, J. F., et al. (2019). The effect of transcutaneous vagus nerve stimulation on fear generalization and subsequent fear extinction. *Neurobiol. Learn. Mem.* 161, 192–201. doi: 10.1016/j.nlm.2019.04.006

Cakmak, Y. O. (2006). Epilepsy, electroacupuncture and the nucleus of the solitary tract. *Acupunct. Med.* 24, 164–168. doi: 10.1136/aim.24.4.164

Cakmak, Y. O., Apaydin, H., Kiziltan, G., Gündüz, A., Ozsoy, B., Olcer, S., et al. (2017). Rapid alleviation of Parkinson's disease symptoms via electrostimulation of intrinsic auricular muscle zones. *Front. Hum. Neurosci.* 11:338. doi: 10.3389/fnhum.2017.00338

Cakmak, Y. O., Cotofana, S., Jäger, C., Morawski, M., Sora, M. C., Werner, M., et al. (2018). Peri-arterial autonomic innervation of the human ear. *Sci. Rep.* 8:11469. doi: 10.1038/s41598-018-29839-z

Chen, J. D. Z., Qian, L., Ouyang, H., and Yin, J. (2003). Gastric electrical stimulation with short pulses reduces vomiting but not dysrhythmias in dogs. *Gastroenterology* 124, 401–409. doi: 10.1053/gast.2003.50048

Chiu, D. T., Crikelair, G. F., and Moss, M. L. (1979). Epigenetic regulation of the shape and position of the auricle in the rat. *Plast. Reconstr. Surg.* 63, 411–417. doi: 10.1097/00006534-197903000-00023

DeGiorgio, C. M., Shewmon, D. A., and Whitehurst, T. (2003). Trigeminal nerve stimulation for epilepsy. *Neurology* 61, 421–422. doi: 10.1212/01.wnl.0000073982.42650.57

Dhond, R. P., Yeh, C., Park, K., Kettner, N., and Napadow, V. (2008). Acupuncture modulates resting state connectivity in default and sensorimotor brain networks. *Pain* 136, 407–418. doi: 10.1016/j.pain.2008.01.011

Diamond, M., Wartmann, C. T., Tubbs, R. S., Shoja, M. M., Cohen-Gadol, A. A., and Loukas, M. (2011). Peripheral facial nerve communications and their clinical implications. *Clin. Anat.* 24, 10–18. doi: 10.1002/ca.21072

Ellis, R. J., and Thayer, J. F. (2010). Music and autonomic nervous system (Dys)function. *Music Percept.* 27, 317–326. doi: 10.1525/mp.2010.27.4.317

Ennis, M., and Aston-Jones, G. (1988). Activation of locus coeruleus from nucleus paragigantocellularis: a new excitatory amino acid pathway in brain. *J. Neurosci.* 8, 3644–3657. doi: 10.1523/jneurosci.08-10-03644.1988

Farmer, D. G. S., Dutschmann, M., Paton, J. F. R., Pickering, A. E., and McAllen, R. M. (2016). Brainstem sources of cardiac vagal tone and respiratory sinus arrhythmia. *J. Physiol.* 594, 7249–7265. doi: 10.1113/JP273164

Ferretti, A., Babiloni, C., Arienzo, D., Del Gratta, C., Rossini, P. M., Tartaro, A., et al. (2007). Cortical brain responses during passive nonpainful median nerve stimulation at low frequencies (0.5–4 Hz): an fMRI study. *Hum. Brain Mapp.* 28, 645–653. doi: 10.1002/hbm.20292

Frangos, E., Ellrich, J., and Komisaruk, B. R. (2015). Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: FMRI evidence in humans. *Brain Stimul.* 8, 624–636. doi: 10.1016/j.brs.2014.11.018

Garcia-Rill, E. (1991). The pedunculopontine nucleus. *Prog. Neurobiol.* 36, 363–389. doi: 10.1016/0301-0082(91)90016-t

Gasser, R. F. (1957). The development of the facial nerve in man. *Ann. Otol. Rhinol. Laryngol.* 76, 37–56. doi: 10.1177/000348946707600103

Geis, G. S., and Wurster, R. D. (1980). Cardiac response during stimulation of the dorsal motor nucleus and nucleus ambiguus in the cat. *Circ. Res.* 46, 606–611. doi: 10.1161/01.res.46.5.606

Heimer, L. (1995). *The Human Brain and Spinal Cord. Functional Neuroanatomy and Dissection Guide.* Verlag New York: Springer.

Hong, G. S., Zillekens, A., Schneiker, B., Pantelis, D., de Jonge, W. J., Schaefer, N., et al. (2019). Non-invasive transcutaneous auricular vagus nerve stimulation prevents postoperative ileus and endotoxemia in mice. *Neurogastroenterol. Motil.* 31:e13501. doi: 10.1111/nmo.13501

Imai, K., Ariga, H., Chen, C., Mantyh, C., Pappas, T. N., and Takahashi, T. (2008). Effects of electroacupuncture on gastric motility and heart rate variability in conscious rats. *Auton. Neurosci.* 138, 91–98. doi: 10.1016/j.autneu.2007.11.003

Jean, A. (2008). Le noyau du faisceau solitaire: aspects neuroanatomiques, neurochimiques et fonctionnels. *Arch. Int. Physiol. Biochim. Biophys.* 99, A3–A52. doi: 10.3109/13813459109145916

Kessel, F., Rosenfield, P., and Anderson, N. (2008). *Expanding the Boundaries of Health and Social Science: Case Studies in Interdisciplinary Innovation.* New York, NY: University Press.

Kiernan, J. A., and Mitchell, R. (1974). Some observations on the innervation of the pinna of the ear of the rat. *J. Anat.* 117, 397–402.

Lang, J. (Ed.) (1983). "Clinical anatomy of the head," in *Neurocranium, Orbit, Craniocervical Regions* (Berlin: Springer-Verlag), 186–203.

Lemon, C. H., and Di Lorenzo, P. M. (2002). Effects of electrical stimulation of the chorda tympani nerve on taste responses in the nucleus of the solitary tract. *J. Neurophysiol.* 88, 2477–2489. doi: 10.1152/jn.00094.2002

Li, X., Yu, B., Sun, Q., Zhang, Y., Ren, M., Zhang, X., et al. (2018). Generation of a whole-brain atlas for the cholinergic system and mesoscopic projectome analysis of basal forebrain cholinergic neurons. *Proc. Natl. Acad. Sci. U S A* 115, 415–420. doi: 10.1073/pnas.1703601115

Limousin, P., Greene, J., Pollak, P., Rothwell, J., Benabid, A. L., and Frackowiak, R. (1997). Changes in cerebral activity pattern due to subthalamic nucleus or internal pallidum stimulation in Parkinson's disease. *Ann. Neurol.* 42, 283–291. doi: 10.1002/ana.410420303

Lingford-Hughes, A., and Kalk, N. (2012). *Core Psychiatry*. 3rd Edn. Toronto: Elsevier.

Liugan, M., Zhang, M., and Cakmak, Y. O. (2018). Neuroprosthetics for auricular muscles: neural networks and clinical aspects. *Front. Neurol.* 8:752. doi: 10.3389/fneur.2017.00752

Maharjan, A., Peng, M., and Cakmak, Y. O. (2019). Non-invasive high frequency median nerve stimulation effectively suppresses olfactory intensity perception in healthy males. *Front. Hum. Neurosci.* 12:533. doi: 10.3389/fnhum.2018.00533

Maslany, S., Crockett, D. P., and Egger, M. D. (1991). Somatotopic organization of the dorsal column nuclei in the rat: transganglionic labelling with B-HRP and WGA-HRP. *Brain Res.* 564, 56–65. doi: 10.1016/0006-8993(91)91351-z

Matthews, B., and Robinson, P. P. (2014). The course of post-ganglionic sympathetic fibres distributed with the trigeminal nerve in the cat. *J. Physiol.* 303, 391–401. doi: 10.1113/jphysiol.1980.sp013294

May, M. (2000). *Anatomy for the Clinician.* 2nd Edn. New York, NY: Thieme Medical.

Mulazimoglu, S., Flury, R., Kapila, S., and Linder, T. (2017). Effects of a sensory branch to the posterior external ear canal: coughing, pain, Ramsay Hunt's syndrome and Hitselberger's sign. *J. Laryngol. Otol.* 131, 329–333. doi: 10.1017/s0022215117000160

Nageris, B., Braverman, I., Kalmanowitz, M., Segal, K., and Frenkiel, S. (2000). Connections of the facial and vestibular nerves: an anatomic study. *J. Otolaryngol.* 29, 159–161.

Netter, F. H. (1999). *The Netter Collection of Medical Illustrations: Nervous System—Anatomy and Physiology.* New York, NY: Elsevier.

Niamtu, J. (2018). "Cosmetic otoplasty and related ear surgery," in *Cosmetic Facial Surgery* (2nd Edn.) ed. J. Niamtu (China: Elsevier Inc.), 473–484.

Nihashi, T., Kakigi, R., Kawakami, O., Hoshiyama, M., Itomi, K., Nakanishi, H., et al. (2001). Representation of the ear in human primary somatosensory cortex. *Neuroimage* 13, 295–304. doi: 10.1006/nimg.2000.0695

Noguchi, E., and Hayashi, H. (1996). Increases in gastric acidity in response to EA stimulation of hindlimb of anesthetized rats. *Jpn. J. Physiol.* 46, 53–58. doi: 10.2170/jjphysiol.46.53

Nomura, S., and Mizuno, N. (1984). Central distribution of primary afferent fibers in the Arnold's nerve (the auricular branch of the vagus nerve): a transganglionic HRP study in the cat. *Brain Res.* 292, 199–205. doi: 10.1016/0006-8993(84)90756-x

Özdoğmuş, Ö., Sezen, O., Kubilay, U., Saka, E., Duman, U., Şan, T., et al. (2004). Connections between the facial, vestibular and cochlear nerve bundles within the internal auditory canal. *J. Anat.* 205, 65–75. doi: 10.1111/j.0021-8782.2004.00313.x

Ozveren, M. F., Ture, U., Ozek, M. M., and Pamir, M. N. (2003). Anatomic landmarks of the glossopharyngeal nerve: a microsurgical anatomic study. *Neurosurgery* 52, 1400–1410. doi: 10.1227/01.neu.0000064807.62571.02

Pantaloni, M., and Sullivan, P. (2000). Relevance of the lesser occipital nerve in facial rejuvenation surgery. *Plast. Reconstr. Surg.* 105, 2594–2599. doi: 10.1097/00006534-200006000-00051

Park, C., and Roh, T. S. (2002). Anatomy and embryology of the external ear and their clinical correlation. *Clin. Plast. Surg.* 29, 155–174. doi: 10.1016/s0094-1298(01)00008-6

Peuker, E. T., and Filler, T. J. (2002). The nerve supply of the human auricle. *Clin. Anat.* 15, 35–37. doi: 10.1002/ca.1089

Reddaway, R. B., Davidow, A. W., Deal, S. L., and Hill, D. L. (2012). Impact of chorda tympani nerve injury on cell survival, axon maintenance, and morphology of the chorda tympani nerve terminal field in the nucleus of the solitary tract. *J. Comp. Neurol.* 2413, 2395–2413. doi: 10.1002/cne.23044

Rhoton, A. L. Jr., O'Leary, J. L., and Ferguson, J. P. (1966). The trigeminal, facial, vagal, and glossopharyngeal nerves in the monkey: afferent connections. *Arch. Neurol.* 14, 530–540. doi: 10.1001/archneur.1966.00470110074010

Schrader, L. M., Cook, I. A., Miller, P. R., Maremont, E. R., and DeGiorgio, C. M. (2011). Trigeminal nerve stimulation in major depressive disorder: first proof of concept in an open pilot trial. *Epilepsy Behav.* 22, 475–478. doi: 10.1016/j.yebeh.2011.06.026

Schuknecht, H. F. (1974). *Pathology of the Ear.* Cambridge, MA: Harvard University.

Segal, K., Lisnyansky, I., Nageris, B., and Feinmesser, R. (1996). Parasympathetic innervation of the salivary glands. *Oper. Tech. Otolaryngol. Head Neck Surg.* 7, 333–338. doi: 10.1016/s1043-1810(96)80005-4

Shu, J., Lui, R. Y., and Huang, X. F. (2005). Efficacy of ear-point stimulation on experimentally induced seizure. *Acupunct. Electrother. Res.* 30, 43–52. doi: 10.3727/036012905815901334

Tada, H., Fujita, M., Harris, M., Tatewaki, M., Nakagawa, K., Yamamura, T., et al. (2003). Neural mechanism of acupuncture-induced gastric relaxations in rats. *Dig. Dis. Sci.* 48, 59–68. doi: 10.1023/a:1021730314068

Tekdemir, I., Aslan, A., and Elhan, A. (1998). A clinico-anatomic study of the auricular branch of the vagus nerve and Arnold's ear-cough reflex. *Surg. Radiol. Anat.* 20, 253–257. doi: 10.1007/s00276-998-0253-5

Tubbs, R. S., Salter, E. G., and Oakes, W. J. (2005). Anatomic landmarks for nerves of the neck: a vade mecum for neurosurgeons. *Neurosurgery* 56, 256–260. doi: 10.1227/01.neu.0000156541.78020.da

Urasaki, E., Wada, S., Yasukouchi, H., and Yokota, A. (1998). Effect of transcutaneous electrical nerve stimulation (TENS) on central nervous system amplification of somatosensory input. *J. Neurol.* 245, 143–148. doi: 10.1007/s004150050194

von Lanz, T., and Wachsmuth, W. (2003). *Lanz/Wachsmuth Praktische Anatomie. Hals: Ein Lehr- und Hilfsbuch der Anatomischen Grundlagen ärztlichen Handelns.* Berlin: Springer.

Wang, J., Irnaten, M., Neff, R. A., Venkatesan, P., Evans, C., Loewy, A. D., et al. (2001). Synaptic and neurotransmitter activation of cardiac vagal neurons in the nucleus ambiguus. *Ann. N Y Acad. Sci.* 940, 237–246. doi: 10.1111/j.1749-6632.2001.tb03680.x

Wang, S., Ren, L., Jia, L., Gu, Z., Fan, X., Chen, W., et al. (2015). Effect of acupuncture at Neiguan (PC 6) on cardiac function using echocardiography in myocardial ischemia rats induced by isoproter-enol. *J. Tradit. Chin. Med.* 35, 653–658. doi: 10.1016/s0254-6272(15)30155-2

Wardman, D. L., Gandevia, S. C., and Colebatch, J. G. (2014). Cerebral, subcortical, and cerebellar activation evoked by selective stimulation of muscle and cutaneous afferents: an fMRI study. *Physiol. Rep.* 2, 1–16. doi: 10.1002/phy2.270

Watanabe, K., Tubbs, R. S., Satoh, S., Zomorodi, A. R., Liedtke, W., Labidi, M., et al. (2016). Isolated deep ear canal pain: possible role of auricular branch of vagus nerve-case illustrations with cadaveric correlation. *World Neurosurg.* 96, 293–301. doi: 10.1016/j.wneu.2016.08.102

Waxenbaum, J. A., and Bordoni, B. (2019). *Anatomy, Head and Neck, Cervical Nerves.* Treasure Island, FL: StatPearls Publishing.

Yakunina, N., Kim, S. S., and Nam, E. C. (2017). Optimization of transcutaneous vagus nerve stimulation using functional MRI. *Neuromodulation* 20, 290–300. doi: 10.1111/ner.12541

Yates, B. J., Grklot, L., Kerman, I. A., Balaban, C. D., and Jakug, J. A. D. M. (1994). Organization of Vestibular inputs to nucleus tractus solitarius and adjacent structures in cat brain stem. *Am. J. Physiol.* 267, R974–R983. doi: 10.1152/ajpregu.1994.267.4.r974

Zhao, B., Liang, L., Jiao, Y., Man, L., Xu, K., Hong, Y., et al. (2019). Transcutaneous auricular vagus nerve stimulation in treating post-stroke insomnia monitored by resting-state fMRI: the first case report. *Brain Stimul.* 12, 824–826. doi: 10.1016/j.brs.2019.02.016

Zilinsky, I., Erdmann, D., Weissman, O., Hammer, N., Sora, M., Schenck, T. L., et al. (2017). Reevaluation of the arterial blood supply of the auricle. *J. Anat.* 230, 315–324. doi: 10.1111/joa.12550

Conflict of Interest: The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Human Neuroscience

ORIGINAL RESEARCH
published: 18 March 2020
doi: 10.3389/fnhum.2020.00077

Transcutaneous Auricular Vagus Nerve Stimulation-Paired Rehabilitation for Oromotor Feeding Problems in Newborns: An Open-Label Pilot Study

Bashar W. Badran[1†], Dorothea D. Jenkins[2†], Daniel Cook[1], Sean Thompson[1], Morgan Dancy[1], William H. DeVries[1], Georgia Mappin[1], Philipp Summers[1], Marom Bikson[3] and Mark S. George[1,4]*

[1]Department of Psychiatry, Medical University of South Carolina, Charleston, SC, United States, [2]Department of Pediatrics, Medical University of South Carolina, Charleston, SC, United States, [3]Department of Biomedical Engineering, City College of New York, New York, NY, United States, [4]Ralph H. Johnson VA Medical Center, Charleston, SC, United States

OPEN ACCESS

Edited by:
Tino Zaehle, Otto von Guericke University Magdeburg, Germany

Reviewed by:
Julian Koenig, Heidelberg University, Germany
Roberta Sclocco, Athinoula A. Martinos Center for Biomedical Imaging and Harvard Medical School, United States

***Correspondence:**
Dorothea D. Jenkins
jenkd@musc.edu

[†]*These authors share first authorship*

Specialty section:
This article was submitted to Brain Imaging and Stimulation, a section of the journal Frontiers in Human Neuroscience

Received: *15 November 2019*
Accepted: *20 February 2020*
Published: *18 March 2020*

Citation:
Badran BW, Jenkins DD, Cook D, Thompson S, Dancy M, DeVries WH, Mappin G, Summers P, Bikson M and George MS (2020) Transcutaneous Auricular Vagus Nerve Stimulation-Paired Rehabilitation for Oromotor Feeding Problems in Newborns: An Open-Label Pilot Study. Front. Hum. Neurosci. 14:77. doi: 10.3389/fnhum.2020.00077

Neonates born premature or who suffer brain injury at birth often have oral feeding dysfunction and do not meet oral intake requirements needed for discharge. Low oral intake volumes result in extended stays in the hospital (>2 months) and can lead to surgical implant and explant of a gastrostomy tube (G-tube). Prior work suggests pairing vagus nerve stimulation (VNS) with motor activity accelerates functional improvements after stroke, and transcutaneous auricular VNS (taVNS) has emerged as promising noninvasive form of VNS. Pairing taVNS with bottle-feeding rehabilitation may improve oromotor coordination and lead to improved oral intake volumes, ultimately avoiding the need for G-tube placement. We investigated whether taVNS paired with oromotor rehabilitation is tolerable and safe and facilitates motor learning in infants who have failed oral feeding. We enrolled 14 infants [11 premature and 3 hypoxic–ischemic encephalopathy (HIE)] who were slated for G-tube placement in a prospective, open-label study of taVNS-paired rehabilitation to increase feeding volumes. Once-daily taVNS was delivered to the left tragus during bottle feeding for 2 weeks, with optional extension. The primary outcome was attainment of oral feeding volumes and weight gain adequate for discharge without G-tube while also monitoring discomfort and heart rate (HR) as safety outcomes. We observed no adverse events related to stimulation, and stimulation-induced HR reductions were transient and safe and likely confirmed vagal engagement. Eight of 14 participants (57%) achieved adequate feeding volumes for discharge without G-tube (mean treatment length: 16 ± 6 days). We observed significant increases in feeding volume trajectories in responders compared with pre-stimulation ($p < 0.05$). taVNS-paired feeding rehabilitation appears safe and may improve oral feeding in infants with oromotor dyscoordination, increasing the rate of discharge without G-tube, warranting larger controlled trials.

Keywords: transcutaneous auricular vagus nerve stimulation, transcutaneous vagus nerve stimulation, vagus nerve stimulation, vagus nerve stimulation, feeding, pediatric rehabilitation, hypoxic–ischemic encephalopathy

INTRODUCTION

In the motor task of feeding, neonates are required to coordinate a complex and rapid sequence of sucking, swallowing, and breathing, all integrated with a typical respiratory rate of 40 breaths per minute. This requires advanced sensorimotor integration of muscles of the face, head, and neck with the myelinated vagal regulation of breathing and heart rate (HR; Porges, 1992; Portales et al., 1997; Suess et al., 2000; Porges and Furman, 2011). Feeding difficulty is the primary reason for delayed hospital discharge in preterm infants with brain dysmaturation or near-term/term infants with hypoxic–ischemic encephalopathy (HIE) who are otherwise clinically stable and ready for discharge (Adamkin, 2006; Lau et al., 2015; Jackson et al., 2016). This increases hospital costs and is associated with a negative impact on long-term neurodevelopment, particularly with receptive and expressive language deficits (Adams-Chapman et al., 2013; Malas et al., 2015). The current standard of treatment for infant oromotor dysfunction consists of occupational or speech therapists feeding infants by mouth (PO) once a day to encourage safe feeding while learning this motor skill. However, many infants do not show improvement by term equivalent age, even after many weeks of rehabilitation with therapists, and have a gastrostomy tube (G-tube) placed for adequate nutrition.

Difficulty learning the motor sequence for oral feeding may be due to brain injury from infection, ischemia, and dysmaturity (Huang et al., 2015; Ismail et al., 2017). This diffuse injury results in less myelination and fewer brainstem–cortical connections (Duerden et al., 2015; Rocha-Ferreira and Hristova, 2016) and may lead to reduced corticobulbar regulation of both vagal activity and the striated muscles of the face, head, and neck (Suess et al., 2000). Atypical neural maturation with prematurity or brain injury also leads to overactive sympathetic inputs into the autonomic nervous system combined with lower parasympathetic vagal tone and persistent brainstem dysmaturity (Heilman et al., 2012; Rocha-Ferreira and Hristova, 2016). Such reactivity and neural dysmaturation make coordinating and learning a complex motor task even more difficult, explaining why the feeding mechanism must be taught through feeding rehabilitation, when it should be a normal reflex.

With improved survival rates of more critically ill neonates, the national rate of G-tube placement has doubled from 2000 to 2012 (Hatch et al., 2018). Complications of G-tube placement and removal often lead to subsequent hospitalizations or procedures after discharge from the nursery (McSweeney et al., 2015; Khalil et al., 2017; Hatch et al., 2018). At the Medical University of South Carolina (MUSC), preterm infants who have not reached full PO feeds by 40-week gestational age (GA) and/or after 40 days of attempting PO feeds have a >90% chance of eventually needing G-tube implantation to achieve full enteral feeds (Ryan and Gehle, 2019). Any therapy that facilitates motor learning and enhances feeding skills would have a significant impact for infants who fail feeding rehabilitation.

Vagus nerve stimulation (VNS) paired with motor activity enhances neuroplasticity, facilitates cortical reorganization and neurogenesis, and improves motor function post stroke (Porter et al., 2012; Engineer et al., 2015; Dawson et al., 2016). Recently, a noninvasive form of VNS known as transcutaneous auricular VNS (taVNS) targeting the auricular branch of the vagus nerve (ABVN) has demonstrated activation of the vagal afferent and efferent networks (Kraus et al., 2013; Garcia et al., 2017; Yakunina et al., 2017; Badran et al., 2018a,c). In patients with limb impairment post stroke or brain injury, pairing taVNS with motor activation can enhance plasticity and improve functional motor recovery (Dawson et al., 2016; Pruitt et al., 2016; Redgrave et al., 2018). This human work extends the large animal literature that demonstrates pairing VNS with a behavioral intervention restores brain function (Hays et al., 2014a,b; Khodaparast et al., 2014, 2016). Therefore, both animal and adult human data support the likely efficacy of VNS-paired with motor rehabilitation.

We applied this model of taVNS paired with a motor behavior to neonates who have failed to learn the oromotor skill of feeding. We conducted a prospective, open-label trial exploring the use of once-daily taVNS-paired rehabilitation training to enhance oral feeding behavior in neonates with oromotor dyscoordination. We hypothesized that taVNS paired with bottle feeding may function in a similar mechanism by enhancing cortical plasticity in neonates with oromotor deficits, resulting in improved acquisition of the sensorimotor skill of feeding. With a favorable safety profile in adults and the ability to treat noninvasively at the bedside, taVNS is an attractive therapeutic option for neuromodulation therapies in this vulnerable population.

MATERIALS AND METHODS

Study Overview

This study was conducted at the MUSC and was approved by the MUSC Institutional Review Board. After obtaining parental consent, we enrolled 14 participants who were consulted for G-tube placement in a prospective, open-label phase 0 trial to determine the feasibility, safety, and potential clinical benefit of a novel taVNS-paired oromotor rehabilitation paradigm in neonates with oromotor dyscoordination. We reported on five of the participants in this trial in an earlier brief communication (Badran et al., 2018b). Our primary clinical outcomes were improved PO feeding volumes and attaining full PO feeds adequate for discharge, thereby avoiding G-tube implantation (**Figure 1**).

Participants

We included infants who were born premature at ≤33 weeks' gestation at birth ($n = 11$) or suffered global HIE ($n = 3$) and who failed to make progress in PO volumes. Importantly, all enrolled participants were clinically determined to require a G-tube due to failure to achieve oral feeds sufficient for discharge from the hospital. Parents of all 14 infants had been approached about G-tube placement by the clinical teams prior to enrollment. Historically at MUSC, these infants would have <10% chance of avoiding a G-tube. We excluded infants who were clinically unstable, were unable to attempt every feed PO, were on

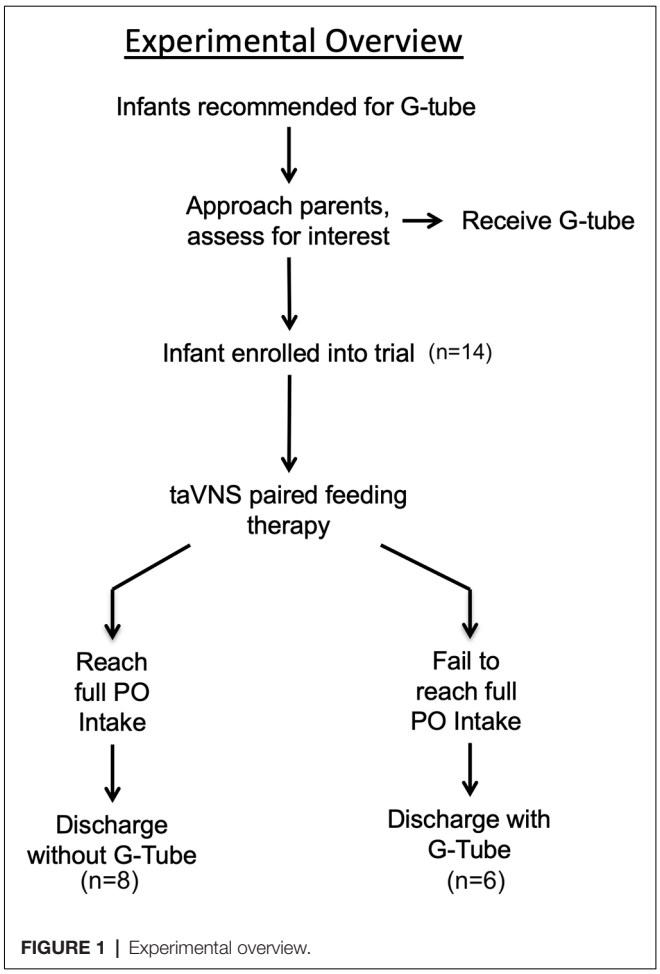


FIGURE 1 | Experimental overview.

significant respiratory support with frequent bradycardia or apnea events, or had cardiomyopathy.

Transcutaneous Auricular Vagus Nerve Stimulation-Paired Feeding Protocol

We delivered taVNS once a day during a bottle feed, timed with observed sucking and swallowing for 30 min or the duration of the feed. Stimulation was paired with nutritive sucking and swallowing and was paused during rest or burping. The treatment period was 2 weeks, with the possibility to continue for an additional 2 weeks if substantial progress was made. If PO feeds had not progressed after 2 weeks of taVNS treatment, the parents and the clinical team made decisions about timing of G-tube placement.

Transcutaneous Auricular Vagus Nerve Stimulation Setup and Technique Refinement

We delivered taVNS using a constant current electrical nerve stimulator (Digitimer DS7AH, Digitimer LTD) connected to custom-designed neonatal ear electrodes (**Figure 2**). Electrodes targeted the anterior wall of the ear canal (anode) and the tragus (cathode). Stimulation was triggered manually for participants 1–7 or *via* a novel closed-loop electromyography (EMG) triggering system for participants 8–14 (Cook et al., 2020 under review, Brain Stimulation). The closed-loop trigger system was developed to more accurately pair stimulation trains with coordinated suck–swallow oromotor activation, to increase ease of use and to decrease operator tasks. Real-time EMG recordings were used to trigger taVNS stimulation based on masseter activation during suck–swallow. EMG leads were placed on the masseter muscle (recording), frontal eminence (reference), and center of the forehead (common).

We also refined the EMG-triggered pulse train for optimal pairing of stimulation with the sensorimotor sequence required for efficient feeding. This includes the pre-motor stage of sensing the nipple in the mouth, expressing and sensing milk on the tongue, and subsequent activation of multiple pharyngeal and hyoid muscles that effect swallowing. Many of these muscles are innervated by branches of the vagus nerve. With a 3-s pulse train following the EMG trigger, sucks that occurred at the end of the taVNS train did not receive stimulation (n = 4 participants). By lengthening the pulse train to 10 s, we achieved better pairing of stimulation with suck bursts (n = 3 participants).

Transcutaneous Auricular Vagus Nerve Stimulation Dosing

Stimulation parameters were as follows: frequency −25 Hz, pulse width −500 μs, and current intensity −0.1 mA below perceptual threshold (PT). We determined PT by increasing the stimulation current in 0.1-mA increments while monitoring for indication that the infant perceived the stimulation, indicated by shrugging, change in facial expression, or fidgety movements. A neonatologist and a technician performed the stimulation. During treatment, infants were fed by occupational or speech therapists, staff, or parents. A custom MATLAB program recorded pulses and current intensity delivered during each session. We recorded PO volume intake during taVNS feed, total daily PO volume, and any adverse events.

Safety Monitoring and Target Engagement

The neonatal and infant pain scale (NIPS) scores (Lawrence et al., 1993; Witt et al., 2016) were recorded at initiation, midway, end, and 5 min after each treatment session. If NIPS scores increased greater than three points or the infant appeared to be sensing the stimulation, we decreased the current intensity by 0.1 mA. We monitored redness and skin irritation at electrode site and HR on bedside monitors for bradycardia, defined per nursery protocol as <80 bpm for 5 s. For target attainment, we recorded the lowest HR within the first 60 s of stimulation, the time to the lowest HR, and the rebound HR, to verify target engagement of vagus nerve using the parasympathetic response as an indicator (Badran et al., 2018c). We also recorded HR in 60-s epochs during taVNS-paired feeds and non-stimulation (control) feeds.

Primary Outcome Measures

The primary safety outcomes were bradycardia events and NIPS score increase of greater than or equal to 3 points due to taVNS stimulation. The primary clinical outcome of this study was a binary endpoint of full oral feeds or G-tube implantation. Responders were participants who were able to increase and maintain full daily PO intake for 4 days (>120 ml/kg/day) and weight gain adequate for discharge (>20 g/day). Infants who

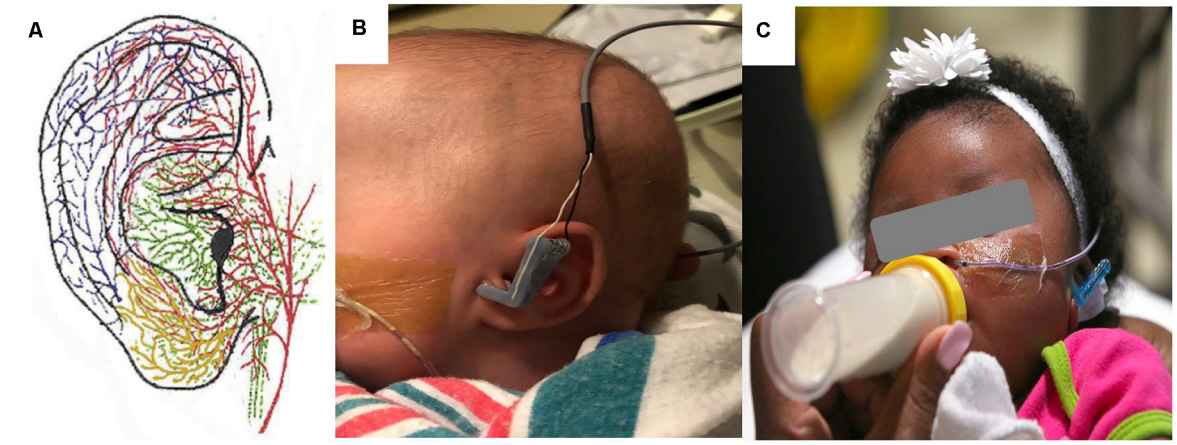


FIGURE 2 | (A) Auricular vagus nerve fibers (He et al., 2012). **(B)** Close-up photo of the left ear with attached custom, 3D-printed transcutaneous auricular vagus nerve stimulation (taVNS) electrodes attached. **(C)** Photo of the taVNS-paired feeding session with stimulation delivered concurrently with bottle feeding (written informed consent was obtained from the legal guardians for the publication of this image).

received G-tubes for inadequate intake after taVNS treatment were classified as non-responders. Other outcomes were rate of increase of daily oral feeding volumes and length of time to achieve full oral feeds.

Statistical Analyses

We analyzed group HR effects that compared HR measured before taVNS (or control) feed, the lowest HR at onset of taVNS (or control) feed, and during taVNS-paired feeding (or control) feeds using a one-way ANOVA. We then investigated the within-individual changes in HR using a paired *t*-test to compare each participant's baseline and the lowest HR prior to feed to the lowest HR during PT within taVNS or control feeds, and unpaired *t*-tests for HR differences across taVNS or control feeds. Behavioral feeding data were analyzed by comparing the slopes of the linear regression generated from the rate of daily PO volume in two different time periods: (1) the 30 days before taVNS; and (2) taVNS-paired feeding period. We compared both between- and within-group subjects in a 2 × 2 design (pre/post taVNS and responder/non-responder). Videofluoroscopic swallow study (VFSS) scores prior to taVNS treatment initiation were compared with treatment period according to response group *via* unpaired *t*-test.

RESULTS

Demographics

We enrolled 11 preterm and 3 near-term/term HIE infants. Clinical characteristics are noted in **Table 1**. Central nervous system (CNS) insults were prevalent (11/14) and consisted of intraventricular hemorrhage (IVH) or cerebellar hemorrhage, white matter infarction or periventricular leukomalacia (PVL), lenticulostriate vasculopathy (LSV), and acute moderate-to-severe HIE. A majority of infants (9/14) had sepsis complicating their neonatal course, which is associated with white matter neuroinflammation and infarction, and worse neurodevelopmental outcomes (Alshaikh et al., 2013; Bakhuizen et al., 2014; Bright et al., 2017; Dubner et al., 2019).

PO feeds were attempted for a mean (SD) of 49 ± 24.3 days before study enrollment in these 14 preterm and HIE infants. At study entry, most preterm infants were more than 44-week GA, well past term equivalent age, and had been trying to learn to feed for more than 40 days, at which point >90% of preterm infants at MUSC have attained full PO feeds (Ryan and Gehle, 2019). Prior to enrollment in this research trial, the clinical team had approached all parents about the need for a G-tube (**Figure 1**).

Thirteen out of 14 infants had clinical studies of videofluoroscopic barium swallow (VFSS, n = 11) or an impedance probe (n = 2) prior to enrollment. Six infants also had upper gastrointestinal (UGI) contrast studies. Eight infants had gastroesophageal reflux documented on one or more of these studies and were treated with histamine or proton pump antagonists. The VFSSs were performed and scored by three pediatric speech language pathologists using the Rosenbek scale (Rosenbek et al., 1996). Mean (SD) penetration and aspiration scores were 6 ± 3 with thin liquids (range 1–8). Six infants had maximum scores of 8, indicating aspiration below vocal folds with no attempt to eject liquid: three of these infants were trialed with thickened feeds prior to beginning the study; two infants continued to attempt with thin maternal breast milk, which could not be adequately thickened during the taVNS treatments; one infant showed dramatic improvement in oral feeding volumes to 100 ml/kg/day after 2 weeks of taVNS treatments but had persistent coughing during feeds and was transitioned to thickened feeds near the end of the treatment course.

TABLE 1 | Infant demographics.

taVNS-treated infants	Preterm (n = 11)	Term HIE (n = 3)
Sex M/F	5/6	0/3
Mean GA at birth (weeks)	28 ± 3	36 ± 0.5
Mean birth weight (g)	1,027 ± 453	2,600 ± 697
Mean GA at enrollment (weeks)	45 ± 5	40 ± 2
Mean days attempting PO before taVNS	57 ± 22	24 ± 10
Sepsis (including NEC, pneumonia, UTI, viral infections)	9	0
CNS abnormalities	8	3
IVH or other intracranial bleed (grade)	6 (grades 1 and 2)	1 (grade 3)
HIE (term HIE stage 2 n = 1), 3 (n = 1); preterm HIE stages not validated)	2	2
White matter infarction or PVL	2	1
Lenticulostriate vasculopathy	1	1
Infants of diabetic mothers	3	1
Hypoglycemia	4	1
Hyperglycemia	4	0
Gastroesophageal reflux requiring treatment	8	0
Aspiration on MBSS	5	1

Note. GA, gestational age; NEC, necrotizing enterocolitis; UTI, urinary tract infection; PVL, periventricular leukomalacia; taVNS, transcutaneous auricular vagus nerve stimulation; HIE, hypoxic–ischemic encephalopathy; GA, gestational age; MBSS, modified barium swallow study.

Safety

We monitored for bradycardia during both PT and during the stimulation-paired feed. There was only one bradycardia adverse event during a taVNS-paired feeding, likely unrelated to the stimulation as it was associated with choking and emesis, and readily rebounded with pausing the bottle feed.

There were no episodes of tragus irritation or redness at the electrode site. Discomfort with stimulation remained low, with the median NIPS scores [interquartile range (IQR)] of 0 (0, 1.0) before, during, and after the taVNS-paired feeding. Out of a total of 228 taVNS-paired feeding sessions, there were 10 sessions (4.3%) during which NIPS scores increased greater than or equal to 3 points from pre-stimulation to during taVNS-paired feeding. In seven instances, the fussiness resolved quickly, and in three instances (1.3%), stimulation current intensity was decreased for a persistent NIPS score increase. In 16 instances (7%), we decreased stimulation when we believed it was possible that the infant was feeling stimulation but did not demonstrate a change in NIPS score. Four feeds were stopped in one infant for excessive fussiness that did not resolve after stopping stimulation, related to reflux (pH probe was in place for one instance).

Heart Rate as a Putative Biomarker of Vagus Target Engagement

We performed a detailed analysis of HR changes in seven consecutive participants (#8–14), averaging 60-s HR data over the 5 min prior to PT and for the first 5 min of treatment. The mean HR before taVNS, compared with the onset of taVNS, revealed non-significant differences in physiology resulting from stimulation. The mean HR during the first 5 min before taVNS-paired feeding was 161 ± 11.7 bpm, compared with 159.6 ± 11.22 bpm during feeding (n = 39 feedings, seven participants, **Figure 3A**). For control feeds without stimulation, mean HR similarly does not change as a function of feeding. The mean HR was 156.7 ± 15.27 bpm for 5 min before the feed and 162.5 ± 15.98 bpm for the first 5 min during the feed, both non-significantly different from taVNS feeds.

The lowest HR was inspected as an indicator of safety profile. We compared the lowest HR before feed and the lowest HR during feeds. For the seven patients during taVNS feeds, the lowest HR before feed was 151.3 ± 15.1, and the lowest HR during onset of taVNS was 142.3 ± 16.9 (p = 0.0005). For control feeds, the lowest HR prior to feed was mean (SD) of 146.2 ± 14.6,

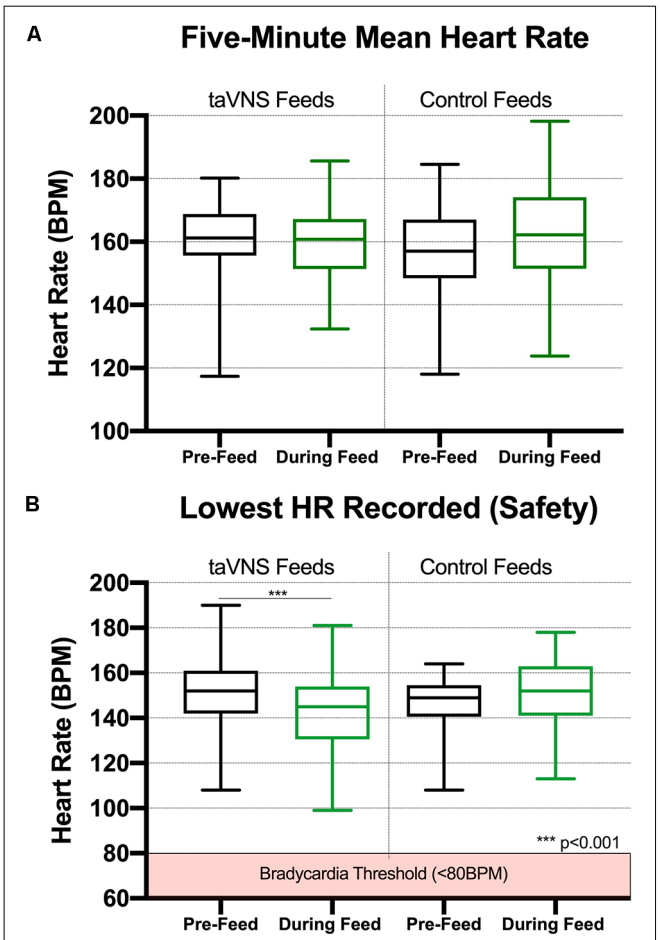


FIGURE 3 | Box and whisker plots for heart rate (HR) data collected during taVNS and control feeds (min to max). **(A)** taVNS with these parameters induces immediate, safe reductions in HR that recover during feeding (n = 7, 39 total feedings in seven participants). For the 5-min epochs prior to perceptual threshold (PT) and during taVNS-paired feeding, HR was averaged in 60-s intervals for a total of 5 min. **(B)** The mean lowest HR during PT was calculated from real-time HR monitor recorded during onset of stimulation to determine the PT. taVNS induced significant reductions in HR compared with those in pre-stimulation baseline ($p < 0.0001$); however, these reductions recovered to baseline levels immediately during the taVNS-paired feeding. There was no significant reduction in HR during control feeds (feeding HR recorded without taVNS administered, n = 7, 19 feeds).

and the lowest HR during feed was mean (SD) = 152.5 ± 15.7, and we found no significant difference between timepoints (**Figure 3B**).

When determining the PT, we consistently observed a decrease in HR with onset of stimulation. The transient HR drop was so common and predictable that we checked impedance and the earlobe contact, and electrode position if no HR decrease was observed. During determination of PT, HR decreased a mean of 20.5 ± 10.6 bpm or 12.6 ± 6.5% of the pre-taVNS HR (n = 105 taVNS sessions). In contrast, during control feeds, the HR decreased from before feed to the lowest HR during feed by a mean of 3.9 ± 6.4 bpm (n = 19 feeds, n = 7 subjects). The HR decrease with onset of taVNS stimulation was significantly greater than the HR change with control feeds ($p < 0.00001$). The comparison of the lowest HR before feed and with the lowest HR with taVNS onset vs. control feeds yielded similar results (**Table 3**).

We observed this HR decrease as a rapid effect after taVNS onset. To determine the time frame of the HR changes, we recorded the time to the lowest HR and HR every 12 s during entire taVNS-paired feeds in three participants (n = 48 sessions). In these three participants, the mean HR decreased 16 ± 9 bpm or 10 ± 3% baseline HR within 26 ± 8 s of stimulation onset, followed by an HR rebound to or above baseline within 60 s from stimulation onset, which was maintained during the taVNS-paired feeding. These measurements were reproducible within and between individuals (**Figures 4A–C**), replicating our group's prior HR findings in an adult human taVNS study (Badran et al., 2018c).

The HR decrease was likely due to vagus target engagement, as it was significantly different than the change in HR before and during control feedings without stimulation. During non-stimulation control feedings, the HR changed by a mean of −2.3 ± 14.0 bpm from HR before feed to the lowest HR during feed (n = 23, *ns*), compared with the rapid, transient mean HR decrease upon stimulation with taVNS (−20.5 ± 10.6 bpm, n = 104 feeds, $p < 0.00001$, t-test).

Feeding Outcomes

Of the 14 participants enrolled, who had all failed to attain feeding after an average of 49 days trying (**Table 1**), eight infants attained full oral feeds with weight gain adequate for discharge from the hospital after a course of taVNS-feeding paired rehabilitation (responders), and six did not receive a G-tube (non-responders). This 57% response rate is higher than our institutional historical controls and published rates for preterm infants (Howe et al., 2007a,b; Jackson et al., 2016; Ryan and Gehle, 2019).

We examined whether the responders were starting to improve oral feeds prior to enrolling in the trial. Although there is day-to-day variability in feeding volumes, the baseline rate of change of daily PO volume, averaged over 5 days immediately prior to taVNS treatment, was not significantly different between responders and non-responders (p = 0.15, **Figure 5**). With taVNS treatment, the rate of change of daily PO volume increased significantly in responders when compared with that in pre-treatment (p = 0.035). In non-responders, the mean rate of change of daily PO feeding volumes did not change from pre-treatment to during treatment (p = 0.29). Responders and non-responders did not differ in the number of taVNS treatments, average current intensity, or total pulses over all treatments (**Table 2**).

TABLE 2 | Clinical condition and treatment characteristics by responders and non-responders.

taVNS-treated infants	Responders n = 8	Non-responders n = 6	p
Preterm (mean GA at birth, birth weight)	6 (27 weeks, 877 g)	5 (29 weeks, 1,107 g)	
Term HIE	2	1	
Male sex	3	2	
Mean days attempting PO pre-taVNS	48 ± 29	49 ± 16	ns
Mean PO volume over 5 days pre-taVNS	52 ± 22 ml/kg/day	45 ± 26 ml/kg/day	ns
Mean # taVNS treatments	16 ± 6	17 ± 3	ns
Average mA current intensity	0.82 ± 0.2	0.75 ± 0.2	ns
Total pulses all treatments (10^5)	2.9 ± 1.7	2.2 ± 0.5	ns
IDM	1	3	
GERD requiring treatment	4	4	
VFSS: mean (SD) PAS scores	6 ± 3	4 ± 3	ns
Aspiration on VFSS	5	1	
Esophagitis	1	2	
Periventricular leukomalacia	1	2	
Lenticulostriate vasculopathy	0	2	

Note. taVNS, transcutaneous auricular vagus nerve stimulation; GA, gestational age; HIE, hypoxic–ischemic encephalopathy; GERD, gastroesophageal reflux disease; VFSS, videofluoroscopic swallow study; IDM, Infants of diabetic mothers; PAS, Penetration-Aspiration scale.

TABLE 3 | Lowest HR for 5 min prior to and during onset of taVNS vs. control feeds (n = 7 subjects).

	Lowest HR before	Lowest HR during	
taVNS-Paired feed	151.3 ± 15.1	142.3 ± 16.9	p = 0.0005
Control feed	146.2 ± 14.6	151.3 ± 15.1	p = 0.2

Note. HR, heart rate; taVNS, transcutaneous auricular vagus nerve stimulation.

The VFSS scores prior to taVNS treatment were not different between response groups (p = 0.3). Among the six infants who demonstrated aspiration below the vocal cords without effort to eject the liquid, five were responders. Of the responders with aspiration, three were taking thickened feeds prior to starting the study, two continued on thin maternal breast milk feeds with pacing, and one infant on breast milk feeds was transitioned to thickened feeds during the taVNS treatment period, after making significant progress to 100 ml/kg/day but demonstrating persistent coughing.

DISCUSSION

In this phase 0 pilot trial, one taVNS-paired feeding per day was safe and well tolerated in infants who had failed to achieve

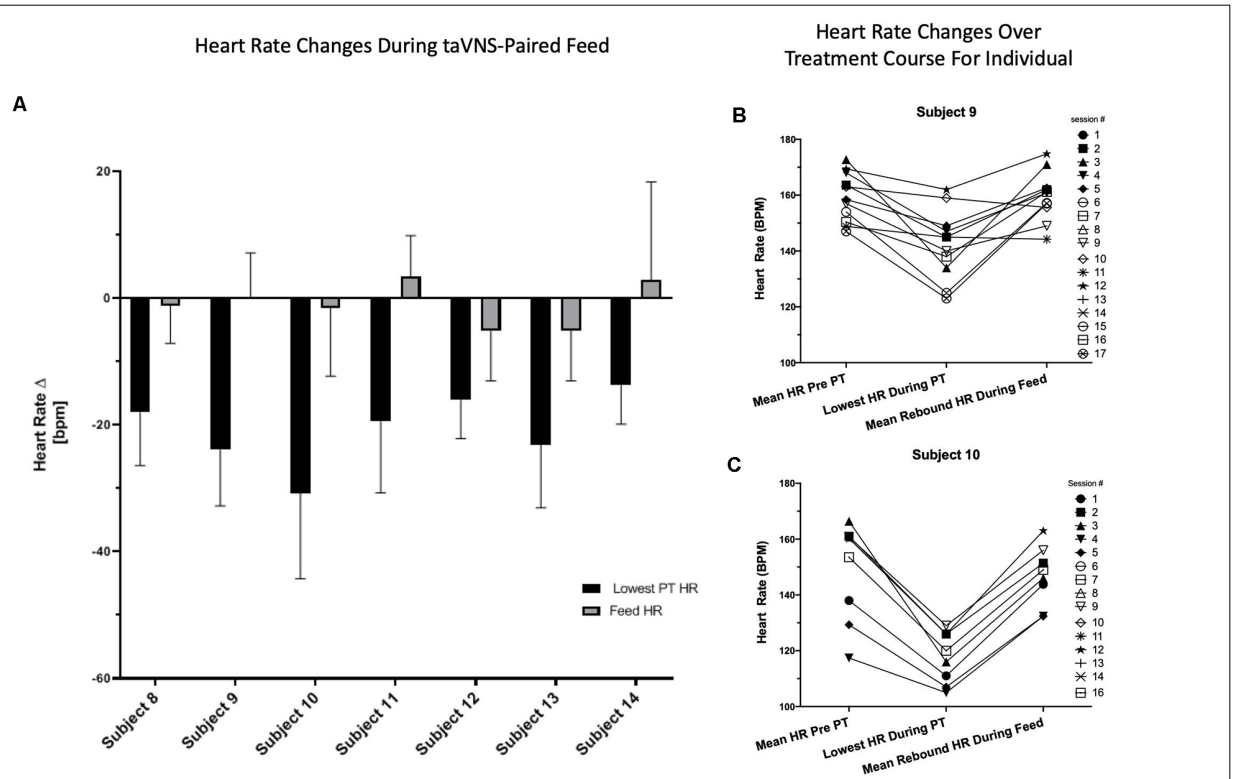


FIGURE 4 | Reproducibility and reliability of individual HR change. **(A)** Individual HR change from baseline with onset of stimulation and during taVNS -paired feeding by individual subject. HR is averaged over all taVNS-paired feedings for each individual subject. **(B,C)** HR data from individual treatment sessions in two representative participants. HR changes are shown for each individual taVNS-paired feeding session over 5 min before and during taVNS-paired feedings and the lowest HR recorded during onset of stimulation with PT determination.

full PO feeds and were referred for G-tube placement. Fifty-seven percent of treated infants were able to take all feeds by mouth within a mean of 16 days of treatment. taVNS-paired oral rehabilitation increased the likelihood of discharge without the need for G-tube implantation than did a historical retrospective comparison cohort who received standard feeding rehabilitation (Ryan and Gehle, 2019). A retrospective study of neonatal feeding outcomes at our institution built a predictive model that shows a minimal chance of reaching the required PO intake for discharge if the neonate has not reached 80 ml/kg/day by the 20th day attempting PO (Ryan and Gehle, 2019). Our cohort attempted PO for a mean of 49 ± 24.3 days prior to taVNS treatment, for which the in-house model predicts a spontaneous recovery rate of less than 10%.

Comparable published data indicate that preterm infants born at 25–32 weeks' gestation, with respiratory complications of bronchopulmonary dysplasia, attained full oral feeds at 38.5 ± 2.8 weeks' gestation, whereas those without BPD did so at 35.5 ± 1.7 weeks' gestation (Howe et al., 2007b). In one large retrospective study of 560 preterm infants born at 32–36 week's gestation, the median time to achieve full oral feeds was 12 days overall (Jackson et al., 2016). Although our taVNS-paired feeding study was non-randomized, our rate of successful attainment of full oral feeds is promising when compared with our historical control data as well as those from other observational studies.

Infants must develop oromotor skills of "suck–swallow" coordination in a particular sequence in order to feed effectively (Lau et al., 2003). Postmature infants (>41-week GA) who were born preterm and are not able to take full feeds by mouth may not be able to start oral feeds during a critical developmental window of oromotor neuroplasticity for learning feeding motor skills (Huang et al., 2015; Ismail et al., 2017). Sick newborns of term age (37- to 41-week GA) who have had critical illnesses frequently do not exhibit a suck–swallow reflex for feeding and may also have to learn this motor sequence, similar to preterm infants.

Both preterm and HIE infants suffer brain injury, triggering excessive stimulation of inflammatory pathways, impairing normal developmental functions of directing neuronal integration and foundational brain circuitry. After birth, the cortex and basal ganglia (BG) undergo significant integrative connectivity associated with shaping of central motor pathways. Disturbance of these processes leads to abnormal connections (Rocha-Ferreira and Hristova, 2016), and along with decreased populations of myelinating cells and inter-neurons, results in brain dysmaturity in preterm infants (Duerden et al., 2015) or overt brain injury in term HIE infants and subsequent motor, cognitive, and neurobehavioral impairments (Rocha-Ferreira and Hristova, 2016). Although postnatally the developing brain is more plastic than the adult brain and thus might be expected to

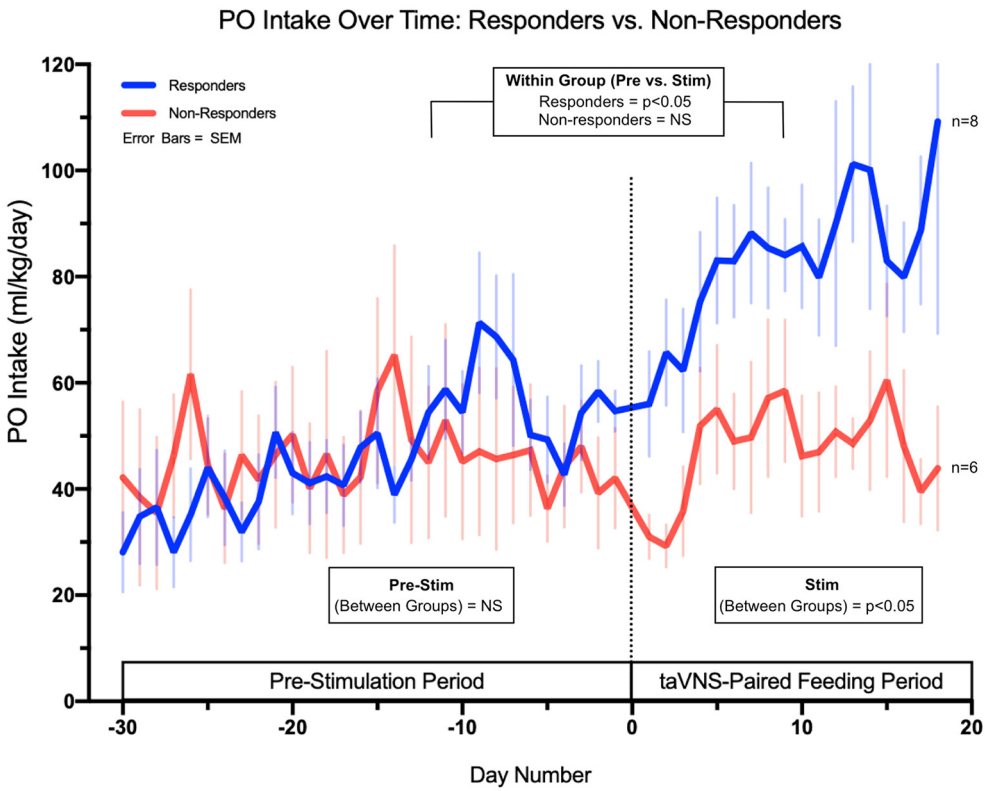


FIGURE 5 | Daily PO intake feeding volumes in ml/kg/day for both responders (full PO feeds without G-tube) and non-responders (G-tube placement). The data demonstrate no significant difference in feeding trajectories between groups in the pre-stimulation phase but significant differences in trajectories between responders and non-responders upon initiation of taVNS-paired feeding.

have better recovery following injury, we engage the developing brain to a very limited extent with clinical rehabilitation therapy while infants are still in the nursery.

The data in both animals and adult humans are convincing in that VNS paired with a stimulus improves functioning (Porter et al., 2012; Khodaparast et al., 2013, 2014, 2016; Dawson et al., 2016; Capone et al., 2017; Meyers et al., 2018). In our neonates with brain dysmaturity or overt brain injury, neuromodulation of abnormal circuits may positively influence neuronal connectivity and neuroplasticity (Kilgard, 2012; Meyers et al., 2018). If we can influence the circuitry early, before motor patterns are fixed, we may improve the developmental deficits that these children experience, starting with feeding delays in the nursery. With approximately 380,000 preterm infants and 4,000 term infants with HIE born in the USA per year, this therapy may translate to a large number of infants and have major impact on their outcomes.

Decreasing G-tube placement and length of stay due to feeding delays is a significant, longer-term goal of these studies. The time to attain full feeds accounted for 90% of variance in length of stay in two reports (Adamkin, 2006; Jackson et al., 2016). Earlier discharge without a G-tube may reduce medical complications for the patients and decrease costs, while offering substantial benefit to families waiting to bring their children home. Beyond feeding and earlier discharge, reinforced plasticity of oromotor function may impact short- and long-term neurodevelopment, particularly language skills.

Even late preterm infants can have difficulty learning to feed. In one retrospective study, one third of 35–36 weeks' gestation infants had feeding problems, and 76% of these had delayed discharge due to poor oromotor coordination (Wang et al., 2004). Also, males may have more difficulty as they have been shown to have later emergence of oral–lingual movements and pharyngeal activity than do females (Miller et al., 2006). In our study, we limited inclusion to preterm infants less than or equal to 33-week GA at birth or near-term to term infants with HIE. In the future, we need to enroll infants of wider GAs, conduct randomized controlled trials with sham stimulation, and enroll sufficient numbers for evaluation of sex differences.

Moreover, if taVNS is successful in the targeted motor behavior of feeding, we will extend investigation of early neuromodulatory therapy in high-risk infants to prevent or mitigate other life-long motor problems, such as cerebral palsy. This taVNS pilot trial, the first in human neonates, may provide a foundation for application of these therapies to infants at high risk for motor problems who have few alternative treatments.

Our HR data suggest that taVNS engages the vagal parasympathetic system. A rapid, transient HR decrease of 12% from baseline at the onset of taVNS stimulation was reproducible and reliable. The observed HR change suggests vagus nerve target

engagement by taVNS-paired feeding in neonates and may be useful in multi-day trials.

Vagal efferent HR change is useful as a stimulation indicator if it is not harmful. Safety is the foremost consideration for treatments in vulnerable infants who are premature or have suffered HIE, who have often recovered from significant respiratory, cardiovascular, or CNS conditions. We did not observe adverse effects of bradycardia or rebound tachycardia that were solely related to stimulation, or any other adverse effects. Mean neonatal discomfort scores measured with the NIPS did not significantly increase during stimulation in over 200 treatment sessions, and we decreased current intensity in only three instances for possible stimulation-induced increase in NIPS scores. No parent withdrew their infant from the study, and parents and staff accepted the treatment as well as tolerated by the infants.

It is unclear why some participants did not respond. Systemic and neuro-inflammation or medications that inhibit synaptic plasticity and learning (e.g., mineralocorticoid receptor inhibitors) may impair learning this motor skill (Favrais et al., 2011; Kuban et al., 2014; Leviton et al., 2016; Kelley et al., 2017). Alternatively, other negative sensory inputs of reflux or esophagitis may counteract positive sensorimotor circuit stimulation (Wingenfeld and Otte, 2019). Half of the non-responders (3/6) and only one of eight responders were infants of diabetic mothers with poor glucose control during pregnancy, which induces a pro-oxidative state for the mother and fetus known to be associated with immune activation, endothelial cell injury, and worse fetal and neonatal outcomes (Teodoro et al., 2013; Durga et al., 2018). A larger sample size may identify specific predictors for responder status leading to a decrease in variance in treatment response.

Further investigation and refinement of treatment parameters will likely also improve treatment response. Although these stimulation parameters were partially optimized in adults and to some extent in this trial, the potential responsiveness of specific circuits is likely determined by the sum as well as timing of stimulatory and inhibitory impulses. For example, we initially tested a pulse-train length of 3 s with the EMG closed-loop system, which proved too short for rhythmic suck–swallow sequences in some infants. We then increased to a 10-s train with each EMG-driven stimulation.

LIMITATIONS

The taVNS methodology was adjusted over the course of this study to more closely pair stimulation with feeding (Cook et al., 2020 in press *Brain Stimulation*). In participants 1–7, researchers activated taVNS manually when the infant was seen to be actively feeding; in participants 8–14, an EMG triggered taVNS-paired stimulation to sucking motor function. The latter would be expected to have better feeding outcomes, if learning depends on the precision of timing the stimulation with the motor activity. Over the study, we also developed several different ear electrodes to maximize contact and minimize the need for readjustment during feeds. All were confirmed by HR and resistance tests to deliver current to the tragus. We did not perform VFSS on every baby and did not explore the physiologic impairments in swallowing or causes of penetration/aspiration in this pilot trial. In future studies, we intend to document specific changes in swallowing function before and after taVNS-paired feeding treatment using a more precise scale adapted for infants (Martin-Harris et al., 2019). We also did not perform sham stimulation, to compare the lowest HR during sham and active taVNS stimulation. However, the HR changes with feeds in the control group indicate that HR usually increases during feeding, compared with the rapid decrease seen with PT in the taVNS feeds.

CONCLUSION

This is the first study investigating taVNS paired with the motor sequence of suck, swallow, and breath to potentially enhance oromotor learning in neonates and infants. In infants who had failed oromotor rehabilitative feeding techniques by therapists, taVNS treatments resulted in 57% achieving full oral feeds adequate for discharge without needing a G-tube. Further, taVNS appeared safe in neonates and infants with no adverse effects. Target engagement may be determined at each session by a brief HR decrease. Further investigations and a randomized trial are needed to confirm our promising results of improved feeding outcomes in infants treated with taVNS.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by MUSC IRB 1. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

BB, DJ, DC, MD, WD, GM, PS, ST, MB, and MG all made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data and participated in drafting, editing, and final approval of this manuscript.

FUNDING

This study was funded by the National MUSC Center of Neuromodulation for Rehabilitation (NC NM4R) and COBRE for Stroke Recovery. The National Center of Neuromodulation for Rehabilitation (NC NM4R) is supported by the Eunice Kennedy Shriver National Institute of Child Health & Human Development of the National Institutes of Health under award number P2CHD086844 and P20GM109040.

REFERENCES

Adamkin, D. H. (2006). Feeding problems in the late preterm infant. *Clin. Perinatol.* 33, 831–837. doi: 10.1016/j.clp.2006.09.003

Adams-Chapman, I., Bann, C. M., Vaucher, Y. E., Stoll, B. J., and Eunice Kennedy Shriver National Institute of Child Health and Human Development Neonatal Research Network. (2013). Association between feeding difficulties and language delay in preterm infants using Bayley Scales of Infant Development-Third Edition. *J. Pediatr.* 163, 680.e1-3–685.e1-3. doi: 10.1016/j.jpeds.2013.03.006

Alshaikh, B., Yusuf, K., and Sauve, R. (2013). Neurodevelopmental outcomes of very low birth weight infants with neonatal sepsis: systematic review and meta-analysis. *J. Perinatol.* 33, 558–564. doi: 10.1038/jp.2012.167

Badran, B. W., Dowdle, L. T., Mithoefer, O. J., LaBate, N. T., Coatsworth, J., Brown, J. C., et al. (2018a). Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) *via* electrical stimulation of the tragus: a concurrent taVNS/fMRI study and review. *Brain Stimul.* 11, 492–500. doi: 10.1016/j.brs.2017.12.009

Badran, B. W., Jenkins, D. D., DeVries, W. H., Dancy, M., Summers, P. M., Mappin, G. M., et al. (2018b). Transcutaneous auricular vagus nerve stimulation (taVNS) for improving oromotor function in newborns. *Brain Stimul.* 11, 1198–1200. doi: 10.1016/j.brs.2018.06.009

Badran, B. W., Mithoefer, O. J., Summer, C. E., LaBate, N. T., Glusman, C. E., Badran, A. W., et al. (2018c). Short trains of transcutaneous auricular vagus nerve stimulation (taVNS) have parameter-specific effects on heart rate. *Brain Stimul.* 11, 699–708. doi: 10.1016/j.brs.2018.04.004

Bakhuizen, S. E., de Haan, T. R., Teune, M. J., van Wassenaer-Leemhuis, A. G., van der Heyden, J. L., van der Ham, D. P., et al. (2014). Meta-analysis shows that infants who have suffered neonatal sepsis face an increased risk of mortality and severe complications. *Acta Paediatr.* 103, 1211–1218. doi: 10.1111/apa.12764

Bright, H. R., Babata, K., Allred, E. N., Erdei, C., Kuban, K. C. K., Joseph, R. M., et al. (2017). Neurocognitive outcomes at 10 years of age in extremely preterm newborns with late-onset bacteremia. *J. Pediatr.* 187, 43.e1–49.e1. doi: 10.1016/j.jpeds.2017.04.045

Capone, F., Miccinilli, S., Pellegrino, G., Zollo, L., Simonetti, D., Bressi, F., et al. (2017). Transcutaneous vagus nerve stimulation combined with robotic rehabilitation improves upper limb function after stroke. *Neural Plast.* 2017:7876507. doi: 10.1155/2017/7876507

Cook, D. N., Thompson, S., Stomberg-Firestein, S., Bikson, M., George, M. S., Jenkins, D. D., et al (2020). Design and validation of a closed-loop, motor-activated auricular vagus nerve stimulation (MAAVNS) system for neurorehabilitation. *Brain Stimulation*. doi: 10.1016/j.brs.2020.02.028

Dawson, J., Pierce, D., Dixit, A., Kimberley, T. J., Robertson, M., Tarver, B., et al. (2016). Safety, feasibility, and efficacy of vagus nerve stimulation paired with upper-limb rehabilitation after ischemic stroke. *Stroke* 47, 143–150. doi: 10.1161/strokeaha.115.010477

Dubner, S. E., Dodson, C. K., Marchman, V. A., Ben-Shachar, M., Feldman, H. M., and Travis, K. E. (2019). White matter microstructure and cognitive outcomes in relation to neonatal inflammation in 6-year-old children born preterm. *Neuroimage Clin.* 23:101832. doi: 10.1016/j.nicl.2019.101832

Duerden, E. G., Foong, J., Chau, V., Branson, H., Poskitt, K. J., Grunau, R. E., et al. (2015). Tract-based spatial statistics in preterm-born neonates predicts cognitive and motor outcomes at 18 months. *AJNR Am. J. Neuroradiol.* 36, 1565–1571. doi: 10.3174/ajnr.a4312

Durga, K. D., Adhisivam, B., Vidya, G., Vishnu Bhat, B., Bobby, Z., and Chand, P. (2018). Oxidative stress and DNA damage in newborns born to mothers with hyperglycemia - a prospective cohort study. *J. Matern. Fetal Neonatal Med.* 31, 2396–2401. doi: 10.1080/14767058.2017.1344630

Engineer, C. T., Engineer, N. D., Riley, J. R., Seale, J. D., and Kilgard, M. P. (2015). Pairing speech sounds with vagus nerve stimulation drives stimulus-specific cortical plasticity. *Brain Stimul.* 8, 637–644. doi: 10.1016/j.brs.2015.01.408

Favrais, G., van de Looij, Y., Fleiss, B., Ramanantsoa, N., Bonnin, P., Stoltenburg-Didinger, G., et al. (2011). Systemic inflammation disrupts the developmental program of white matter. *Ann. Neurol.* 70, 550–565. doi: 10.1002/ana.22489

Garcia, R. G., Lin, R. L., Lee, J., Kim, J., Barbieri, R., Sclocco, R., et al. (2017). Modulation of brainstem activity and connectivity by respiratory-gated auricular vagal afferent nerve stimulation (RAVANS) in migraine patients. *Pain* 158, 1461–1472. doi: 10.1097/j.pain.0000000000000930

Hatch, L. D., Scott, T. A., Walsh, W. F., Goldin, A. B., Blakely, M. L., and Patrick, S. W. (2018). National and regional trends in gastrostomy in very low birth weight infants in the USA: 2000–2012. *J. Perinatol.* 38, 1270–1276. doi: 10.1038/s41372-018-0145-4

Hays, S. A., Khodaparast, N., Hulsey, D. R., Ruiz, A., Sloan, A. M., Rennaker, R. L., et al. (2014a). Vagus nerve stimulation during rehabilitative training improves functional recovery after intracerebral hemorrhage. *Stroke* 45, 3097–3100. doi: 10.1161/strokeaha.114.006654

Hays, S. A., Khodaparast, N., Ruiz, A., Sloan, A. M., Hulsey, D. R., Rennaker, R. L., et al. (2014b). The timing and amount of vagus nerve stimulation during rehabilitative training affect poststroke recovery of forelimb strength. *Neuroreport* 25, 676–682. doi: 10.1097/wnr.0000000000000154

He, W., Wang, X., Shi, H., Shang, H., Li, L., Jing, X., et al. (2012). Auricular acupuncture and vagal regulation. *Evid. Based Complement. Alternat. Med.* 2012:786839. doi: 10.1155/2012/786839

Heilman, K. J., Connolly, S. D., Padilla, W. O., Wrzosek, M. I., Graczyk, P. A., and Porges, S. W. (2012). Sluggish vagal brake reactivity to physical exercise challenge in children with selective mutism. *Dev. Psychopathol.* 24, 241–250. doi: 10.1017/s0954579411000800

Howe, T. H., Sheu, C. F., Hinojosa, J., Lin, J., and Holzman, I. R. (2007a). Multiple factors related to bottle-feeding performance in preterm infants. *Nurs. Res.* 56, 307–311. doi: 10.1097/01.nnr.0000289498.99542.dd

Howe, T. H., Sheu, C. F., and Holzman, I. R. (2007b). Bottle-feeding behaviors in preterm infants with and without bronchopulmonary dysplasia. *Am. J. Occup. Ther.* 61, 378–383. doi: 10.5014/ajot.61.4.378

Huang, X., Stodieck, S. K., Goetze, B., Cui, L., Wong, M. H., Wenzel, C., et al. (2015). Progressive maturation of silent synapses governs the duration of a critical period. *Proc. Natl. Acad. Sci. U S A* 112, E3131–E3140. doi: 10.1073/pnas.1506488112

Ismail, F. Y., Fatemi, A., and Johnston, M. V. (2017). Cerebral plasticity: windows of opportunity in the developing brain. *Eur. J. Paediatr. Neurol.* 21, 23–48. doi: 10.1016/j.ejpn.2016.07.007

Jackson, B. N., Kelly, B. N., McCann, C. M., and Purdy, S. C. (2016). Predictors of the time to attain full oral feeding in late preterm infants. *Acta Paediatr.* 105, e1–e6. doi: 10.1111/apa.13227

Kelley, M. H., Wu, W. W., Lei, J., McLane, M., Xie, H., Hart, K. D., et al. (2017). Functional changes in hippocampal synaptic signaling in offspring survivors of a mouse model of intrauterine inflammation. *J. Neuroinflammation* 14:180. doi: 10.1186/s12974-017-0951-1

Khalil, S. T., Uhing, M. R., Duesing, L., Visotcky, A., Tarima, S., and Nghiem-Rao, T. H. (2017). Outcomes of infants with home tube feeding: comparing nasogastric vs. gastrostomy tubes. *JPEN J. Parenter. Enteral Nutr.* 41, 1380–1385. doi: 10.1177/0148607116670621

Khodaparast, N., Hays, S. A., Sloan, A. M., Fayyaz, T., Hulsey, D. R., Rennaker, R. L., et al. (2014). Vagus nerve stimulation delivered during motor rehabilitation improves recovery in a rat model of stroke. *Neurorehabil. Neural Repair* 28, 698–706. doi: 10.1177/1545968314521006

Khodaparast, N., Hays, S. A., Sloan, A. M., Hulsey, D. R., Ruiz, A., Pantoja, M., et al. (2013). Vagus nerve stimulation during rehabilitative training improves forelimb strength following ischemic stroke. *Neurobiol. Dis.* 60, 80–88. doi: 10.1016/j.nbd.2013.08.002

Khodaparast, N., Kilgard, M. P., Casavant, R., Ruiz, A., Qureshi, I., Ganzer, P. D., et al. (2016). Vagus nerve stimulation during rehabilitative training improves forelimb recovery after chronic ischemic stroke in rats. *Neurorehabil. Neural Repair* 30, 676–684. doi: 10.1177/1545968315616494

Kilgard, M. P. (2012). Harnessing plasticity to understand learning and treat disease. *Trends Neurosci.* 35, 715–722. doi: 10.1016/j.tins.2012.09.002

Kraus, T., Kiess, O., Hösl, K., Terekhin, P., Kornhuber, J., and Forster, C. (2013). CNS BOLD fMRI effects of sham-controlled transcutaneous electrical nerve stimulation in the left outer auditory canal-a pilot study. *Brain Stimul.* 6, 798–804. doi: 10.1016/j.brs.2013.01.011

Kuban, K. C., O'Shea, T. M., Allred, E. N., Paneth, N., Hirtz, D., Fichorova, R. N., et al. (2014). Systemic inflammation and cerebral palsy risk in extremely preterm infants. *J. Child Neurol.* 29, 1692–1698. doi: 10.1177/0883073813513335

Lau, C., Bhat, K., Potak, D., and Schanler, R. J. (2015). Oral feeding assessment predicts length of hospital stay in late preterm infants. *J. Pediatr. Mother Care* 1:102. doi: 10.19104/japm.2016.102

Lau, C., Smith, E. O., and Schanler, R. J. (2003). Coordination of suck-swallow and swallow respiration in preterm infants. *Acta Paediatr.* 92, 721–727. doi: 10.1111/j.1651-2227.2003.tb00607.x

Lawrence, J., Alcock, D., McGrath, P., Kay, J., MacMurray, S. B., and Dulberg, C. (1993). The development of a tool to assess neonatal pain. *Neonatal Netw.* 12, 59–66.

Leviton, A., Allred, E. N., Fichorova, R. N., Kuban, K. C., Michael O'Shea, T., Dammann, O., et al. (2016). Systemic inflammation on postnatal days 21 and 28 and indicators of brain dysfunction 2years later among children born before the 28th week of gestation. *Early Hum. Dev.* 93, 25–32. doi: 10.1016/j.earlhumdev.2015.11.004

Malas, K., Trudeau, N., Chagnon, M., and McFarland, D. H. (2015). Feeding-swallowing difficulties in children later diagnosed with language impairment. *Dev. Med. Child Neurol.* 57, 872–879. doi: 10.1111/dmcn.12749

Martin-Harris, B., Carson, K. A., Pinto, J. M., and Lefton-Greif, M. A. (2019). BaByVFSSImP((c)) a novel measurement tool for videofluoroscopic assessment of swallowing impairment in bottle-fed babies: establishing a standard. *Dysphagia* 35, 90–98. doi: 10.1007/s00455-019-10008-x

McSweeney, M. E., Kerr, J., Jiang, H., and Lightdale, J. R. (2015). Risk factors for complications in infants and children with percutaneous endoscopic gastrostomy tubes. *J. Pediatr.* 166, 1514.e1–1519.e1. doi: 10.1016/j.jpeds.2015.03.009

Meyers, E. C., Solorzano, B. R., James, J., Ganzer, P. D., Lai, E. S., Rennaker, R. L., et al. (2018). Vagus nerve stimulation enhances stable plasticity and generalization of stroke recovery. *Stroke* 49, 710–717. doi: 10.1161/strokeaha.117.019202

Miller, J. L., Macedonia, C., and Sonies, B. C. (2006). Sex differences in prenatal oral-motor function and development. *Dev. Med. Child Neurol.* 48, 465–470. doi: 10.1017/s0012162206001009

Porges, S. W. (1992). Vagal tone: a physiologic marker of stress vulnerability. *Pediatrics* 90, 498–504.

Porges, S. W., and Furman, S. A. (2011). The early development of the autonomic nervous system provides a neural platform for social behavior: a polyvagal perspective. *Infant Child Dev.* 20, 106–118. doi: 10.1002/icd.688

Portales, A. L., Porges, S. W., Doussard-Roosevelt, J. A., Abedin, M., Lopez, R., Young, M. A., et al. (1997). Vagal regulation during bottle feeding in low-birthweight neonates: support for the gustatory-vagal hypothesis. *Dev. Psychobiol.* 30, 225–233. doi: 10.1002/(sici)1098-2302(199704)30:3<225::aid-dev5>3.0.co;2-r

Porter, B. A., Khodaparast, N., Fayyaz, T., Cheung, R. J., Ahmed, S. S., Vrana, W. A., et al. (2012). Repeatedly pairing vagus nerve stimulation with a movement reorganizes primary motor cortex. *Cereb. Cortex* 22, 2365–2374. doi: 10.1093/cercor/bhr316

Pruitt, D. T., Schmid, A. N., Kim, L. J., Abe, C. M., Trieu, J. L., Choua, C., et al. (2016). Vagus nerve stimulation delivered with motor training enhances recovery of function after traumatic brain injury. *J. Neurotrauma* 33, 871–879. doi: 10.1089/neu.2015.3972

Redgrave, J. N., Moore, L., Oyekunle, T., Ebrahim, M., Falidas, K., Snowdon, N., et al. (2018). Transcutaneous auricular vagus nerve stimulation with concurrent upper limb repetitive task practice for poststroke motor recovery: a pilot study. *J. Stroke Cerebrovasc. Dis.* 27, 1998–2005. doi: 10.1016/j.jstrokecerebrovasdis.2018.02.056

Rocha-Ferreira, E., and Hristova, M. (2016). Plasticity in the neonatal brain following hypoxic-ischaemic injury. *Neural Plast.* 2016:4901014. doi: 10.1155/2016/4901014

Rosenbek, J. C., Robbins, J. A., Roecker, E. B., Coyle, J. L., and Wood, J. L. (1996). A penetration-aspiration scale. *Dysphagia* 11, 93–98. doi: 10.1007/bf00417897

Ryan, R. M. R. V., and Gehle, D. (2019). "Can we predict who will need a feeding gastrostomy tube in premature babies at discharge from the NICU?" in *Pedaitric Academic Societies meeting* (Baltimore).

Suess, P. E., Alpan, G., Dulkerian, S. J., Doussard-Roosevelt, J., Porges, S. W., and Gewolb, I. H. (2000). Respiratory sinus arrhythmia during feeding: a measure of vagal regulation of metabolism, ingestion and digestion in preterm infants. *Dev. Med. Child Neurol.* 42, 169–173. doi: 10.1017/s001216220000030x

Teodoro, J. S., Gomes, A. P., Varela, A. T., Duarte, F. V., Rolo, A. P., and Palmeira, C. M. (2013). Uncovering the beginning of diabetes: the cellular redox status and oxidative stress as starting players in hyperglycemic damage. *Mol. Cell. Biochem.* 376, 103–110. doi: 10.1007/s11010-012-1555-9

Wang, M. L., Dorer, D. J., Fleming, M. P., and Catlin, E. A. (2004). Clinical outcomes of near-term infants. *Pediatrics* 114, 372–376. doi: 10.1542/peds.114.2.372

Wingenfeld, K., and Otte, C. (2019). Mineralocorticoid receptor function and cognition in health and disease. *Psychoneuroendocrinology* 105, 25–35. doi: 10.1016/j.psyneuen.2018.09.010

Witt, N., Coynor, S., Edwards, C., and Bradshaw, H. (2016). A guide to pain assessment and management in the neonate. *Curr. Emerg. Hosp. Med. Rep.* 4, 1–10. doi: 10.1007/s40138-016-0089-y

Yakunina, N., Kim, S. S., and Nam, E. C. (2017). Optimization of transcutaneous vagus nerve stimulation using functional MRI. *Neuromodulation* 20, 290–300. doi: 10.1111/ner.12541

Conflict of Interest: BB, DJ, DC and MG have pending patents on the methods described in this manuscript.

The remaining 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.

frontiers in Psychology

ORIGINAL RESEARCH
published: 15 April 2020
doi: 10.3389/fpsyg.2020.00551

Transcutaneous Vagus Nerve Stimulation Does Not Affect Verbal Memory Performance in Healthy Volunteers

Ann Mertens[1], Lien Naert[2], Marijke Miatton[1], Tasha Poppa[3,4], Evelien Carrette[1], Stefanie Gadeyne[1], Robrecht Raedt[1], Paul Boon[1] and Kristl Vonck[1]*

[1] 4Brain, Department of Neurology, Institute for Neuroscience, Ghent University Hospital, Ghent, Belgium, [2] Department of Experimental Psychology, Ghent University, Ghent, Belgium, [3] Ghent Experimental Psychiatry (GHEP) Lab, Department of Psychiatry, Ghent University Hospital, Ghent, Belgium, [4] Department of Psychology, University of Southern California, Los Angeles, CA, United States

OPEN ACCESS

Edited by:
Tino Zaehle,
Otto von Guericke University Magdeburg, Germany

Reviewed by:
Marius Keute,
Tübingen University Hospital, Germany
Carlos Ventura-Bort,
University of Potsdam, Germany

****Correspondence:***
Ann Mertens
Ann.Mertens@ugent.be

Specialty section:
This article was submitted to Cognition, a section of the journal Frontiers in Psychology

Received: *10 January 2020*
Accepted: *09 March 2020*
Published: *15 April 2020*

Citation:
Mertens A, Naert L, Miatton M, Poppa T, Carrette E, Gadeyne S, Raedt R, Boon P and Vonck K (2020) Transcutaneous Vagus Nerve Stimulation Does Not Affect Verbal Memory Performance in Healthy Volunteers. Front. Psychol. 11:551. doi: 10.3389/fpsyg.2020.00551

Introduction: Invasive vagus nerve stimulation (VNS) improves word recognition memory in patients with epilepsy. Recent studies with transcutaneous VNS (tVNS) have also shown positive effects on various subdomains of cognitive functioning in healthy volunteers. In this randomized, controlled, crossover study, we investigated the effect of tVNS on a word recognition memory paradigm in healthy volunteers to further investigate the potential of tVNS in the treatment of cognitive disorders.

Methods: We included 41 healthy participants aged between 18 and 30 years (young age group) and 24 healthy participants aged between 45 and 80 years (older age group). Each participant completed a word recognition memory paradigm during three different conditions: true tVNS, sham, and control. During true tVNS, stimulation was delivered at the cymba conchae. Sham stimulation was delivered by stimulating the earlobe. In the control condition, no stimulation was given. In each condition, participants were asked to remember highlighted words from three test paragraphs. Accuracy scores were calculated for immediate recall after each test paragraph and for delayed recognition at the end of the paradigm. We hypothesized that highlighted words from paragraphs in the true tVNS condition would be more accurately recalled and/or recognized compared to highlighted words from paragraphs in the sham or control condition.

Results: In this randomized study, tVNS did not affect the accuracy scores for immediate recall or delayed recognition in both age groups. The younger group showed significantly higher accuracy scores than the older group. The accuracy scores improved over time, and the most recently learned words were better recognized. Participants rated true tVNS as significantly more painful; however, pain was not found to affect accuracy scores.

Conclusion: In this study, tVNS did not affect verbal memory performance in healthy volunteers. Our results could not replicate the positive effects of invasive VNS on word recognition memory in epilepsy patients. Future research with the aim of improving cognitive function should focus on the rational identification of optimized and individualized stimulation settings primarily in patients with cognitive deficits.

Keywords: transcutaneous vagus nerve stimulation, verbal memory performance, word recognition memory paradigm, cognition, immediate recall, delayed recognition

INTRODUCTION

There is an ever-increasing scientific interest in the vagus nerve as a potential target for memory modulation. Age-related declines are seen in short-term memory functioning and in free recall (retrieval) probably due to general slowing, reduced processing resources, loss of inhibitory functions, and lack of cognitive control (Luo and Craik, 2008). However, the brain is able to reorganize itself during aging, learning, and following damage, a process defined as neural plasticity. This concept has stimulated the development of new treatment options for cognitive decline, aiming to enhance this plastic potential (Duffau, 2006). The formation of declarative or explicit memory requires three essential processes: learning-encoding, consolidation-storage, and retrieval (Tulving, 1983). After information is perceived, it enters the memory system through the short-term memory function (Baddeley and Hitch, 1974) in which a small amount of information can be held active, as long as attention to the stimulus is maintained. Subsequently, information is stored in the long-term memory system, depending on the depth and elaboration of processing of the information. Retrieval of the stored information refers to the activation of the correct information from the long-term memory into the short-term memory, while suppressing the incorrect information (Shiffrin and Steyvers, 1997). It has been well documented that arousal shortly following a learning experience, during the process of memory consolidation, can modulate the storage of information (Cahill and McGaugh, 1996; McGaugh, 1966; McGaugh, 2015). Although this process has not been fully elucidated, preclinical research suggests that the vagus nerve plays a crucial role in transmitting the signals of peripheral neuromodulators associated with arousal to brain structures involved in memory storage (Williams and McGaugh, 1993; Nogueira et al., 1994; Hassert et al., 2004).

In 1999, Clark et al. (1999) demonstrated that stimulation of the vagus nerve, by means of an implanted device to treat drug-resistant epilepsy patients, was able to significantly enhance verbal memory performance when stimulation was delivered during the consolidation phase of a memory task. In 2006, Ghacibeh et al. (2006) found that VNS improved the retention of information by enhancing consolidation rather than by affecting memory retrieval, concluding that VNS specifically interacts with the processes underlying memory consolidation.

The vagus nerve projects to the nucleus of the solitary tract and consequently activates the noradrenergic neurons in the locus coeruleus and cholinergic neurons in the nucleus basalis, resulting in the release of norepinephrine (NE) and acetylcholine in wide areas of the cortex (Gu, 2002; Hassert et al., 2004; Roosevelt et al., 2006; Follesa et al., 2007; Nichols et al., 2011; Raedt et al., 2011). NE subsequently causes a release of serotonin by activating alpha-1-adrenergic receptors in the dorsal raphe nucleus (Manta et al., 2009). These neurotransmitters are known to facilitate neural plasticity, a key mechanism in many behavioral and cognitive processes (Gu, 2002; Duffau, 2006). Other neurotransmitters presumably involved in the mechanism of action of VNS are gamma-aminobutyric acid (GABA) and aspartate (Hammond et al., 1992; Ben-Menachem et al., 1995). Long-term potentiation is considered the major cellular mechanism of memory formation. As NE is known to facilitate this early long-term potentiation through the activation of beta-noradrenergic receptors, the VNS-induced NE release has been proposed as a possible mechanism of modulating memory performance (Harley, 2007; Mueller et al., 2008). These findings have given rise to an increasing interest in neuromodulation as a potential treatment for cognitive disorders. Currently available treatment options for cognitive dysfunction, including pharmacotherapy and psychosocial interventions, have shown limited effects on cognition (Perng et al., 2018). Based on the potential of VNS to modulate memory formation and the positive effects seen in epilepsy patients, VNS has been investigated as a potential treatment option for conditions associated with cognitive decline with promising results (Sjogren et al., 2002; Merrill et al., 2006).

Recently, noninvasive treatment options have gained interest, aiming to achieve the same effects as invasive VNS without the need for an invasive procedure. Transcutaneous vagus nerve stimulation (tVNS) represents a noninvasive neurostimulation modality that targets the receptive field of the auricular branch of the vagus nerve, located at the outer part of the ear (Ellrich, 2019). Functional imaging studies have shown that tVNS leads to activation of intracranial structures similar to the ones activated by invasive VNS, suggesting potential for evoking similar effects in a less invasive manner (Yakunina et al., 2017). Seeking to replicate the cognitive effects of invasive VNS seen in patients, several clinical studies have investigated the modulatory effect of tVNS on cognitive functioning in healthy volunteers (Jacobs et al., 2015; Sellaro et al., 2015, 2018; Steenbergen et al., 2015; Beste et al., 2016; Colzato et al., 2017, 2018a,b; Jongkees et al., 2018). Recent studies demonstrated that tVNS affected post-error slowing (Sellaro et al., 2015), response selection functions (Steenbergen et al., 2015; Jongkees et al., 2018), response speed when two actions were executed in succession (Steenbergen et al., 2015), divergent thinking (Colzato et al., 2018a), and emotion recognition (Colzato et al., 2017; Sellaro et al., 2018). tVNS also

significantly influenced inhibitory control processes (Beste et al., 2016) and decreased flow experience during a task (Colzato et al., 2018b). A study by Jacobs et al. (2015) was the first study to investigate the effect of tVNS on memory performance. They demonstrated that a single session of tVNS enhanced associative memory performance in older healthy volunteers, measured by means of an associative face–name memory task.

In this study, we aimed to replicate the positive effect of invasive VNS on verbal memory performance seen in epilepsy patients. Therefore, we investigated whether tVNS is able to improve verbal memory in younger as well as older healthy participants by applying stimulation during the consolidation phase of a word recognition memory paradigm. We hypothesized that tVNS, as compared to sham stimulation and control, would enhance verbal memory performance.

MATERIALS AND METHODS

Participants

The effect of tVNS on memory function was investigated in healthy volunteers belonging to two different age groups: young individuals between 18 and 30 years and older individuals between 45 and 80 years. Forty-one participants were included in the young age group and 24 participants in the older age group. Participants were recruited through flyers and an online recruitment system. Subjects were excluded in case of a history of cardiac disease, substance abuse or dependence, treatment with psychoactive drugs, a history of neurological or psychiatric disorders, pregnancy, and presence of an active implanted device (e.g., pacemaker, VNS, cochlear implant) or cerebral shunt. In the older age group, cognitive status was examined with the Montreal Cognitive Assessment (MoCA) test battery. Participants with a MoCA score lower than 24 were also excluded. Written informed consent was obtained from each participant before the beginning of the experimental session. Participants were instructed to have a light breakfast or lunch on the day of the experimental session and to avoid caffeine 2 h before. During the experimental session, the participant was not allowed to eat. Drinks were limited to water. Before conducting the experimental session, each participant was asked to fill out a demographic questionnaire. At the end of the experimental session, subjects received a gift certificate with a value of 20 euro for participating.

The study protocol was reviewed and approved by the ethics committee of Ghent University Hospital and conformed to the ethical standards of the Declaration of Helsinki.

Procedure

This (sham-)controlled, randomized, crossover, within-subjects study investigated the effect of tVNS on verbal memory performance in healthy volunteers. An overview of the study protocol is presented in **Figure 1**. First, the investigator and participant went through the inclusion criteria and informed consent form. Participants were included in the study after signing the informed consent form. Second, demographic data were collected through a questionnaire. For the participants in the older age group, cognitive functioning was also evaluated by administering the MoCA. After the appropriate tVNS amplitude was chosen (according to the threshold method or set to 0.5 mA), each subject conducted the word recognition memory paradigm in three different conditions: true stimulation, sham stimulation (active control), and no stimulation (control). At the end of the paradigm, a word recognition task was performed. A washout period of 30 min was implemented between experimental conditions and before the recognition

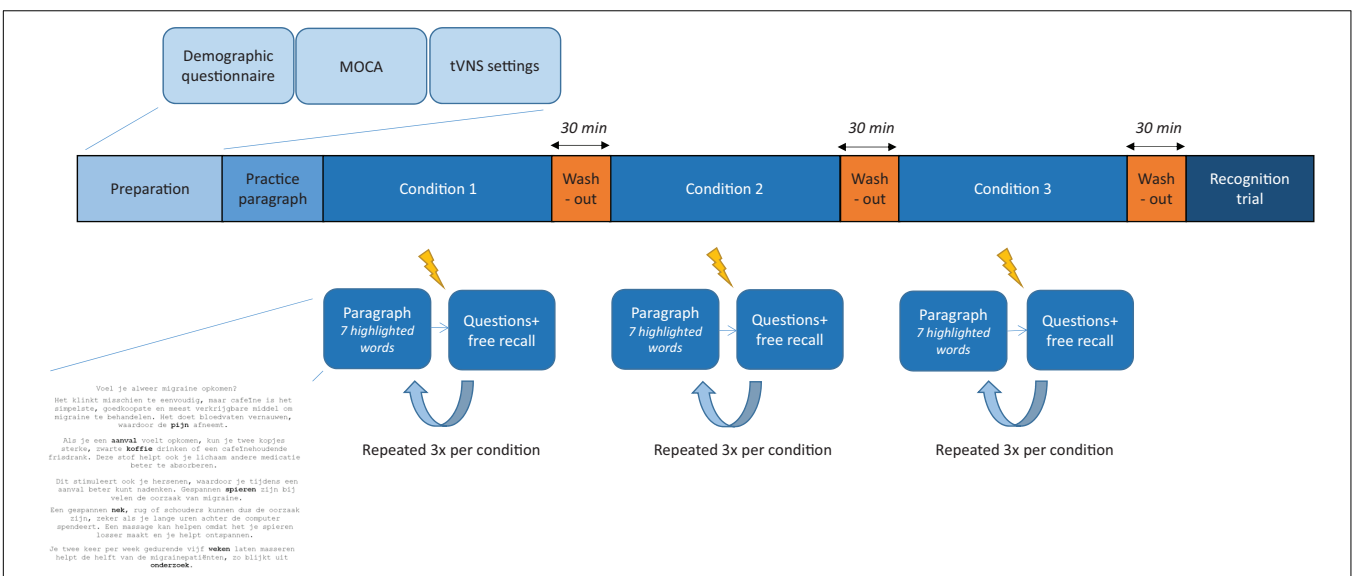


FIGURE 1 | Overview of the study. After preparation of the experimental session and one practice paragraph, the word recognition memory paradigm is conducted. Three experimental conditions are used, separated by a washout period of 30 min. Stimulation (⚡) is delivered 2 min after reading a paragraph and followed by two questions and a free recall task. This is repeated three times per condition. At the end of the paradigm, participants perform a recognition test.

task. During these breaks, participants were asked to perform a relaxing activity. The order of experimental conditions was randomized across subjects. All the experimental sessions were conducted at the Neurology Department at Ghent University Hospital in a neutral examination room. The experimental session lasted 3 h including breaks and could take place in the morning or in the afternoon, depending on the availability of the participant.

Transcutaneous Vagus Nerve Stimulation

Stimulation was delivered by means of the NEMOS® tVNS device (Cerbomed, Erlangen, Germany) which targets the cutaneous receptive field of the auricular branch of the vagus nerve at the outer ear. This external device consists of an auricular electrode connected to a control unit. The electrode is attached to an earplug to ensure that the electrode is placed on the cymba conchae. Sham stimulation was delivered by inverting the earplug and placing the electrode on the earlobe. Stimulation at this location will cause the same tingling sensation but will not activate the vagus nerve (Peuker and Filler, 2002; Kraus et al., 2013).

Based on previously published tVNS protocols (Steenbergen et al., 2015; Colzato et al., 2018a,b), stimulation intensity was set to 0.5 mA in 16 participants. As perceived and tolerated stimulation intensity varies across participants, we decided to set the stimulation intensity to the maximum tolerated output in the other 49 participants by using the threshold method. Before the beginning of the memory task, stimulation was increased in steps of 0.10 mA until the participant felt a tingling sensation. Stimulation was then further increased until the participant reported pain and finally decreased 0.10 mA below the pain threshold. This stimulation output was noted and used throughout the experimental session for both the sham and true tVNS conditions. Stimulation was delivered for 30 s during the consolidation phase of the memory task. Frequency was set to 25 Hz and pulse width to 250 μs.

The participants were informed that stimulation would be given during the experimental session by means of the tVNS device. Possible side effects of stimulation were discussed (e.g., pain, redness of the skin, itching) (Mertens et al., 2018). The participants were not informed about the type of stimulation (sham versus true) and expected outcome.

Word Recognition Memory Paradigm

The memory task in this study (**Figure 1**) was based on the word recognition memory paradigm in the study by Clark et al. (1999). It was designed with E-prime software (Psychology Software Tools Inc., Pittsburgh, PA, United States) and conducted on a laptop with a 14-inch screen (Dell, Windows 7). A short introduction was given by the investigator before starting the memory task. Written instructions were displayed on the laptop during the experimental session. Participants were instructed to silently read fragments of text paragraphs displayed on the screen. A practice paragraph was given before the beginning of the memory task to familiarize the participant with the testing procedures. The paragraphs were chosen from the "wablieft krant," an online journal known for its low difficulty level. One paragraph was divided into five to six fragments, displayed separately on the computer screen. Participants could continue to the next fragment by pressing the space bar. In each paragraph, seven words were highlighted. Participants were asked to read the paragraph thoroughly and memorize the highlighted words. Two minutes after finishing reading a paragraph, stimulation was delivered during 30 s. No stimulation was delivered in the control condition. In accordance to the study by Clark et al. (1999), stimulation was delivered after 2 min in order to stimulate during the consolidation phase of memory formation. Immediately after stimulation, participants were asked to answer two questions on the content of the paragraph and to write down as many of the highlighted words as possible (immediate free recall). Afterwards, participants were asked to rate pain during the stimulation. In the first 25 participants, a Likert scale from 1 to 9 was used. However, as participants reported difficulties in using this scale, the Wong–Baker FACES Pain Rating Scale ranging from 0 to 10 was used for the remaining 40 participants. The pain ratings of the first 25 participants were converted to fit the new scale. Three consecutive paragraphs with associated questions were merged into one text file. For each experimental condition, one text file was used, leading to a total of 21 highlighted words to be remembered and six questions to be answered per condition. Each subject conducted the paradigm sequentially in three conditions: true tVNS, sham, and control (no stimulation), summing up to nine paragraphs, 63 highlighted words, and 18 questions throughout the experimental session. Both the order of text files and conditions were randomized across subjects. A 30-min break was added after each condition to ensure washout between the different conditions. After completing all three conditions and adding a last break of 30 min, the recognition task was performed. During this final task, all 63 highlighted words as well as 63 related words and 63 non-related words were displayed on the computer screen in a randomized order. Each participant was asked to recognize target words and distinguish them from non-target words by pressing a green button when a target word was displayed and pressing a red button for a non-target word.

Outcome Measures

Primary outcome measures were accuracy scores on the immediate recall tests (after each test paragraph) and accuracy scores on the delayed recognition test (at the end of the paradigm). Regarding delayed recognition accuracy scores, only correct categorization of the highlighted words (hits) was compared. The categorization of related and unrelated novel words was not considered. We hypothesized that highlighted words from paragraphs in the true tVNS condition would be more accurately recalled and/or recognized compared to highlighted words from paragraphs in the sham or control condition.

Data Analysis

For each participant, immediate recall scores were calculated by the investigator based on the number of correct words the participant noted after each paragraph. This resulted in a mean accuracy score (percentage) for each stimulation condition. Delayed recognition scores were calculated using R statistical

software (R Core Team, 2017), resulting in accuracy scores for correct categorization of the highlighted words (hit or miss) on a single trial level.

The data analysis was conducted using R with lme4 (Bates et al., 2015) to perform generalized linear mixed effects (GLMEs) analyses. In case the dependent variable was dichotomous (categorization accuracy for delayed recognition test), we used logistic regression analyses. Both for the fixed and random effects, the chi-square statistics and the corresponding p-values were acquired by the likelihood ratio test. The dependent variable was the accuracy on the immediate recall and delayed recognition test. The independent variable was the stimulation condition (tVNS, sham, and control). The study order (block 1, block 2, block 3), the text file (file1, file2, file3), the stimulation intensity, pain score, gender, age, and years of education were also taken into account, as well as the MoCA score for the older group. As our dataset is relatively small, R failed to converge when making a full model with all the fixed and random effects. Therefore, we chose to start from the null model with a random intercept for subject and compare it with the model with the effect at test. This was done for all variables of interest for both the fixed effects and the random effects. Hereby, we can test if a variable is an important predictor in its own right, independent of the presence of any other variables. A significance level of $p < 0.05$ was adopted for all statistical tests.

Data analysis was conducted for both age groups separately as well as for all participants together.

RESULTS

Demographics

Forty-one participants (20 males) were included in the young age group with a mean age of 22.20 years ($\pm$1.97) and mean years of education of 15.44 years ($\pm$2.12). In the older age group, 24 participants were included, of whom seven were males. The mean age was 55.13 years ($\pm$6.59), and the mean years of education were 15.21 years ($\pm$2.05). The younger and the older age groups did not significantly differ from each other on gender [χ^2 (1, N = 65) = 1.66, p = 0.20] and years of education [Welch's t(49.21) = 0.43, p = 0.67].

Stimulation and Pain Report

The mean stimulation intensity was 0.54 mA ($\pm$0.21) in the younger group and 0.57 mA ($\pm$0.12) in the older group. Both groups did not significantly differ in stimulation intensity [Welch's t(62.99) = 0.35, p = 0.72]. Stimulation intensity had a significant effect on reported pain level [χ^2 (1, N = 65) = 7.82, p = 0.0051], with significantly higher pain scores after lower stimulation. There was a trend for higher reported pain levels in the younger group (1.08 $\pm$ 1.71) compared to the older group (0.57 $\pm$ 1.10), but this difference was not significant [χ^2 (1, N = 65) = 3.70, p = 0.055]. A significant effect of experimental condition on pain reports [χ^2 (1, N = 65) = 10.31, p = 0.0013] was found, showing that true tVNS led to a significantly higher pain score than sham [Welch's t(123.22) = 2.68, p = 0.0083], and sham led to a significantly higher pain score than the control condition [Welch's t(113.13) = 2.83, p = 0.0055].

Immediate Recall

We hypothesized to find an effect of experimental condition on immediate recall scores, more specifically, higher immediate recall accuracy scores for the true tVNS condition compared to the sham and control conditions. We first analyzed the data of the younger and older groups separately and then compared both groups.

Young Age Group

The mean accuracy score on the immediate recall test was 85.64% ($\pm$11.81%). We found no main effect of experimental condition [χ^2 (1, N = 41) = 0.37, p = 0.83] (**Figure 2**). There was also no main effect of order [χ^2 (1, N = 41) = 0.011, p = 0.92] (**Figure 3**). There was a significant main effect for the specific text they had to memorize, with highest accuracy scores for text file 2 [χ^2 (1, N = 41) = 14.27, p = 0.0008]. There were no significant random effects. There was no effect of pain report, stimulation intensity (**Figure 4**), age, gender, or education level on accuracy.

Old Age Group

The mean accuracy score on the immediate recall test was 77.31% ($\pm$16.43%). We found no main effect of experimental condition [χ^2 (1, N = 24) = 2.56, p = 0.11] (**Figure 2**). There was a significant main effect of order [χ^2 (1, N = 24) = 14.76, p = 0.00012], showing that the accuracy improved over time (**Figure 3**). Similar to the younger group, a significant main effect was found for the specific text they had to memorize, with highest accuracy scores for text file 2 [χ^2 (1, N = 24) = 21.07, $p < 0.001$]. There was an effect of age on accuracy [χ^2 (1, N = 24) = 24.35, $p < 0.0001$], showing higher accuracy scores for younger participants. There was also a significant effect of MoCA score on accuracy [χ^2 (1, N = 24) = 4.88, p = 0.027], showing that participants with a higher MoCA score obtained higher recall scores. There were no significant random effects. There was no effect of pain reports, stimulation intensity (**Figure 4**), gender, or years of education on the immediate recall score.

Both Age Groups

The mean accuracy score on the immediate recall test when combining both datasets was 82.56% ($\pm$14.24%). Age group (young versus old) had a significant main effect on recall accuracy, with significantly higher scores in the young age group [χ^2 (1, N = 65) = 8.55, p = 0.0034]. Regarding the effects of experimental condition on accuracy, we found no main effect of condition [χ^2 (1, N = 65) = 1.16, p = 0.28]. A significant main effect of order was found [χ^2 (1, N = 65) = 4.66, p = 0.031], showing that the accuracy improved over time. There was also a significant main effect for the specific text they had to memorize, with highest accuracy scores for text file 2 [χ^2 (1, N = 65) = 32.62, $p < 0.0001$]. There were no significant random effects. There was no effect of pain report, stimulation intensity, gender, or education level on accuracy.

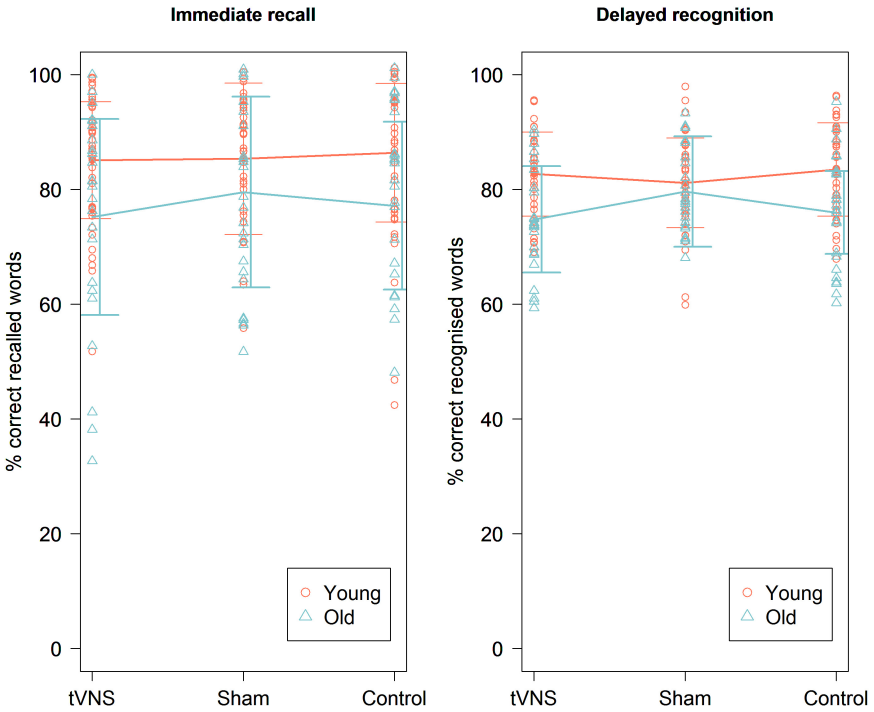


FIGURE 2 | Immediate recall **(left)** and delayed recognition **(right)** accuracy scores in percentage for the three experimental conditions for the young and old age group. There was no significant effect of experimental condition on immediate recall and delayed recognition scores in both age groups. Line plots represent mean scores. Error bars represent standard error.

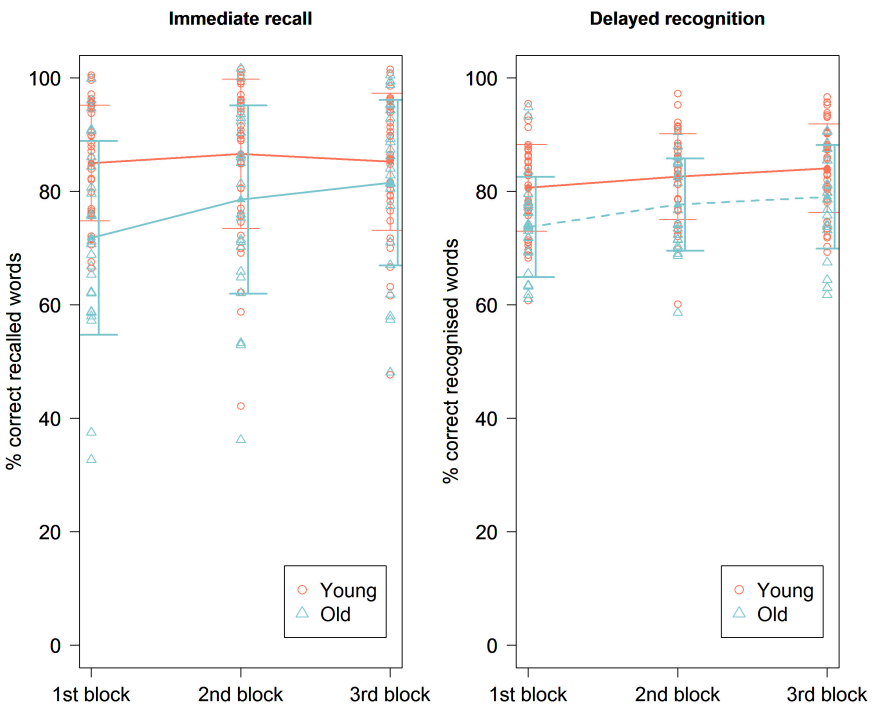


FIGURE 3 | Immediate recall **(left)** and delayed recognition **(right)** accuracy scores in percentage during the three blocks of the experimental session for the young and the old group. A significant effect of order was seen on immediate recall scores in the old age group and on delayed recognition scores in both age groups, showing significantly higher scores toward the end of the paradigm. Line plots represent mean scores. Error bars represent standard error.

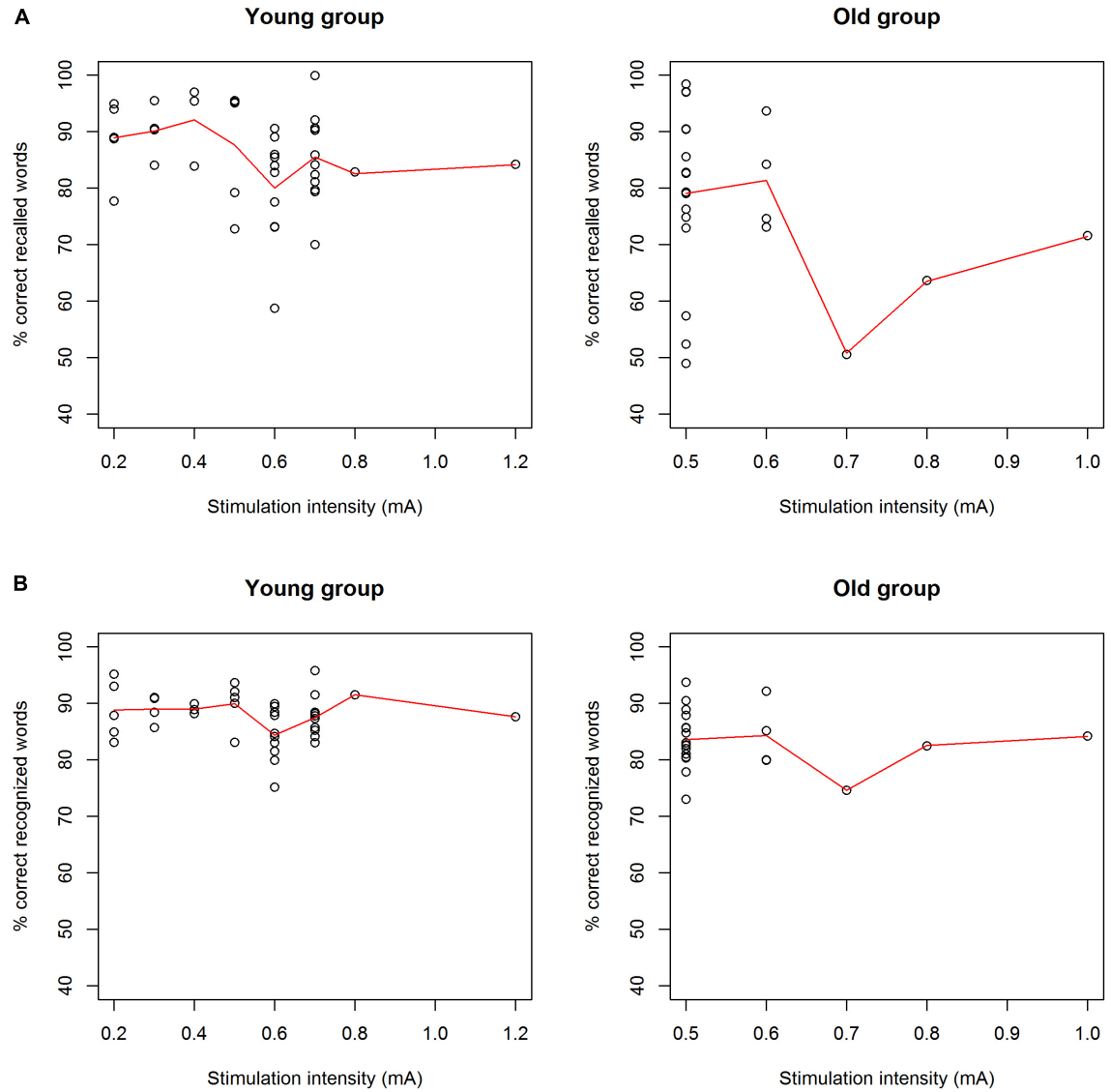


FIGURE 4 | (A) Correlation between immediate recall scores and stimulation intensity for the young group (left) and the old group (right). There was no correlation between stimulation intensity and immediate recall scores in both age groups. **(B)** Correlation between delayed recognition scores and stimulation intensity for the young group (left) and the old group (right). There was no correlation between stimulation intensity and delayed recognition scores in both age groups.

Delayed Recognition

During the recognition task, delayed recognition accuracy scores were obtained for correct categorization of the highlighted words. The categorization of related and unrelated novel words was not considered. We hypothesized that higher accuracy scores on the delayed recognition task would be associated with the true tVNS condition as compared to the sham and control conditions. We first analyzed the data of the younger and older groups separately and then compared both groups.

Young Age Group

The mean accuracy score on the delayed recognition test was 73.17% (±11.26%). No significant main effect of experimental condition was found [χ^2 (1, N = 41) = 0.01, p = 0.90] (**Figure 2**). There was a significant main effect of order [χ^2 (1, N = 41) = 12.72, p = 0.00036], showing a recency effect where the most recent learned words are better recognized (**Figure 3**). There was also a significant random effect of order, showing that the strength of this recency effect differed across participants [χ^2 (1, N = 41) = 7.18, p = 0.028]. There was no significant effect of text. We also found no effect of stimulation intensity (**Figure 4**), pain report, age, gender, or educational level on accuracy scores.

Old Age Group

The mean accuracy score on the delayed recognition test was 65.67% (±13.29%). No significant main effect of experimental condition was found (χ^2 (1, N = 24) = 0.99, p = 0.32) (**Figure 2**). There was a significant main effect of order

TABLE 1 | Overview of study characteristics of this clinical study and previous research investigating the effect of VNS on memory performance.

	This study	Clark et al. (1999)	Jacobs et al. (2015)
Study design	Within-subjects	Within-subjects	Within-subjects
Study population	Healthy volunteers young age group old age group	Epilepsy patients	Healthy older volunteers
Sample size	65 41 young age group 24 old age group	10	30
Memory function	Immediate recall Delayed recognition	Immediate recall Delayed recognition	Name–face association
Device	tVNS (NEMOS)	Invasive VNS	tVNS (TENStem)
Stimulation parameters			
Output current	According to threshold	0.5–1.5 mA	5 mA
Frequency	25 Hz	30 Hz	8 Hz
Pulse width	250 μs	500 μs	200 μs
Duration	30 s	30 s	17 min
Washout period	30 min	unknown	>7 days
Number of conditions	3 (tVNS, sham, control)	2 (VNS, sham)	2 (tVNS, sham)

[χ^2 (1, N = 24) = 25.79, $p < 0.0001$], showing again a recency effect (**Figure 3**) as well as a significant random effect of order [χ^2 (1, N = 24) = 13.75, $p = 0.0010$]. No significant effect of text was found. A trend was seen toward an effect of age on accuracy scores showing that the younger participants are, the higher their score is [χ^2 (1, N = 24) = 2.86, $p = 0.091$]. There was no effect of stimulation intensity (**Figure 4**), pain report, gender, educational level, or MoCA score on accuracy scores.

Both Age Groups

The mean accuracy score on the delayed recognition test was 70.40% (±12.49%). No significant main effect of experimental condition was found [χ^2 (1, N = 65) = 0.18, $p = 0.67$]. Also for both groups combined, a significant main effect of order [χ^2 (1, N = 65) = 34.12, $p < 0.0001$] as well as a significant random effect of order [χ^2 (1, N = 65) = 16.86, $p = 0.00022$] was found, showing a recency effect that differed across participants. There was a significant effect of text on accuracy scores [χ^2 (1, N = 65) = 7.41, $p = 0.025$] with significantly lower scores on text file 1. A significant effect of group [χ^2 (1, N = 65) = 13.21, $p = 0.00028$] was seen, showing that the younger group scored significantly higher on the delayed recognition test than the older group. No effect of stimulation intensity, pain report, gender, or educational level on accuracy scores was seen.

Power Analysis

A *post hoc* power analysis was conducted as the effect size could not be established before the beginning of the clinical study. The effect size (tVNS compared to control) was $d = 0.19$ for the immediate recall and $d = 0.03$ for the delayed recognition. By means of the package WebPower (Zhang and Yuan, 2018) in R, we used a function specifically for regression models to determine the sample size with a power of 0.8 and alpha set to 0.05. Regarding the immediate recall, a sample size of 43 participants was required to obtain a power of 80%. For the delayed recognition, a sample size of 263 participants was required to obtain a power of 80%.

DISCUSSION

While VNS was previously shown to improve performance on memory paradigms, we did not find a significant effect of tVNS on verbal memory performance in young and older healthy participants. Differences in the study methodology may underlie the different outcomes with regard to the effects of VNS on memory function (see also **Table 1**).

We investigated healthy participants, while in the study by Clark et al. (1999), the effect of VNS on memory performance was evaluated in epilepsy patients. It has been shown that epilepsy is associated with cognitive comorbidities including memory impairment (Butler and Zeman, 2008; Helmstaedter and Witt, 2017). A lower baseline performance in epilepsy patients may be more prone to improvement as compared to healthy volunteers, in whom the verbal memory performance test cannot be further improved, a feature described as "the ceiling effect." The study by Jacobs et al. (2015) also included only healthy volunteers, but inclusion was restricted to older individuals with a higher mean age compared to our participants in the old age group (60.57 years ± 2.54 versus 55.13 years ± 6.59), with potentially lower baseline memory scores again more susceptible to improvement.

In our study, a noninvasive device for targeting the vagus nerve was used. However, more effective stimulation of the vagal afferent pathway may be achieved when the vagus nerve is targeted directly by means of an implanted device. The optimal stimulation location and parameters of tVNS have not been elucidated. We chose to target the cymba conchae as this region is exclusively innervated by the auricular branch of the vagus nerve (Peuker and Filler, 2002), and stimulation at this location produced a significant activation of intracranial structures similarly affected by invasive VNS (Yakunina et al., 2017).

Previous research has shown that moderate levels of stimulation were most efficient for improving memory performance, whereas low and high levels of stimulation caused no improvement or even deterioration, visualized by an

inverted U-curve (Clark et al., 1999). In this study, we did not find a significant correlation between stimulation intensity and accuracy scores on immediate recall and delayed recognition (**Figure 4**). Participants who could tolerate higher output currents did not perform better than participants receiving a lower stimulation intensity. The previously described inverted U-curve could also not be confirmed by our results. However, we do emphasize that we did not investigate the effect of different stimulation intensities within subjects as was conducted in the study by Clark et al. (1999). The optimal stimulation intensity of tVNS for improving memory performance remains to be elucidated. Therefore, it is possible that subjects in this study were not stimulated at individually optimized levels of intensity.

Stimulation was delivered for only 30 s during the consolidation phase of a memory task, analogous to the invasive VNS protocol in Clark et al. (1999). However, 30 s of tVNS may be insufficient for a noninvasive device to effectively stimulate the vagal afferent pathway. As long-term potentiation is considered the most important mechanism of memory formation, longer and more repetitive stimulation of the vagus nerve might be required to effectively modulate hippocampal processes. In addition, some participants only tolerated very low output currents which could have been too low to sufficiently activate vagal afferent fibers. In the study by Jacobs et al. (2015), a significant effect on associative memory was found with a different tVNS device that continuously stimulated the inner side of the tragus during 17 min. The longer stimulation duration used in the Jacobs et al. (2015) study may prove more effective at modulating memory performance. However, it could also be possible that tVNS only interacts with specific memory functions, such as associative memory, and is not able to improve immediate recall or delayed recognition. In 2016, Burger et al. investigated the effect of tVNS on fear extinction, a process that is also highly dependent on memory formation (Burger et al., 2016, 2017, 2018; Verkuil et al., 2017). A significant acceleration of fear extinction learning was seen after tVNS; however, this did not lead to better retention of extinction memory. By further investigating the mechanism of action of (t)VNS, potential targets of memory function and optimal conditions for intervention could be identified.

In this study, we used a relatively short interval between the conditions. Thirty minutes may have been too short to ensure complete washout. Due to the setup of the word recognition memory paradigm, all conditions had to be conducted in one experimental session on the same day. To date, studies investigating the enduring effects of invasive VNS on NE show inconsistent results; some authors describe completely transient effects (Roosevelt et al., 2006), while others demonstrated elevated NE levels up to 2 h after stimulation (Hassert et al., 2004). To our knowledge, the enduring effect of tVNS has not been studied.

In contrast to previous research, we compared true tVNS to both a sham stimulation and control condition. The use of sham stimulation by means of stimulating the earlobe is under discussion (Keute et al., 2018; Rangon, 2018). Not including a sham stimulation would lead to blinding issues as participants can clearly distinguish the true condition from the control. A sham stimulation is also necessary to ensure that effects are caused by activating the vagal trajectory and not merely by the sensation of electrical current through the trigeminal nerve (Keute et al., 2018). As true tVNS did not significantly alter verbal memory performance compared to both a sham and control condition, we concluded that these results were not confounded by insufficient blinding or by sham-induced activation. Participants did report higher pain scores during true tVNS than sham, which could possibly impact their performance. However, we did not find a significant effect of pain on accuracy scores in both age groups.

A limitation of this study is the sample size. A *post hoc* power analysis indicated that the sample size of this study was sufficient to reliably investigate the effect of tVNS on immediate recall but should be extended to 263 participants for delayed recognition. This should be considered when interpreting the results for delayed recognition.

Although no effect of stimulation was found in this study, several experimental and demographic factors were identified that significantly affected verbal memory performance.

In the older age group, a practice effect was found with a significant increase in immediate recall accuracy scores throughout the paradigm. This practice effect was not seen in the younger age group. In all volunteers, highlighted words that were presented at the last condition of the paradigm were more easily recognized than highlighted words at the beginning, demonstrating a recency effect. A significant effect of text file was also found in both age groups, indicating that the highlighted words in some paragraphs could be more easily remembered than others. This effect was unexpected as all paragraphs were chosen from the same online journal involving health-related topics, and the highlighted words were controlled for frequency and concreteness ratings (Fliessbach et al., 2006; Keuleers et al., 2010; Lohnas and Kahana, 2013; Brysbaert et al., 2014). As we counterbalanced the order of intervention (active tVNS was randomly delivered as first, second, or third intervention) and text files across participants, these practice, recency, and text effects should not have interfered with our results. These findings emphasize the difficulties in designing a reliable neuropsychological study and the importance of counterbalancing conditions and test versions across participants.

Gender and years of education did not have an effect on memory performance. In the older age group, a higher MoCA score improved accuracy scores on immediate recall. Delayed recognition scores also seemed to increase with higher MoCA score, but this effect was not significant. Only in the older age group was a significant correlation between age and accuracy score on immediate recall found, with lower test scores as age increased. When comparing both age groups, we found significantly higher accuracy scores on immediate recall as well as delayed recognition in the young age group compared to the older age group. These findings demonstrate that aging above 45 years significantly reduces verbal memory performance.

This study does not find evidence that noninvasive targeting of the vagus nerve improves verbal memory performance in young and older healthy volunteers. Methodological issues potentially underlying the absence of effects have been discussed.

Further research to investigate the potential of targeting vagus nerve fibers noninvasively to improve cognitive function is required. As optimal stimulation parameters have not been elucidated, future research should focus on the effect of different stimulation settings in an individualized way in order to define the most efficient stimulation parameters.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics committee of Ghent University Hospital, Ghent, Belgium. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

AM was responsible for data acquisition and drafting of the manuscript. LN conducted the data processing and statistical analysis. MM, TP, EC, SG, RR, PB, and KV proofread the manuscript. The experimental sessions were conducted under the supervision of MM and KV.

FUNDING

This research was supported by a grant from the "Geneeskundige Stichting Koningin Elisabeth" (GSKE). AM is supported by an "Aspirant" grant of the "Fonds voor Wetenschappelijk Onderzoek" (FWO) Flanders. LN is supported by the Ghent University Research Fund (BOF). TP is supported by the National Science Foundation Graduate Research Fellowship Program. EC is supported by a research grant of Ghent University Hospital. PB is supported by grants of the "Fonds voor Wetenschappelijk Onderzoek" (FWO) Flanders, the Ghent University Research Fund (BOF), Ghent University Hospital, and E-Epilepsy (EU). RR and KV have been funded by the BOF-UGent, special research fund from Ghent University Hospital.

REFERENCES

Baddeley, A. D., and Hitch, G. (1974). "Working memory," in *The Psychology of Learning and Motivation*, ed. G. Bower (New York, NY: Academic Press), 47–89.

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed-effects models usinglme4. *J. Stat. Softw.* 67, 1–48.

Ben-Menachem, E., Hamberger, A., Hedner, T., Hammond, E. J., Uthman, B. M., Slater, J., et al. (1995). Effects of vagus nerve stimulation on amino acids and other metabolites in the CSF of patients with partial seizures. *Epilepsy Res.* 20, 221–227. doi: 10.1016/0920-1211(94)00083-9

Beste, C., Steenbergen, L., Sellaro, R., Grigoriadou, S., Zhang, R., Chmielewski, W., et al. (2016). Effects of concomitant stimulation of the GABAergic and norepinephrine system on inhibitory control – a study using transcutaneous vagus nerve stimulation. *Brain Stimul.* 9, 811–818. doi: 10.1016/j.brs.2016.07.004

Brysbaert, M., Stevens, M., De Deyne, S., Voorspoels, W., and Storms, G. (2014). Norms of age of acquisition and concreteness for 30,000 Dutch words. *Acta Psychol. (Amst).* 150, 80–84. doi: 10.1016/j.actpsy.2014.04.010

Burger, A. M., Van Diest, I., van der Does, W., Hysaj, M., Thayer, J. F., Brosschot, J. F., et al. (2018). Transcutaneous vagus nerve stimulation and extinction of prepared fear: a conceptual non-replication. *Sci. Rep.* 8:11471. doi: 10.1038/s41598-018-29561-w

Burger, A. M., Verkuil, B., Fenlon, H., Thijs, L., Cools, L., Miller, H. C., et al. (2017). Mixed evidence for the potential of non-invasive transcutaneous vagal nerve stimulation to improve the extinction and retention of fear. *Behav. Res. Ther.* 97, 64–74. doi: 10.1016/j.brat.2017.07.005

Burger, A. M., Verkuil, B., Van Diest, I., Van der Does, W., Thayer, J. F., and Brosschot, J. F. (2016). The effects of transcutaneous vagus nerve stimulation on conditioned fear extinction in humans. *Neurobiol. Learn Mem.* 132, 49–56. doi: 10.1016/j.nlm.2016.05.007

Butler, C. R., and Zeman, A. Z. (2008). Recent insights into the impairment of memory in epilepsy: transient epileptic amnesia, accelerated long-term forgetting and remote memory impairment. *Brain* 131(Pt 9), 2243–2263. doi: 10.1093/brain/awn127

Cahill, L., and McGaugh, J. L. (1996). Modulation of memory storage. *Curr. Opin. Neurobiol.* 6, 237–242. doi: 10.1016/s0959-4388(96)80078-x

Clark, K. B., Naritoku, D. K., Smith, D. C., Browning, R. A., and Jensen, R. A. (1999). Enhanced recognition memory following vagus nerve stimulation in human subjects. *Nat. Neurosci.* 2, 94–98. doi: 10.1038/4600

Colzato, L. S., Ritter, S. M., and Steenbergen, L. (2018a). Transcutaneous vagus nerve stimulation (tVNS) enhances divergent thinking. *Neuropsychologia* 111, 72–76. doi: 10.1016/j.neuropsychologia.2018.01.003

Colzato, L. S., Sellaro, R., and Beste, C. (2017). Darwin revisited: the vagus nerve is a causal element in controlling recognition of other's emotions. *Cortex* 92, 95–102. doi: 10.1016/j.cortex.2017.03.017

Colzato, L. S., Wolters, G., and Peifer, C. (2018b). Transcutaneous vagus nerve stimulation (tVNS) modulates flow experience. *Exp. Brain Res.* 236, 253–257. doi: 10.1007/s00221-017-5123-0

Duffau, H. (2006). Brain plasticity: from pathophysiological mechanisms to therapeutic applications. *J. Clin. Neurosci.* 13, 885–897. doi: 10.1016/j.jocn.2005.11.045

Ellrich, J. (2019). Transcutaneous auricular vagus nerve stimulation. *J. Clin. Neurophysiol.* 36, 437–442. doi: 10.1097/WNP.0000000000000576

Fliessbach, K., Weis, S., Klaver, P., Elger, C. E., and Weber, B. (2006). The effect of word concreteness on recognition memory. *Neuroimage* 32, 1413–1421. doi: 10.1016/j.neuroimage.2006.06.007

Follesa, P., Biggio, F., Gorini, G., Caria, S., Talani, G., Dazzi, L., et al. (2007). Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. *Brain Res.* 1179, 28–34. doi: 10.1016/j.brainres.2007.08.045

Ghacibeh, G. A., Shenker, J. I., Shenal, B., Uthman, B. M., and Heilman, K. M. (2006). The influence of vagus nerve stimulation on memory. *Cogn. Behav. Neurol.* 19, 119–122.

Gu, Q. (2002). Neuromodulatory transmitter systems in the cortex and their role in cortical plasticity. *Neuroscience* 111, 815–835. doi: 10.1016/s0306-4522(02)00026-x

Hammond, E. J., Uthman, B. M., Wilder, B. J., Ben-Menachem, E., Hamberger, A., Hedner, T., et al. (1992). Neurochemical effects of vagus nerve stimulation in humans. *Brain Res.* 583, 300–303. doi: 10.1016/s0006-8993(10)80038-1

Harley, C. W. (2007). Norepinephrine and the dentate gyrus. *Prog. Brain Res.* 163, 299–318. doi: 10.1016/s0079-6123(07)63018-0

Hassert, D. L., Miyashita, T., and Williams, C. L. (2004). The effects of peripheral vagal nerve stimulation at a memory-modulating intensity on norepinephrine

output in the basolateral amygdala. *Behav. Neurosci.* 118, 79–88. doi: 10.1037/0735-7044.118.1.79

Helmstaedter, C., and Witt, J. A. (2017). Epilepsy and cognition – a bidirectional relationship? *Seizure* 49, 83–89. doi: 10.1016/j.seizure.2017.02.017

Jacobs, H. I., Riphagen, J. M., Razat, C. M., Wiese, S., and Sack, A. T. (2015). Transcutaneous vagus nerve stimulation boosts associative memory in older individuals. *Neurobiol. Aging* 36, 1860–1867. doi: 10.1016/j.neurobiolaging.2015.02.023

Jongkees, B. J., Immink, M. A., Finisguerra, A., and Colzato, L. S. (2018). Transcutaneous vagus nerve stimulation (tVNS) enhances response selection during sequential action. *Front. Psychol.* 9:1159. doi: 10.3389/fpsyg.2018.01159

Keuleers, E., Brysbaert, M., and New, B. (2010). SUBTLEX-NL: a new measure for Dutch word frequency based on film subtitles. *Behav. Res. Methods* 42, 643–650. doi: 10.3758/BRM.42.3.643

Keute, M., Ruhnau, P., and Zaehle, T. (2018). Reply to "Reconsidering sham in transcutaneous vagus nerve stimulation studies". *Clin. Neurophys. J. Int. Fed. Clin. Neurophys.* 129, 2503–2504. doi: 10.1016/j.clinph.2018.09.001

Kraus, T., Kiess, O., Hosl, K., Terekhin, P., Kornhuber, J., and Forster, C. (2013). CNS BOLD fMRI effects of sham-controlled transcutaneous electrical nerve stimulation in the left outer auditory canal – a pilot study. *Brain Stimul.* 6, 798–804. doi: 10.1016/j.brs.2013.01.011

Lohnas, L. J., and Kahana, M. J. (2013). Parametric effects of word frequency in memory for mixed frequency lists. *J. Exp. Psychol. Learn. Mem. Cogn.* 39, 1943–1946. doi: 10.1037/a0033669

Luo, L., and Craik, F. I. (2008). Aging and memory: a cognitive approach. *Can. J. Psychiatry* 53, 346–353.

Manta, S., Dong, J., Debonnel, G., and Blier, P. (2009). Enhancement of the function of rat serotonin and norepinephrine neurons by sustained vagus nerve stimulation. *J. Psychiatry Neurosci.* 34, 272–280.

McGaugh, J. L. (1966). Time-dependent processes in memory storage. *Science* 153, 1351–1358. doi: 10.1126/science.153.3742.1351

McGaugh, J. L. (2015). Consolidating memories. *Annu. Rev. Psychol.* 66, 1–24. doi: 10.1146/annurev-psych-010814-014954

Merrill, C. A., Jonsson, M. A., Minthon, L., Ejnell, H., Silander, H., Blennow, K., et al. (2006). Vagus nerve stimulation in patients with Alzheimer's disease: additional follow-up results of a pilot study through 1 year. *J. Clin. Psychiatry* 67, 1171–1178. doi: 10.4088/jcp.v67n0801

Mertens, A., Raedt, R., Gadeyne, S., Carrette, E., Boon, P., and Vonck, K. (2018). Recent advances in devices for vagus nerve stimulation. *Expert Rev. Med. Devices* 15, 527–539. doi: 10.1080/17434440.2018.1507732

Mueller, D., Porter, J. T., and Quirk, G. J. (2008). Noradrenergic signaling in infralimbic cortex increases cell excitability and strengthens memory for fear extinction. *J. Neurosci.* 28, 369–375. doi: 10.1523/JNEUROSCI.3248-07.2008

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

Nogueira, P. J., Tomaz, C., and Williams, C. L. (1994). Contribution of the vagus nerve in mediating the memory-facilitating effects of substance P. *Behav. Brain Res.* 62, 165–169. doi: 10.1016/0166-4328(94)90024-8

Perng, C.-H., Chang, Y.-C., and Tzang, R.-F. (2018). The treatment of cognitive dysfunction in dementia: a multiple treatments meta-analysis. *Psychopharmacology* 235, 1571–1580. doi: 10.1007/s00213-018-4867-y

Peuker, E. T., and Filler, T. J. (2002). The nerve supply of the human auricle. *Clin. Anat.* 15, 35–37. doi: 10.1002/ca.1089

R Core Team (2017). *R: A Language and Environment for Statistical Computing*. Available at: https://www.R-project.org/ (accessed December 2, 2019).

Raedt, R., Clinckers, R., Mollet, L., Vonck, K., El Tahry, R., Wyckhuys, T., et al. (2011). Increased hippocampal noradrenaline is a biomarker for efficacy of vagus nerve stimulation in a limbic seizure model. *J. Neurochem.* 117, 461–469. doi: 10.1111/j.1471-4159.2011.07214.x

Rangon, C. M. (2018). Reconsidering sham in transcutaneous vagus nerve stimulation studies. *Clin. Neurophys. J. Int. Fed. Clin. Neurophys.* 129, 2501–2502. doi: 10.1016/j.clinph.2018.08.027

Roosevelt, R. W., Smith, D. C., Clough, R. W., Jensen, R. A., and Browning, R. A. (2006). Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. *Brain Res.* 1119, 124–132. doi: 10.1016/j.brainres.2006.08.048

Sellaro, R., de Gelder, B., Finisguerra, A., and Colzato, L. S. (2018). Transcutaneous vagus nerve stimulation (tVNS) enhances recognition of emotions in faces but not bodies. *Cortex* 99, 213–223. doi: 10.1016/j.cortex.2017.11.007

Sellaro, R., van Leusden, J. W., Tona, K. D., Verkuil, B., Nieuwenhuis, S., and Colzato, L. S. (2015). Transcutaneous vagus nerve stimulation enhances post-error slowing. *J. Cogn. Neurosci.* 27, 2126–2132. doi: 10.1162/jocn_a_00851

Shiffrin, R. M., and Steyvers, M. (1997). A model for recognition memory: REM-retrieving effectively from memory. *Psychon. Bull. Rev.* 4, 145–166. doi: 10.3758/BF03209391

Sjogren, M. J., Hellstrom, P. T., Jonsson, M. A., Runnerstam, M., Silander, H. C., and Ben-Menachem, E. (2002). Cognition-enhancing effect of vagus nerve stimulation in patients with Alzheimer's disease: a pilot study. *J. Clin. Psychiatry* 63, 972–980. doi: 10.4088/jcp.v63n1103

Steenbergen, L., Sellaro, R., Stock, A. K., Verkuil, B., Beste, C., and Colzato, L. S. (2015). Transcutaneous vagus nerve stimulation (tVNS) enhances response selection during action cascading processes. *Eur. Neuropsychopharmacol.* 25, 773–778. doi: 10.1016/j.euroneuro.2015.03.015

Tulving, E. (1983). *Elements of Episodic Memory*. Oxford: Oxford university Press.

Verkuil, B., Burger, A. M., van Diest, I., Vervliet, B., van der Does, W., Thayer, J. F., et al. (2017). Transcutaneous vagal nerve stimulation to promote the extinction of fear. *Brain Stimul.* 10:395. doi: 10.1016/j.brat.2017.07.005

Williams, C. L., and McGaugh, J. L. (1993). Reversible lesions of the nucleus of the solitary tract attenuate the memory-modulating effects of posttraining epinephrine. *Behav. Neurosci.* 107, 955–962. doi: 10.1037/0735-7044.107.6.955

Yakunina, N., Kim, S. S., and Nam, E. C. (2017). Optimization of transcutaneous vagus nerve stimulation using functional MRI. *Neuromodulation* 20, 290–300. doi: 10.1111/ner.12541

Zhang, Z., and Yuan, K.-H. (2018). *Practical Statistical Power Analysis Using Webpower and R*. Granger, IN: ISDSA Press.

Conflict of Interest: EC has received travel and registration grants from Elekta Neuromag Oy to participate in conferences and workshops on MEG. PB has received consultancy and speaker fees from UCB Pharma, LivaNova, Medtronic, and Eisai. KV and PB have received speaker fees from Livanova. PB, KV, and RR have received free devices for research studies in normal volunteers and preclinical studies from LivaNova, Cerbomed, Neurosigma, Medtronic.

The remaining 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.

frontiers in Human Neuroscience

ORIGINAL RESEARCH
published: 18 June 2020
doi: 10.3389/fnhum.2020.00206

Effect of Short-Term Transcutaneous Vagus Nerve Stimulation (tVNS) on Brain Processing of Food Cues: An Electrophysiological Study

Martina A. Obst[1], Marcus Heldmann[1,2], Helena Alicart[3], Marc Tittgemeyer[4,5] and Thomas F. Münte[1,2]**

[1]Department of Neurology, University of Lübeck, Lübeck, Germany, [2]Institute of Psychology II, University of Lübeck, Lübeck, Germany, [3]Cognition and Brain Plasticity Group, University of Barcelona, Barcelona, Spain, [4]Max-Planck-Institute for Metabolism Research, Cologne, Germany, [5]Cluster of Excellence in Cellular Aging and Aging-Associated Diseases (CECAD), Cologne, Germany

OPEN ACCESS

Edited by:
Tino Zaehle,
Otto von Guericke University Magdeburg, Germany

Reviewed by:
Lihua Sun,
Turku PET Centre, Finland
Ann Mertens,
Ghent University, Belgium

***Correspondence:**
Martina A. Obst
martina.a.obst@gmail.com
Thomas F. Münte
thomas.muente@neuro.uni-luebeck.de

Specialty section:
This article was submitted to Brain Imaging and Stimulation, a section of the journal Frontiers in Human Neuroscience

Received: 25 February 2020
Accepted: 07 May 2020
Published: 18 June 2020

Citation:
Obst MA, Heldmann M, Alicart H, Tittgemeyer M and Münte TF (2020) Effect of Short-Term Transcutaneous Vagus Nerve Stimulation (tVNS) on Brain Processing of Food Cues: An Electrophysiological Study. Front. Hum. Neurosci. 14:206. doi: 10.3389/fnhum.2020.00206

Background: The vagus nerve plays an important role in the regulation of food intake. Modulating vagal activity *via* electrical stimulation (VNS) in patients and animal studies caused changes in food intake, energy metabolism, and body weight. However, the moderating impact of cognitive processes on VNS effects on eating behavior has not been investigated so far.

Hypothesis: We hypothesized that transcutaneous VNS (tVNS) affects food intake by altering cognitive functions relevant to the processing of food-related information.

Methods: Using a repeated-measurement design, we applied tVNS and a sham stimulation for 2 h on two different days in normal-weight subjects. We recorded standard scalp EEG while subjects watched food and object pictures presented in an oddball task. We analyzed the event-related potentials (ERPs) P1, P2, N2, and LPP and also examined the amount of consumed food and eating duration in a free-choice test meal.

Results: Significant differences between stimulations were observed for the P1, P2, and N2 amplitudes. However, we found no tVNS-dependent modulation of food intake nor a specific food-related stimulation effect on the ERPs. Further analyses revealed a negative relationship between P2 amplitude and food intake for the sham stimulation. Significant effects are additionally confirmed by Bayesian statistics.

Conclusion: Our study demonstrates tVNS' impact on visual processing. Since the effects were similar between food and object stimuli, a general effect on visual perceptual processing can be assumed. More detailed investigations of these effects and their relationship with food intake and metabolism seem reasonable for future studies.

Keywords: tVNS, food, ERP, P2 and N2, brain stimulation, human, healthy, Bayesian

INTRODUCTION

The obesity epidemic in developed countries is one of the most pressing health problems. Between 1975 and 2016, the worldwide prevalence of obesity almost tripled in adults, whereas in children and adolescents (5–19 years), it increased nearly five-fold [World Health Organization (WHO), 2020]. In contrast, current treatment approaches, such as behavioral interventions, pharmacological treatments, and bariatric surgery, show limited effectiveness, are costly, or are burdened with side effects (Butryn et al., 2011; Kakkar and Dahiya, 2015; Al-Najim et al., 2018). Effective treatment options for the management of body weight are relevant because increased body weight is associated with various diseases (Afshin et al., 2017). Therefore, additional treatment modalities are urgently needed.

Brain stimulation techniques might represent such an alternative. Possible procedures currently include invasive approaches such as deep brain stimulation (DBS) and vagus nerve stimulation (VNS) and non-invasive options like transcranial magnetic stimulation (TMS), transcranial DC stimulation (tDCS), and transcutaneous VNS (tVNS). The scarce evidence on these techniques in the treatment of obesity has been reviewed recently (McClelland et al., 2013; Gorgulho et al., 2014; Val-Laillet et al., 2015; Göbel et al., 2017; Johnson and Wilson, 2018). Remarkable effects on food intake and body weight were shown by DBS of the hypothalamus and nucleus accumbens, regions associated with energy homeostasis and reward processing, respectively (albeit in single cases or small case series). Yet, the TMS and tDCS affected food craving, but no evidence exists so far that these two techniques also influence food intake and/or body weight. VNS also showed promising effects in animal studies, but the results of human (replication) studies are inconsistent so far (Pelot and Grill, 2018).

However, the modulation of vagal nerve afferents using brain stimulation techniques appears to be promising for a variety of reasons. De Lartigue (2016) reviewed evidence that vagal afferent neurons provide a satiety signal to the brain but lose sensitivity to peripheral signals in obesity, leading to further ingestion of palatable food. Disrupting vagal afferent neurons can lead to hyperphagia and weight gain. Several studies which used invasive vagal nerve stimulation to modulate these processes reported effects on body weight, metabolism, and fat tissue activity in animal models (Roslin and Kurian, 2001; Sobocki et al., 2002, 2006; Bugajski et al., 2007; Gil et al., 2011; Banni et al., 2012; Han et al., 2015). In human patient studies targeting refractory epilepsy and treatment-resistant depression, weight loss has been reported (Burneo et al., 2002; Bodenlos et al., 2007; Pardo et al., 2007; Abubakr and Wambacq, 2008; Vijgen et al., 2013; Ghani et al., 2015).

Using functional MRI, it has been shown that VNS leads to widespread activation in several brainstem regions (ipsilateral NTS, bilateral spinal trigeminal nucleus, dorsal raphe, locus coeruleus, and contralateral parabrachial area, Chae et al., 2003). In particular, the parabrachial area projects to a variety of structures comprising the hypothalamus, the insular cortex, the limbic system, and frontal regions such as the lateral prefrontal cortex (Van Bockstaele et al., 1999). Those regions have been shown to be activated by VNS as well (Lomarev et al., 2002; Chae et al., 2003). Since the insular cortex and hypothalamus are known for their involvement in the regulation of ingestive behavior (Sainsbury and Zhang, 2012; De Silva et al., 2012), both structures are candidates for the mediation of the potential effect of VNS on body weight and food intake. Moreover, it is assumed that VNS induces brain satiety signals by mimicking anorexigenic hormonal signals transmitted by vagal afferents; this ultimately leads to decreased food consumption (animal: Val-Laillet et al., 2010; Banni et al., 2012; human: Bodenlos et al., 2007). In addition, it was shown that the satiety status modulates various cognitive functions which occur in the cognitive processing of food information (Carbine et al., 2018). However, it is still unclear whether VNS also affects these cognitive functions in addition to metabolic and neuronal effects.

A recent development is tVNS that operates *via* electrodes placed in the outer ear (cymba conchae) that stimulate the auricular branch of the vagus nerve which projects to the nucleus tractus solitarii (NTS, Ellrich, 2011). Just like VNS, tVNS showed a similar activation pattern in the aforementioned brainstem and (sub)cortical brain regions (Frangos et al., 2015), leading to the assumption that tVNS could carry the same potential for modulating body weight and food intake behavior.

As tVNS has been shown to impact cognitive functions (Jacobs et al., 2015; Colzato et al., 2018; Sellaro et al., 2018) including action selection (Jongkees et al., 2018) and cognitive control (Fischer et al., 2018), this technique appears to be suitable to study the relevance of alterations in the cognitive processing of food information and the impact on food intake.

Cognitive functions are an essential part of the regulation of food consumption. They constantly integrate metabolic signals indicating homeostatic requirements, motivational needs, and external information by neurocognitive processes (Ferrario et al., 2016). Using event-related potentials (ERPs), it has been shown that being in a hungry state already affects early attentional functions during visual processing of food-related information resulting in higher P1 and N1 amplitudes (Plihal et al., 2001; Schacht et al., 2016). Indicated by varying P2, P3, and late positive potential (LPP) amplitudes, the homeostatic status also impacts selective and higher-order attentional processes to food-related information (Carbine et al., 2018). Based on the known tendency of organisms to approach food, an increase in the food-associated Nogo-N2 amplitude can be seen as an indicator for inhibiting the immanent approach tendencies toward food items (Watson and Garvey, 2013; Kong et al., 2015; Carbine et al., 2017). In some studies, the cognitive control-related N2 component correlates negatively with the body mass index (BMI) and food consumption (Carbine et al., 2018). This suggests that a decline in inhibitory control may lead to increased food consumption and, accordingly, to increased body weight. Similarly, a positive correlation between BMI and P2 has been found which points to an increased allocation of attentional resources to food items in people with a high BMI (Carbine et al., 2018). However, only one out of ten studies reported a relationship between increased LPP and elevated BMI (Versace et al., 2016) indicating that cognitive processes are just one of many factors which influence ingestive behavior.

To gain a first insight into a potential effect of tVNS on food-related cognition and ingestive behavior, we conducted the current study in healthy participants receiving stimulation of the cymba conchae or sham stimulation at the outer upper ear for about 120 min. This was done in a blinded crossover design after having fasted from 6 pm the day before. During sham and verum stimulation, the varying processing of food and object pictures (control visual stimuli) was assessed using event-related potentials (ERPs).

Based on previous findings, we were expecting a differential effect of tVNS on ERPs to food vs. object pictures. We tested effects on different ERP components (N1, P1, N2, P2, P3, and LPP), as in the absence of previous similar tVNS studies we could not formulate a more specific hypothesis. Furthermore, food intake was assessed after the ERP session to test whether tVNS leads to a reduction in calorie intake. Statistical analyses were performed in terms of frequential and Bayesian statistics to get a reliable estimate of the effects.

MATERIALS AND METHODS

Subjects

The procedures were approved by the local ethics committee prior to the study. Thirty-one healthy, right-handed subjects (15 women) were recruited *via* mailing lists from the university community and gave written informed consent to participate. All participants were compensated for their effort. Thirty participants exercised regularly between 1 and 7 h per week. Five participants indicated that they had been dieting at one point, but none of the participants was currently on a diet. Five participants were smokers (all <10 cigarettes per day). As visible in **Table 1**, the BMI for all subjects was within the normal range. It turned out that the women were distributed evenly across the cycle ruling this out as a nuisance factor in our results. Exclusion criteria were the presence of any psychological (e.g., depressive episode, eating disorders), neurological (e.g., seizure, migraine), and/or somatic (e.g., cardiac arrhythmia, diabetes) disorders; a BMI greater than 30 kg/m^2; an age above 40 years; and any kind of irregular sleep cycle (sleeping disorder, shift-working) and/or diet style (low-carb, vegetarian/vegan, weight reduction diet). All subjects had a normal or corrected-to-normal vision.

Procedure

Participants took part in two sessions (experimental stimulation, control stimulation) which were spaced 1 week apart. The order of the sessions was counterbalanced across participants. The participants were required to abstain from eating beginning at 18:00 h on the evening before the experimental sessions. They appeared either at 8:00 h or at 10:00 h in the laboratory. Participants indicated their subjective hungriness on a visual analog scale of 15 cm length. As visible in **Table 1**, there was no difference in the stated hungriness rating between each session (within-subject) as well as in terms of the measurements starting time (8 am vs. 10 am; between-subject). Also, the starting time point did not influence the eating duration in the final test meal.

Transcutaneous Vagal Nerve Stimulation

Before the application of the EEG cap and ECG electrodes, a titanium/titanium–iridium ear electrode (Nemos®, Cerbomed, Germany) was applied to the cymba conchae of the left ear to stimulate the auricular branch of the vagus nerve transcutaneously (experimental condition, **Figure 1A**). As a control stimulation, the outer upper ear was targeted (**Figure 1B**). This region does not contain vagal afferents (Berthoud and Neuhuber, 2000). In both conditions, stimulation was performed with 0.6 mA and a frequency of 25 Hz and a biphasic impulse interval (30 s stimulation, 30 s pause). This stimulation leads to a tingling sensation at the ear. To enhance the contact between skin and electrode, a contact spray was used (TIGA-TRONIC, Tiga-med, Ronneburg, Germany). The total duration of the stimulation was 1.9 h (SD 0.2) in both conditions.

Visual Stimuli and Procedure

After the application of all electrodes, participants were seated in a comfortable chair in front of a video monitor (viewing distance 90 cm). They viewed a sequence of 140 pictures (duration 1,000 ms, intertrial interval 2,400–2,700 ms), subtending 20 (height) by 20 (width) degrees of visual angle. The picture set comprised 70 different food (sweet and savory, high and low caloric) and 70 different object pictures (e.g., household objects, items from nature) that were repeated seven times in a random order in seven blocks of approximately 8 min in duration (**Figure 2**). Between blocks, short breaks were given to allow the participants to stretch and move. Each block contained

TABLE 1 | Descriptive sample statistics with mean (M) and standard deviation (SD) as well as statistical significance (p).

variables	mean (SD)	mean (SD)	test-statistic	*p*-value
Age (years)	23.0 (2.0)			
BMI (kg/m^2)	22.5 (1.7)			
sportive Activity (week)	3.3 (1.7)			
	Day 1	**Day 2**		
Hungriness (VAS)	8.5 (3.6)	8.9 (3.2)	$z^{w}_{(32)} = -0.6$	0.556
	8 am Group	**10 am Group**		
Hungriness (VAS)	8.8 (3.2)	8.6 (3.7)	$z^{mw}_{(34,28)} = 0.17$	0.865
	tVNS and 8 am	**tVNS and 10 am**		
Eating Duration (h)	0.22 (0.1)	0.19 (0.1)	$z^{mw}_{(16,13)} = 0.15$	0.124
	sham and 8 am	**sham and 10 am**		
Eating Duration (h)	0.21 (0.1)	0.21 (0.1)	$z^{mw}_{(16,13)} = 0.15$	0.878

z^{w} = Wilcoxon sign rank test for paired samples (two-sided). z^{mw} = Mann-Whitney-Wilcoxon test for unpaired samples (two-sided). n = 31; females = 15.

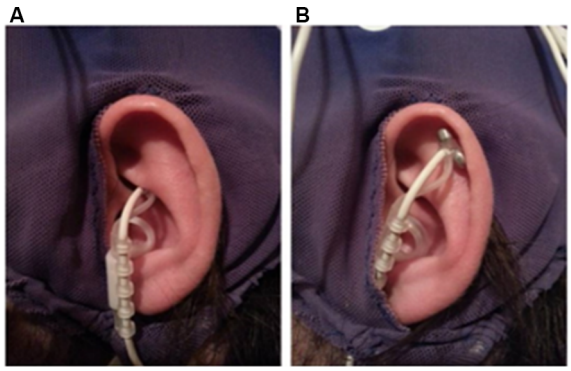


FIGURE 1 | Positions of the ear-electrode (NEMOS®, Cerbomed Erlangen-Germany). **(A)** Transcutaneous stimulation of the auricular branch of the vagus nerve (tVNS), **(B)** stimulation of the auricular scaphoid fossa (sham). Stimulation parameters: 25 Hz, biphasic, 30 s ON and 30 s OFF interval, current intensity of 0.6 mA so that a tingly was perceptible.

10 pictures (both object and food items) that contained a small green square (1 × 1 cm) that could appear anywhere within the picture. The participant's task was to screen each picture for the presence of the green square and to press a button whenever a square was detected. This ensured attentive processing of all pictures.

Food Intake/Standardized Meal

After removing EEG and ear electrodes, the participants received a standardized breakfast containing a variety of foods typical for a continental breakfast (no meat; **Supplementary Table S1**). They were instructed to eat as much and as long as they wanted until they were satiated. To calculate the consumed amount of food, we calculated the difference between the food's weight before and after breakfast. Also, the duration of food consumption was documented. For the analysis, the energy density of the consumed food was calculated using the *fddb database*.[1]

EEG Recording and Analysis

The electroencephalogram was recorded from 29 scalp channels (FP1, FP2, F3, F4, C3, C4, P3, P4, O1, O2, F7, F8, T7, T8, P7, P8, FZ, CZ, PZ, FC1, FC2, CP1, CP2, PO3, PO4, FC5, FC6, CP5, CP6) referenced to the nose tip (bandpass of 0.01–50 Hz, 500 samples/s). Ocular fixation was verified by recordings of the horizontal EOG. Trials which were contaminated by eye blinks were detected by vertical electrooculogram.

EEG data were processed using EEGlab (Delorme and Makeig, 2004) and ERPlab (Lopez-Calderon and Luck, 2014) implemented in MatLab R2017a (MathWorks Inc.). First, frequencies below 0.1 Hz and above 48 Hz were filtered out. Next, the data were divided into 3,000-ms epochs starting 1,000 ms before the onset of the picture presentation. Independent component analysis (ICA) was used to remove ocular and muscle artifacts from the data. Briefly summarized, ICA separates the signal into different statistically independent sources. Sources identified as artifactual were removed (Bell and Sejnowski, 1995; Makeig et al., 1996). Additionally, artifact-contaminated epochs were rejected based on the identification of peak-to-peak amplitudes exceeding 130 μV and visual inspection. Less than 20% of the epochs were rejected per participant. The remaining epochs were used to calculate an average ERP per subject and condition.

For statistical testing, mean amplitudes with a baseline of −100 to 0 ms were calculated using component-specific time windows for early (P1 100–140 ms, N1 140–180 ms), middle (P2 210–260 ms, N2 260–360 ms), and late components (LPP 400–600 ms). P1 and P2 mean amplitudes were calculated for the occipital–parietal scalp regions (P3, P4, PO3, PO4, O1, O2), N1 and N2 mean amplitudes for midline electrode positions (FZ, CZ, PZ), and the mean amplitudes LPP at the electrode sites C3, C4, P3, P4, O1, and O2.

Each component was analyzed separately. For P1, P2, and LPP, a 3 (gradient: parietal, occipital–parietal, occipital) × 2 (hemisphere: left vs. right) × 2 (stimulation: tVNS vs. sham) × 2 (picture: food vs. object) repeated-measures ANOVA and for the N1 and N2 components a 3 (electrode: FZ, CZ, PZ) × 2 (stimulation: tVNS vs. Sham) × 2 (picture: food vs. object) repeated-measures ANOVA were calculated using IBM SPSS (Version 22). All tests were conducted two-sided at the 5% significance level and adjusted for multiple comparisons using Bonferroni correction. Effect sizes were reported as partial η^2 for ANOVAs and Hedges g for *post hoc* paired-sample t-tests. To assess stimulation effects on food consumption, two-sided paired-sample t-tests on a 0.05 alpha level were conducted in SPSS and effects sizes are reported (Hedges g).

Following Colzato et al. (2018) and Warren et al. (2019), we additionally used Bayesian statistics (Kennedy, 2015; Wagenmakers et al., 2016, 2018) to further evaluate the significance of effects. In contrast to frequential statistics, one benefit of Bayesian statistics is the calculation of the Bayes factor (BF) representing the strength of evidence for the null (H0) and the alternative hypothesis (H1) given by the empirical data. Bayesian statistics is also known to be more conservative when testing for the alternative hypothesis (Gelman et al., 2012; Wagenmakers et al., 2018) and can accordingly be seen as a lower limit of the effect's strength, providing further evidence for the validity of the reported findings.

Bayesian paired-sample *t*-tests for *post hoc* analysis of the ANOVA effects were calculated using the JASP software package (JASP v0.8.6.0).[2] For these statistics, the prior probability was defined by a Cauchy distribution (default setting) and the BF (H1|H0) is reported. The BF(H1|H0) is the ratio that quantifies the likelihood of H1 over H0; i.e., a $BF_{(10)}$ of three means that H1 is three times more likely (based on the empirical data) than H0. According to Kass and Raftery (1995), a BF between one and three indicates anecdotal, between three and ten moderate, between 10 and 30 strong, and between 30 and 100 very strong evidence for H1. Potential interrelations between electrophysiological effects and food consumption were tested using Pearson correlation and Bayesian correlation pairs, respectively.

[1]https://fddb.info

[2]https://jasp-stats.org/

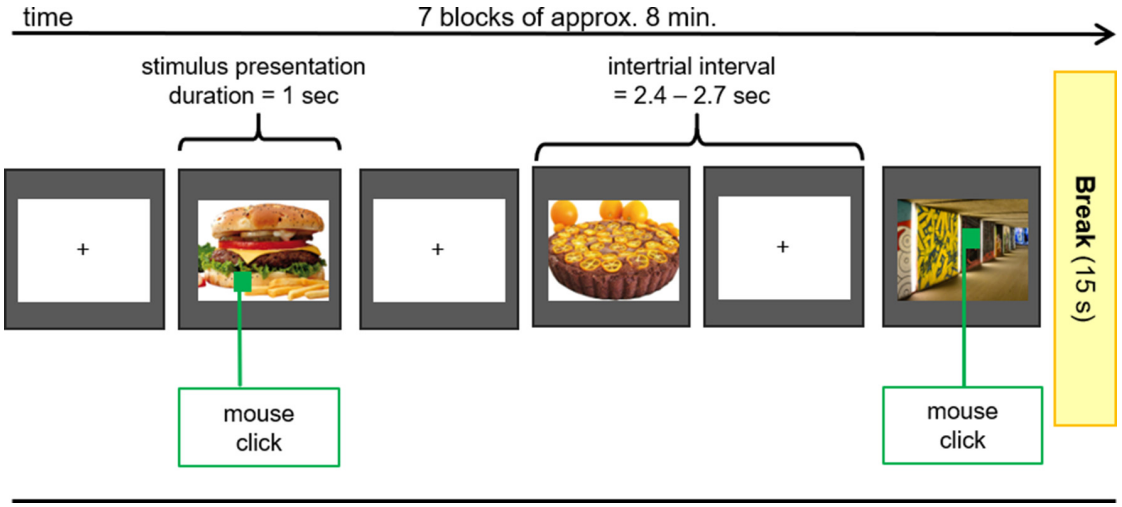


FIGURE 2 | EEG Oddball-Paradigm. During the EEG-Measurements pictures of food with high and low caloric content as well as unexceptional objects (control) were presented to the subjects. If the presented picture contained a green square, participants had to press the right mouse button with the index finger of the right hand as fast as possible.

RESULTS

Electrophysiological Results

Descriptive statistics (mean, standard error, and 95% confidence interval values) are reported in **Table 2**.

The visual inspection of the ERPs (**Figure 3A**) reveals the most pronounced effect for the P1 amplitudes at occipital electrode sites. The main effect stimulation reached significance ($F_{(1,30)} = 5.36$, $p = 0.028$, $\eta^2_{par} = 0.15$) revealing lower amplitudes in the tVNS condition. The corresponding $BF_{(10)}$ for this effect is 1.91 (**Figure 4A**). Food pictures evoked higher P1 amplitudes than object pictures did, although this difference reached only a statistical trend level ($F_{(1,30)} = 3.98$, $p = 0.055$, $\eta^2_{par} = 0.12$). There was no significant interaction between the stimulation and picture factor ($F_{(1,30)} = 0.12$, $p = 0.732$, $\eta^2_{par} < 0.01$) or any other factor.

Regarding the N1 component (**Figure 3B**), visual inspection suggested lower N1 amplitudes in response to food compared to object pictures and likewise higher amplitudes after tVNS compared to the control condition. Statistical analysis revealed, however, that the main effects for the factors picture and stimulation and their interaction were not significant. There was a trend effect for the interaction between the factors electrode × picture × stimulation ($F_{(2,29)} = 3.22$, $p = 0.054$, $\eta^2_{par} = 0.10$). Exploratory *post hoc* ANOVA showed a significant stimulation main effect only at PZ electrode ($F_{(1,30)} = 4.93$, $p = 0.034$, $\eta^2_{par} = 0.14$) with larger N1 amplitudes in the tVNS ($M = 3.30$, $SD = 0.70$) compared to the sham condition ($M = 3.73$, $SD = 0.63$). Inspected with Bayesian statistics, this main stimulation effect reached a $BF_{(10)}$ of 1.6 (**Figure 4B**).

Analyzing the later components P2 and N2 both ANOVAs revealed a significant stimulation main effect indicating smaller P2 amplitudes (**Figure 3A**) but higher N2 amplitudes (**Figure 3B**) for tVNS (P2: $F_{(1,30)} = 6.21$, $p = 0.018$, $\eta^2_{par} = 0.17$; N2: $F_{(1,30)} = 7.20$, $p = 0.012$, $\eta^2_{par} = 0.19$). *Post hoc*

TABLE 2 | Descriptive statistic for component analysis.

		picture				stimulation		
		M(SE)	*p*	$CI_{Difference}$		M(SE)	*p*	$CI_{Difference}$
P1	food	6.61 (0.61)	0.055[t]	−0.01 0.86	*tVNS*	6.18 (0.61)	0.028*	−0.78 −0.05
	object	6.20 (0.60)			*Sham*	6.63 (0.60)		
N1	food	0.67 (0.43)	0.587	−0.20 0.34	*tVNS*	0.50 (0.45)	0.119	−0.60 0.07
	object	0.60 (0.41)			*Sham*	0.77 (0.40)		
P2	food	15.94 (1.13)	<0.001***	1.36 2.38	*tVNS*	14.05 (1,12)	0.018*	−1.85 −0.18
	object	13.63 (1.02)			*Sham*	15.07 (1.05)		
N2	food	0.88 (0.56)	<0.001***	2.75 3.39	*tVNS*	−0.91 (0.58)	0.012*	−0.89 −0.12
	object	−2.19 (0.55)			*Sham*	−0.40 (0.53)		
LPP	food	7.26 (0.57)	<0.001***	0.62 1.23	*tVNS*	6.48 (0.59)	0.059[t]	−1.30 0.03
	object	6.34 (0.58)			*sham*	7.12 (0.62)		

*Significance of two-sided paired sample t-test: *p < 0.05, ***p < 0.001. [t]Statistical trend at 10% significance level. Mean (M), standard error (SE), significance (p) and 95%-confidence interval. n = 31.*

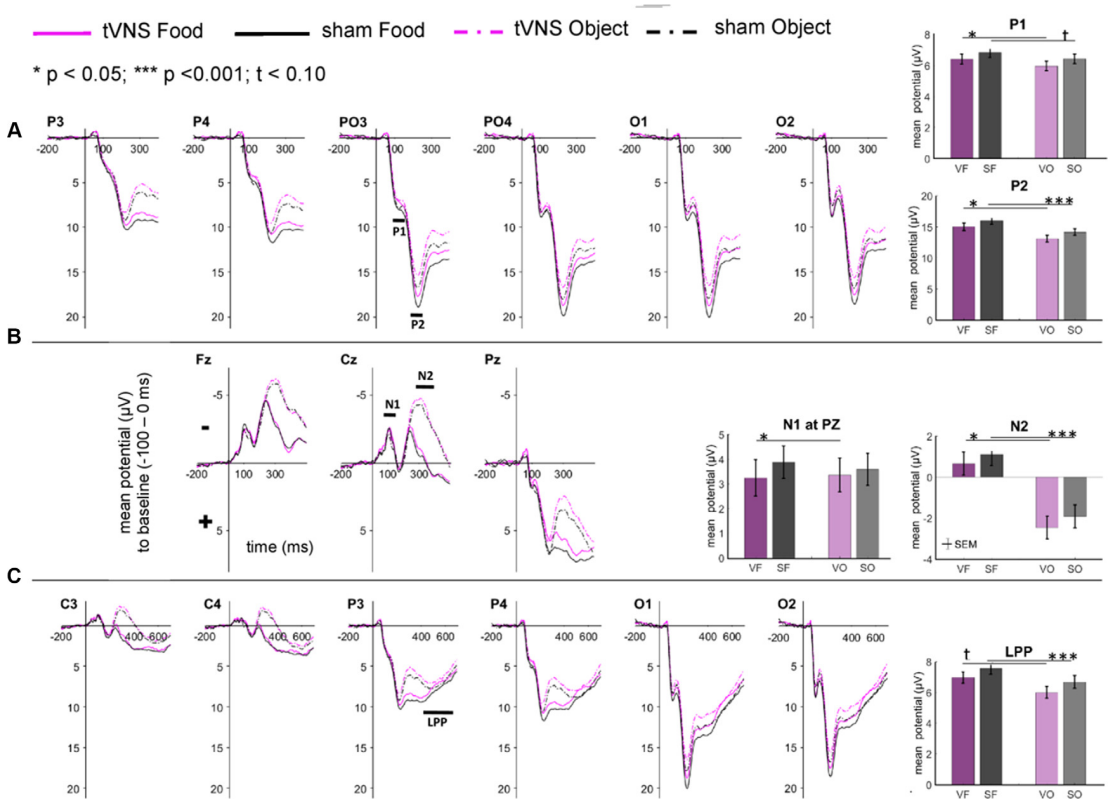


FIGURE 3 | Effects of tVNS on ERPs calculated with frequential statistics. Channel **(A)** shows positive potentials (P1 and P2), channel **(B)** negative potentials (N1 and N2) and channel **(C)** LPP potential. Line plots: mean change in electrical potentials (µV) in relation to a baseline (−100 to stimulus onset) over the time (x-axis) are shown for the conditions (lines). Bar plots: mean potential for each task condition pooled across electrodes. Abbreviations describe task conditions (stimulation and picture type; VF = tVNS Food, SF = Sham Food, VO = tVNS Object, SO = Sham Object). Horizontal lines signify statistical main effects. $^{t}p < 0.10$, $^{*}p < 0.05$, $^{***}p < 0.001$.

testing of these stimulation main effects showed small (P2, $BF_{(10)}$ = 2.66) to moderate (N2, $BF_{(10)}$ = 3.88) effect sizes (**Figures 4C,D**). Moreover, food pictures evoked higher P2 but smaller N2 amplitudes compared to object pictures (P2: $F_{(1,30)}$ = 57.05, $p < 0.001$, η^2_{par} = 0.66; N2: $F_{(1,30)}$ = 384.32 $p < 0.001$, η^2_{par} = 0.93). The interaction between the stimulation and any other factor was not significant for the P2, nor the N2-component (P2: $F_{(1,30)}$ = 0.22, p = 0.643, η^2_{par} = 0.01; N2: $F_{(1,30)}$ = 0.22, p = 0.644, η^2_{par} = 0.01).

Larger LPPs (**Figure 3C**) were observed in response to food compared to object pictures ($F_{(1,30)}$ = 37.52 $p \leq 0.001$, η^2_{par} = 0.56). In the tVNS condition, LPP amplitudes were smaller than in the sham condition but the difference reached only a statistically trend level ($F_{(1,30)}$ = 3.87 p = 0.059, η^2_{par} = 0.11). The uncertainty is also reflected by a $BF_{(01)}$ of 0.91 for the null and $BF_{(10)}$ of 1.04 for the alternative hypothesis (see **Figure 4E**). No further significant interactions between the stimulation and any other factor were observed.

Results on Food Consumption

The total food intake (in kcal); the consumption of protein, fat, and carbohydrates; and the duration of food consumption are given in **Table 3**. No stimulation effects were found, neither for the general food consumption nor the eaten amount of protein, fat, or carbohydrates.

Correlation of Electrophysiological Stimulation Effects With Food Consumption

As expected, the number of consumed kilocalories correlated positively with the BMI in the control condition, but only as a statistical trend effect (r = 0.35, p = 0.057, see **Figure 5A**). Interestingly, this correlation was even weaker in the tVNS condition (r = 0.29, p = 0.112). As displayed in **Figure 5B**, P2 amplitudes (pooled across task conditions) and BMI correlated negatively in both stimulation conditions (tVNS: r = −0.40, p = 0.028; sham: r = −0.41, p = 0.021) while the amount of food intake (see **Figure 5C** and **Supplementary Figure S1**) correlated with the P2 amplitudes in the sham (r = −0.38 p = 0.035) but not in the tVNS condition (r = −0.291, p = 0.112). Corrected for multiple testing (0.05/5 = 0.01), all correlations missed statistical significance. However, the found relations are supported by the corresponding BFs (food intake and BMI: tVNS = 0.75, sham = 1.27; BMI and P2: tVNS = 2.27, sham = *2.85*; food intake and P2: tVNS = 0.7, sham = 2.27).

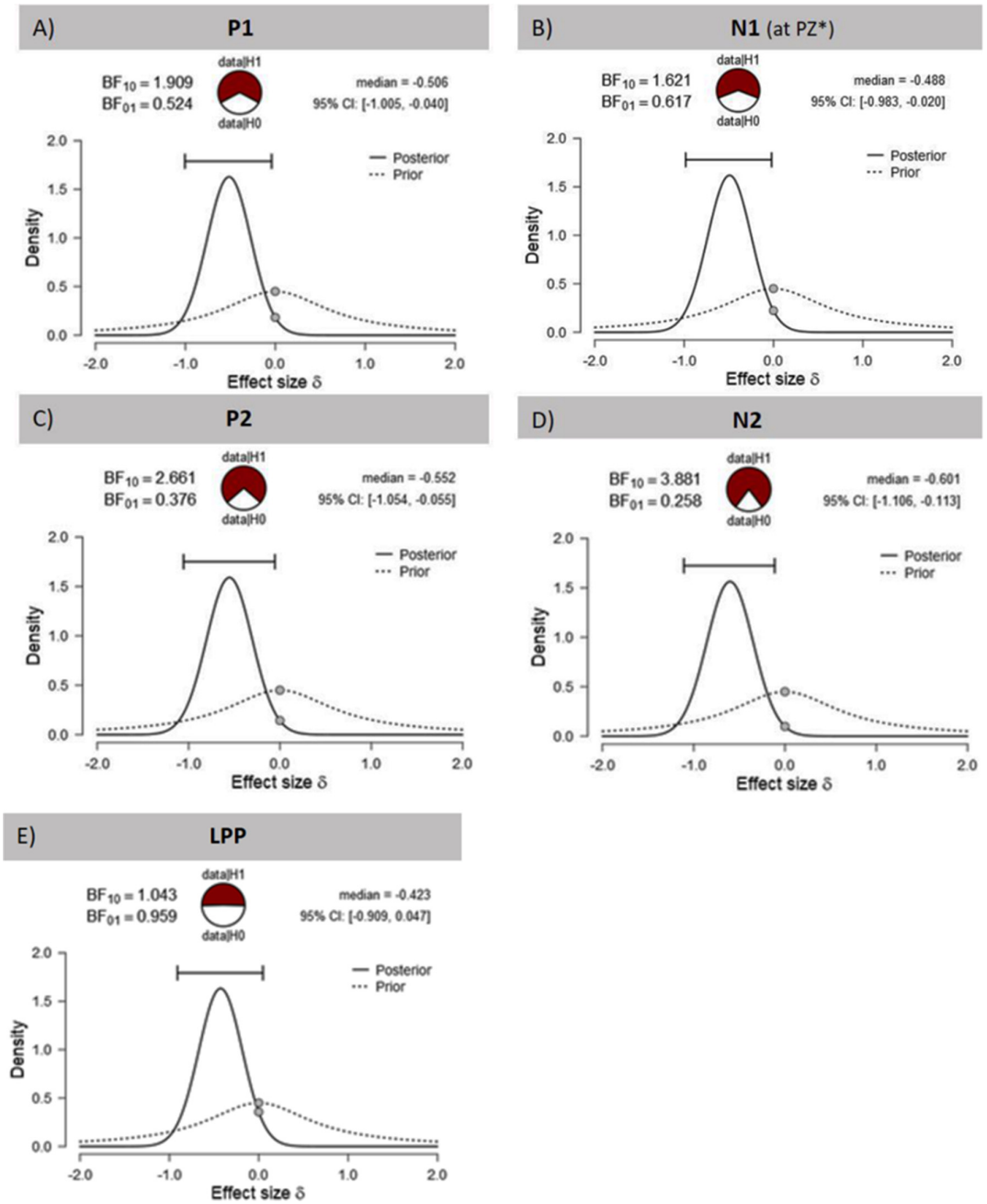


FIGURE 4 | Effects of tVNS on ERPs calculated with Bayesian statistics. Graphics show the prior and posterior probability density for the stimulation main effect on **(A,B)** early latency phase components P1 and N1, **(C,D)** on middle latency phase components P2 and N2 and **(E)** on late latency phase component LPP. CI describes the Bayesian credible interval. $BF_{(10)}$ is the Bayes factor in favour to the alternative hypothesis (H1) and $BF_{(01)}$ in favor of the null hypothesis. *Result of exploratory analysis.

DISCUSSION

The present study used event-related potentials to test tVNS' impact on food-related cognitive functions and whether these changes mediate food intake.

As hypothesized, our study shows that tVNS indeed has an impact on EEG components related to the processing of visual stimuli. TVNS significantly decreased P1 and P2 amplitudes and increased N2 amplitudes compared to sham stimulation. A similar trend was also seen for the LPP component. Against our assumptions, stimulation effects were food-stimulus-unspecific given that none of the expected interactions between stimulation condition and picture type reached significance. We also could not find any effects of tVNS on food intake. However, we did find

TABLE 3 | Descriptive food consumption statistics.

Food consumption	mdn (IQR) tVNS	mdn (IQR) Sham	$t_{(30)}$	p	g	$BF_{(10)}$
kCal (g)	1,307 (891–1,454)	1,144 (960–1,335)	0.10	0.92	0.02	0.19
Proteins (g)	39 (32–49)	39 (28–46)	0.20	0.49	0.26	0.20
Fat (g)	48 (36–59)	48 (41–59)	0.71	0.85	0.07	0.24
Carbohydrates (g)	144 (102–177)	138 (112–164)	0.94	0.36	0.39	0.29

Medians (mdn), inter quartile ratio (IQR), test statistic (t, p) and effect size (Hedges' g) and Bayes Factor (BF) for consumed kilocalories (kcal) and food ingredients for both conditions (tVNS, sham), n = 31.

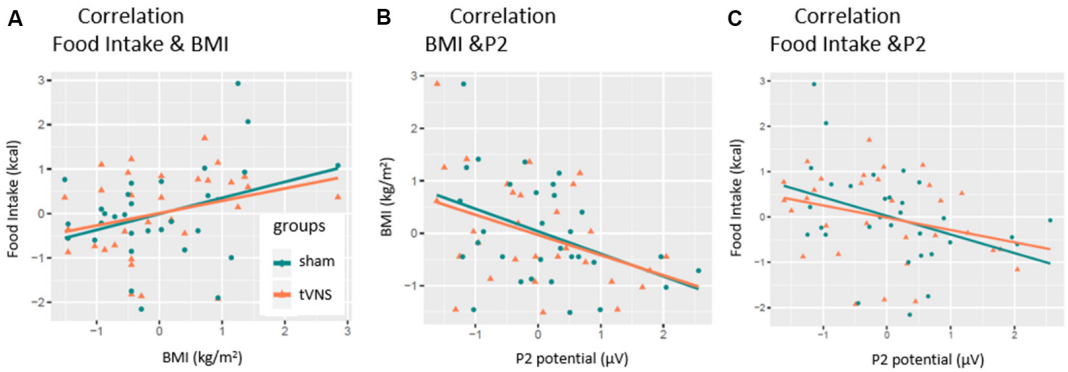


FIGURE 5 | Correlation of P2-amplitude and behavioral outcomes. P2 amplitudes are pooled across electrodes and task conditions. Significant relations were found for **(A)** only sham condition. **(B)** Both conditions. **(C)** Only sham condition.

a significant relationship between P2 amplitudes and the food consumption in sham but not with tVNS.

The reliability of our results is supported by the previously reported differential processing of food and object items (Plihal et al., 2001; Schacht et al., 2016; Carbine et al., 2018). The significant stimulation main effects are additionally confirmed by the results of Bayesian statistics. However, while large effect sizes were calculated using frequential statistics (η^2_{par} range: 0.14–0.19), the results of Bayesian statistics indicated small to medium effect sizes ($BF_{(10)}$ range: 1.6–3.88). It can therefore be assumed that the actual effect sizes are in the middle, since the results of Bayesian statistics can be regarded as the lower limit.

Stimulation effects on P1 and N1 suggest that tVNS already modulates initial sensory and visual attentional functions (Hillyard and Anllo-Vento, 1998; Bernat et al., 2001). More specifically, the reduced P1 and increased N1 potentials can be speculated to represent improved flexibility in attentional directing and/or allocation by reducing the costs of disengaging (P1) and an increased benefit in guiding attention (N1; Luck et al., 1990). This is corroborated by a study in which cervical VNS increased N1 amplitude in a working memory task in epilepsy patients (Sun et al., 2017).

P2 has been reported to be elevated in obese subjects (Nijs et al., 2010; Carbine et al., 2018), as well as in restraint-eaters in a hungry state (Plihal et al., 2001; Hachl et al., 2003), and to be decreased in the latter group after food intake. Thus, P2 was interpreted to indicate the arousing value of food (Sänger, 2019). Considering these findings, the decreased P2 amplitudes in the tVNS condition might indicate that tVNS reduces the processing of external stimuli unspecifically.

However, in our study, we also found a negative correlation between P2 amplitudes and food intake but only in the control group. This correlation is interesting because it implies a second factor by which the P2 component can be modulated—the blood glucose level. Previous research described reduced P2 amplitudes during hypoglycemic states in a food-unrelated cognitive task (Schultes et al., 2005; Svaldi et al., 2010). Presuming that the (preceding) blood glucose level influences the amount of food intake (during homeostatic eating), it is justified to infer (from our correlation) that the higher food intake after the examination indicates lower blood glucose levels (reflected by the P2 amplitudes) at the beginning of our examination. Importantly, that relation was not significant in the tVNS condition (see **Figure 5**), pointing to the discussed impact of tVNS on food metabolism and the modulation of satiety signaling (Banni et al., 2012; Malbert et al., 2017). This interpretation is in line with the increased P2 amplitudes observed in obese individuals since obesity is known to manifest higher blood glucose levels (Spiegelman et al., 1992). Taken together, our results imply that the P2 amplitudes are not only moderated by arousal—a fast switching reaction toward stimuli—but also by basic metabolic states. Unfortunately, we did not examine further somatic state variables as well as food intake-related behavior (hungriness, craving, wanting, liking; Berridge and Robinson, 1998) more closely.

The increased N2 amplitudes in the tVNS condition might tentatively suggest that tVNS enhances inhibitory processes and/or conflict monitoring (Carretié et al., 2004; Jonkman et al., 2007; Folstein and Van Petten, 2008) given prior findings on this component. This is in line with prior assumptions that (t)VNS modulates the NE-hormone release in the brain that is associated with neural inhibition (Henry, 2002; Fornai et al., 2011). Moreover, effects on conflict processing have already been reported by Fischer et al. (2018). This interpretation should be tested further in dedicated studies.

While visual inspection suggested a less pronounced LPP in the tVNS condition, this effect was neither robust with conventional statistics (p = 0.059) nor with Bayes statistics ($BF_{(01)}$ = 1; $BF_{(10)}$ = 1).

Finally, the fact that we did not find any effects on food intake can be explained by at least three reasons. First, in the present study normal-weight individuals were tested. However, Pardo et al. (2007) suggested that the VNS effects could be weight-dependent; the higher the initial body weight, the stronger the VNS effect on body weight. Second, we applied tVNS for approximately 2 h but studies showing effects on food intake (and body weight) stimulated much longer (weeks or months). Therefore, the time of stimulation could have been too short to reveal behavioral effects and we recommend long-term trials. Third, despite hunger, food intake is driven by a variety of reasons for example by habits or as a strategy for emotion regulation (Davidson et al., 2019). While habits should be constant in each participant, the personal stress level could have been varied. However, both could have overlaid possible effects on food intake—while the top-down regulated habits ("I always eat only a croissant in the morning") overwrite bottom-up salience allocation and/or hunger feelings and therefore the assumed tVNS effects, the evaluation of the stress level before the test meal could have been a valuable covariate. Therefore, food intake behavior and the states of participants should be surveyed more precisely.

To summarize, while our study failed to reveal an effect of short-term tVNS on food consumption and differential processing of food pictures (i.e., no interaction between tVNS and picture type), a general effect on several ERP components was found that indicates a possible influence on attentional and inhibitory aspects in visual perception processes. We, therefore, suggest two lines of research for future studies. First, given the reported effects on weight in long-term invasive VNS, a longer-term intervention study seems to be justified. Second, the potential effects of tVNS on attentional and inhibitory cognitive functions need to be examined using dedicated paradigms. This information might also be important to judge potential side effects of this method of non-invasive brain stimulation.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of University of Lübeck. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

Experimental design was developed by MH, MT, and TM. Data were collected by MO and HA. Statistical analysis was performed by MO under supervision of MH and TM. Bayesian analysis was performed by MO. Manuscript was written by MO, MH, and TM. All authors read and approved the final manuscript.

FUNDING

This work was supported by the Deutsche Forschungsgemeinschaft (DFG; grant number MU 1311/11-3 and BMBF-grant 01GO0202). We also acknowledge financial support by Land Schleswig-Holstein within the funding programme Open Access Publikationsfonds.

ACKNOWLEDGMENTS

We also want to thank Dr. Arkan Al-Zubaidi and Marius Möller for very helpful discussions as well as Dr. Fränze Kibowski for proofreading.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnhum.2020.00206/full#supplementary-material.

REFERENCES

Abubakr, A., and Wambacq, I. (2008). Long-term outcome of vagus nerve stimulation therapy in patients with refractory epilepsy. *J. Clin. Neurosci.* 15, 127–129. doi: 10.1016/j.jocn.2007.07.083

Afshin, A., Forouzanfar, M. H., Reitsma, M. B., Sur, P., Estep, K., Lee, A., et al. (2017). Health effects of overweight and obesity in 195 countries over 25 years. *N. Engl. J. Med.* 377, 13–27. doi: 10.1056/NEJMoa1614362

Al-Najim, W., Docherty, N. G., and Le Roux, C. W. (2018). Food intake and eating behavior after bariatric surgery. *Physiol. Rev.* 98, 1113–1141. doi: 10.1152/physrev.00021.2017

Banni, S., Carta, G., Murru, E., Cordeddu, L., Giordano, E., Marrosu, F., et al. (2012). Vagus nerve stimulation reduces body weight and fat mass in rats. *PLoS One* 7:e44813. doi: 10.1371/journal.pone.0044813

Bell, A., and Sejnowski, T. (1995). Information-maximization approach to blind separation and blind deconvolution. *Neural Comput.* 7, 1129–1159. doi: 10.1162/neco.1995.7.6.1129

Bernat, E., Bunce, S., and Shevrin, H. (2001). Event-related brain potentials differentiate positive and negative mood adjectives during both supraliminal and subliminal visual processing. *Int. J. Psychophysiol.* 42, 11–34. doi: 10.1016/s0167-8760(01)00133-7

Berridge, K. C., and Robinson, T. E. (1998). What is the role of dopamine in reward: hedonics, learning, or incentive salience? *Brain Res. Rev.* 28, 308–367.

Berthoud, H. R., and Neuhuber, W. L. (2000). Functional and chemical anatomy of the afferent vagal system. *Auton. Neurosci.* 85, 1–17. doi: 10.1016/S1566-0702(00)00215-0

Bodenlos, J. S., Kose, S., Borckardt, J. J., Nahas, Z., Shaw, D., O'Neil, P. M., et al. (2007). Vagus nerve stimulation acutely alters food craving in adults with depression. *Appetite* 48, 145–153. doi: 10.1016/j.appet.2006.07.080

Bugajski, A. J., Gil, K., Ziomber, A., Zurowski, D., Zaraska, W., and Thor, P. J. (2007). Effect of long-term vagal stimulation on food intake and body weight during diet induced obesity in rats. *J. Physiol. Pharmacol.* 58, 5–12.

Burneo, J. G., Faught, E., Knowlton, R., Morawetz, R., and Kuzniecky, R. (2002). Weight loss associated with vagus nerve stimulation. *Neurology* 59, 463–464. doi: 10.1212/wnl.59.3.463

Butryn, M. L., Webb, V., and Wadden, T. A. (2011). Behavioral treatment of obesity. *Psychiatr. Clin. North Am.* 34, 841–859. doi: 10.1016/j.psc.2011.08.006

Carbine, K. A., Christensen, E., LeCheminant, J. D., Bailey, B. W., Tucker, L. A., and Larson, M. J. (2017). Testing food-related inhibitory control to high- and low-calorie food stimuli: electrophysiological responses to high-calorie food stimuli predict calorie and carbohydrate intake. *Psychophysiology* 54, 982–997. doi: 10.1111/psyp.12860

Carbine, K. A., Rodeback, R., Modersitzki, E., Miner, M., LeCheminant, J. D., and Larson, M. J. (2018). The utility of event-related potentials (ERPs) in understanding food-related cognition: a systematic review and recommendations. *Appetite* 128, 58–78. doi: 10.1016/j.appet.2018.05.135

Carretié, L., Hinojosa, J. A., Martín-Loeches, M., Mercado, F., and Tapia, M. (2004). Automatic attention to emotional stimuli: neural correlates. *Hum. Brain Mapp.* 22, 290–299. doi: 10.1002/hbm.20037

Chae, J. H., Nahas, Z., Lomarev, M., Denslow, S., Lorberbaum, J. P., Bohning, D. E., et al. (2003). A review of functional neuroimaging studies of vagus nerve stimulation (VNS). *J. Psychiatr. Res.* 37, 443–455. doi: 10.1016/s0022-3956(03)00074-8

Colzato, L. S., Ritter, S. M., and Steenbergen, L. (2018). Transcutaneous vagus nerve stimulation (TVNS) enhances divergent thinking. *Neuropsychol.* 111, 72–76. doi: 10.1016/j.neuropsychologia.2018.01.003

Davidson, T. L., Jones, S., Roy, M., and Stevenson, R. J. (2019). The cognitive control of eating and body weight: it's more than what you "think". *Front. Psychol.* 10:62. doi: 10.3389/fpsyg.2019.00062

De Lartigue, G. (2016). Role of the vagus nerve in the development and treatment of diet-induced obesity. *J. Physiol.* 594, 5791–5815. doi: 10.1113/JP271538

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

De Silva, A., Salem, V., Matthews, P. M., and Dhillo, W. S. (2012). The use of functional MRI to study appetite control in the CNS. *Exp. Diabetes Res.* 2012:764017. doi: 10.1155/2012/764017

Ellrich, J. (2011). Transcutaneous vagus nerve stimulation. *Eur. Neurol. Rev.* 6, 254–256. doi: 10.17925/ENR.2011.06.04.254

Ferrario, C. R., Labouèbe, G., Liu, S., Nieh, E. H., Routh, V. H., Xu, S., et al. (2016). Homeostasis meets motivation in the battle to control food intake. *J. Neurosci.* 36, 11469–11481. doi: 10.1523/JNEUROSCI.2338-16.2016

Fischer, R., Ventura-Bort, C., Hamm, A., and Weymar, M. (2018). Transcutaneous vagus nerve stimulation (tVNS) enhances conflict-triggered adjustment of cognitive control. *Cogn. Affect. Behav. Neurosci.* 18, 680–693. doi: 10.3758/s13415-018-0596-2

Folstein, J. R., and Van Petten, C. (2008). Influence of cognitive control and mismatch on the N2 component of the ERP: a review. *Psychophysiology* 45, 152–170. doi: 10.1111/j.1469-8986.2007.00602.x

Fornai, F., Ruffoli, R., Giorgi, F. S., and Paparelli, A. (2011). The role of locus coeruleus in the antiepileptic activity induced by vagus nerve stimulation. *Eur. J. Neurosci.* 33, 2169–2178. doi: 10.1111/j.1460-9568.2011.07707.x

Frangos, E., Ellrich, J., and Komisaruk, B. (2015). Non-invasive access to the vagus nerve central projections *via* electrical stimulation of the external ear: fMRI evidence in humans. *Brain Stimul.* 8, 624–636. doi: 10.1016/j.brs.2014.11.018

Gelman, A., Hill, J., and Yajima, M. (2012). Why we (usually) don't have to worry about multiple comparisons. *J. Res. Educ. Effect.* 5, 189–211. doi: 10.1080/19345747.2011.618213

Ghani, S., Vilensky, J., Turner, B., Tubbs, R. S., and Loukas, M. (2015). Meta-analysis of vagus nerve stimulation treatment for epilepsy: correlation between device setting parameters and acute response. *Childs Nerv. Syst.* 31, 2291–2304. doi: 10.1007/s00381-015-2921-1

Gil, K., Bugajski, A., and Thor, P. (2011). Electrical vagus nerve stimulation decreases food consumption and weight gain in rats fed a high-fat diet. *J. Physiol. Pharmacol.* 62, 637–646.

Göbel, C. H., Tronnier, V. M., and Münte, T. F. (2017). Brain stimulation in obesity. *Int. J. Obes.* 41, 1721–1727. doi: 10.1038/ijo.2017.150

Gorgulho, A. A., Pereira, J. L. B., Krahl, S., Lemaire, J. J., and De Salles, A. (2014). Neuromodulation for eating disorders. Obesity and anorexia. *Neurosurg. Clin. N. Am.* 25, 147–157. doi: 10.1016/j.nec.2013.08.005

Hachl, P., Hempel, C., and Pietrowsky, R. (2003). ERPs to stimulus identification in persons with restrained eating behavior. *Int. J. Psychophysiol.* 49, 111–121. doi: 10.1016/s0167-8760(03)00099-0

Han, L., Jian-Bin, Z., Chen, X., Qing-Qing, T., Wei-Xing, S., Jing-Zhu, Z., et al. (2015). Effects and mechanisms of auricular vagus nerve stimulation on high-fat-diet-induced obese rats. *Nutrition* 31, 1416–1422. doi: 10.1016/j.nut.2015.05.007

Henry, T. R. (2002). Therapeutic mechanisms of vagus nerve stimulation. *Neurology* 59, S3–S14. doi: 10.1212/wnl.59.6_suppl_4.s3

Hillyard, S. A., and Anllo-Vento, L. (1998). Event-related brain potentials in the study of visual selective attention. *Proc. Natl. Acad. Sci. U S A* 95, 781–787. doi: 10.1073/pnas.95.3.781

Jacobs, H. I. L., Riphagen, J. M., Razat, C. M., Wiese, S., and Sack, A. T. (2015). Transcutaneous vagus nerve stimulation boosts associative memory in older individuals. *Neurobiol. Aging* 36, 1860–1867. doi: 10.1016/j.neurobiolaging.2015.02.023

Johnson, R. L., and Wilson, C. G. (2018). A review of vagus nerve stimulation as a therapeutic intervention. *J. Inflamm. Res.* 11, 203–213. doi: 10.2147/jir.s163248

Jongkees, B. J., Immink, M. A., Finisguerra, A., and Colzato, L. S. (2018). Transcutaneous vagus nerve stimulation (tVNS) Enhances response selection during sequential action. *Front. Psychol.* 9:1159. doi: 10.3389/fpsyg.2018.01159

Jonkman, L. M., Sniedt, F. L. F., and Kemner, C. (2007). Source localization of the Nogo-N2: a developmental study. *Clin. Neurophysiol.* 118, 1069–1077. doi: 10.1016/j.clinph.2007.01.017

Kass, R. E., and Raftery, A. E. (1995). Bayes factors. *J. Am. Stat. Asso.* 90, 773–795. doi: 10.1080/01621459.1995.10476572

Kakkar, A. K., and Dahiya, N. (2015). Drug treatment of obesity: current status and future prospects. *Eur. J. Intern. Med.* 26, 89–94. doi: 10.1016/j.ejim.2015.01.005

Kennedy, J. E. (2015). Beware of inferential errors and low power with Bayesian analyses: power analysis is needed for confirmatory research. *J. Parapsychol.* 79, 53–64.

Kong, F., Zhang, Y., and Chen, H. (2015). Inhibition ability of food cues between successful and unsuccessful restrained eaters: a two-choice oddball task. *PLoS One* 10:e0120522. doi: 10.1371/journal.pone.0133942

Lomarev, M., Denslow, S., Nahas, Z., Chae, J. H., George, M. S., and Bohning, D. E. (2002). Vagus nerve stimulation (VNS) synchronized BOLD fMRI suggests that VNS in depressed adults has frequency/dose dependent effects. *J. Psychiatr. Res.* 36, 219–227. doi: 10.1016/s0022-3956(02)00013-4

Lopez-Calderon, J., and Luck, S. J. (2014). ERPLAB: an open-source toolbox for the analysis of event-related potentials. *Front. Hum. Neurosci.* 8:213. doi: 10.3389/fnhum.2014.00213

Luck, S. J., Heinze, H. J., Mangun, G. R., and Hillyard, S. A. (1990). Visual event-related potentials indexed focused attention within bilateral stimulus arrays. II. Functional dissociation of P1 and N1 components. *Electroencephalogr. Clin. Neurophysiol.* 75, 528–542. doi: 10.1016/0013-4694(90)90139-b

Malbert, C. H., Picq, C., Divoux, J. L., Henry, C., Horowitz, M., Love, J., et al. (2017). Obesity-Associated alterations in glucose metabolism are reversed by chronic bilateral stimulation of the abdominal vagus nerve. *Diabetes* 66, 848–857. doi: 10.2337/db16-0847

Makeig, S., Bell, A. J., Jung, T.-P., and Sejnowski, T. J. (1996). "Independent component analysis of electroencephalographic data," in *Advances in Neural Infomation Processing Systems* (Vol. 8), eds D. Touretzky, M. Mozer and M. Hasselmo (Cambridge, MA: MIT Press), pp. 145–151.

McClelland, J., Bozhilova, N., Campbell, I., and Schmidt, U. (2013). A systematic review of the effects of neuromodulation on eating and body weight:

evidence from human and animal studies. *Eur. Eat. Disord. Rev.* 21, 436–455. doi: 10.1002/erv.2256

Nijs, I. M. T., Franken, I. H. A., and Muris, P. (2010). Food-related Stroop interference in obese and normal-weight individuals: behavioral and electrophysiological indices. *Eat. Behav.* 11, 258–265. doi: 10.1016/j.eatbeh.2010.07.002

Pardo, J. V., Sheikh, S. A., Kuskowski, M. A., Surerus-Johnson, C., Hagen, M. C., Lee, J. T., et al. (2007). Weight loss during chronic, cervical vagus nerve stimulation in depressed patients with obesity: an observation. *Int. J. Obes.* 31, 1756–1759. doi: 10.1038/sj.ijo.0803666

Pelot, N. A., and Grill, W. M. (2018). Effects of vagal neuromodulation on feeding behavior. *Brain Res.* 1693, 180–187. doi: 10.1016/j.brainres.2018.02.003

Plihal, W., Haenschel, C., Hachl, P., Born, J., and Pietrowsky, R. (2001). The effect of food deprivation on ERP during identification of tachistoscopically presented food-related words. *J. Psychophysiol.* 15, 163–172. doi: 10.1027/0269-8803.15.3.163

Roslin, M., and Kurian, M. (2001). The use of electrical stimulation of the vagus nerve to treat morbid obesity. *Epilepsy Behav.* 2, S11–S16. doi: 10.1006/ebeh.2001.0213

Sainsbury, A., and Zhang, L. (2012). Role of the hypothalamus in the neuroendocrine regulation of body weight and composition during energy deficit. *Obes. Rev.* 13, 234–257. doi: 10.1111/j.1467-789x.2011.00948.x

Sänger, J. (2019). Can't take my eyes off you—How task irrelevant pictures of food influence attentional selection. *Appetite* 133, 313–323. doi: 10.1016/j.appet.2018.11.030

Schacht, A., €uczak, A., Pinkpank, T., Vilgis, T., and Sommer, W. (2016). The valence of food in pictures and on the plate: impacts on brain and body. *Int. J. Gastr. Food Sci.* 5–6, 33–40. doi: 10.1016/j.ijgfs.2016.11.002

Schultes, B., Kern, W., Oltmanns, K., Peters, A., Gais, S., Fehm, H. L., et al. (2005). Differential adaptation of neurocognitive brain functions to recurrent hypoglycemia in healthy men. *Psychoneuroendocrinology* 30, 149–161. doi: 10.1016/j.psyneuen.2004.06.007

Sellaro, R., de Gelder, B., Finisguerra, A., and Colzato, L. S. (2018). Transcutaneous vagus nerve stimulation (tVNS) enhances recognition of emotions in faces but not bodies. *Cortex* 99, 213–223. doi: 10.1016/j.cortex.2017.11.007

Sobocki, J., Fourtanier, G., Estany, J., and Otal, P. (2006). Does vagal nerve stimulation affect body composition and metabolism? Experimental study of a new potential technique in bariatric surgery. *Surgery* 139, 209–216. doi: 10.1016/j.surg.2005.06.025

Sobocki, J., Thor, P., Krolczyk, G., Uson, J., Diaz-Guemes, I., and Lipinski, M. (2002). The cybergut. An experimental study on permanent microchip neuromodulation for control of gut function. *Acta Chir. Belg.* 102, 68–70. doi: 10.1080/00015458.2002.11679268

Spiegelman, D., Israel, R. G., Bouchard, C., and Willett, W. C. (1992). Absolute fat mass, percent body fat and body-fat distribution: which is the real determinant of blood pressure and serum glucose? *Am. J. Clin. Nutr.* 55, 1033–1044. doi: 10.1093/ajcn/55.6.1033

Sun, L., Peräkylä, J., Holm, K., Haapasalo, J., Lehtimäki, K., Ogawa, K. H., et al. (2017). Vagus nerve stimulation improves working memory performance. *J. Clin. Exp. Neuropsychol.* 39, 954–964. doi: 10.1080/13803395.2017.1285869

Svaldi, J., Tuschen-Caffier, B., Peyk, P., and Blechert, J. (2010). Information processing of food pictures in binge eating disorder. *Appetite* 55, 685–694. doi: 10.1016/j.appet.2010.10.002

Val-Laillet, D., Aarts, E., Weber, B., Ferrari, M., Quaresima, V., Stoeckel, L. E., et al. (2015). Neuroimaging and neuromodulation approaches to study eating behavior and prevent and treat eating disorders and obesity. *Neuroimage Clin.* 8, 1–31. doi: 10.1016/j.nicl.2015.03.016

Val-Laillet, D., Biraben, A., Randuineau, G., and Malbert, C. H. (2010). Chronic vagus nerve stimulation decreased weight gain, food consumption and sweet craving in adult obese minipigs. *Appetite* 55, 245–252. doi: 10.1016/j.appet.2010.06.008

Van Bockstaele, E. J., Peoples, J., and Valentino, R. J. (1999). Anatomic basis for differential regulation of the rostrolateral peri- locus coeruleus region by limbic afferents. *Biol. Psychiatry* 46, 1352–1363. doi: 10.1016/s0006-3223(99)00213-9

Versace, F., Kypriotakis, G., Basen-Engquist, K., and Schembre, S. M. (2016). Heterogeneity in brain reactivity to pleasant and food cues: evidence of sign-tracking in humans. *Soc. Cogn. Affect. Neurosci.* 11, 604–611. doi: 10.1093/scan/nsv143

Vijgen, G. H. E. J., Bouvy, N. D., Leenen, L., Rijkers, K., Cornips, E., Majoie, M., et al. (2013). Vagus nerve stimulation increases energy expenditure: relation to brown adipose tissue activity. *PLoS One* 8:e77221. doi: 10.1371/journal.pone.0077221

Wagenmakers, E. J., Marsman, M., Jamil, T., Ly, A., Verhagen, J., Love, J., et al. (2018). Bayesian inference for psychology. Part I: theoretical advantages and practical ramifications. *Psychon. Bull. Rev.* 25, 35–57. doi: 10.3758/s13423-017-1343-3

Wagenmakers, E. J., Verhagen, J., and Ly, A. (2016). How to quantify the evidence for the absence of a correlation. *Behav. Res. Methods* 48, 413–426. doi: 10.3758/s13428-015-0593-0

Warren, C. M., Tona, K. D., Ouwerkerk, L., van Paridon, J., Poletiek, F., van Steenbergen, H., et al. (2019). The neuromodulatory and hormonal effects of transcutaneous vagus nerve stimulation as evidenced by salivary alpha amylase, salivary cortisol, pupil diameter, and the P3 event-related potential. *Brain Stimulation* 12, 635–642. doi: 10.1016/j.brs.2018.12.224

Watson, T. D., and Garvey, K. T. (2013). Neurocognitive correlates of processing food-related stimuli in a Go/No-go paradigm. *Appetite* 71, 40–47. doi: 10.1016/j.appet.2013.07.007

World Health Organization (WHO). (2020). Obesity and overweight. Available online at: https://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight.

Conflict of Interest: 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.

frontiers in Psychology

ORIGINAL RESEARCH
published: 09 July 2020
doi: 10.3389/fpsyg.2020.01276

Transcutaneous Vagus Nerve Stimulation (tVNS) Improves High-Confidence Recognition Memory but Not Emotional Word Processing

Manon Giraudier, Carlos Ventura-Bort and Mathias Weymar**

Department of Biological Psychology and Affective Science, University of Potsdam, Potsdam, Germany

Previous clinical research found that invasive vagus nerve stimulation (VNS) enhanced word recognition memory in epileptic patients, an effect assumed to be related to the activation of brainstem arousal systems. In this study, we applied non-invasive transcutaneous auricular VNS (tVNS) to replicate and extend the previous work. Using a single-blind, randomized, between-subject design, 60 healthy volunteers received active or sham stimulation during a lexical decision task, in which emotional and neutral stimuli were classified as words or non-words. In a subsequent recognition memory task (1 day after stimulation), participants' memory performance on these words and their subjective memory confidence were tested. Salivary alpha-amylase (sAA) levels, a putative indirect measure of central noradrenergic activation, were also measured before and after stimulation. During encoding, pleasant words were more accurately detected than neutral and unpleasant words. However, no tVNS effects were observed on task performance or on overall sAA level changes. tVNS also did not modulate overall recognition memory, which was particularly enhanced for pleasant emotional words. However, when hit rates were split based on confidence ratings reflecting familiarity- and recollection-based memory, higher recollection-based memory performance (irrespective of emotional category) was observed during active stimulation than during sham stimulation. To summarize, we replicated prior findings of enhanced processing and memory for emotional (pleasant) words. Whereas tVNS showed no effects on word processing, subtle effects on recollection-based memory performance emerged, which may indicate that tVNS facilitates hippocampus-mediated consolidation processes.

Keywords: transcutaneous vagus nerve stimulation, salivary alpha-amylase, emotion, words, episodic memory, recognition, recollection, confidence

OPEN ACCESS

Edited by:
Claudia Gianelli, University Institute of Higher Studies in Pavia, Italy

Reviewed by:
Sander Nieuwenhuis, Leiden University, Netherlands
Giuseppe Curcio, University of L'Aquila, Italy

****Correspondence:***
Manon Giraudier
giraudier@uni-potsdam.de
Mathias Weymar
mathias.weymar@uni-potsdam.de

Specialty section:
This article was submitted to Cognition, a section of the journal Frontiers in Psychology

Received: *10 March 2020*
Accepted: *15 May 2020*
Published: *09 July 2020*

Citation:
Giraudier M, Ventura-Bort C and Weymar M (2020) Transcutaneous Vagus Nerve Stimulation (tVNS) Improves High-Confidence Recognition Memory but Not Emotional Word Processing. Front. Psychol. 11:1276. doi: 10.3389/fpsyg.2020.01276

1. INTRODUCTION

One of the core aspects of adaptive behavior is the detection and remembrance of emotionally salient information. Ample evidence suggests that emotional content impacts various stages of processing, from initial encoding to later long-term retrieval (Dolcos et al., 2017, 2020b). For instance, emotionally salient events capture more attentional resources and are detected more

efficiently than their neutral counterparts (Dolan, 2002). This effect has been demonstrated across a range of paradigms, including visual search (Fox et al., 2000), spatial cueing (Armony and Dolan, 2002), dot-probe (Mogg et al., 1997), and passive viewing tasks (Schupp et al., 2006), and using a variety of stimuli, such as emotional facial expressions (Fox et al., 2000; Schupp et al., 2004), images (Öhman et al., 2001; Armony and Dolan, 2002; Weymar et al., 2011), and non-verbal vocalizations (Sauter and Eimer, 2010). Similarly, for lexical material, an overall advantage for emotional (pleasant and unpleasant) words is consistently reported in lexical decision and reading tasks (Eviatar and Zaidel, 1991; Ortigue et al., 2004; Kanske and Kotz, 2007; Herbert et al., 2008; Schacht and Sommer, 2009; Scott et al., 2009; for a review see Kissler et al., 2009). These emotional benefits in attention may further influence memory formation and consolidation (Dolcos and Cabeza, 2002; Kensinger, 2009; Lang and Bradley, 2010), particularly during sleep (Wagner et al., 2006; Payne et al., 2008; Nishida et al., 2009), leading to increased memory retrieval for emotionally relevant compared with neutral information (Bradley et al., 1992; Weymar and Hamm, 2013; Dolcos et al., 2020b). Previous studies demonstrated a memory advantage for emotional images (Hamann et al., 1999), even 1 year after encoding, as evidenced by higher recall rates (Bradley et al., 1992) and recognition rates (Dolcos et al., 2005; Weymar et al., 2011), but also better memory for other material, including stories (Cahill and McGaugh, 1995), faces (Righi et al., 2012), and words (Phelps et al., 1997; Doerksen and Shimamura, 2001; Kensinger and Corkin, 2003). In addition to enhanced memory accuracy, emotional events are also retrieved more vividly, with more subjective confidence, and with enhanced recollective experience (i.e., recollection, the conscious retrieval of specific contextual details of the encoding episode) than for neutral events (Ochsner, 2000; Sharot et al., 2004; Dolcos et al., 2005; Weymar et al., 2009, 2010; Rimmele et al., 2012), which are often remembered with less confidence and less contextual information (i.e., familiarity) (D'Argembeau and Van der Linden, 2004; Sharot et al., 2007). A particular characteristic of the mnemonic advantage for emotionally relevant material is that it seems to be particularly sensitive to arousal (exciting vs. calming) rather than valence (pleasant vs. unpleasant), leading to prioritized perception and memory of emotionally (pleasant and unpleasant) arousing information at the expense of neutral, less-relevant information (Mather and Sutherland, 2011; Mather et al., 2016).

An influential neuroscientific theory (McGaugh, 2015) derived from animal and human studies suggests that better memory for unpleasant and pleasant events is related to the interaction between emotion-specific regions (e.g., amygdala) and memory-related regions (e.g., hippocampus) and is mediated by the afferent influence of stress hormones (epinephrine and glucocorticoids from adrenal glands) released during and after emotionally arousing experiences (Cahill and McGaugh, 1998; McIntyre et al., 2012; McGaugh, 2015). Critically, one of the pathways by means of which the stress hormones project to the amygdala consists of the afferent fibers of the vagus nerve (McIntyre et al., 2012). Specifically, the release of epinephrine in the adrenal gland modulates the activity of the vagus nerve, which consequently exerts influence on the locus coeruleus (LC) via the nucleus of the solitary tract (Van Bockstaele et al., 1999; McIntyre et al., 2012). The LC is the main source of noradrenergic neurons in the brain, and its activation favors the release of norepinephrine (NE) in a variety of cortical and subcortical brain areas, including the amygdala and hippocampus. In turn, activity of the LC may facilitate the formation, consolidation, and retrieval of emotional memories (Sterpenich et al., 2006; Groch et al., 2011; McIntyre et al., 2012; Clewett et al., 2018). Giving support to this assumption, prior research in animals and humans using implanted vagus nerve stimulation (VNS) found that invasive vagus nerve activation enhances long-term memory for inhibitory avoidance in rats (Clark et al., 1998) and modulates word recognition memory in humans (Clark et al., 1999), an effect assumed to be modulated by the LC-NE system (Hassert et al., 2004).

Recently, transcutaneous vagus nerve stimulation (tVNS) has been introduced as a novel brain stimulation tool that can activate the vagus nerve—in a non-invasive fashion—via the auricular branch (Van Leusden et al., 2015). Indeed, recent brain imaging studies have shown that tVNS modulates activity in the LC and areas innervated by this region, including the insula, amygdala, hippocampus, and thalamus (Dietrich et al., 2008; Kraus et al., 2013; Yakunina et al., 2017); tVNS also increased P300b amplitudes (Rufener et al., 2018; Ventura-Bort et al., 2018; Lewine et al., 2019; but see also Warren et al., 2019), an attention-related event-related potential (ERP) component putatively associated with phasic activity of the LC-NE system (Nieuwenhuis et al., 2005), and salivary alpha-amylase (sAA) levels (Ventura-Bort et al., 2018; Warren et al., 2019; but see also Koenig et al., 2019), an indirect marker of endogenous noradrenergic activation in the brain (Chatterton et al., 1996; Warren et al., 2017). Additionally, tVNS was found to modulate a variety of other cognitive and affective processes, such as cognitive control (Sellaro et al., 2015, 2018; Steenbergen et al., 2015; Fischer et al., 2018; Keute et al., 2019), associative memory (Jacobs et al., 2015), fear extinction (Burger et al., 2016; Szeska et al., 2020), and emotion recognition (Colzato et al., 2017), which may be related to the activation of LC-mediated NE following tVNS.

In the current study, we continued this line of research on the impact of non-invasive vagal stimulation on cognitive and affective functions by directly focusing on its effect on emotional encoding and memory. To extend the work of Clark et al. (1999), who found enhanced word recognition memory following VNS, we used emotional and neutral words as stimulus material. In a single-blind, randomized, between-subject design, healthy participants received either tVNS or sham stimulation while performing a lexical decision task with words and non-words. As in prior studies (Ventura-Bort et al., 2018; Warren et al., 2019), we measured sAA levels before and after stimulation to assess changes in noradrenergic activation following tVNS. One day later, participants performed a surprise recognition memory task in which previously encoded words and new words were presented. In the lexical decision task, we expected to replicate the advantage of lexical access for emotionally relevant information (e.g., Schacht and Sommer, 2009; Scott et al., 2009)

as indicated by enhanced accuracy rates and reduced response times for emotional (unpleasant and pleasant) words than for neutral words. Given that emotional words undergo prioritized processing over neutral words as indicated by previous ERP studies (e.g., Schacht and Sommer, 2009), and assuming that tVNS increases the arousal level, which may enhance perception and memory for salient emotional information (at the expense of less-relevant neutral information) (Mather and Sutherland, 2011; Mather et al., 2016), we expected that tVNS would lead to higher rates of accuracy and reduced response times particularly for emotional words. In the recognition memory task, we predicted enhanced recognition performance for emotional compared to neutral words, reflected in greater discrimination accuracy and shorter response times. Because emotional stimuli are often associated with (hippocampus-driven) recollection-based memory processes (e.g., Doerksen and Shimamura, 2001 and Kensinger and Corkin, 2003 for words, and Ochsner, 2000, Dolcos et al., 2005, Weymar et al., 2009, Weymar et al., 2010, and Dolcos et al., 2020a for scenes), we speculated that tVNS, compared to sham stimulation, would particularly increase memory for emotional words that were retrieved with high subjective confidence (Yonelinas, 2001; Wixted and Stretch, 2004; Weymar et al., 2009). As a marker of endogenous noradrenergic activation, increased sAA levels were expected after tVNS compared to sham stimulation.

2. MATERIALS AND METHODS

2.1. Participants

Sixty-one healthy psychology students (47 female; M_{age} = 23.39 years, SD_{age} = 4.67 years) from the University of Potsdam participated in the study for course credits. All participants provided informed written consent for the experimental protocol, which was approved by the ethics committee of the University of Potsdam and in accordance with the Declaration of Helsinki. All participants were native German speakers with normal or corrected-to-normal vision (54 right-handed). Prior to the first session, participants were phone-screened and invited to participate upon passing the following exclusion criteria: neurological or psychiatric disorders, brain surgery, use of medication or drugs, pregnancy, history of migraine or epilepsy, cardiac diseases, metal pieces in the body (e.g., pacemaker), and active implants or physical alterations in the ear (e.g., cochlear implant). One participant was excluded from the analyses for medical reasons, leaving a final sample of sixty participants (46 female; M_{age} = 23.45 years, SD_{age} = 4.87 years; 53 right-handed).

2.2. Procedure

2.2.1. Stimulus Materials

Overall, 400 German words were selected from the Berlin Affective Word List Reloaded (BAWL-R) database (Võ et al., 2009). Based on their normative ratings, which ranged from −3 (very unpleasant; e.g. Grab/coffin) to 3 (very pleasant; e.g. Geschenk/gift) for valence and from 1 (low arousal; e.g. Eimer/bucket) to 5 (high arousal; e.g. Irrsinn/insanity) for arousal, we selected three different categories ($M_{valence}$ = −1.50, $SD_{valence}$ = 0.55, $M_{arousal}$ = 3.59, $SD_{arousal}$ = 0.38), neutral ($M_{valence}$ = 0.11, $SD_{valence}$ = 0.29, $M_{arousal}$ = 2.23, $SD_{arousal}$ = 0.29), and pleasant ($M_{valence}$ = 1.32, $SD_{valence}$ = 0.55, $M_{arousal}$ = 3.33, $SD_{arousal}$ = 0.27). The collection of all 400 words was divided into two sets of 200 stimuli each. The sets were matched on the basis of hedonic valence, arousal, and various lexical and sublexical variables, including word imageability, word length (numbers of letters, phonemes, syllables), word frequency, and orthographic neighborhood density (*ps* > 0.13). One set of words (100 emotional and 100 neutral) was presented in a lexical decision task and served as the basis for generating 200 non-words by randomly substituting one letter at a random position in each of the given words (e.g., *Eimer* → *Eimej*; *Grab* → *Grkb*). The same set of old words was presented in an explicit recognition memory task together with the set of 200 new words.

2.2.2. Encoding Session: Lexical Decision Task

The study consisted of two experimental sessions (lexical decision task and recognition memory task), which took place on two consecutive days (see **Figure 1**). During encoding, participants received either active stimulation or sham stimulation for 23 min while performing a lexical decision task in a sound-attenuated, dimly lit room. Subjects were familiarized with the experimental protocols (though no mention of a memory task was made) and randomly assigned to one of the two experimental groups (active stimulation or sham stimulation). Before undergoing stimulation, participants' heart rate, blood pressure (systolic and diastolic), and sAA levels were measured. Subjects were familiarized with the stimulator device, and an individual stimulation intensity was determined for each participant to ensure a maximum strong stimulation without pain (for the procedure see Ventura-Bort et al., 2018). Participants were stimulated before (5 min), during (13 min), and after (5 min) the lexical decision task.

Each trial began with a fixation cross presented in the middle of the screen for an interval that varied randomly between 770 and 1,770 ms. Thereafter, a letter string (i.e., a word or non-word) was presented until a response was given or until a time-out of 1 s. Participants were asked to indicate as quickly and accurately as possible if the presented string was a German word by pressing a response button with their index finger of the dominant hand; if a non-word was presented, participants were instructed not to respond. The strings were presented in two blocks of 200 trials each in a pseudorandomized order to control for confounding effects of word order. The order of blocks was randomized across participants, and the sequence of trials in each block was pseudorandomized under the constraints that words with identical arousal levels and valence levels were not presented more than two times in a row. The same word type (word or non-word) was not presented more than three times in a row. After stimulation, participants' sAA levels, heart rate, and blood pressure were measured a second time and they completed a tVNS adverse effects questionnaire in which they had to indicate, on a 7-point scale (1 being *not at all* and 7 being *very much*), how much they had experienced headache, nausea, dizziness, neck pain, muscle contractions in the neck, stinging sensations under the electrodes, skin irritation in the ear, fluctuations in

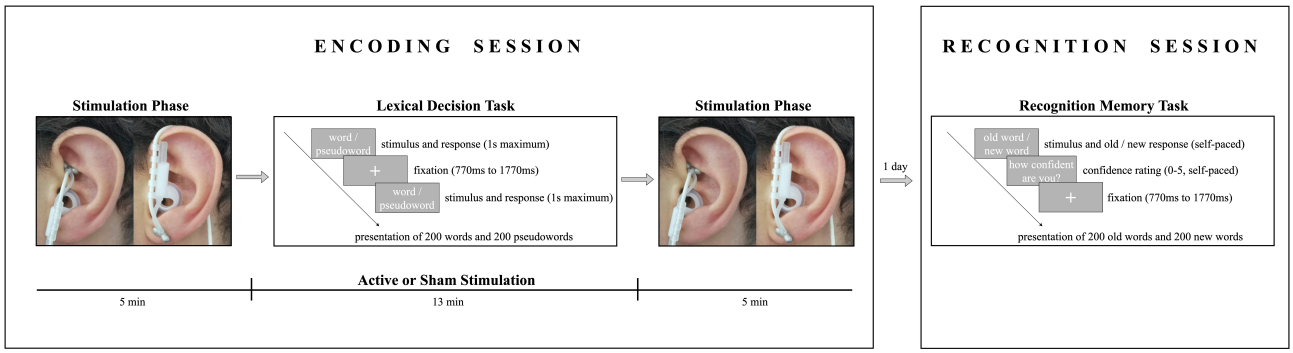


FIGURE 1 | Schema illustrating the encoding and recognition procedures. In the encoding session, participants received either active stimulation to the cymba conchae **(left)** or sham stimulation to the earlobe **(right)** before, during, and after a lexical decision task. In the lexical decision task, participants indicated if the presented string was a German word by pressing a response button, or if it was a non-word by not responding. In the recognition session one day after encoding, participants indicated whether or not the presented string was a word that they had seen during the lexical decision task and were asked to indicate their recognition confidence on a scale from 0 (not confident) to 5 (absolutely confident).

mental concentration or feelings, and other unpleasant feelings or adverse effects.

2.2.3. Recognition Session: Explicit Recognition Memory Task

One day after encoding, participants performed an old/new recognition task with self-paced response and a rating-based recognition confidence task on a 6-point scale (see **Figure 1**). Each trial began with a fixation cross presented in the middle of the screen for an interval that varied randomly between 770 and 1,770 ms, followed by a string that was presented until a response was given. The words were presented in two blocks of 200 trials each in a pseudorandomized order to control for confounding effects of word order. Participants were presented one word at a time and were asked to decide whether they had previously seen the word during encoding (old word) or not (new word) by pressing the corresponding response button on a keyboard. Hand assignment for the response buttons for old and new words was counterbalanced across participants. Following the old/new judgment, participants were asked to rate their confidence in memory by pressing the corresponding key in a Likert scale ranging from 0 (not confident) to 5 (absolutely confident) in order to assess the contribution of recollection and familiarity-based memory.

2.3. Transcutaneous Vagus Nerve Stimulation

In the current study, a single-blind, active stimulation-sham stimulation, randomized between-subject design was employed. Transcutaneous VNS was applied by stimulating the auricular branch of the vagus nerve using a recently engineered and non-invasive device (Cerbomed GmbH, Erlangen, Germany). The tVNS stimulator consists of two electrodes connected to a wired neurostimulating device; for the active stimulation it was placed in the left cymba conchae, an area innervated by the auricular branch of the vagus nerve, and for the sham stimulation it was placed on the left earlobe, an area that has been found to be free of cutaneous vagal innervation (Peuker and Filler, 2002; Ellrich et al., 2011) (see **Figure 1**). Stimulation alternated between on and off phases every 30 s and was delivered with a pulse width of 200–300 ms at 25 Hz. The stimulation intensity was determined individually for each participant by applying increasing and decreasing sequences of 10 s stimulation trials. Participants were asked to give direct feedback on how they perceived each stimulation intensity on a 10-point scale ranging from no perception (1) and light tingling (3) to strong tingling (6) and pain (10). The increasing sequence started from an intensity of 0.1 mA and increased stepwise in 0.1 mA increments until the subject reported a slight feeling of pain (corresponding to a subjective sensation of 9 on the scale). Before starting the decreasing series, the same intensity was repeated and then decreased stepwise in 0.1 mA increments until a subjective sensation of 6 or below was experienced (cf. Ventura-Bort et al., 2018). This protocol was repeated twice and the average of the intensities rated as 8 was used as the stimulation threshold. The individual stimulation intensities varied from 0.5 to 2.5 mA for the sham (earlobe) stimulation group ($M_{sham} = 1.31$, $SD_{sham} = 0.50$) and from 0.5 to 3.5 mA for the active (cymba conchae) stimulation group ($M_{active} = 1.48$, $SD_{active} = 0.59$). Stimulation intensities did not differ significantly between the groups [$t(1, 58) = 1.08$, $p = 0.28$, $d = 0.28$].

2.4. Autonomic Measures

To investigate the effects of tVNS on autonomic reactivity, subjects' heart rate and blood pressure (diastolic and systolic) were measured using the Intelli Wrap Manschette M500 device (Omron Healthcare, Medizintechnik Handelsgesellschaft mbH, Mannheim, Germany). In addition, their sAA levels were measured as a potential marker of endogenous noradrenergic activity (Warren et al., 2017). This was done by collecting saliva samples using cotton Salivette sampling devices (Sarstedt, Nümbrecht, Germany). Participants were asked to chew the swab from the Salivette for 60 s to activate salivation. The samples were

stored frozen and later sent to the Dresden LabService GmbH for sAA analysis (Thoma et al., 2012).

2.5. Statistical Analyses

To investigate the tolerability and adverse effects of tVNS, separate *t*-tests comparing active stimulation and sham stimulation were conducted. To evaluate the effects of tVNS on heart rate, blood pressure, and sAA levels, separate analysis of variance (ANOVA) tests were carried out using the factors Time (pre, post) and Stimulation (active, sham). The log transformation was applied to the right-skewed sAA data to achieve a normal distribution. Repeated-measures ANOVA tests including the within-subject factor Valence (unpleasant, neutral, pleasant) and the between-subject factor Stimulation (active, sham) were used to investigate the effects of tVNS on the accuracy and response time of the lexical decision task. Non-words were not included in the analyses as they are of little theoretical importance. *Post hoc* analyses were conducted using the Bonferroni correction (Wright, 1992). Similarly, to assess the effects of tVNS on recognition memory, 3 (Valence) × 2 (Stimulation) repeated-measures ANOVA tests were used for the discrimination index $P_r = p(\text{hit}) - p(\text{false alarm})$ (Snodgrass and Corwin, 1988) and the bias index $B_r = \frac{p(\text{false alarm})}{p(1-P_r)}$ (with $B_r > 0.5$ indicating liberal response bias and $B_r < 0.5$ conservative response bias). For the assessment of confidence in memory, analysis of the distribution of the confidence ratings revealed that a confidence rating of 5 occurred most frequently for correctly identified old words (hits) (40.73%). Evidence suggests that familiarity-based memory judgments increase gradually as a function of recognition confidence, whereas recollection-based memory judgments are generally associated with high-confidence memory judgments (Wixted and Stretch, 2004). Therefore, the proportion of hit rates based on their confidence ratings was calculated to assess the roles of recollection and familiarity in memory (Weymar et al., 2009; Rimmele et al., 2012). Those words that were correctly identified as old words and that received confidence ratings of 5 were classified into a *high-confidence hit rate* category, whereas all remaining words that were correctly identified as old words and that received lower confidence ratings were put into a *low-confidence hit rate* category. Recognition memory based on confidence ratings was analyzed using a three-way ANOVA with the within-subject factors Response Type (high-confidence hit rate, low-confidence hit rate) and Valence (unpleasant, neutral pleasant) and the between-subject factor Stimulation (active, sham).

3. RESULTS

3.1. Side Effects of tVNS

Overall, subjective ratings indicated that the side effects of stimulation were low ($N = 60$, $M = 2.02$, SD = 0.88); see **Table 1**. Statistical analyses of subjective ratings indicated no significant differences between the active stimulation condition and the sham stimulation condition in any of the symptoms assessed ($ps > 0.09$), suggesting that side effects were minimal and comparable for the two types of stimulation.

TABLE 1 | Mean subjective ratings (with standard errors in parentheses) for the stimulation side effects in the active stimulation condition and the sham stimulation condition.

	Active	Sham	*p*-value
Headache	1.83 (1.42)	2.03 (1.38)	0.58
Nausea	1.17 (0.75)	1.30 (0.75)	0.49
Dizziness	1.67 (1.15)	2.27 (1.55)	0.09
Neck pain	1.80 (1.49)	1.30 (0.75)	0.11
Neck contraction	2.27 (1.87)	1.93 (1.46)	0.44
Stinging sensation	2.87 (1.94)	2.80 (1.73)	0.93
Ear irritation	1.77 (1.45)	1.93 (1.55)	0.67
Concentration	2.97 (1.65)	3.47 (1.68)	0.25
Fluctuation of feelings	1.53 (1.01)	1.73 (1.34)	0.51
Unpleasant feelings	1.67 (1.15)	2.23 (1.45)	0.10

Ratings were scored on a seven-point scale, with 1 being not at all and 7 very much.

3.2. Autonomic Results

Table 2 provides an overview of the outcomes for the autonomic measures and salivary data during encoding. Statistical analyses revealed a main effect of Time for systolic blood pressure [$F_{(1, 114)} = 6.23$, $p = 0.01$, $\eta_p^2 = 0.05$], which suggests a decrease during encoding. This effect was not observed for diastolic blood pressure [$F_{(1, 114)} = 2.23$, $p = 0.14$, $\eta_p^2 = 0.02$] or for heart rate ($F < 1$). No main effect of Stimulation was observed for either systolic blood pressure ($F < 1$), diastolic blood pressure ($F < 1$), or heart rate ($F < 1$). No interaction effect of Time and Stimulation was found for either systolic blood pressure ($F < 1$), diastolic blood pressure ($F < 1$), or heart rate ($F < 1$), suggesting that stimulation had no significant impact on the autonomic measures over time. For sAA levels, no significant main effect of Time [$F_{(1, 58)} = 2.1$, $p = 0.14$, $\eta_p^2 = 0.03$], Stimulation ($F < 1$), or interaction between Time and Stimulation ($F < 1$) was found.

3.3. Behavioral Results

3.3.1. Lexical Decision Task

Behavioral results from the lexical decision task are presented in **Table 3**. Detection accuracy was modulated by the emotional content of words [$F_{(2, 116)} = 12.62$, $p < 0.001$, $\eta_p^2 = 0.18$] and was higher for pleasant words than for unpleasant words [$t(59) = -3.38$, $p < 0.001$, $d = -0.29$] and neutral words [$t(59) = 5.13$, $p < 0.001$, $d = 0.5$]. No differences were observed between unpleasant and neutral words [$t(59) = 0.63$, $p = 0.53$, $d = 0.03$]; see **Figure 2A**. No main effect of Stimulation ($F < 1$) or interaction with Valence ($F < 1$) was observed. For response times, a significant main effect of Valence [$F_{(2, 116)} = 48.06$, $p < 0.001$, $\eta_p^2 = 0.45$] revealed longer response times for unpleasant words than for pleasant words [$t(59) = 8.04$, $p < 0.001$, $d = 0.57$] and neutral words [$t(59) = 8.34$, $p < 0.001$, $d = 0.55$]; no difference in response times was found between pleasant and neutral words [$t(59) = -0.32$, $p = 0.75$, $d = -0.02$; see **Figure 2B**]. No main effect of Stimulation ($F < 1$) or interaction (F < 1) was observed.

TABLE 2 | Means (with standard deviations in parentheses) of the autonomic and salivary measures before and after active stimulation and sham stimulation in the encoding session (lexical decision task).

		Heart rate (bpm)	Systolic blood pressure (mmHg)	Diastolic blood pressure (mmHg)	Alpha-amylase [log(μkatal/l)]
Active					
	Pre	73.97 (13.98)	116.13 (11.48)	77.43 (8.33)	4.46 (0.71)
	Post	71.10 (8.99)	107.97 (10.66)	73.40 (7.62)	4.46 (0.64)
Sham					
	Pre	72.73 (11.97)	116.10 (9.76)	77.93 (7.14)	4.52 (0.84)
	Post	70.33 (7.93)	108.50 (12.07)	73.80 (6.48)	4.24 (0.91)

TABLE 3 | Means (with standard deviations in parentheses) of the performance in the lexical decision task as a function of valence and stimulation.

		Lexical decision task		
		Hits	False alarms	Response time (s)
Active				
	Unpleasant	0.94 (0.06)	0.03 (0.02)	0.57 (0.10)
	Neutral	0.94 (0.04)	0.02 (0.02)	0.55 (0.09)
	Pleasant	0.96 (0.04)	0.03 (0.02)	0.55 (0.09)
Sham				
	Unpleasant	0.93 (0.09)	0.05 (0.04)	0.57 (0.11)
	Neutral	0.93 (0.06)	0.03 (0.04)	0.55 (0.09)
	Pleasant	0.96 (0.05)	0.04 (0.04)	0.55 (0.09)

3.3.2. Recognition Memory

Results from the behavioral performance in the explicit recognition task are presented in **Table 4**. Memory accuracy, as measured by the discrimination index P_r, was modulated by the emotional content of words [$F_{(2,\ 116)} = 10.65$, $p < 0.001$, $\eta_p^2 = 0.15$]. Pleasant words were better discriminated than unpleasant [$t(59) = -3.95$, $p < 0.001$, $d = -0.5$] and neutral words [$t(59) = 4.39$, $p < 0.001$, $d = 0.45$]; no difference in P_r was observed between unpleasant and neutral words [$t(59) = -0.35$, $p = 0.73$, $d = -0.04$; see **Figure 2C**]. No main effect of Stimulation ($F < 1$) or interaction (F < 1) was found, however. For the bias index B_r, a main effect of Valence was observed [$F_{(2,\ 116)} = 40.91$, $p < 0.001$, $\eta_p^2 = 0.41$], indicating a more liberal response bias for emotional than for neutral words ($ps < 0.001$). No main effect of Stimulation or interaction (F < 1) was observed. A main effect of Valence was also observed for hit rates [$F_{(2,\ 116)} = 44.83$, $p < 0.001$, $\eta_p^2 = 0.44$], revealing increased hit rates for both unpleasant [$t(59) = 6.41$, $p < 0.001$, $d = 0.56$] and pleasant words [$t(59) = 9.25$, $p < 0.001$, $d = 0.72$] compared to neutral words (see **Figure 2D**).

3.3.3. Recognition Memory Based on Confidence Ratings

When the proportion of hit rates based on subjects' confidence ratings were taken into account, a main effect of Response Type [$F_{(1,\ 58)} = 15.02$, $p < 0.001$, $\eta_p^2 = 0.76$] and a significant interaction between Response Type and Stimulation [$F_{(1,\ 58)} = 4.04$, $p = 0.049$, $\eta_p^2 = 0.46$] were observed, showing that active stimulation increased hit rates for words that were remembered with higher subjective confidence, as opposed to sham stimulation [$t(58) = 2.03$, $p = 0.046$, $d = 0.52$]. The reverse effect was observed for trials with low recognition confidence [$t(58) = -2.03$, $p = 0.046$, $d = -0.52$]; see **Figures 2E,F**. The results revealed a significant interaction of Response Type and Valence [$F_{(1,\ 116)} = 54.09$, $p < 0.001$, $\eta_p^2 = 0.48$], indicating increased high-confidence hit rates for both unpleasant words [$t(59) = -7.36$, $p < 0.001$, $d = -0.48$] and pleasant words [$t(59) = -11.03$, $p < 0.001$, $d = -0.59$] compared to neutral words. The reverse effect was observed for trials with low recognition confidence, which showed increased hit rates for neutral words remembered with low confidence than for unpleasant [$t(59) = 7.36$, $p < 0.001$, $d = 0.48$] or pleasant words [$t(59) = 11.03$, $p < 0.001$, $d = 0.59$].

4. DISCUSSION

In the present study, we investigated the impact of tVNS on emotional word processing (lexical decision task) and later recognition memory (old/new task). As an indirect marker of noradrenergic activation, sAA levels were measured before and after tVNS. As expected, emotion modulated word processing and memory. Pleasant words were better identified and remembered than neutral and unpleasant ones. However, tVNS showed no effects on word processing and overall emotional recognition memory performance. In addition, tVNS did not produce the expected sAA level increase, compared to sham stimulation. However, when high and low confidence ratings were considered, tVNS, compared to sham stimulation, increased the proportion of hit rates for words that were remembered with high confidence, irrespective of emotional category, suggesting an effect of tVNS on recollection-based memory performance.

4.1. Effects of Emotion on Word Processing and Recognition Memory but No Effect of tVNS

Replicating prior studies using lexical decision tasks (Kanske and Kotz, 2007; Schacht and Sommer, 2009; Scott et al., 2009; Kousta et al., 2011), we found that emotional word contents were better accessed than neutral ones. In the current study,

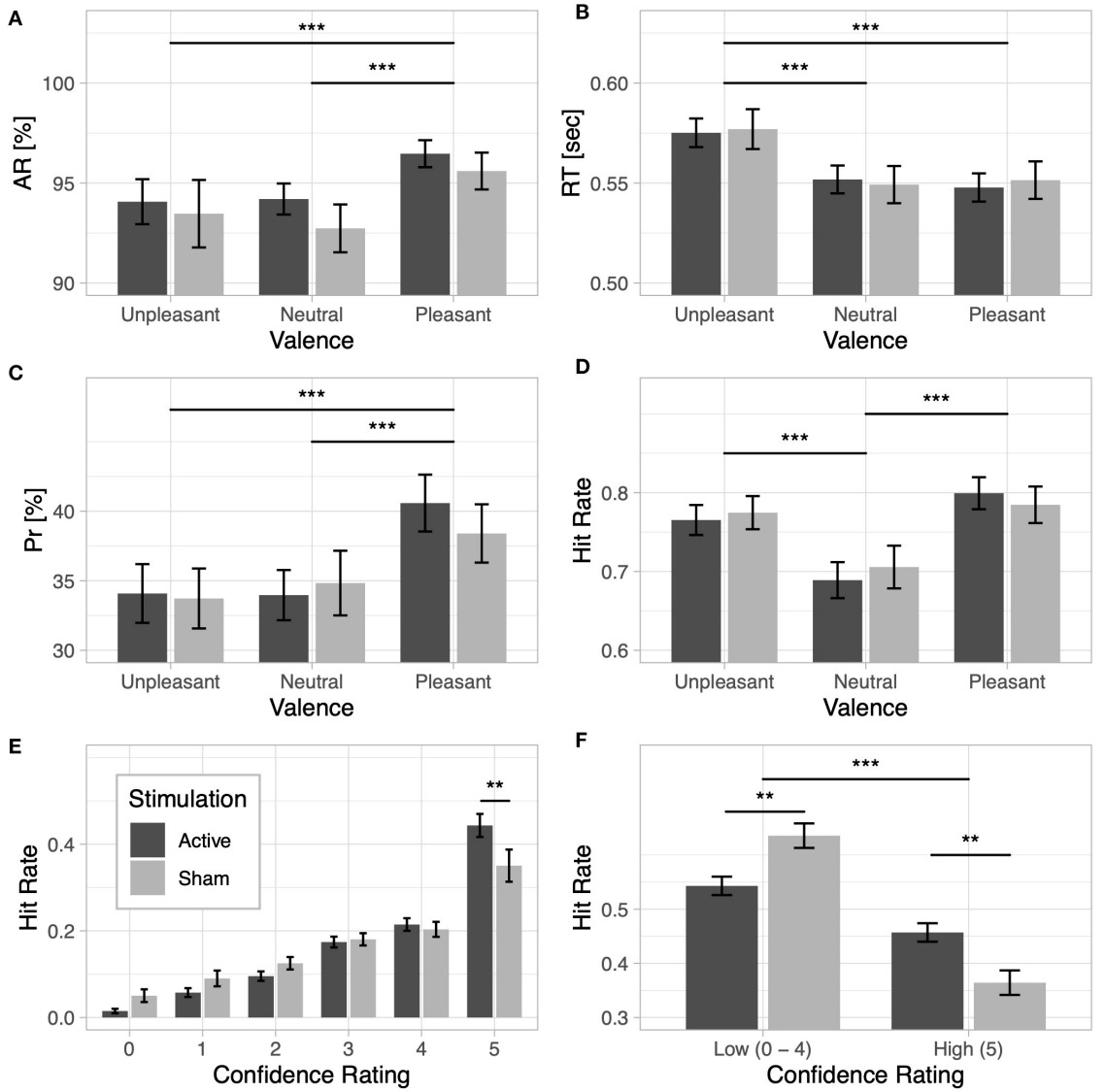


FIGURE 2 | Lexical decision task: **(A)** accuracy rate (AR) and **(B)** response time (RT) for unpleasant, neutral, and pleasant words under the active and sham stimulation conditions. Recognition memory: **(C)** discrimination index (Pr) and **(D)** hit rate based on valence for the active and sham stimulation conditions. **(E)** Recognition confidence: hit rate based on confidence responses for the active and sham stimulation conditions. **(F)** Recognition memory based on confidence ratings: hit rate based on high-confidence category (words that received confidence ratings of 5) and low-confidence category (words that received confidence ratings from 0 to 4) for the active and sham stimulation conditions; note that the low-confidence hit rate category is the cumulative hit rate for ratings 0–4. Two asterisks indicate a *p*-value smaller than 0.01 and three asterisks indicate a *p*-value smaller than 0.001.

however, we found an advantage for pleasant words, reflected in higher detection accuracy and shorter response times than for unpleasant words. Valence differences in word processing have also been reported in previous studies employing similar tasks (Herbert et al., 2006; Estes and Adelman, 2008; Nasrallah et al., 2009; Kissler and Koessler, 2011; Citron et al., 2014). It has been proposed that word frequency (Kahan and Hely, 2008; Scott et al., 2009) is one of the most important factors influencing emotional effects on word recognition, which may explain the amount of variance in word recognition latencies and accuracies across studies (for a review see Kuperman et al., 2014). Some authors argue that although emotional (both pleasant and unpleasant) words convey emotionally salient information, particularly unpleasant stimuli capture and hold attention in early processing because of their potentially threatening nature and relevance to survival (see the automatic vigilance hypothesis of Pratto and John, 1991). Therefore it could be that, in comparison with pleasant and neutral material, unpleasant words engage more attentional resources, thereby reducing the amount of resources available for lexical decision processes. Alternatively,

TABLE 4 | Means (with standard deviations in parentheses) of the recognition memory performance and hit rate confidence ratings as a function of valence and stimulation.

		Recognition memory				Recognition memory based on confidence ratings	
		Hits	**False alarms**	**P_r index**	**B_r index**	**Familiarity-based hit rate**	**Recollection-based hit rate**
Active							
	Unpleasant	0.76 (0.10)	0.42 (0.11)	0.34 (0.12)	0.64 (0.15)	0.51 (0.16)	0.49 (0.16)
	Neutral	0.69 (0.12)	0.35 (0.10)	0.34 (0.10)	0.54 (0.15)	0.61 (0.15)	0.38 (0.15)
	Pleasant	0.80 (0.11)	0.39 (0.11)	0.41 (0.11)	0.66 (0.17)	0.50 (0.15)	0.50 (0.15)
Sham							
	Unpleasant	0.77 (0.11)	0.44 (0.13)	0.34 (0.12)	0.66 (0.16)	0.62 (0.22)	0.38 (0.22)
	Neutral	0.71 (0.15)	0.36 (0.10)	0.35 (0.13)	0.56 (0.17)	0.70 (0.20)	0.30 (0.20)
	Pleasant	0.78 (0.13)	0.40 (0.12)	0.38 (0.11)	0.66 (0.18)	0.59 (0.21)	0.41 (0.21)

however, because detection accuracy and response times were observed to be faster for pleasant than for unpleasant material (in line with Herbert et al., 2006; Estes and Adelman, 2008; Kissler et al., 2009; Kissler and Koessler, 2011; Citron et al., 2014), this could also indicate facilitated processing for positive compared to neutral concepts, either owing to higher interconnectivity in the mental lexicon (i.e., mental dictionary that contains information such as word meaning and syntactic characteristics, among other aspects) (Ashby et al., 1999) or, which may be more likely, because pleasant words are perceived as being more motivationally relevant than unpleasant ones (Citron et al., 2014). Particularly during low levels of emotional activation or arousal (see the theory of Cacioppo et al., 1999), there seems to be a preference for positive information that mediates approach motivation (in contrast to a preference for negative information that leads to defense motivation at high levels of arousal). This latter theoretical view seems reasonable for explaining the present results, since induced arousal is often lower for words than for faces or scenes (see also the recent ERP evidence in Bayer and Schacht, 2014, showing early positivity bias for words as compared to faces and scenes).

We also found better long-term memory discrimination for pleasant words than for the other semantic categories. Given the results from the lexical decision task, this indicates that prioritized processing during encoding for pleasant contents may have promoted deeper consolidation processes, which produced enhanced long-term memory performance (Weymar et al., 2012; for a review see Cross et al., 2018). As for the encoding data, previous research on recognition memory also found a memory advantage for pleasant words (Herbert et al., 2008). However, it should be noted that arousal-specific effects or even unpleasant enhancing effects have also been reported (Kensinger and Corkin, 2003; Weymar and Hamm, 2013; Weymar et al., 2014). The inconsistent effects of arousal and valence on recognition memory across studies may, however, be a result of differences in arousal and valence levels of the stimuli used in experiments and may also be related to differences in lexico-semantic variables, such as the frequency, concreteness, imageability, age of acquisition, and familiarity of words (Scott et al., 2009; Kousta et al., 2011), which also likely lead to differences in word processing studies. Interestingly, though, besides valence-specific effects in overall memory, we found enhanced memory for both pleasant and unpleasant words when high-confidence responses were taken into account, which replicates many studies showing that the memory-enhancing effect of emotion is mediated by the process of recollection rather than familiarity (Ochsner, 2000; Kensinger and Corkin, 2003; Dolcos et al., 2004, 2005; Sharot et al., 2004, 2007; Weymar et al., 2009; Rimmele et al., 2012). The results of the present study also show that qualitative memory retrieval does not necessarily depend on the level of word processing during encoding (lexical decision task).

Critically, and relevant to the main research question, active tVNS, as compared to sham stimulation, did not affect lexical decision performance and recognition memory performance for emotional and neutral words, which is contrary to our expectations. From animal models it has been suggested that stimulation of vagal afferents leads to activation of the LC-NE system, which increases arousal levels in the brain. Arousal, in turn, was expected to directly facilitate amygdala function, by promoting on the one hand the processing of highly relevant information but also by influencing regions that support memory consolidation, such as the hippocampus (Mather and Sutherland, 2011; Mather et al., 2016). In contrast to prior studies (Ventura-Bort et al., 2018; Warren et al., 2019), however, we found no evidence in the overall analysis of enhanced sAA levels after tVNS, compared to sham stimulation (Koenig et al., 2019). Assuming that changes in sAA levels reflect changes in central noradrenergic levels (Chatterton et al., 1996; Warren et al., 2017), the lack of an increase in the sAA level may point toward an insufficient activation of the arousal-modulated LC-NE system in the present study that made enhanced emotional word processing and subsequent memory storage unlikely. Similarly, although our material was emotionally laden, as indicated by the ratings and the expected enhancement observed in the lexical decision and recognition memory tasks, arousal levels are usually lower for words and faces than for affective scenes (Lang et al., 1998; Bradley, 2000). Thus, emotional words themselves may not have sufficiently triggered arousal to produce a significant impact of tVNS on processing and memory. This argument is also substantiated by preliminary data from our lab (Weymar

et al., 2019) showing that tVNS can indeed facilitate recognition memory when emotional scenes are used, which was also found to be related to an increase in sAA levels. Another potentially important factor that may have led to no changes in sAA levels relates to the stimulation duration and protocol of the current study. Prior studies showing an increase in sAA levels after tVNS used continuous stimulation or stimulated for longer periods of time (more than the 23 min in the current study) (Ventura-Bort et al., 2018; Warren et al., 2019). It could therefore be that the stimulation protocol used in the current study was not optimal for increasing arousal as reflected in changes of sAA levels. It should be noted, however, that, irrespective of a potential central noradrenergic activation by tVNS, the reliability of elevated alpha-amylase levels as a biomarker (for recent findings see Warren et al., 2017) has not yet been sufficiently proven (e.g., Nater and Rohleder, 2009; Bosch et al., 2011). Therefore, additional variables are clearly needed to study the involvement of NE in emotion-cognition interactions.

4.2. Effects of tVNS on Recollection-Based Memory

Despite finding no tVNS effects on overall memory, tVNS modulated recognition confidence. When the proportion of hit rates based on low and high confidence ratings were considered, reflecting familiarity- and recollection-based memory (Wixted and Stretch, 2004), respectively, higher recollection performance was observed during tVNS compared to sham stimulation, partly supporting our a priori hypothesis. This result may indicate that tVNS facilitates memory consolidation, resulting in a greater recollective experience of past memories irrespective of their emotional category. This finding is partially in line with the clinical study of Clark et al. (1999) using invasive VNS in epileptic patients, which showed better memory for words, suggesting that vagus nerve activation modulates word memory formation in humans.

Previous studies have shown that the hippocampus plays an important role in recollection (for a review see Brown and Aggleton, 2001, or Eichenbaum et al., 2007). For instance, patients with selective hippocampal damage exhibit more pronounced deficits on tasks related to recollection processes, such as associative and source memory tasks (Holdstock et al., 2005; Gold et al., 2006; for a review see Yonelinas et al., 2010). Further evidence comes from animal studies showing that selective hippocampal damage impaired recollection but spared familiarity-based odor recognition in rats (Fortin et al., 2004; Sauvage et al., 2008; for a review see Rugg and Vilberg, 2013) and from fMRI studies showing hippocampal involvement primarily in recollection processes in healthy humans (Brown and Aggleton, 2001; Ranganath and Ritchey, 2012; for a review see Eichenbaum et al., 2007). The enhanced recollection-based memory for items encoded under tVNS may therefore indicate that tVNS modulates, to some extent, hippocampal activation (Roosevelt et al., 2006; Raedt et al., 2011). Although the underlying neural circuitry is not well-understood, previous research has shown that tVNS most likely activates NE secretion in the LC, which projects to a wide variety of cortical and subcortical regions, including not only the amygdala but also the hippocampus (Van Bockstaele et al., 1999; McIntyre et al., 2012). Giving support to this assumption, prior research in animals using implanted vagus nerve stimulators found that invasive VNS increases LC firing rates in rats (Dorr and Debonnel, 2006), hippocampal activity (Roosevelt et al., 2006; Raedt et al., 2011), and long-term memory for inhibitory avoidance (Clark et al., 1998). Similarly, Jacobs et al. (2015) found that tVNS, compared to sham simulation, increases associative memory in older humans. Although the noradrenergic activation of the amygdala is fundamental for memory-enhancing effects of emotion, recent studies indicate that the LC can facilitate memory processes through an amygdala-independent path, projecting directly to the hippocampus (Mello-Carpes and Izquierdo, 2013). It might therefore be that the non-specific effects of tVNS on word recollection memory are due to a lesser involvement of the amygdala in the hippocampus-mediated memory consolidation process (which may partly be driven by the nature of the stimulus material; see Kensinger and Schachter, 2006). Altogether, the current results indicate that tVNS can facilitate hippocampus-mediated, recollection-based memory.

4.3. Conclusion

To summarize, the present study has replicated prior research showing enhanced processing and memory for emotional (pleasant) words (particularly when based on high subjective confidence). Although tVNS showed no effects on word processing, subtle effects on recollection-based memory (high-confidence memory) were found, which may indicate that tVNS facilitates hippocampus-mediated consolidation processes. The potential of tVNS to improve memory in individuals (cf. Jacobs et al., 2015) supports its relevance for future research. For example, tVNS may be used to enhance memory consolidation during sleep, which is also related to hippocampus (Moroni et al., 2007) and LC activity (Eschenko et al., 2012). Furthermore, tVNS may be relevant in clinical applications, for instance as a therapeutic tool (and in combination with emerging neuroscientific approaches; see e.g., Dennis and Thompson, 2014 and Vecchio et al., 2015) in cognitive aging and for the treatment of a number of neuropsychiatric disorders associated with cognitive impairment, such as depression or Alzheimer's disease (Broncel et al., 2020).

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethikkommission Universität Potsdam. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MG and MW conceived the idea and discussed the design of the study. MG programmed the experiment and collected and analyzed the data. All authors discussed the results and contributed to writing the manuscript.

FUNDING

For funding of the publication fees we acknowledge the support of the Deutsche Forschungsgemeinschaft (DFG) and Open Access Publication Fund of the University of Potsdam.

REFERENCES

Armony, J. L., and Dolan, R. J. (2002). Modulation of spatial attention by fear-conditioned stimuli: an event-related fMRI study. *Neuropsychologia* 40, 817–826. doi: 10.1016/S0028-3932(01)00178-6

Ashby, F. G., Isen, A. M., and Turken, A. U. (1999). A neuropsychological theory of positive affect and its influence on cognition. *Psychol. Rev*. 106:529. doi: 10.1037/0033-295X.106.3.529

Bayer, M., and Schacht, A. (2014). Event-related brain responses to emotional words, pictures, and faces-a cross-domain comparison. *Front. Psychol*. 5:1106. doi: 10.3389/fpsyg.2014.01106

Bosch, J. A., Veerman, E. C., de Geus, E. J., and Proctor, G. B. (2011). α-amylase as a reliable and convenient measure of sympathetic activity: don't start salivating just yet! *Psychoneuroendocrinology* 36, 449–453. doi: 10.1016/j.psyneuen.2010.12.019

Bradley, M. M. (2000). "Emotion and motivation," in *Handbook of Psychophysiology*, eds J. T. Cacioppo, L. G. Tassinary, and G. G. Berntson (Cambridge University Press), 602–642.

Bradley, M. M., Greenwald, M. K., Petry, M. C., and Lang, P. J. (1992). Remembering pictures: pleasure and arousal in memory. *J. Exp. Psychol. Learn. Mem. Cogn*. 18:379. doi: 10.1037/0278-7393.18.2.379

Broncel, A., Bocian, R., Kłos-Wojtczak, P., Kulbat-Warycha, K., and Konopacki, J. (2020). Vagal nerve stimulation as a promising tool in the improvement of cognitive disorders. *Brain Res. Bull*. 155, 37–47. doi: 10.1016/j.brainresbull.2019.11.011

Brown, M. W., and Aggleton, J. P. (2001). Recognition memory: what are the roles of the perirhinal cortex and hippocampus? *Nat. Rev. Neurosci*. 2:51. doi: 10.1038/35049064

Burger, A. M., Verkuil, B., Van Diest, I., Van der Does, W., Thayer, J. F., and Brosschot, J. F. (2016). The effects of transcutaneous vagus nerve stimulation on conditioned fear extinction in humans. *Neurobiol. Learn. Mem*. 132, 49–56. doi: 10.1016/j.nlm.2016.05.007

Cacioppo, J. T., Gardner, W. L., and Berntson, G. G. (1999). The affect system has parallel and integrative processing components: form follows function. *J. Pers. Soc. Psychol*. 76:839. doi: 10.1037/0022-3514.76.5.839

Cahill, L., and McGaugh, J. L. (1995). A novel demonstration of enhanced memory associated with emotional arousal. *Conscious. Cogn*. 4, 410–421. doi: 10.1006/ccog.1995.1048

Cahill, L., and McGaugh, J. L. (1998). Mechanisms of emotional arousal and lasting declarative memory. *Trends Neurosci*. 21, 294–299. doi: 10.1016/S0166-2236(97)01214-9

Chatterton, R. T. Jr., Vogelsong, K. M., Lu, Y.-C., Ellman, A. B., and Hudgens, G. A. (1996). Salivary α-amylase as a measure of endogenous adrenergic activity. *Clin. Physiol*. 16, 433–448. doi: 10.1111/j.1475-097X.1996.tb00731.x

Citron, F. M., Weekes, B. S., and Ferstl, E. C. (2014). Arousal and emotional valence interact in written word recognition. *Lang. Cogn. Neurosci*. 29, 1257–1267. doi: 10.1080/23273798.2014.897734

Clark, K., Smith, D., Hassert, D., Browning, R., Naritoku, D., and Jensen, R. (1998). Posttraining electrical stimulation of vagal afferents with concomitant vagal efferent inactivation enhances memory storage processes in the rat. *Neurobiol. Learn. Mem*. 70, 364–373. doi: 10.1006/nlme.1998.3863

Clark, K. B., Naritoku, D. K., Smith, D. C., Browning, R. A., and Jensen, R. A. (1999). Enhanced recognition memory following vagus nerve stimulation in human subjects. *Nat. Neurosci*. 2:94. doi: 10.1038/4600

Clewett, D. V., Huang, R., Velasco, R., Lee, T.-H., and Mather, M. (2018). Locus coeruleus activity strengthens prioritized memories under arousal. *J. Neurosci*. 38, 1558–1574. doi: 10.1523/JNEUROSCI.2097-17.2017

Colzato, L. S., Sellaro, R., and Beste, C. (2017). Darwin revisited: The vagus nerve is a causal element in controlling recognition of other's emotions. *Cortex* 92, 95–102. doi: 10.1016/j.cortex.2017.03.017

Cross, Z. R., Santamaria, A., and Kohler, M. (2018). Attention and emotion-enhanced memory: a systematic review and meta-analysis of behavioural and neuroimaging evidence. *bioRxiv [Preprint]* 273920. doi: 10.1101/273920

D'Argembeau, A., and Van der Linden, M. (2004). Influence of affective meaning on memory for contextual information. *Emotion* 4:173. doi: 10.1037/1528-3542.4.2.173

Dennis, E. L., and Thompson, P. M. (2014). Functional brain connectivity using fMRI in aging and alzheimer's disease. *Neuropsychol. Rev*. 24, 49–62. doi: 10.1007/s11065-014-9249-6

Dietrich, S., Smith, J., Scherzinger, C., Hofmann-Preiß, K., Freitag, T., Eisenkolb, A., et al. (2008). A novel transcutaneous vagus nerve stimulation leads to brainstem and cerebral activations measured by functional MRI/funktionelle magnetresonanztomographie zeigt aktivierungen des hirnstamms und weiterer zerebraler strukturen unter transkutaner vagusnervstimulation. *Biomed. Eng*. 53, 104–111. doi: 10.1515/BMT.2008.022

Doerksen, S., and Shimamura, A. P. (2001). Source memory enhancement for emotional words. *Emotion* 1:5. doi: 10.1037/1528-3542.1.1.5

Dolan, R. J. (2002). Emotion, cognition, and behavior. *Science* 298, 1191–1194. doi: 10.1126/science.1076358

Dolcos, F., and Cabeza, R. (2002). Event-related potentials of emotional memory: encoding pleasant, unpleasant, and neutral pictures. *Cogn. Affect. Behav. Neurosci*. 2, 252–263. doi: 10.3758/CABN.2.3.252

Dolcos, F., Katsumi, Y., Bogdan, P., Shen, C., Jun, S., Buetti, S., et al. (2020a). The impact of focused attention on subsequent emotional recollection: a functional MRI investigation. *Neuropsychologia* 138:107338. doi: 10.1016/j.neuropsychologia.2020.107338

Dolcos, F., Katsumi, Y., Moore, M., Berggren, N., de Gelder, B., Derakshan, N., et al. (2020b). Neural correlates of emotion-attention interactions: from perception, learning, and memory to social cognition, individual differences, and training interventions. *Neurosci. Biobehav. Rev*. 108, 559–601. doi: 10.1016/j.neubiorev.2019.08.017

Dolcos, F., Katsumi, Y., Weymar, M., Moore, M., Tsukiura, T., and Dolcos, S. (2017). Emerging directions in emotional episodic memory. *Front. Psychol*. 8:1867. doi: 10.3389/fpsyg.2017.01867

Dolcos, F., LaBar, K. S., and Cabeza, R. (2004). Interaction between the amygdala and the medial temporal lobe memory system predicts better memory for emotional events. *Neuron* 42, 855–863. doi: 10.1016/S0896-6273(04)00289-2

Dolcos, F., LaBar, K. S., and Cabeza, R. (2005). Remembering one year later: role of the amygdala and the medial temporal lobe memory system in retrieving emotional memories. *Proc. Natl. Acad. Sci. U.S.A*. 102, 2626–2631. doi: 10.1073/pnas.0409848102

Dorr, A. E., and Debonnel, G. (2006). Effect of vagus nerve stimulation on serotonergic and noradrenergic transmission. *J. Pharmacol. Exp. Therapeut*. 318, 890–898. doi: 10.1124/jpet.106.104166

Eichenbaum, H., Yonelinas, A. P., and Ranganath, C. (2007). The medial temporal lobe and recognition memory. *Annu. Rev. Neurosci*. 30, 123–152. doi: 10.1146/annurev.neuro.30.051606.094328

Ellrich, J. (2011). Transcutaneous vagus nerve stimulation. Eur Neurol Rev 6, 262–264. doi: 10.17925/ENR.2011.06.04.254

Eschenko, O., Magri, C., Panzeri, S., and Sara, S. J. (2012). Noradrenergic neurons of the locus coeruleus are phase locked to cortical up-down states during sleep. *Cereb. Cortex* 22, 426–435. doi: 10.1093/cercor/bhr121

Estes, Z., and Adelman, J. S. (2008). Automatic vigilance for negative words in lexical decision and naming: comment on larsen, mercer, and balota (2006). *Emotion* 8, 441–444. doi: 10.1037/1528-3542.8.4.441

Eviatar, Z., and Zaidel, E. (1991). The effects of word length and emotionality on hemispheric contribution to lexical decision. *Neuropsychologia* 29, 415–428. doi: 10.1016/0028-3932(91)90028-7

Fischer, R., Ventura-Bort, C., Hamm, A., and Weymar, M. (2018). Transcutaneous vagus nerve stimulation (TVNS) enhances conflict-triggered adjustment of cognitive control. *Cogn. Affect. Behav. Neurosci.* 18, 680–693. doi: 10.3758/s13415-018-0596-2

Fortin, N. J., Wright, S. P., and Eichenbaum, H. (2004). Recollection-like memory retrieval in rats is dependent on the hippocampus. *Nature* 431, 188–191. doi: 10.1038/nature02853

Fox, E., Lester, V., Russo, R., Bowles, R., Pichler, A., and Dutton, K. (2000). Facial expressions of emotion: Are angry faces detected more efficiently? *Cogn. Emot.* 14, 61–92. doi: 10.1080/026999300378996

Gold, J. J., Hopkins, R. O., and Squire, L. R. (2006). Single-item memory, associative memory, and the human hippocampus. *Learn. Mem.* 13, 644–649. doi: 10.1101/lm.258406

Groch, S., Wilhelm, I., Diekelmann, S., Sayk, F., Gais, S., and Born, J. (2011). Contribution of norepinephrine to emotional memory consolidation during sleep. *Psychoneuroendocrinology* 36, 1342–1350. doi: 10.1016/j.psyneuen.2011.03.006

Hamann, S. B., Ely, T. D., Grafton, S. T., and Kilts, C. D. (1999). Amygdala activity related to enhanced memory for pleasant and aversive stimuli. *Nat. Neurosci.* 2:289. doi: 10.1038/6404

Hassert, D., Miyashita, T., and Williams, C. (2004). The effects of peripheral vagal nerve stimulation at a memory-modulating intensity on norepinephrine output in the basolateral amygdala. *Behav. Neurosci.* 118:79. doi: 10.1037/0735-7044.118.1.79

Herbert, C., Junghofer, M., and Kissler, J. (2008). Event related potentials to emotional adjectives during reading. *Psychophysiology* 45, 487–498. doi: 10.1111/j.1469-8986.2007.00638.x

Herbert, C., Kissler, J., Junghöfer, M., Peyk, P., and Rockstroh, B. (2006). Processing of emotional adjectives: evidence from startle EMG and ERPs. *Psychophysiology* 43, 197–206. doi: 10.1111/j.1469-8986.2006.00385.x

Holdstock, J., Mayes, A., Gong, Q., Roberts, N., and Kapur, N. (2005). Item recognition is less impaired than recall and associative recognition in a patient with selective hippocampal damage. *Hippocampus* 15, 203–215. doi: 10.1002/hipo.20046

Jacobs, H. I., Riphagen, J. M., Razat, C. M., Wiese, S., and Sack, A. T. (2015). Transcutaneous vagus nerve stimulation boosts associative memory in older individuals. *Neurobiol. Aging* 36, 1860–1867. doi: 10.1016/j.neurobiolaging.2015.02.023

Kahan, T. A., and Hely, C. D. (2008). The role of valence and frequency in the emotional stroop task. *Psychon. Bull. Rev.* 15, 956–960. doi: 10.3758/PBR.15.5.956

Kanske, P., and Kotz, S. A. (2007). Concreteness in emotional words: ERP evidence from a hemifield study. *Brain Res.* 1148, 138–148. doi: 10.1016/j.brainres.2007.02.044

Kensinger, E. A. (2009). What factors need to be considered to understand emotional memories? *Emot. Rev.* 1, 120–121. doi: 10.1177/1754073908100436

Kensinger, E. A., and Corkin, S. (2003). Memory enhancement for emotional words: are emotional words more vividly remembered than neutral words? *Mem. Cogn.* 31, 1169–1180. doi: 10.3758/BF03195800

Kensinger, E. A., and Schachter, D. L. (2006). Processing emotional pictures and words: effects of valence and arousal. *Cogn. Affect. Behav. Neurosci.* 6, 110–126. doi: 10.3758/CABN.6.2.110

Keute, M., Boehere, L., Ruhnau, P., Heinze, H.-J., and Zaehle, T. (2019). Transcutaneous vagus nerve stimulation (TVNS) and the dynamics of visual bistable perception. *Front. Neurosci.* 13:227. doi: 10.3389/fnins.2019.00227

Kissler, J., Herbert, C., Winkler, I., and Junghofer, M. (2009). Emotion and attention in visual word processing–an erp study. *Biol. Psychol.* 80, 75–83. doi: 10.1016/j.biopsycho.2008.03.004

Kissler, J., and Koessler, S. (2011). Emotionally positive stimuli facilitate lexical decisions–an ERP study. *Biol. Psychol.* 86, 254–264. doi: 10.1016/j.biopsycho.2010.12.006

Koenig, J., Parzer, P., Haigis, N., Liebemann, J., Jung, T., Resch, F., et al. (2019). Effects of acute transcutaneous vagus nerve stimulation on emotion recognition in adolescent depression. *Psychol. Med.* doi: 10.1017/S0033291719003490. [Epub ahead of print].

Kousta, S.-T., Vigliocco, G., Vinson, D. P., Andrews, M., and Del Campo, E. (2011). The representation of abstract words: why emotion matters. *J. Exp. Psychol. Gen.* 140:14. doi: 10.1037/a0021446

Kraus, T., Kiess, O., Hösl, K., Terekhin, P., Kornhuber, J., and Forster, C. (2013). CNS bold fMRI effects of sham-controlled transcutaneous electrical nerve stimulation in the left outer auditory canal-a pilot study. *Brain Stimul.* 6, 798–804. doi: 10.1016/j.brs.2013.01.011

Kuperman, V., Estes, Z., Brysbaert, M., and Warriner, A. B. (2014). Emotion and language: valence and arousal affect word recognition. *J. Exp. Psychol. Gen.* 143:1065. doi: 10.1037/a0035669

Lang, P. J., and Bradley, M. M. (2010). Emotion and the motivational brain. *Biol. Psychol.* 84, 437–450. doi: 10.1016/j.biopsycho.2009.10.007

Lang, P. J., Bradley, M. M., and Cuthbert, B. N. (1998). Emotion, motivation, and anxiety: brain mechanisms and psychophysiology. *Biol. Psychiatry* 44, 1248–1263. doi: 10.1016/S0006-3223(98)00275-3

Lewine, J. D., Paulson, K., Bangera, N., and Simon, B. J. (2019). Exploration of the impact of brief noninvasive vagal nerve stimulation on EEG and event-related potentials. *Neuromodul. Technol. Neural Interface* 22, 564–572. doi: 10.1111/ner.12864

Mather, M., Clewett, D., Sakaki, M., and Harley, C. W. (2016). Norepinephrine ignites local hot spots of neuronal excitation: How arousal amplifies selectivity in perception and memory. *Behav. Brain Sci.* 39:e200. doi: 10.1017/S0140525X15000667

Mather, M., and Sutherland, M. R. (2011). Arousal-biased competition in perception and memory. *Perspect. Psychol. Sci.* 6, 114–133. doi: 10.1177/1745691611400234

McGaugh, J. L. (2015). Consolidating memories. *Annu. Rev. Psychol.* 66, 1–24. doi: 10.1146/annurev-psych-010814-014954

McIntyre, C. K., McGaugh, J. L., and Williams, C. L. (2012). Interacting brain systems modulate memory consolidation. *Neurosci. Biobehav. Rev.* 36, 1750–1762. doi: 10.1016/j.neubiorev.2011.11.001

Mello-Carpes, P. B., and Izquierdo, I. (2013). The nucleus of the solitary tract → nucleus paragigantocellularis → locus coeruleus → ca1 region of dorsal hippocampus pathway is important for consolidation of object recognition memory. *Neurobiol. Learn. Mem.* 100, 56–63. doi: 10.1016/j.nlm.2012.12.002

Mogg, K., Bradley, B. P., De Bono, J., and Painter, M. (1997). Time course of attentional bias for threat information in non-clinical anxiety. *Behav. Res. Therapy* 35, 297–303. doi: 10.1016/S0005-7967(96)00109-X

Moroni, F., Nobili, L., Curcio, G., De Carli, F., Fratello, F., Marzano, C., et al. (2007). Sleep in the human hippocampus: a stereo-EEG study. *PLoS ONE* 2:e867. doi: 10.1371/journal.pone.0000867

Nasrallah, M., Carmel, D., and Lavie, N. (2009). Murder, she wrote: enhanced sensitivity to negative word valence. *Emotion* 9:609. doi: 10.1037/a0016305

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

Nieuwenhuis, S., Aston-Jones, G., and Cohen, J. D. (2005). Decision making, the p3, and the locus coeruleus-norepinephrine system. *Psychol. Bull.* 131:510. doi: 10.1037/0033-2909.131.4.510

Nishida, M., Pearsall, J., Buckner, R. L., and Walker, M. P. (2009). REM sleep, prefrontal theta, and the consolidation of human emotional memory. *Cereb. Cortex* 19, 1158–1166. doi: 10.1093/cercor/bhn155

Ochsner, K. N. (2000). Are affective events richly recollected or simply familiar? The experience and process of recognizing feelings past. *J. Exp. Psychol. Gen.* 129:242. doi: 10.1037/0096-3445.129.2.242

Öhman, A., Flykt, A., and Esteves, F. (2001). Emotion drives attention: detecting the snake in the grass. *J. Exp. Psychol. Gen.* 130:466. doi: 10.1037/0096-3445.130.3.466

Ortigue, S., Michel, C. M., Murray, M. M., Mohr, C., Carbonnel, S., and Landis, T. (2004). Electrical neuroimaging reveals early generator modulation to emotional words. *Neuroimage* 21, 1242–1251. doi: 10.1016/j.neuroimage.2003.11.007

Payne, J. D., Stickgold, R., Swanberg, K., and Kensinger, E. A. (2008). Sleep preferentially enhances memory for emotional components of scenes. *Psychol. Sci.* 19, 781–788. doi: 10.1111/j.1467-9280.2008.02157.x

Peuker, E. T., and Filler, T. J. (2002). The nerve supply of the human auricle. *Clin. Anat*. 15, 35–37. doi: 10.1002/ca.1089

Phelps, E. A., LaBar, K. S., and Spencer, D. D. (1997). Memory for emotional words following unilateral temporal lobectomy. *Brain Cogn*. 35, 85–109. doi: 10.1006/brcg.1997.0929

Pratto, F., and John, O. P. (1991). Automatic vigilance: the attention-grabbing power of negative social information. *J. Pers. Soc. Psychol*. 61:380. doi: 10.1037/0022-3514.61.3.380

Raedt, R., Clinckers, R., Mollet, L., Vonck, K., El Tahry, R., Wyckhuys, T., et al. (2011). Increased hippocampal noradrenaline is a biomarker for efficacy of vagus nerve stimulation in a limbic seizure model. *J. Neurochem*. 117, 461–469. doi: 10.1111/j.1471-4159.2011.07214.x

Ranganath, C., and Ritchey, M. (2012). Two cortical systems for memory-guided behaviour. *Nat. Rev. Neurosci*. 13:713. doi: 10.1038/nrn3338

Righi, S., Marzi, T., Toscani, M., Baldassi, S., Ottonello, S., and Viggiano, M. (2012). Fearful expressions enhance recognition memory: electrophysiological evidence. *Acta Psychol*. 139, 7–18. doi: 10.1016/j.actpsy.2011.09.015

Rimmele, U., Davachi, L., and Phelps, E. A. (2012). Memory for time and place contributes to enhanced confidence in memories for emotional events. *Emotion* 12:834. doi: 10.1037/a0028003

Roosevelt, R. W., Smith, D. C., Clough, R. W., Jensen, R. A., and Browning, R. A. (2006). Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. *Brain Res*. 1119, 124–132. doi: 10.1016/j.brainres.2006.08.048

Rufener, K. S., Geyer, U., Janitzky, K., Heinze, H.-J., and Zaehle, T. (2018). Modulating auditory selective attention by non-invasive brain stimulation: Differential effects of transcutaneous vagal nerve stimulation and transcranial random noise stimulation. *Eur. J. Neurosci*. 48, 2301–2309. doi: 10.1111/ejn.14128

Rugg, M. D., and Vilberg, K. L. (2013). Brain networks underlying episodic memory retrieval. *Curr. Opin. Neurobiol*. 23, 255–260. doi: 10.1016/j.conb.2012.11.005

Sauter, D. A., and Eimer, M. (2010). Rapid detection of emotion from human vocalizations. *J. Cogn. Neurosci*. 22, 474–481. doi: 10.1162/jocn.2009.21215

Sauvage, M. M., Fortin, N. J., Owens, C. B., Yonelinas, A. P., and Eichenbaum, H. (2008). Recognition memory: opposite effects of hippocampal damage on recollection and familiarity. *Nat. Neurosci*. 11, 16–18. doi: 10.1038/nn2016

Schacht, A., and Sommer, W. (2009). Time course and task dependence of emotion effects in word processing. *Cogn. Affect. Behav. Neurosci*. 9, 28–43. doi: 10.3758/CABN.9.1.28

Schupp, H. T., Flaisch, T., Stockburger, J., and Junghöfer, M. (2006). Emotion and attention: event-related brain potential studies. *Prog. Brain Res*. 156, 31–51. doi: 10.1016/S0079-6123(06)56002-9

Schupp, H. T., Öhman, A., Junghöfer, M., Weike, A. I., Stockburger, J., and Hamm, A. O. (2004). The facilitated processing of threatening faces: an ERP analysis. *Emotion* 4:189. doi: 10.1037/1528-3542.4.2.189

Scott, G. G., O'Donnell, P. J., Leuthold, H., and Sereno, S. C. (2009). Early emotion word processing: evidence from event-related potentials. *Biol. Psychol*. 80, 95–104. doi: 10.1016/j.biopsycho.2008.03.010

Sellaro, R., de Gelder, B., Finisguerra, A., and Colzato, L. S. (2018). Transcutaneous vagus nerve stimulation (TVNS) enhances recognition of emotions in faces but not bodies. *Cortex* 99, 213–223. doi: 10.1016/j.cortex.2017.11.007

Sellaro, R., van Leusden, J. W., Tona, K.-D., Verkuil, B., Nieuwenhuis, S., and Colzato, L. S. (2015). Transcutaneous vagus nerve stimulation enhances post-error slowing. *J. Cogn. Neurosci*. 27, 2126–2132. doi: 10.1162/jocn_a_00851

Sharot, T., Delgado, M. R., and Phelps, E. A. (2004). How emotion enhances the feeling of remembering. *Nat. Neurosci*. 7:1376. doi: 10.1038/nn1353

Sharot, T., Verfaellie, M., and Yonelinas, A. P. (2007). How emotion strengthens the recollective experience: a time-dependent hippocampal process. *PLoS ONE* 2:e1068. doi: 10.1371/journal.pone.0001068

Snodgrass, J. G., and Corwin, J. (1988). Pragmatics of measuring recognition memory: applications to dementia and amnesia. *J. Exp. Psychol. Gen*. 117:34. doi: 10.1037/0096-3445.117.1.34

Steenbergen, L., Sellaro, R., Stock, A.-K., Verkuil, B., Beste, C., and Colzato, L. S. (2015). Transcutaneous vagus nerve stimulation (TVNS) enhances response selection during action cascading processes. *Eur. Neuropsychopharmacol*. 25, 773–778. doi: 10.1016/j.euroneuro.2015.03.015

Sterpenich, V., D'Argembeau, A., Desseilles, M., Balteau, E., Albouy, G., Vandewalle, G., et al. (2006). The locus ceruleus is involved in the successful retrieval of emotional memories in humans. *J. Neurosci*. 26, 7416–7423. doi: 10.1523/JNEUROSCI.1001-06.2006

Szeska, C., Richter, J., Wendt, J., Weymar, M., and Hamm, A. O. (2020). Promoting long-term inhibition of human fear responses by non-invasive transcutaneous vagus nerve stimulation during extinction training. *Sci. Rep*. 10, 1–16. doi: 10.1038/s41598-020-58412-w

Thoma, M. V., Kirschbaum, C., Wolf, J. M., and Rohleder, N. (2012). Acute stress responses in salivary alpha-amylase predict increases of plasma norepinephrine. *Biol. Psychol*. 91, 342–348. doi: 10.1016/j.biopsycho.2012.07.008

Van Bockstaele, E. J., Peoples, J., and Telegan, P. (1999). Efferent projections of the nucleus of the solitary tract to peri-locus coeruleus dendrites in rat brain: Evidence for a monosynaptic pathway. *J. Comp. Neurol*. 412, 410–428. doi: 10.1002/(SICI)1096-9861(19990927)412:3<410::AID-CNE3>3.0.CO;2-F

Van Leusden, J. W., Sellaro, R., and Colzato, L. S. (2015). Transcutaneous vagal nerve stimulation (TVNS): a new neuromodulation tool in healthy humans? *Front. Psychol*. 6:102. doi: 10.3389/fpsyg.2015.00102

Vecchio, F., Miraglia, F., Curcio, G., Altavilla, R., Scrascia, F., Giambattistelli, F., et al. (2015). Cortical brain connectivity evaluated by graph theory in dementia: a correlation study between functional and structural data. *J. Alzheimers Dis*. 45, 745–756. doi: 10.3233/JAD-142484

Ventura-Bort, C., Wirkner, J., Genheimer, H., Wendt, J., Hamm, A. O., and Weymar, M. (2018). Effects of transcutaneous vagus nerve stimulation (TVNS) on the p300 and alpha-amylase level: a pilot study. *Front. Hum. Neurosci*. 12:202. doi: 10.3389/fnhum.2018.00202

Võ, M. L., Conrad, M., Kuchinke, L., Urton, K., Hofmann, M. J., and Jacobs, A. M. (2009). The berlin affective word list reloaded (BAWL-R). *Behav. Res. Methods* 41, 534–538. doi: 10.3758/BRM.41.2.534

Wagner, U., Hallschmid, M., Rasch, B., and Born, J. (2006). Brief sleep after learning keeps emotional memories alive for years. *Biol. Psychiatry* 60, 788–790. doi: 10.1016/j.biopsych.2006.03.061

Warren, C. M., Tona, K. D., Ouwerkerk, L., Van Paridon, J., Poletiek, F., van Steenbergen, H., et al. (2019). The neuromodulatory and hormonal effects of transcutaneous vagus nerve stimulation as evidenced by salivary alpha amylase, salivary cortisol, pupil diameter, and the p3 event-related potential. *Brain Stimul*. 12, 635–642. doi: 10.1016/j.brs.2018.12.224

Warren, C. M., van den Brink, R. L., Nieuwenhuis, S., and Bosch, J. A. (2017). Norepinephrine transporter blocker atomoxetine increases salivary alpha amylase. *Psychoneuroendocrinology* 78, 233–236. doi: 10.1016/j.psyneuen.2017.01.029

Weymar, M., Bradley, M. M., Hamm, A. O., and Lang, P. J. (2014). Encoding and reinstatement of threat: recognition potentials. *Neurobiol. Learn. Mem*. 107, 87–92. doi: 10.1016/j.nlm.2013.11.005

Weymar, M., and Hamm, A.O. (2013). "Electrophysiological signature of emotional memories," in *Hurting Memories and Beneficial Forgetting*, eds M. Linden and K. Rutkowski (Elsevier Insights), 21–35. doi: 10.1016/B978-0-12-398393-0.00002-X

Weymar, M., Löw, A., and Hamm, A. O. (2011). Emotional memories are resilient to time: evidence from the parietal ERP old/new effect. *Hum. Brain Mapp*. 32, 632–640. doi: 10.1002/hbm.21051

Weymar, M., Löw, A., Melzig, C. A., and Hamm, A. O. (2009). Enhanced long-term recollection for emotional pictures: evidence from high-density ERPs. *Psychophysiology* 46, 1200–1207. doi: 10.1111/j.1469-8986.2009.00869.x

Weymar, M., Löw, A., Schwabe, L., and Hamm, A. O. (2010). Brain dynamics associated with recollective experiences of emotional events. *Neuroreport* 21, 827–831. doi: 10.1097/WNR.0b013e32833d180a

Weymar, M., Schwabe, L., Löw, A., and Hamm, A. O. (2012). Stress sensitizes the brain: Increased processing of unpleasant pictures after exposure to acute stress. *J. Cogn. Neurosci*. 24, 1511–1518. doi: 10.1162/jocn_a_00174

Weymar, M., Ventura-Bort, C., Wirkner, J., Genheimer, H., Wendt, J., and Hamm, A. (2019). "Effects of transcutaneous vagus nerve stimulation (TVNS) on selective attention and emotional episodic memory: findings from ERP research," in *Psychophysiology*, Vol. 56 (Hoboken, NJ: Wiley), S12'12.

Wixted, J. T., and Stretch, V. (2004). In defense of the signal detection interpretation of remember/know judgments. *Psychon. Bull. Rev.* 11, 616–641. doi: 10.3758/BF03196616

Wright, S. P. (1992). Adjusted p-values for simultaneous inference. *Biometrics* 48, 1005–1013. doi: 10.2307/2532694

Yakunina, N., Kim, S. S., and Nam, E.-C. (2017). Optimization of transcutaneous vagus nerve stimulation using functional MRI. *Neuromodul. Technol. Neural Interface* 20, 290–300. doi: 10.1111/ner.12541

Yonelinas, A. P. (2001). Components of episodic memory: the contribution of recollection and familiarity. *Philos. Trans. R. Soc. Lond. Ser. B Biol Sci.* 356, 1363–1374. doi: 10.1098/rstb.2001.0939

Yonelinas, A. P., Aly, M., Wang, W.-C., and Koen, J. D. (2010). Recollection and familiarity: examining controversial assumptions and new directions. *Hippocampus* 20, 1178–1194. doi: 10.1002/hipo.20864

Conflict of Interest: 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.

frontiers in Psychology

PERSPECTIVE
published: 29 July 2020
doi: 10.3389/fpsyg.2020.01659

Vagus Nerve Stimulation as a Gateway to Interoception

Albertyna Paciorek[1] and Lina Skora[2,3]*

[1] Faculty of Psychology, University of Warsaw, Warsaw, Poland, [2] Sackler Centre for Consciousness Science, University of Sussex, Brighton, United Kingdom, [3] School of Psychology, University of Sussex, Brighton, United Kingdom

The last two decades have seen a growing interest in the study of interoception. Interoception can be understood as a hierarchical phenomenon, referring to the body-to-brain communication of internal signals, their sensing, encoding, and representation in the brain, influence on other cognitive and affective processes, and their conscious perception. Interoceptive signals have been notoriously challenging to manipulate in experimental settings. Here, we propose that this can be achieved through electrical stimulation of the vagus nerve (either in an invasive or non-invasive fashion). The vagus nerve is the main pathway for conveying information about the internal condition of the body to the brain. Despite its intrinsic involvement in interoception, surprisingly little research in the field has used Vagus Nerve Stimulation to explicitly modulate bodily signals. Here, we review a range of cognitive, affective and clinical research using Vagus Nerve Stimulation, showing that it can be applied to the study of interoception at each level of its hierarchy. This could have considerable implications for our understanding of the interoceptive dimension of cognition and affect in both health and disease, and lead to development of new therapeutic tools.

Keywords: interoception, vagus nerve, vagus nerve stimulation, VNS, tVNS, transcutaneous vagus nerve stimulation

OPEN ACCESS

Edited by:
Mathias Weymar,
University of Potsdam, Germany

Reviewed by:
Franziska Labrenz,
Essen University Hospital, Germany
Christoph Benke,
University of Marburg, Germany

****Correspondence:***
Albertyna Paciorek
albertyna.paciorek@psych.uw.edu.pl

Specialty section:
This article was submitted to Cognition, a section of the journal Frontiers in Psychology

Received: *02 April 2020*
Accepted: *18 June 2020*
Published: *29 July 2020*

Citation:
Paciorek A and Skora L (2020) Vagus Nerve Stimulation as a Gateway to Interoception. Front. Psychol. 11:1659. doi: 10.3389/fpsyg.2020.01659

INTRODUCTION

Interoception pertains to receiving, encoding, and representation of internal bodily signals in the brain, as well as their perception (Cameron, 2001; Craig, 2002; Critchley et al., 2004). Through interoception we know when our heart is beating fast, when we need to take a deep breath, and when we are hungry, thirsty, hot, cold, nauseous, tired, or alert. It encompasses both the non-conscious bodily signals themselves and our conscious perception of them. Growing research has shown interoception not only to be crucial for homeostasis and allostasis (acute change to achieve homeostasis), but also central in a range of cognitive and emotional processes, including memory, decision-making, emotional processing, social interactions, and even consciousness, body ownership and a sense of self (Critchley et al., 2001; Dunn et al., 2010; Shah et al., 2017; Berntson et al., 2018; Critchley and Garfinkel, 2018). By their nature, internal bodily processes are notoriously difficult to manipulate in experimental settings. The vagus nerve, the main cranial nerve in the human body known to be central in relaying visceral signals to the brain, is naturally implicated in interoception (Critchley and Harrison, 2013; Quadt et al., 2019; Yoris et al., 2019). Yet surprisingly little research in this area has used vagus nerve stimulation (VNS) to modulate bodily signaling. So far, to the best of our knowledge, only one recent study has explicitly related VNS to interoception (Villani et al., 2019). Here, we review the accumulated cognitive and clinical research on VNS and propose that this technique can indeed be used to modulate a wide range of interoception-related processes.

The vagus nerve (cranial nerve X) is the longest and one of the most widely distributed nerves in the body (*vagus* in Latin means "wandering"). As part of the parasympathetic division of the autonomic nervous system, the vagus nerve conveys information between visceral organs (i.e., organs located in the thoracic and abdominal cavities, including the heart) and the brain (Berthoud and Neuhuber, 2000; Craig, 2002; Critchley and Harrison, 2013). About 80% of the vagus nerve is composed of afferent fibers, projecting to the nucleus tractus solitarii (NTS) in the medulla, before being relayed further to other brainstem nuclei (broadly implicated in homeostatic control) and higher-order structures, including the thalamus, hippocampus, amygdala, and insula (Goehler et al., 2000; Saper, 2002). VNS involves electrical stimulation of the afferent fibers of the vagus nerve, either in an invasive variant (where the stimulator is implanted in the patient's body) or a non-invasive variant where the stimulation is delivered transcutaneously through the auricular branch of the vagus nerve (tVNS, aVNS, or taVNS). Both VNS and tVNS reliably induce activation in the same brain areas (Narayanan et al., 2002; Frangos et al., 2015; Badran et al., 2018a). These areas, and their projections, and especially the insula, have been shown to be implicated in interoception (Craig, 2002).

Interoception has been conceptually divided into hierarchical levels (Garfinkel et al., 2015; Quadt et al., 2018), from low-level visceral afferents and their preconscious effects on cognitive or affective processes to psychological dimensions and metacognitive awareness of internal bodily processes. Because the vagus nerve is the main pathway relaying visceral signals into the brain, we propose that (t)VNS could affect interoception at each of those levels. Recent years have also seen a growing interest in the role of interoception in mental illness, proposing that interoceptive dysfunction can contribute to impairments in social, cognitive, behavioral, affective, and somatic processes associated with certain psychopathologies such as anxiety, depression, eating disorders, addiction, or post-traumatic stress disorder (PTSD) (Paulus and Stein, 2010; Harshaw, 2015; Khalsa et al., 2018). Those dysfunctions can occur at any level of interoceptive processing. Proposed mechanisms include aberrant processing of the afferent signal (e.g., noisy inputs) or weight given to its representations (e.g., overweighted interoceptive signal affecting subsequent evaluation of affective stimuli), abnormal expectations about bodily states, attentional or cognitive biases (e.g., hypervigilance to bodily states), or psychological biases (e.g., weak insight into one's bodily state and its relevance to environmental context) (Khalsa et al., 2018; Petzschner et al., 2017; Gu et al., 2019).

This paper provides an overview of the research on the effects of VNS on interoception-related processes (both cognitive and clinical), from the lowest to highest level, and relates those findings to embodied models of brain function.

LOW-LEVEL AFFERENT INTEROCEPTIVE SIGNAL

The low-level visceral signal, such as baroreceptor activity or blood oxygenation, is not consciously perceived unless there is a problem. However, according to embodied, interoception-based accounts of the conscious self, access to and evaluation of the signal from the physical body is necessary for the conscious "self" to arise in the first place (Seth et al., 2012; Seth, 2013; Cleeremans et al., 2020). Stimulated NTS, the projection area of the vagus nerve, activates the dorsal raphe and other areas known to control alertness (George et al., 2000). For this reason, VNS was proposed as a fruitful therapy for disorders of sleep or even consciousness (George et al., 2000; Naritoku et al., 2003), and was subsequently shown to promote improved sleep in rats (Rong et al., 2019) and human adults (Bretherton et al., 2019). Impressively Corazzol et al. (2017), in a single case study, demonstrated that long-term VNS may improve patient condition even after years in persistent unresponsive wakefulness (vegetative state), warranting future research in this area. This finding corroborates the proposal that a sense of conscious self is embodied – the experience of owning and identifying with one's own body, and the experience of first-person perspective, are directly associated with multi-level representations of physiological condition of the body (Seth et al., 2012; Seth, 2013; Cleeremans et al., 2020). As such, (t)VNS may play a pivotal role in helping to restore this conscious, embodied sensation. This hypothesis could be further reinforced if classical paradigms exploring the sense of self, such as the rubber-hand or full-body illusion or sense of agency, proved to be modulated by (t)VNS.

In the cognitive literature, a demonstration that VNS directly manipulates the read-outs of basic visceral afferent signals has yet to be given. The most promising candidate is the modulation of Heartbeat Evoked Potential (HEP) amplitude. The cardiac signal is one of the most widely explored low-level afferent signals, reflected in electroencephalography as an event-related potential synchronized with cardiac R-peaks. HEP has been shown to predict individual heartbeat perception (Pollatos et al., 2005; Terhaar et al., 2012), and was proposed to reflect interoceptive belief updating (Ainley et al., 2016) and affective predictions (Gentsch et al., 2018; Marshall et al., 2018). It has also been shown to be modulated by internal (vs. external) attentional focus, proposed to reflect interoceptive precision (Petzschner et al., 2019). The HEP has been shown to originate in the insula, the brain region assumed to be key for interoception (Park et al., 2017), and activated through (t)VNS (e.g., Badran et al., 2018a). Interestingly, Park et al. (2017) confirmed the HEP's functional role in self-consciousness by demonstrating HEP modulations as a response to an experimentally-induced altered sense of self-identification (full-body illusion). This result supports the proposal that interoceptive information is a crucial substrate of the sense of self and body ownership (Sierra and David, 2011; Critchley and Harrison, 2013; Crucianelli et al., 2018). Additionally, the sense of body ownership has been shown to be impaired after insula lesions (Karnath, 2005; Gandola et al., 2012; Moro et al., 2016). Given that (t)VNS reliably activates the interoceptive network (including the insula), it could constitute a tool for manipulating low-level interoceptive signals and their read-outs, such as the HEP, for research into consciousness, sense of self and body ownership.

PRECONSCIOUS IMPACTS ON COGNITIVE PROCESSES

While the previous, lowest level of the interoceptive hierarchy was concerned with the mere communication and detection of bodily afferents, the second, preconscious level refers to the early stages of central processing and pertains to the impact the afferent visceral signals have on cognitive and affective processing. The cardiac signal in particular has been shown to play a part in emotion processing (Bechara and Naqvi, 2004). There is evidence that processing of emotional faces is enhanced at systole compared to diastole (Garfinkel and Critchley, 2016), and the learning of fearful face-name pairs is better at systole than at diastole (interestingly, just in people with heightened interoceptive ability, and not for happy or neutral faces; Pfeifer et al., 2017). Higher interoceptive ability (encompassing perception and confidence in one's perception of bodily signals; see next section) correlates with recognition memory for emotional pictures (Pollatos and Schandry, 2008) and words (Werner et al., 2010). "Gut feeling" is commonly used to mean intuition, pointing to a popular sensation of visceral signals influencing decisions. Adaptive behavior and optimal decision-making have indeed been proposed to be supported, or even guided, by visceral signals, where the physiological state of the body provides a reference frame for homeostatic or motivational value of given choice options (Damasio, 1994; Bechara, 2004; Gu and FitzGerald, 2014; Maniscalco and Rinaman, 2018). Although the (t)VNS literature has not focused on interoception, studies have shown that this technique modulates similar interoceptive processes to those mentioned above. Emerging evidence suggests that tVNS can improve facial emotion recognition (Colzato et al., 2017a), though, interestingly, not from entire bodies (Sellaro et al., 2018). Elsewhere, preliminary experimental evidence suggests that VNS can improve decision-making on the Iowa Gambling Task (Martin et al., 2004).

Another example of a physical state influencing cognitive function is the phenomenon of memory enhancement during somatosensory arousal. Traumatic memories are remembered particularly vividly. This phenomenon is crucially reliant on vagal transmission of various neuromodulators (Flood and Morley, 1988; Williams and Jensen, 1993; Nogueira et al., 1994; Talley et al., 2002). VNS may induce a state similar to arousal, most likely linked to secretion of noradrenaline and acetylcholine in the brain, neurotransmitters known to mediate attention (Martino et al., 2007; Klinkenberg et al., 2011). Studies have shown that (t)VNS may improve declarative memory retention (Clark et al., 1999; Ghacibeh et al., 2006; Jacobs et al., 2015; Broncel et al., 2020; Giraudier et al., 2020), even in patients with Alzheimer's Disease (Clark et al., 1999; Sjogren et al., 2002; Merrill et al., 2006). Other authors have found mixed results in single-session studies (see Vonck et al., 2014 for review), depending on the stimulation settings (0.5 mA being optimal). Clinical interventions have capitalized on this memory-enhancing effect of (t)VNS to strengthen the formation of adaptive memories and behaviors after brain damage, e.g., "targeted plasticity" interventions (Hays et al., 2013). Interestingly, there is mixed evidence whether long-term VNS may lead to general memory improvement, which was not observed in those undergoing chronic VNS treatment for epilepsy, but was in depression (Aaronson et al., 2013; Vonck et al., 2014). Vonck et al. (2014) point out that cognitive dysfunctions are inherent to clinical depression and known to improve with improvement of depressive symptoms. This ties in with accounts of depression linking it to malfunctioning interoceptive evaluation of bodily signals (Barrett and Simmons, 2015; Quadt et al., 2018). Chronic aberrant interoceptive processing has maladaptive allostatic consequences for dealing with stress and, in particular, inflammation, which frequently cooccurs with depression. Indeed (Howland, 2014) points that the relationship between depression and inflammation may be mediated by the vagus nerve.

Malfunctioning interoceptive evaluation of bodily signals is also implicated in anxiety and panic. It may result from distorted interoceptive learning, when a benign sensation is experienced in the context of an initial panic attack, resulting in rapid conditioning. Overriding such strongly conditioned responses remains a challenge for most therapies. Fear extinction, a removal of conditioned response, is a gold standard therapy for PTSD (Genheimer et al., 2017), yet for many it remains not entirely effective, calling for its enhancement, potentially with VNS or tVNS. So far, however, the results are mixed, with promising results of VNS on fear extinction in rats (Peña et al., 2013, 2014; Noble et al., 2017; Souza et al., 2019, 2020), and with varied success of tVNS in humans (Burger et al., 2016, 2017; Genheimer et al., 2017; Szeska et al., 2020), which may depend on particular stimulation parameters (Hansen, 2019).

PSYCHOLOGICAL DIMENSIONS: INTEROCEPTIVE ACCURACY, SENSIBILITY, AND AWARENESS

Psychological dimensions of interception refer to the conscious perception of bodily signals (Garfinkel et al., 2015). These dimensions have been conceptualized across three levels: interoceptive accuracy (referring to objective accuracy in perceiving a bodily signal, e.g., one's own heartbeat), sensibility (subjective beliefs about the ability to perceive own bodily sensations), and awareness (the correspondence between accuracy and confidence, i.e., a metacognitive aspect of interoceptive ability). Interoceptive accuracy and awareness are typically quantified with performance on bodily signal perception tasks, such as heartbeat counting or detection tasks (e.g., Schandry, 1981). Those who perform well on heartbeat detection tasks also tend to experience greater arousal and higher HEP amplitudes for emotional pictures (Herbert et al., 2007). Performance on such tasks is shown to correlate with the intensity of one's own emotions (Wiens et al., 2000), perception of others' emotions (Terasawa et al., 2014), and decision-making (Werner et al., 2009; Kandasamy et al., 2016), although the causal relations are still unclear.

The role of tVNS on the psychological dimensions of interoception was directly tested by Villani et al. (2019). They found tVNS to improve participants' ability to correctly identify (but not count) their own heartbeats (interoceptive accuracy). Furthermore, participants reported higher confidence in their decisions under tVNS, but this did not lead to enhanced interoceptive awareness. This promising result, in light of the range of phenomena shown to be improved in individuals with higher interoceptive accuracy outlined above, offers an avenue for further research.

Deficits in interoceptive processing at this level are related to poor processing or evaluation of own bodily signals (e.g., impaired insight into one's internal state and its relevance to environmental context; e.g., Paulus and Stein, 2010; Petzschner et al., 2017), and can predict emotional and affective psychopathology. Individuals with alexithymia, an impairment in recognizing one's emotions which often cooccurs with autism (ASC), show reduced interoceptive accuracy (Ernst et al., 2013), as well as reduced brain activation in the insular cortex (Bird et al., 2010). Evidence for a reduction in interoceptive accuracy in the autistic population is mixed (Quadt et al., 2018), and correlated with co-existing alexithymia (Shah et al., 2016). Interestingly, autistic individuals tend to have elevated confidence (sensibility) relative to their performance accuracy (Garfinkel et al., 2016). A similar discrepancy, termed "interoceptive trait prediction error," has been found in anxiety disorders (Paulus and Stein, 2006). Direct interventions with tVNS have been proposed for autism (Engineer et al., 2017) and anxiety (George et al., 2008). tVNS has been shown to alleviate some symptoms of ASC in epileptic patients with this comorbidity (see Levy et al., 2010; Jin and Kong, 2017 for review), which we hypothesize to be modulated by improvement in their interpretative processing. More research is needed to correlate treatment-induced change in ASC symptomatology and interoceptive accuracy, sensibility, and awareness.

Aberrant performance on interoceptive tasks (usually lower accuracy) has been shown in eating disorders (ED), such as anorexia nervosa (Pollatos et al., 2008; Van den Bergh et al., 2017), along with impaired ability to differentiate hunger and satiety, and reduced response to emotional states (Fassino et al., 2004). Together these characteristics suggest weakened interoceptive processing. Vagus nerve stimulation is slowly being recognized as a potential treatment to regulate food craving (Wernicke et al., 1993; Boveja and Widhany, 2003) proposed that vagal signal suppression could be helpful in treating obesity, signal stimulation in anorexia, and intermittent stimulation in bulimia, though experimental research is still needed. Further work might also identify whether the regulation of food cravings leads to changes in performance on interoceptive tasks.

Finally, pain has also been described in terms of interoceptive processing (Craig, 2003; Khalsa et al., 2009), with the vagus nerve crucial in relaying somatosensory sensations. Pain is usually caused by the activation of nociceptors and nociceptive pathways (Meyer et al., 2006), but it is also known to occur without corresponding activity, and nociceptors can be active without the sensation of pain (e.g., lack of reported pain by soldiers during battle, despite severe injuries), and be modulated by psychological state (Melzack et al., 1982; Mariana von Mohr, 2019). Deficits in interoceptive accuracy have been reported in patients with fibromyalgia (Duschek et al., 2015), lower back pain (Mehling et al., 2013) and migraines (see (Lernia et al., 2016) for review). VNS has been shown to modulate the sensation of pain in fibromyalgia (Lange et al., 2011) and migraines (Barbanti et al., 2015). Pain perception has been also reported to be reduced in patients treated with VNS for depression (Borckardt et al., 2005). This points toward the conclusion that vagus nerve stimulation affects interoceptive processing of pain, though more research is needed to elucidate the causes of individual differences in response to treatment.

METACOGNITIVE LEVEL

The final, metacognitive level represents an executive dimension. It refers to one's ability to flexibly switch between attending to and utilizing interoceptive and exteroceptive information in an adaptive manner. Though direct tests on such tasks are yet to be done, there is promising evidence that tVNS facilitates attentional switching in conflict situations, such as a number version of the Simon task (Fischer et al., 2018), allows rapid attentional adaptation (Colzato et al., 2017b), and improves response selection in sequential action (Jongkees et al., 2018). In the clinical domain, tVNS has been shown to reduce temper outbursts in Prader-Willi Syndrome (Manning et al., 2019), further reinforcing the notion that it improves interoceptive processing at the high level implicated in executive control.

A VNS influence on metacognition may also account for the rare adverse side effect observed in 4 epilepsy patients, namely hallucinations and psychosis (De Herdt et al., 2003), most likely caused by acutely increased alertness and decreased sedation through VNS. Patients with mild or severe intellectual disability may be specifically prone to the development of these symptoms. We suggest that in cases where cognitive resources for the appraisal of peripheral, somatosensory information are impaired, augmenting the signal strength can lead to systemic overload and malfunction.

DISCUSSION

The range of processes which can be affected by stimulating the vagus nerve points to the sheer scale of visceral influence. The presented literature can be unified under the theoretical frameworks which posit that affective and cognitive states are continuously interpreted through, and biased by, the body's internal states. While the primary function of interoceptive signals is to feed the brain a continuous stream of information on the internal state of the organism so as to ensure survival, there is increasing consensus that they also fundamentally inform motivational states, adaptive behavior and emotion (Damasio, 2010; Critchley and Harrison, 2013; Seth, 2013; Barrett and Simmons, 2015; Critchley and Garfinkel, 2018; Seth and Tsakiris, 2018). According to this view, the brain interprets its current environmental challenges in light of the concurrent state of the

body. The evidence of VNS influence on higher-order processes can thus lend support to models of brain function that assume a causal visceral dimension.

The successful application of VNS in clinical settings, briefly outlined here in the context of interoception, also corroborates the idea that a number of clinical conditions may have an interoceptive dimension. The visceral contribution has been noted for anxiety, depression, and PTSD, and many of the conditions discussed above have overlapping symptoms (Khalsa et al., 2018). For example, (Howland, 2014) pointed out that the relationship between depression, inflammation, metabolic syndrome, and heart disease may be mediated by the vagus nerve. VNS research can lead to greater understanding of the interoceptive dimension in clinical conditions, giving rise to future treatments.

While VNS has been enjoying increasing clinical success and is a promising tool for interoceptive manipulation, it is noteworthy that the optimal experimental protocols are still a work in progress, and should be carefully considered on an individual basis. Considerations under ongoing debate include optimal stimulation locations on the ear (Burger and Verkuil, 2018), potential differences in signal paths between VNS and tVNS, and the longevity of the effect after stimulation ceases. It seems that although cognitive effects of VNS may be detectable after short periods of stimulation, even 20 min (e.g., Colzato et al., 2017b), attenuation of certain clinical symptoms may require much longer stimulation durations (e.g., Manning et al., 2019) point to reduction in number and severity of temper outbursts in Prader-Willi Syndrome with 4 h/daily stimulation (as recommended for epilepsy), applied for 6–9 months, but a prompt return of the symptoms in all 5 participants when the stimulation was subsequently reduced to 2 h. Researchers should also consider making age related adjustments, e.g., (Koo et al., 2001) point out that in children younger than 12 stimulation settings may require a higher stimulus current or longer pulse along with lower stimulus frequency than adults (e.g., stimulation at 20 Hz or lower instead of e.g., 30 Hz), as a child's vagus nerve has slower conduction velocity. The effects of VNS on heart rate need to be considered as well. (Badran et al., 2018b) have shown that certain settings (pulse width 500 μs and frequency 10 Hz) are likely to lower the heart rate.

As for limitations of using VNS to modulate interoception, VNS cannot afford selective targeting of aspects of the interoceptive signal which may be of specific interest (e.g., heart, stomach, breath). As such, protocols should be designed to capitalize on VNS's capacity to manipulate the strength of the afferent signal. Functional neuroimaging studies could be particularly amenable to this. For a thorough treatment of the limitations, see Kaniusas et al. (2019).

To conclude, vagus nerve stimulation affords a novel experimental approach to studying interoception and its role in cognitive and emotional processes and disorders at increasing levels of complexity. With developments in the method and optimal protocols gaining momentum, vagus nerve stimulation may prove a fruitful new gateway to interoception.

AUTHOR CONTRIBUTIONS

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

FUNDING

AP gratefully acknowledged the funding from the National Science Centre in Poland (Grant No. 2016/21/D/HS2/02493) as well as the contribution from the Faculty of Psychology, University of Warsaw. LS gratefully acknowledged the funding from the Dr. Mortimer and Theresa Sackler Foundation, which funds the Sackler Centre for Consciousness Science, as well as funding from School of Psychology at the University of Sussex.

ACKNOWLEDGMENTS

We thank the community of implicit learning researchers, especially the organizers of the 8th Implicit Learning Seminar in Tromso in 2019 (Elisabeth Norman & Axel Cleeremans). We met and began discussing these ideas there.

REFERENCES

Aaronson, S. T., Carpenter, L. L., Conway, C. R., Reimherr, F. W., Lisanby, S. H., Schwartz, T. L., et al. (2013). Vagus nerve stimulation therapy randomized to different amounts of electrical charge for treatment-resistant depression: acute and chronic effects. *Brain Stimul.* 6, 631–640. doi: 10.1016/j.brs.2012.09.013

Ainley, V., Apps, M. A. J., Fotopoulou, A., and Tsakiris, M. (2016). 'Bodily precision': a predictive coding account of individual differences in interoceptive accuracy. *Philos. Trans. R. Soc. B Biol. Sci.* 371:20160003. doi: 10.1098/rstb.2016.0003

Badran, B. W., Dowdle, L. T., Mithoefer, O. J., LaBate, N. T., Coatsworth, J., Brown, J. C., et al. (2018a). Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: a concurrent taVNS/fMRI study and review. *Brain Stimul.* 11, 492–500. doi: 10.1016/j.brs.2017.12.009

Badran, B. W., Mithoefer, O. J., Summer, C. E., LaBate, N. T., Glusman, C. E., Badran, A. W., et al. (2018b). Short trains of transcutaneous auricular vagus nerve stimulation (taVNS) have parameter-specific effects on heart rate. *Brain Stimul.* 11, 699–708. doi: 10.1016/j.brs.2018.04.004

Barbanti, P., Grazzi, L., Egeo, G., Padovan, A. M., Liebler, E., and Bussone, G. (2015). Non-invasive vagus nerve stimulation for acute treatment of high-frequency and chronic migraine: an open-label study. *J. Headache Pain* 16, 61. doi: 10.1186/s10194-015-0542-4

Barrett, L. F., and Simmons, W. K. (2015). Interoceptive predictions in the brain. *Nat. Rev. Neurosci.* 16, 419–429. doi: 10.1038/nrn3950

Bechara, A. (2004). The role of emotion in decision-making: Evidence from neurological patients with orbitofrontal damage. *Brain Cogn.* 55, 30–40. doi: 10.1016/j.bandc.2003.04.001

Bechara, A., and Naqvi, N. (2004). Listening to your heart: interoceptive awareness as a gateway to feeling. *Nat. Neurosci.* 7, 102–103. doi: 10.1038/nn0204-102

Berntson, G., Gianaros, P. J., and Tsakiris, M. (2018). "Interoception and the autonomic nervous system: bottom-up meets top-down," in *Interoceptive Mind: From Homeostasis to Awareness*, eds M. Tsakiris and H. De Preester (Oxford: Oxford University Press), 3–25.

Berthoud, H.-R., and Neuhuber, W. L. (2000). Functional and chemical anatomy of the afferent vagal system. *Auton. Neurosci.* 85, 1–17. doi: 10.1016/s1566-0702(00)00215-0

Bird, G., Silani, G., Brindley, R., White, S., Frith, U., and Singer, T. (2010). Empathic brain responses in insula are modulated by levels of alexithymia but not autism. *Brain* 133, 1515–1525. doi: 10.1093/brain/awq060

Borckardt, J. J., Kozel, F. A., Anderson, B., Walker, A., and George, M. S. (2005). Vagus nerve stimulation affects pain perception in depressed adults. *Pain Res. Manag.* 10, 9–14. doi: 10.1155/2005/256472

Boveja, B. R., and Widhany, A. (2003). Method and System for Providing Therapy for bulimia/Eating Disorders by Providing Electrical Pulses to Vagus nerve(s). Washington, DC: U.S. Patent and Trademark Office. U.S. Patent No US 2005/0192644 A1. Available online at: https://patents.google.com/patent/US20050192644

Bretherton, B., Atkinson, L., Murray, A., Clancy, J., Deuchars, S., and Deuchars, J. (2019). Effects of transcutaneous vagus nerve stimulation in individuals aged 55 years or above: potential benefits of daily stimulation. *Aging* 11, 4836–4857. doi: 10.18632/aging.102074

Broncel, A., Bocian, R., Kłos-Wojtczak, P., Kulbat-Warycha, K., and Konopacki, J. (2020). Vagal nerve stimulation as a promising tool in the improvement of cognitive disorders. *Brain Res. Bull.* 155, 37–47. doi: 10.1016/j.brainresbull.2019.11.011

Burger, A. M., and Verkuil, B. (2018). Transcutaneous nerve stimulation via the tragus: are we really stimulating the vagus nerve? *Brain Stimula.* 11, 945–946. doi: 10.1016/j.brs.2018.03.018

Burger, A. M., Verkuil, B., Diest, I. V., der Does, W. V., Thayer, J. F., and Brosschot, J. F. (2016). The effects of transcutaneous vagus nerve stimulation on conditioned fear extinction in humans. *Neurobiol. Learn. Mem.* 132, 49–56. doi: 10.1016/j.nlm.2016.05.007

Burger, A. M., Verkuil, B., Fenlon, H., Thijs, L., Cools, L., Miller, H. C., et al. (2017). Mixed evidence for the potential of non-invasive transcutaneous vagal nerve stimulation to improve the extinction and retention of fear. *Behav. Res. Ther.* 97, 64–74. doi: 10.1016/j.brat.2017.07.005

Cameron, O. G. (2001). Interoception: the inside StoryA model for psychosomatic processes. *Psychosom. Med.* 63, 697–710. doi: 10.1097/00006842-200109000-00001

Clark, K. B., Naritoku, D. K., Smith, D. C., Browning, R. A., and Jensen, R. A. (1999). Enhanced recognition memory following vagus nerve stimulation in human subjects. *Nat. Neurosci.* 2, 94–98. doi: 10.1038/4600

Cleeremans, A., Achoui, D., Beauny, A., Keuninckx, L., Martin, J.-R., Muñoz-Moldes, S., et al. (2020). Learning to be conscious. *Trends Cogn. Sci.* 24, 112–123. doi: 10.1016/j.tics.2019.11.011

Colzato, L. S., Sellaro, R., and Beste, C. (2017a). Darwin revisited: the vagus nerve is a causal element in controlling recognition of others emotions. *Cortex* 92, 95–102. doi: 10.1016/j.cortex.2017.03.017

Colzato, L. S., Wolters, G., and Peifer, C. (2017b). Transcutaneous vagus nerve stimulation (tVNS) modulates flow experience. *Exp. Brain Res.* 236, 253–257. doi: 10.1007/s00221-017-5123-0

Corazzol, M., Lio, G., Lefevre, A., Deiana, G., Tell, L., André-Obadia, N., et al. (2017). Restoring consciousness with vagus nerve stimulation. *Curr. Biol.* 27, R994–R996. doi: 10.1016/j.cub.2017.07.060

Craig, A. D. B. (2003).). Interoception: the sense of the physiological condition of the body. *Curr. Opin. iNeurobiol.* 13, 500–505. doi: 10.1016/s0959-4388(03)00090-4

Craig, B. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nat. Rev. Neurosci.* 3, 655–666. doi: 10.1038/nrn894

Critchley, H. D., and Garfinkel, S. N. (2018). The influence of physiological signals on cognition. *Curr. Opin. Behav. Sci.* 19, 13–18. doi: 10.1016/j.cobeha.2017.08.014

Critchley, H. D., and Harrison, N. A. (2013). Visceral Influences on Brain and Behavior. *Neuron* 77, 624–638. doi: 10.1016/j.neuron.2013.02.008

Critchley, H. D., Mathias, C. J., and Dolan, R. J. (2001). Neuroanatomical basis for first- and second-order representations of bodily states. *Nat. Neurosci.* 4, 207–212. doi: 10.1038/84048

Critchley, H. D., Wiens, S., Rotshtein, P., Öhman, A., and Dolan, R. J. (2004). Neural systems supporting interoceptive awareness. *Nat. Neurosci.* 7, 189–195. doi: 10.1038/nn1176

Crucianelli, L., Krahe, C., Jenkinson, P. M., and Fotopoulou, A. K. (2018). Interoceptive ingredients of body ownership: affective touch and cardiac awareness in the rubber hand illusion. *Cortex* 104, 180–192. doi: 10.1016/j.cortex.2017.04.018

Damasio, A. (1994). *Descartes' Error*. New York, NY: Vintage.

Damasio, A. (2010). *Self Comes to Mind: Constructing the Conscious Self*. New York, NY: Random House.

De Herdt, V., Boon, P., Vonck, K., Goossens, L., Nieuwenhuis, L., Paemeleire, K., et al. (2003). Are psychotic symptoms related to vagus nerve stimulation in epilepsy patients? *Acta Neurol. Belg.* 103, 170–175.

Dunn, B. D., Galton, H. C., Morgan, R., Evans, D., Oliver, C., Meyer, M., et al. (2010). Listening to your heart. *Psychol. Sci.* 21, 1835–1844. doi: 10.1177/0956797610389191

Duschek, S., Montoro, C. I., and del Paso, G. A. R. (2015). Diminished interoceptive awareness in fibromyalgia syndrome. *Behav. Med.* 43, 100–107. doi: 10.1080/08964289.2015.1094442

Engineer, C. T., Hays, S. A., and Kilgard, M. P. (2017). Vagus nerve stimulation as a potential adjuvant to behavioral therapy for autism and other neurodevelopmental disorders. *J. Neurodev. Disord.* 9:20. doi: 10.1186/s11689-017-9203-z

Ernst, J., Böker, H., Hättenschwiler, J., Schüpbach, D., Northoff, G., Seifritz, E., et al. (2013). The association of interoceptive awareness and alexithymia with neurotransmitter concentrations in insula and anterior cingulate. *Soc. Cogn. Affect. Neurosci.* 9, 857–863. doi: 10.1093/scan/nst058

Fassino, S., Pierò, A., Gramaglia, C., and Abbate-Daga, G. (2004). Clinical psychopathological and personality correlates of interoceptive awareness in anorexia nervosa. Bulimia nervosa and Obesity. *Psychopathology* 37, 168–174. doi: 10.1159/000079420

Fischer, R., Ventura-Bort, C., Hamm, A., and Weymar, M. (2018). Transcutaneous vagus nerve stimulation (tVNS) enhances conflict-triggered adjustment of cognitive control. *Cogn. Affect. Behavi. Neurosci.* 18, 680–693. doi: 10.3758/s13415-018-0596-2

Flood, J. F., and Morley, J. E. (1988). Effects of bombesin and gastrin-releasing peptide on memory processing. *Brain Res.* 460, 314–322. doi: 10.1016/0006-8993(88)90375-7

Frangos, E., Ellrich, J., and Komisaruk, B. R. (2015). Non-invasive access to the vagus nerve central projections via electrical stimulation of the external Ear: fMRI Evidence in humans. *Brain Stimul.* 8, 624–636. doi: 10.1016/j.brs.2014.11.018

Gandola, M., Invernizzi, P., Sedda, A., Ferrè, E. R., Sterzi, R., Sberna, M., et al. (2012). An anatomical account of somatoparaphrenia. *Cortex* 48, 1165–1178. doi: 10.1016/j.cortex.2011.06.012

Garfinkel, S. N., and Critchley, H. D. (2016). Threat and the body: how the heart supports fear processing. *Trends Cogn. Sci.* 20, 34–46. doi: 10.1016/j.tics.2015.10.005

Garfinkel, S. N., Seth, A. K., Barrett, A. B., Suzuki, K., and Critchley, H. D. (2015). Knowing your own heart: distinguishing interoceptive accuracy from interoceptive awareness. *Biolo. Psychol.* 104, 65–74. doi: 10.1016/j.biopsycho.2014.11.004

Garfinkel, S. N., Tiley, C., OKeeffe, S., Harrison, N. A., Seth, A. K., and Critchley, H. D. (2016). Discrepancies between dimensions of interoception in autism: implications for emotion and anxiety. *Biol. Psychol.* 114, 117–126. doi: 10.1016/j.biopsycho.2015.12.003

Genheimer, H., Andreatta, M., Asan, E., and Pauli, P. (2017). Reinstatement of contextual conditioned anxiety in virtual reality and the effects of transcutaneous vagus nerve stimulation in humans. *Scie. Rep.* 7:17886. doi: 10.1038/s41598-017-18183-3

Gentsch, A., Sel, A., Marshall, A. C., and Schütz-Bosbach, S. (2018). Affective interoceptive inference: evidence from heart-beat evoked brain potentials. *Hum. Brain Mapp.* 40, 20–33. doi: 10.1002/hbm.24352

George, M. S., Sackeim, H. A., Rush, A. J., Marangell, L. B., Nahas, Z., Husain, M. M., et al. (2000). Vagus nerve stimulation: a new tool for brain research and therapy. *Biol. Psychiatry* 47, 287–295. doi: 10.1016/s0006-3223(99)00308-x

George, M. S., Ward, H. E., Ninan, P. T., Pollack, M., Nahas, Z., Anderson, B., et al. (2008). A pilot study of vagus nerve stimulation (VNS) for treatment-resistant anxiety disorders. *Brain Stimul.* 1, 112–121. doi: 10.1016/j.brs.2008.02.001

Ghacibeh, G. A., Shenker, J. I., Shenal, B., Uthman, B. M., and Heilman, K. M. (2006). Effect of vagus nerve stimulation on creativity and cognitive flexibility. *Epilepsy Behav.* 8, 720–725. doi: 10.1016/j.yebeh.2006.03.008

Giraudier, M., Ventura-Bort, C., and Weymar, M. (2020). Transcutaneous vagus nerve stimulation improves high confidence recognition memory but not emotional word processing. *Front. Psychol.* 11:1276. doi: 10.3389/fpsyg.2020.01276

Goehler, L. E., Gaykema, R. P. A., Hansen, M. K., Anderson, K., Maier, S. F., and Watkins, L. R. (2000). Vagal immune-to-brain communication: a visceral chemosensory pathway. *Auton. Neurosci.* 85, 49–59. doi: 10.1016/s1566-0702(00)00219-8

Gu, X., and FitzGerald, T. H. B. (2014). Interoceptive inference: homeostasis and decision making. *Trends Cogni. Sci.* 18, 269–270. doi: 10.1016/j.tics.2014.02.001

Gu, X., FitzGerald, T. H. B., and Friston, K. (2019). Modelling subjective belief states in computational psychiatry: interoceptive inference as candidate framework. *Psychopharmacology* 236, 2405–2412. doi: 10.1007/s00213-019-05300-5

Hansen, N. (2019). Memory reinforcement and attenuation by activating the human locus coeruleus via transcutaneous vagus nerve stimulation. *Front. Neurosci.* 12:955. doi: 10.3389/fnins.2018.00955

Harshaw, C. (2015). Interoceptive dysfunction: toward an integrated framework for understanding somatic and affective disturbance in depression. *Psychol. Bull.* 141, 311–363. doi: 10.1037/a0038101

Hays, S. A., Rennaker, R. L., and Kilgard, M. P. (2013). "Targeting Plasticity with vagus nerve stimulation to treat neurological disease," in *Changing Brains - Applying Brain Plasticity to Advance and Recover Human Ability*, eds M. Nahum, M. Merzenich, and T. van Vleet (Amsterdam: Elsevier), 275–299. doi: 10.1016/b978-0-444-63327-9.00010-2

Herbert, B. M., Pollatos, O., and Schandry, R. (2007). Interoceptive sensitivity and emotion processing: an EEG study. *Int. J. Psychophysiol.* 65, 214–227. doi: 10.1016/j.ijpsycho.2007.04.007

Howland, R. H. (2014). Vagus nerve stimulation. *Curr. Behav. Neurosci. Rep.* 1, 64–73. doi: 10.1007/s40473-014-0010-5

Jacobs, H. I. L., Riphagen, J. M., Razat, C. M., Wiese, S., and Sack, A. T. (2015). Transcutaneous vagus nerve stimulation boosts associative memory in older individuals. *Neurobiol. Aging* 36, 1860–1867. doi: 10.1016/j.neurobiolaging.2015.02.023

Jin, Y., and Kong, J. (2017). Transcutaneous vagus nerve stimulation: a promising method for treatment of autism spectrum disorders. *Front. Neurosci.* 10:609. doi: 10.3389/fnins.2016.00609

Jongkees, B. J., Immink, M. A., Finisguerra, A., and Colzato, L. S. (2018). Transcutaneous vagus nerve stimulation (tVNS) enhances response selection during sequential action. *Front. Psychol.* 9:1159. doi: 10.3389/fpsyg.2018.01159

Kandasamy, N., Garfinkel, S. N., Page, L., Hardy, B., Critchley, H. D., Gurnell, M., et al. (2016). Interoceptive ability predicts survival on a london trading floor. *Sci. Rep.* 6:32986. doi: 10.1038/srep32986

Kaniusas, E., Kampusch, S., Tittgemeyer, M., Panetsos, F., Gines, R. F., Papa, M., et al. (2019). Current directions in the auricular vagus nerve stimulation i a physiological perspective. *Front. Neurosci.* 13:854. doi: 10.3389/fnins.2019.00854

Karnath, H.-O. (2005). Awareness of the functioning of ones own limbs mediated by the insular cortex? *J. Neurosci.* 25, 7134–7138. doi: 10.1523/jneurosci.1590-05.2005

Khalsa, S. S., Adolphs, R., Cameron, O. G., Critchley, H. D., Davenport, P. W., Feinstein, J. S., et al. (2018). Interoception and mental health: a roadmap. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* 3, 501–513. doi: 10.1016/j.bpsc.2017.12.004

Khalsa, S. S., Rudrauf, D., Feinstein, J. S., and Tranel, D. (2009). The pathways of interoceptive awareness. *Nat. Neurosci.* 12, 1494–1496. doi: 10.1038/nn.2411

Klinkenberg, I., Sambeth, A., and Blokland, A. (2011). Acetylcholine and attention. *Behav. Brain Res.* 221, 430–442. doi: 10.1016/j.bbr.2010.11.033

Koo, B., Ham, S. D., Sood, S., and Tarver, B. (2001). Human vagus nerve electrophysiology. *J. Clin. Neurophysiol.* 18, 429–433. doi: 10.1097/00004691-200109000-00007

Lange, G., Janal, M. N., Maniker, A., FitzGibbons, J., Fobler, M., Cook, D., et al. (2011). Safety and efficacy of vagus nerve stimulation in fibromyalgia: a phase I/II proof of concept trial. *Pain Med.* 12, 1406–1413. doi: 10.1111/j.1526-4637.2011.01203.x

Lernia, D. D., Serino, S., Cipresso, P., and Riva, G. (2016). Ghosts in the machine. interoceptive modeling for chronic pain treatment. *Front. Neurosci.* 10:314. doi: 10.3389/fnins.2016.00314

Levy, M. L., Levy, K. M., Hoff, D., Amar, A. P., Park, M. S., Conklin, J. M., et al. (2010). Vagus nerve stimulation therapy in patients with autism spectrum disorder and intractable epilepsy: results from the vagus nerve stimulation therapy patient outcome registry. *J. Neurosurg. Pediatr.* 5, 595–602. doi: 10.3171/2010.3.peds09153

Maniscalco, J. W., and Rinaman, L. (2018). Vagal interoceptive modulation of motivated behavior. *Physiology* 33, 151–167. doi: 10.1152/physiol.00036.2017

Manning, K. E., Beresford-Webb, J. A., Aman, L. C. S., Ring, H. A., Watson, P. C., Porges, S. W., et al. (2019). Transcutaneous vagus nerve stimulation (t-VNS): a novel effective treatment for temper outbursts in adults with Prader-Willi Syndrome indicated by results from a non-blind study. *PLoS One* 14:e0223750. doi: 10.1371/journal.pone.0223750

Mariana von Mohr, A. F. (2019). "The cutaneous borders of interoception: active and social inference of pain and pleasure on the skin," in *The Interoceptive Mind: From Homeostasis to Awareness*, eds M. Tsakiris and H. De Preester (Oxford: Oxford University Press).

Marshall, A. C., Gentsch, A., Schröder, L., and Schütz-Bosbach, S. (2018). Cardiac interoceptive learning is modulated by emotional valence perceived from facial expressions. *Soc. Cogn. Affect. Neurosci.* 13, 677–686. doi: 10.1093/scan/nsy042

Martin, C. O., Denburg, N. L., Tranel, D., Granner, M. A., and Bechara, A. (2004). The effects of vagus nerve stimulation on decision-making. *Cortex* 40, 605–612. doi: 10.1016/s0010-9452(08)70156-4

Martino, B. D., Strange, B. A., and Dolan, R. J. (2007). Noradrenergic neuromodulation of human attention for emotional and neutral stimuli. *Psychopharmacology* 197, 127–136. doi: 10.1007/s00213-007-1015-5

Mehling, W., Daubenmier, J., Price, C., and Acree, M. (2013). Self-reported interoceptive awareness in primary care patients with past or current low back pain. *J. Pain Res.* 6, 403–418. doi: 10.2147/jpr.s42418

Melzack, R., Wall, P. D., and Ty, T. C. (1982). Acute pain in an emergency clinic: latency of onset and descriptor patterns related to different injuries. *Pain* 14, 33–43. doi: 10.1016/0304-3959(82)90078-1

Merrill, C. A., Jonsson, M. A. G., Minthon, L., Ejnell, H., Silander, H., C-son Silander, H., et al. (2006). Vagus nerve stimulation in patients with Alzheimers Disease. *J. Clin. Psychiatry* 67, 1171–1178. doi: 10.4088/jcp.v67n0801

Meyer, R. A., Ringkamp, M., Campbell, J. N., and Raja, S. N. (2006). "Peripheral mechanisms of cutaneous nociception," in *Wall and Melzacks Textbook of Pain*, eds S. B. McMahon, M. Koltzenburg, I. Tracey, and D. Turk (Amsterdam: Elsevier), 3–34. doi: 10.1016/b0-443-07287-6/50006-0

Moro, V., Pernigo, S., Tsakiris, M., Avesani, R., Edelstyn, N. M. J., Jenkinson, P. M., et al. (2016). Motor versus body awareness: voxel-based lesion analysis in anosognosia for hemiplegia and somatoparaphrenia following right hemisphere stroke. *Cortex* 83, 62–77. doi: 10.1016/j.cortex.2016.07.001

Narayanan, J. T., Watts, R., Haddad, N., Labar, D. R., Li, P. M., and Filippi, C. G. (2002). Cerebral activation during vagus nerve stimulation: a functional MR Study. *Epilepsia* 43, 1509–1514. doi: 10.1046/j.1528-1157.2002.16102.x

Naritoku, D. K., Jensen, R. A., Browning, R. A., Clark, K. B., Smith, D. C., Terry, R. S. Jr., et al. (2003). *Methods for Improving Learning or Memory by Vagus Nerve Stimulation*. Washington, DC: U.S. Patent and Trademark Office. US Patent No: US 6,556,868 B2. Available online at: https://patents.google.com/patent/US6556868

Noble, L. J., Gonzalez, I. J., Meruva, V. B., Callahan, K. A., Belfort, B. D., Ramanathan, K. R., et al. (2017). Effects of vagus nerve stimulation on extinction of conditioned fear and post-traumatic stress disorder symptoms in rats. *Transl. Psychiatry* 7:e1217. doi: 10.1038/tp.2017.191

Nogueira, P. J. C., Tomaz, C., and Williams, C. L. (1994). Contribution of the vagus nerve in mediating the memory-facilitating effects of substance P. *Behav. Brain Res.* 62, 165–169. doi: 10.1016/0166-4328(94)90024-8

Park, H.-D., Bernasconi, F., Salomon, R., Tallon-Baudry, C., Spinelli, L., Seeck, M., et al. (2017). Neural sources and underlying mechanisms of neural responses to heartbeats and their role in bodily self-consciousness: an intracranial EEG Study. *Cereb. Cortex* 28, 2351–2364. doi: 10.1093/cercor/bhx136

Paulus, M. P., and Stein, M. B. (2006). An insular view of anxiety. *Biol. Psychiatry* 60, 383–387. doi: 10.1016/j.biopsych.2006.03.042

Paulus, M. P., and Stein, M. B. (2010). Interoception in anxiety and depression. *Brain Struct. Funct.* 214, 451–463. doi: 10.1007/s00429-010-0258-9

Peña, D. F., Childs, J. E., Willett, S., Vital, A., McIntyre, C. K., and Kroener, S. (2014). Vagus nerve stimulation enhances extinction of conditioned fear and modulates plasticity in the pathway from the ventromedial prefrontal cortex to the amygdala. *Front. Behav. Neurosci.* 8:327. doi: 10.3389/fnbeh.2014.00327

Peña, D. F., Engineer, N. D., and McIntyre, C. K. (2013). Rapid remission of conditioned fear expression with extinction training paired with vagus nerve stimulation. *Biol. Psychiatry* 73, 1071–1077. doi: 10.1016/j.biopsych.2012.10.021

Petzschner, F. H., Weber, L. A., Gard, T., and Stephan, K. E. (2017). Computational psychosomatics and computational psychiatry: toward a joint framework for differential diagnosis. *Biol. Psychiatry* 82, 421–430. doi: 10.1016/j.biopsych.2017.05.012

Petzschner, F. H., Weber, L. A., Wellstein, K. V., Paolini, G., Do, C. T., and Stephan, K. E. (2019). Focus of attention modulates the heartbeat evoked potential. *NeuroImage* 186, 595–606. doi: 10.1016/j.neuroimage.2018.11.037

Pfeifer, G., Garfinkel, S. N., van Praag, C. D. G., Sahota, K., Betka, S., and Critchley, H. D. (2017). Feedback from the heart: emotional learning and memory is controlled by cardiac cycle interoceptive accuracy and personality. *Biol. Psychol.* 126, 19–29. doi: 10.1016/j.biopsycho.2017.04.001

Pollatos, O., Kirsch, W., and Schandry, R. (2005). On the relationship between interoceptive awareness emotional experience, and brain processes. *Cogn. Brain Res.* 25, 948–962. doi: 10.1016/j.cogbrainres.2005.09.019

Pollatos, O., Kurz, A.-L., Albrecht, J., Schreder, T., Kleemann, A. M., Schöpf, V., et al. (2008). Reduced perception of bodily signals in anorexia nervosa. *Eat. Behav.* 9, 381–388. doi: 10.1016/j.eatbeh.2008.02.001

Pollatos, O., and Schandry, R. (2008). Emotional processing and emotional memory are modulated by interoceptive awareness. *Cogn. Emot.* 22, 272–287. doi: 10.1080/02699930701357535

Quadt, L., Critchley, H. D., and Garfinkel, S. N. (2018). The neurobiology of interoception in health and disease. *Ann. N. Y. Acad. Sci.* 1428, 112–128. doi: 10.1111/nyas.13915

Quadt, L., Critchley, H. D., and Garfinkel, S. N. (2019). "Interoception and emotion: Shared mechanisms and clinical implications," in *The Interoceptive Mind. From Homeostasis to Awareness*, eds M. Tsakaris and H. De Preester (Oxford: Oxford University Press), 123–143.

Rong, P., Luo, M., Li, L., Zhang, J., Guo, X., Zhao, B., et al. (2019). Sleep electroencephalography power spectral response to transcutaneous auricular vagus nerve stimulation on insomnia rats. *Heart Mind* 3, 55–62. doi: 10.4103/hm.hm_51_19

Saper, C. B. (2002). The central autonomic nervous system: conscious visceral perception and autonomic pattern generation. *Annu. Rev. Neurosci.* 25, 433–469. doi: 10.1146/annurev.neuro.25.032502.111311

Schandry, R. (1981). Heart beat perception and emotional experience. *Psychophysiology* 18, 483–488. doi: 10.1111/j.1469-8986.1981.tb02486.x

Sellaro, R., de Gelder, B., Finisguerra, A., and Colzato, L. S. (2018). Transcutaneous vagus nerve stimulation (tVNS) enhances recognition of emotions in faces but not bodies. *Cortex* 99, 213–223. doi: 10.1016/j.cortex.2017.11.007

Seth, A. K. (2013). Interoceptive inference emotion, and the embodied self. *Trends Cogn. Sci.* 17, 565–573. doi: 10.1016/j.tics.2013.09.007

Seth, A. K., Suzuki, K., and Critchley, H. D. (2012). An interoceptive predictive coding model of conscious presence. *Front. Psychol.* 2:395. doi: 10.3389/fpsyg.2011.00395

Seth, A. K., and Tsakiris, M. (2018). Being a beast machine: the somatic basis of selfhood. *Trends Cogn. Sci.* 22, 969–981. doi: 10.1016/j.tics.2018.08.008

Shah, P., Catmur, C., and Bird, G. (2017). From heart to mind: linking interoception emotion, and theory of mind. *Cortex* 93, 220–223. doi: 10.1016/j.cortex.2017.02.010

Shah, P., Hall, R., Catmur, C., and Bird, G. (2016). Alexithymia not autism, is associated with impaired interoception. *Cortex* 81, 215–220. doi: 10.1016/j.cortex.2016.03.021

Sierra, M., and David, A. S. (2011). Depersonalization: a selective impairment of self-awareness. *Conscious. Cogn.* 20, 99–108. doi: 10.1016/j.concog.2010.10.018

Sjogren, M. J. C., Hellstrom, P. T. O., Jonsson, M. A. G., Runnerstam, M., Silander, H. C., and Ben-Menachem, E. (2002). Cognition-enhancing effect of vagus nerve stimulation in patients with Alzheimer's Disease. *J. Clin. Psychiatry* 63, 972–980. doi: 10.4088/jcp.v63n1103

Souza, R. R., Robertson, N. M., Mathew, E., Tabet, M. N., Bucksot, J. E., Pruitt, D. T., et al. (2020). Efficient parameters of vagus nerve stimulation to enhance extinction learning in an extinction-resistant rat model of PTSD. *Prog. Neuro Psychopharmacol. Biol. Psychiatry* 99:109848. doi: 10.1016/j.pnpbp.2019.109848

Souza, R. R., Robertson, N. M., Pruitt, D. T., Gonzales, P. A., Hays, S. A., Rennaker, R. L., et al. (2019). Vagus nerve stimulation reverses the extinction impairments in a model of PTSD with prolonged and repeated trauma. *Stress* 22, 509–520. doi: 10.1080/10253890.2019.1602604

Szeska, C., Richter, J., Wendt, J., Weymar, M., and Hamm, A. O. (2020). Promoting long-term inhibition of human fear responses by non-invasive transcutaneous vagus nerve stimulation during extinction training. *Sci. Rep.* 10:1529. doi: 10.1038/s41598-020-58412-w

Talley, C. P., Clayborn, H., Jewel, E., McCarty, R., and Gold, P. E. (2002). Vagotomy attenuates effects of l-glucose but not of d-glucose on spontaneous alternation performance. *Physiol. Behav.* 77, 243–249. doi: 10.1016/s0031-9384(02)00850-8

Terasawa, Y., Moriguchi, Y., Tochizawa, S., and Umeda, S. (2014). Interoceptive sensitivity predicts sensitivity to the emotions of others. *Cogn. Emot.* 28, 1435–1448. doi: 10.1080/02699931.2014.888988

Terhaar, J., Viola, F. C., Bär, K.-J., and Debener, S. (2012). Heartbeat evoked potentials mirror altered body perception in depressed patients. *Clin. Neurophysiol.* 123, 1950–1957. doi: 10.1016/j.clinph.2012.02.086

Van den Bergh, O. V., Witthöft, M., Petersen, S., and Brown, R. J. (2017). Symptoms and the body: taking the inferential leap. *Neurosci. Biobehav. Rev.* 74, 185–203. doi: 10.1016/j.neubiorev.2017.01.015

Villani, V., Tsakiris, M., and Azevedo, R. T. (2019). Transcutaneous vagus nerve stimulation improves interoceptive accuracy. *Neuropsychologia* 134:107201. doi: 10.1016/j.neuropsychologia.2019.107201

Vonck, K., Raedt, R., Naulaerts, J., Vogelaere, F. D., Thiery, E., Roost, D. V., et al. (2014). Vagus nerve stimulation…25 years later! What do we know about the effects on cognition? *Neurosci. Biobehav. Rev.* 45, 63–71. doi: 10.1016/j.neubiorev.2014.05.005

Werner, N. S., Jung, K., Duschek, S., and Schandry, R. (2009). Enhanced cardiac perception is associated with benefits in decision-making. *Psychophysiology* 46, 1123–1129. doi: 10.1111/j.1469-8986.2009.00855.x

Werner, N. S., Peres, I., Duschek, S., and Schandry, R. (2010). Implicit memory for emotional words is modulated by cardiac perception. *Biol. Psychol.* 85, 370–376. doi: 10.1016/j.biopsycho.2010.08.008

Wernicke, J. F., Terry, R. S. Jr., and Baker, R. G. (1993). *Treatment of Eating Disorders by Nerve Stimulation*. Washington, DC: U.S. Patent and Trademark Office. US Patent No 5, 104. Available online at: https://patents.google.com/patent/US5263480A/en

Wiens, S., Mezzacappa, E. S., and Katkin, E. S. (2000). Heartbeat detection and the experience of emotions. *Cogn. Emot.* 14, 417–427. doi: 10.1080/026999300378905

Williams, C. L., and Jensen, R. A. (1993). Effects of vagotomy on leu-enkephalin-induced changes in memory storage processes. *Physiol. Behav.* 54, 659–663. doi: 10.1016/0031-9384(93)90073-o

Yoris, A., García, A. M., Salamone, P. C., Sedeño, L., García-Cordero, I., and Ibáñez, A. (2019). "Cardiac interoception in neurological conditions and its relevance for dimensional approaches," in *Interoceptive Mind: From Homeostasis to Awareness*, eds M. Tsakaris and H. De Preester (Oxford: Oxford University Press), 197–211.

Conflict of Interest: 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.

frontiers in Psychology

ORIGINAL RESEARCH
published: 17 September 2020
doi: 10.3389/fpsyg.2020.570196

Stress and Tinnitus; Transcutaneous Auricular Vagal Nerve Stimulation Attenuates Tinnitus-Triggered Stress Reaction

Jukka Ylikoski[1,2,3], Marika Markkanen[4], Ulla Pirvola[5], Jarmo Antero Lehtimäki[1,2], Matti Ylikoski[1,2], Zou Jing[6], Saku T. Sinkkonen[3] and Antti Mäkitie[3]*

[1] Helsinki Ear Institute, Helsinki, Finland, [2] Salustim Group Inc., Kempele, Finland, [3] Department of Otolaryngology-Head & Neck Surgery, University of Helsinki and Helsinki University Hospital, Helsinki, Finland, [4] Department of Anatomy, University of Helsinki, Helsinki, Finland, [5] Molecular and Integrative Biosciences Research Program, University of Helsinki, Helsinki, Finland, [6] Department of Otolaryngology-Head and Neck Surgery, Center for Otolaryngology-Head & Neck Surgery of Chinese PLA, Changhai Hospital, Second Military Medical University, Shanghai, China

OPEN ACCESS

Edited by:
Tino Zaehle, Otto von Guericke University Magdeburg, Germany

Reviewed by:
Marcus Heldmann, Department of Neurology, University of Lübeck, Germany
Derrick L. Hassert, Trinity Christian College, United States

***Correspondence:**
Marika Markkanen
marika.markkanen@helsinki.fi

Specialty section:
This article was submitted to Cognition, a section of the journal Frontiers in Psychology

Received: *06 June 2020*
Accepted: *26 August 2020*
Published: *17 September 2020*

Citation:
Ylikoski J, Markkanen M, Pirvola U, Lehtimäki JA, Ylikoski M, Jing Z, Sinkkonen ST and Mäkitie A (2020) Stress and Tinnitus; Transcutaneous Auricular Vagal Nerve Stimulation Attenuates Tinnitus-Triggered Stress Reaction. Front. Psychol. 11:570196. doi: 10.3389/fpsyg.2020.570196

Introduction: Tinnitus can become a strong stressor for some individuals, leading to imbalance of the autonomous nervous system with reduction of parasympathetic activity. It can manifest itself as sleep disturbances, anxiety and even depression. This condition can be reversed by bioelectrical vagal nerve stimulation (VNS). Conventional invasive VNS is an approved treatment for epilepsy and depression. Transcutaneous VNS (taVNS) stimulating the auricular branch of the vagus nerve has been shown to activate the vagal pathways similarly as an implanted VNS. Therefore, taVNS might also be a therapeutic alternative in health conditions such as tinnitus-related mental stress (TRMS). This retrospective study in 171 TRMS patients reports the clinical features, psychophysiological characteristics, and results of the heart rate variability (HRV) tests before and after test-taVNS. This study also reports the therapy outcomes of 113 TRMS patients treated with taVNS, in combination with standard tinnitus therapy.

Methods: Diagnostic tinnitus and hearing profiles were defined. To detect possible cardiac adverse effects, test-taVNS with heart rate monitoring as well as pre- and post-stimulation HRV tests were performed. Daily taVNS home therapy was prescribed thereafter. To assess therapeutic usefulness of taVNS, 1-year follow-up outcome was studied. Results of HRV tests were retrospectively analyzed and correlated to diagnostic data.

Results: The large majority of patients with TRMS suffer from associated symptoms such as sleep disturbances and anxiety. Baseline HRV data showed that more than three quarters of the 171 patients had increased sympathetic activity before test-taVNS. Test-taVNS shifted mean values of different HRV parameters toward increased parasympathetic activity in about 80% of patients. Test-taVNS did not cause any cardiac or other side effects. No significant adverse effects were reported in follow-up questionnaires.

Conclusion: TRMS is an example of a stress condition in which patients may benefit from taVNS. As revealed by HRV, test-taVNS improved parasympathetic function, most efficiently in patients with a low starting HRV level. Our tinnitus treatment program, including taVNS, effectively alleviated tinnitus stress and handicap. For wider clinical use, there is a great need for more knowledge about the optimal methodology and parameters of taVNS.

Keywords: stress, tinnitus, patients, parasympathetic, vagus, neuromodulation

INTRODUCTION

All our unconscious bodily functions are controlled by the autonomic nervous system (ANS), particularly by the CAN (Benarroch, 1993). The most common cause for the dysfunction of CAN is stress, the major cause of deteriorating health conditions and illnesses. CAN initially reacts to stressor effects with sympathetic fight/flight response that is restored back to normal by the parasympathetic nervous systemś relax/digest response (Selye, 1950). Many illnesses result from the inability of the parasympathetic activity to restore the ANS balance (for review see McEwen, 2000; McEwen and Akil, 2020). These two circuits, sympathetic and parasympathetic systems, are constantly interacting. This interaction is reflected by HRV that, hence, is a read out of ANS balance. HRV may consequently serve as a measure of stress (Akselrod et al., 1981; Thayer et al., 2012). As the vagal system with the vagus nerve in front is responsible for parasympathetic activity, neuromodulation via VNS can serve as targeted treatment in stressful conditions. VNS has been conventionally performed for more than two decades to treat severe epilepsy and depression by applying an electrode surgically implanted to the cervical trunk of the vagus nerve. More recently, it has been shown by electrophysiological and neuroimaging studies that taVNS of the ABVN activates central vagal pathways similarly as VNS with an implanted electrode (Kraus et al., 2007; Dietrich et al., 2008; Frangos et al., 2015; Yakunina et al., 2017; Badran et al., 2018a).

ANS imbalance is most often a result of the individual's exposure to concurrent stressors. Therefore, the stress-triggered clinical picture is often very heterogeneous. On the contrary, tinnitus (ringing in the ears) as a stressor usually results in a relatively regular SR in otherwise healthy individuals. Therefore, individuals with TRMS seem to be an optimal target group for investigations of the effects of taVNS on stress in patients.

Tinnitus is considered to be generated in the auditory periphery (cochlea-cochlear nerve), detected in the subcortical centers according to the lines of pattern recognition principles, and perceived and evaluated in the auditory cortex with significant participation of the limbic system and prefrontal and other cortical areas (Jastreboff, 1990). Tinnitus sound itself usually constitutes only minor symptoms. However, tinnitus connected with fear (that it is maintained or even growing worse) and threat (that it is a sign of a serious illness) leads to automatic negative thoughts, developing a SR (arousal) that may potentiate sleep problems, anxiety and depression. The end result all of this is a vicious cycle, SR worsening tinnitus and increased tinnitus worsening stress.

This was taken into consideration in the neurophysiological tinnitus model by Jastreboff (1990) and it formed the basis for the development of the TRT program (Jastreboff and Hazell, 1993). The target of TRT therapy is the stress-arousal caused by tinnitus and it leads to distress that prevents habituation. The goal of TRT is to remove the negative perception of tinnitus from patient's consciousness, thereby facilitating habituation. Furthermore, the rationale of TRT is to attenuate the conditioned stress-response (arousal) with associated sympathovagal imbalance by stimulating the parasympathetic system (Jastreboff and Hazell, 1993). TRT is a program consisting of diagnostics, instructive counseling and sound therapy, each acting in concert with the aim to stimulate the parasympathetic system.

Plans for this study were started after Engineer et al. (2011) reported that maladaptive neuronal plasticity of the central auditory system, thought to be behind tinnitus in an animal tinnitus model, can be reversed through (invasive) VNS. It was clear that conventional (invasive) VNS would not be the optimal treatment for patients with tinnitus. Therefore, we first had to develop a method and device for noninvasive VNS (taVNS). When the device development was completed, we had to start to test-use it particularly for cardiac safety.

This retrospective study reports the clinical, audiological and psychophysical diagnostic results in a historical group of 171 patients managed for TMRS. The study also reports the results of the 1-year follow-up outcome study with patients treated with taVNS. Because stress levels of patients needed to be measured and because of the novelty of taVNS and the potential cardiac complications reported by VNS, the HRV test and test-taVNS with HR monitoring were considered obligatory. We performed HRV test both before and after test-taVNS. Our data show that taVNS is safe and improves parasympathetic activity and, in conjunction with TRT, attenuates tinnitus severity based on results of symptom questionnaires.

Abbreviations: ABVN, auricular branch of the vagus nerve; AF, atrial fibrillation; ANS, autonomous nervous system; CAN, central autonomic network; CNS, central nervous system; ECG, electrocardiogram; HR, heart rate; HRV, heart rate variability; MIT, music-induced tinnitus; NIHD, noise-induced hearing disorder; NIT, noise-induced tinnitus; NTS, nucleus tractus solitarius; SR, stress reaction; taVNS, transcutaneous VNS; TCPT, tinnitus care pathway technology; THI, tinnitus handicap inventory; TRMS, tinnitus-related mental stress; TRT, tinnitus retraining therapy; VAS, visual analog scale; VNS, vagal nerve stimulation; VSEP, vagal somatosensory evoked potential.

PATIENTS AND METHODS

The present series consists of 171 consecutive patients (67 female, 104 male, mean age 49 years, range 17–84) who visited the Tinnitus Clinic of Helsinki Ear Institute between November 2014 and December 2017 due to annoying tinnitus. We have developed our own modification of TRT that is named TCPT. Its main constituents include diagnostic profiling of tinnitus and hearing, counseling, sound therapy, and a sleep module. In order to strengthen parasympathetic activation, taVNS was added to the program in 2014. In addition to other diagnostics, HRV tests and a 15–60 min test-taVNS were performed and, as adjunctive to other TCPT therapy, a taVNS device was prescribed as home-therapy if tinnitus had been defined as moderate or severe (THI, questionnaire score higher than 34/100). Some patients with lower THI scores were also instructed for taVNS treatment if they showed special interest in the device or complained of being particularly stressed. At the initial office visit, extensive otological and audiological examinations with profiling of tinnitus and hearing were done using different structured diagnostic forms and questionnaires. For clinical evaluation of the subjective severity of tinnitus annoyance and associated symptoms such as sleep disturbance and anxiety levels were quantified based on VAS questions.

Each patient's stress level was evaluated by HRV testing. The patient was then (for safety reason) test-stimulated with the Salustim taVNS device (Helsinki Ear Institute) continuously for 15–60 min after which a new HRV test was performed. We have earlier shown by magnetoencephalography that the amplitudes of auditory N1m responses in the auditory cortex are reduced by using this taVNS method (Lehtimäki et al., 2013). As there were no adverse effects related to the test-taVNS, all tested patients were then instructed to use the taVNS device at home 60–90 min per day. The long-term therapeutic outcomes of our first 113 patients were studied with structured questionnaires about 1 year after the first visit. Such follow-up data was possible to obtain during office visits scheduled according to the TCPT program or by telephone interviews in 78 patients (69%) (54 males and 59 females; age range from 18 to 84 years).

HRV Test and Heart Rate Monitoring During Test-taVNS

The main aim of HR monitoring was to detect possible cardiac side-effects during the first 15–60 min of taVNS stimulation. Other reasons were to measure the mental stress level of patients and to collect HRV data for later analyses to study whether HRV could be used for selection of presumably taVNS-responding patients. In this study, we report the results of HRV analyses and specifically correlate HRV results to clinical data in a total of 171 patients. We have previously described in detail our HRV testing procedure (Ylikoski et al., 2017). Briefly, for analyzing the dynamics of HRV signals (R-R intervals), the eMotion HRV measurement system (Mega Electronics Ltd., Kuopio, Finland) was used. Stress test with a HRV scan was performed with the patient breathing with a parasympathetic stimulating respiratory rate during which the R-R interval variability was registered by wrist electrodes with a one-lead ECG. In the eMotion HRV measurement, artifacts and interruptions were eliminated with high-end technology (and disposable surface electrodes) (Malik, 1996; Tarvainen et al., 2009). Only tests with 100% measurement quality in ECG monitoring were used for evaluation. The following HRV parameters were measured during the 1-min deep-breathing test (one-min DBT): mean HR, amplitude and ratio of HR oscillation (E-I difference, E/I ratio) (E-I = difference between the highest and the lowest HR within a breathing cycle), RMSSD (root mean square of the successive differences), SDNN (standard deviation of the R-R intervals), and Power LF (LF = low frequency; 0.04–0.15 Hz). One-min DBT was followed by 5-min short-term HRV (s-HRV) where the HRV parameters HR, SD1 (width of the Poincare plot, reflecting short-term variability), SD2 (length of the Poincare plot, reflecting short-term variability), SDNN, Stress Index, Power HF (HF = high frequency; 0.15–0.4 Hz), Power LF, Power VLF (very low frequency), and Total Power were determined as well. Parameters were compared through correlation analysis and agreement analysis by Bland-Altman plots. The results of HRV tests were evaluated on the basis of eMotion tests performed in normal individuals (Camm et al., 1996; Tarvainen et al., 2009; Weinschenk et al., 2016).

Vagal Somatosensory Evoked Potential Test

In order to biomonitor the electrical stimulation of ABVN, we measured the VSEP response (Fallgatter et al., 2003). We used EGI GTEN 100 EEG system with 8 kHz collection rate (256 electrodes) (Palva and Palva, 2018) and with different stimulation parameters. We used uni- or bipolar pulses, pulse width 100–560 microseconds, frequencies 1, 2, 4, 8, 15, 20, 25, 30 Hz, amplitude at or just below the pain threshold, and delivered 200–500 epochs at each setting.

taVNS

We used the taVNS instrument consisting of one ear clip electrode connected to a wired TENS-neurostimulating device (Salustim, Helsinki Ear Institute). The clip-electrode was placed on the tragus of the left ear. The clinical efficacy of taVNS requires activation of the thick myelinated afferent fibers of the vagus nerve. Fibers of a sensory peripheral nerve, such as the ABVN, mediate touch sensation. Consequently, the stimulus intensity of taVNS will be adjusted to a level above the individual's detection threshold and clearly below the individual's pain threshold. The taVNS device offers a stimulus intensity between 0.1 and 30 mA with a stimulation frequency of 25 Hz and pulse duration of 250 μs. After the individual adjustments the level of stimulation in patients ranged from 0.3 to 3.0 mA. The 15–60 min continuous test stimulation was performed under medical supervision in the office, with continuous HR monitoring. After the initial test stimulation, patients were instructed to use the taVNS device at home for 60–90 min daily, 5 days a week.

Statistical Analyses

Statistical analyses were performed using GraphPad Prism 8 (GraphPad Software, La Jolla, CA, United States). Normality of data was tested with D'Agostino & Pearson test. The non-parametric Wilcoxon's matched pairs signed rank test was used to estimate the *p*-values between pre/post -taVNS data, as all data did not follow a normal distribution. Comparison between age groups was also done using the non-parametric Kolmogorov–Smirnov test. Comparison between super-responders vs. non -responders was done using unpaired two-tailed Student's *t*-test as the data followed a normal distribution. Data is presented as mean ± standard deviation (SD). Statistical significance was set up at $P < 0.05$. Bonferroni adjustment were performed for multiple comparisons by dividing the initial significance level of 0.05 by the number of tests to obtain a modified significance level. Treatment effect sizes were calculated by Cohen's *d* as the difference in means, divided by the pooled standard deviation of the two means (Cohen's, 1988). The magnitude of Cohen's *d* can be expressed as small (0.2), moderate (0.5), and large (0.8).

RESULTS

Baseline Values

All the patients had clinically relevant tinnitus; chronic in two thirds, subchronic in about 20% and acute (tinnitus duration less than 3 months) in about 15%. In acute cases, most patients had visited our tinnitus clinic one to 6 weeks after the start or worsening of tinnitus. The most common cause of tinnitus was acoustic overstimulation ("NIT", 47%), usually of a result of exposure to loud music in a festival or restaurant. About one third of NIT cases was defined as music-induced tinnitus (MIT). The MIT patients were usually professional or hobby musicians. Other causes were self-reported stress (6%), otitis media (4%) and other causes such as flight travel (2%). The cause of tinnitus was unknown in 40% of cases (**Figure 1A**).

Otological examinations were normal and there were no audiometrically detectable acute hearing impairment, except in five patients that had been exposed to shooting noise. They had a mild (10–25 dB) dip-type hearing loss either at 4 or 6 kHz. Two thirds of patients showed normal or age-related hearing loss in pure tone audiometry. Tinnitus was high-pitched (8 kHz or higher) in two thirds (66%) and >4 kHz in about 85% of cases. The most common tinnitus frequency was detected between 7 and 9 kHz (27%), followed by 11–14 kHz (19%), 9–11 kHz (14%) and 5–7 kHz (13%) (**Figure 1B**).

The mean THI was 55, and it was between 34 and 100 in 81% of patients indicating moderate or severe tinnitus (**Figure 2**). Tinnitus was frequently associated with sleep disturbances (92%) and anxiety (96%) (**Figure 2**). Both sleep disturbances and anxiety were severe or very severe (above 50/100 in VAS in more than half of the patients (57% and 54%, respectively). It may be noteworthy that the patients were typically stressed because, prior to visiting us, they had visited general practitioners or ENT specialists and had received negative counseling ("nothing can be done", "you just have to learn to live with it"). One third of patients had a history or ongoing therapy of depression, ranging from mild (12%) to moderate (18%) to severe (3%).

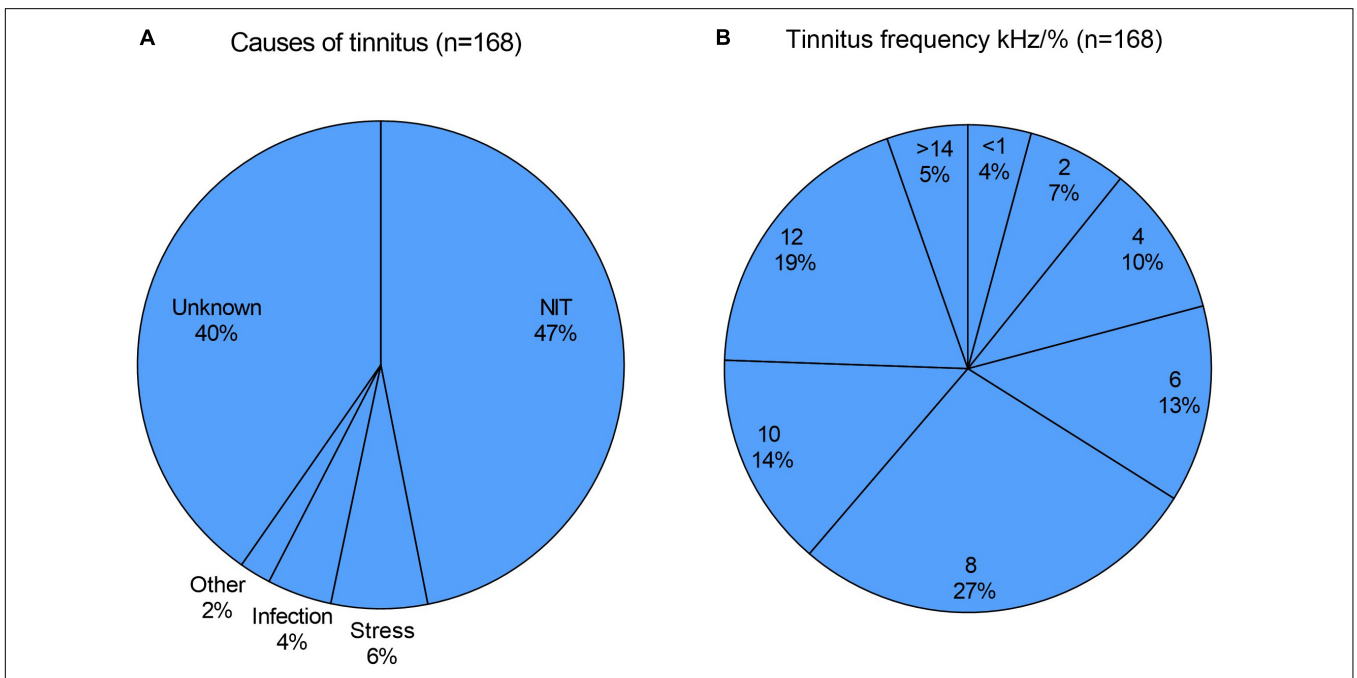


FIGURE 1 | (A) The most common cause of tinnitus was acoustic overstimulation (noise-induced tinnitus, NIT; 47%), followed by self-reported stress (6%), otitis media (4%) and other causes (2%). The cause was unknown in 40%. **(B)** The most common tinnitus frequency was between 7 and 9 kHz (27%), (marked as 8), followed by 11–14 kHz (19%), (marked as 12), 9–11 kHz (14%), (marked as 10), 5–7 kHz (13%), (marked as 6). In 9 patients (5%) tinnitus frequency was >14.0 kHz, in 17 patients (10 %) between 3 and 5 kHz (marked as 4), in 11 patients (7 %) between 1 and 3 kHz (marked as 2) and in seven patients (4%) < 1.0 kHz.

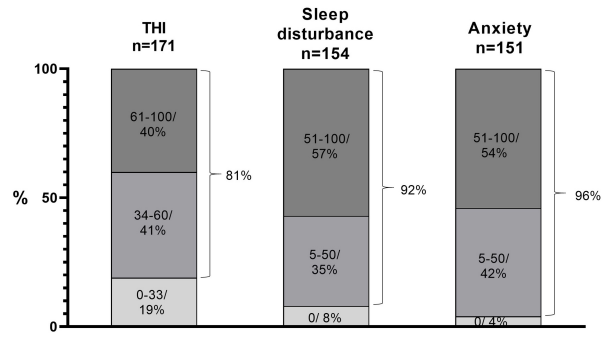


FIGURE 2 | THI was between 34 and 100 in 81% of patients. The mean THI value was 55 and the mean VAS-scaled (0–100) values of both loudness and annoyance were 55. Tinnitus was associated with sleep disturbances in 92% and with anxiety in 96%, both being severe or very severe (>50/100 in VAS scale) in 57% and 54% of patients, respectively.

HRV Tests and Results of Test-taVNS

The main aim of the initial test-taVNS was to ascertain the cardiac safety of the method. We found no cardiac adverse effects in our 171 patients. This applies actually to more than 250 taVNS patients treated so far by us: none of them reported cardiac or other serious side-effects during the first test-taVNS or during home treatment. Several of our patients have used the taVNS device regularly, practically daily for 2–3 years, some up to 5 years.

Baseline data from 1-min DBT-HRV and 5-min short-term HRV (s-HRV) showed that more than three quarters of TRMS patients had increased sympathetic activity before test-taVNS. This was deduced from the stress index (data not shown) and the HRV age, which was in this patient population approximately 16 years higher than the mean chronological age. **Table 1** shows the mean values of different HRV parameters, considered to describe the vasovagal tone and the changes of HRV parameters immediately after the 15–60 min test-taVNS. The taVNS significantly increased all HRV parameters: the mean R-R interval in 81%, Flexibility = E-I (difference between the highest and the lowest heart rate within a breathing cycle) in 63%, Dynamics = RMSSD, (root of the mean square of successive differences) in 69%, Tone = mean HR in DBT in 68% of patients, and decreased the HRV age by 9 years (**Table 1**).

From our previous experience (Ylikoski et al., 2017) and from the results of the current study, we deduced that R-R interval, HRV age and RMSSD are the most useful markers for stimulation changes in HRV (**Table 1**). RMSSD is mainly related to beat-to-beat variations reflecting parasympathetic output (Faber et al., 1996). In practice, the change in vasovagal tone is best illustrated by the HRV age that was determined by algorithms based on values of HRV parameter values, as described by Weinschenk et al. (2016).

If in cases where R-R interval was decreased (in 19%) after test-taVNS, the results of HRV age were taken into consideration, either the HRV age or R-R interval showed increased parasympathetic activity in more than 95% of cases.

To compare the changes in R-R interval, RMSSD and HRV age with the age of patients, we selected the youngest patients (age < 41 years) for one group (n = 41) and the oldest patients (age > 63 years) for another group (n = 21) (**Table 2**, **Figure 3**). Test-taVNS increased all the three HRV parameters much more often in patients of the older group: R-R interval in 86% in >63 group, 73% in < 41 group, RMSSD in 81% in >63 group, 66% in < 41 group and HRV age in 81% in >63 group, 58% in < 41 group (**Table 2**). The taVNS-induced numerical increases of the three HRV parameters were also greater in the older patients. However, only the RMSSD changes reached statistical significance (**Table 2**, **Figure 3**). This indicates that taVNS is more efficient in older patients, a result that could be explained by lower starting levels of HRV in this group due to age-related decline in parasympathetic activity. The magnitude of taVNS-induced changes has been shown to be higher in individuals with lower starting HRV level (Bretherton et al., 2019).

TABLE 1 | Mean HRV test data of 171 patients with TRMS.

	R-R-interval	HRV-age	Flexibility %	Dynamic % (RMSSD)	Tone %
	pre/post taVNS	pre/post taVNS/chronol	pre/post taVNS	pre/post taVNS	pre/post taVNS
Mean	815/868	65/56/49	32/44	32/49	38/49
SD	141/140	22/23/16	28/30	30/34	30/30
p-Value	<0,0001	<0,0001	<0,0001	<0,0001	<0,0001
Cohen's *d*	0,377	0,400	0,414	0,530	0,367
taVNS induced change	+52 (6.4%)	−9 (13.7%)	+11 (34.5%)	+18 (53.2%)	+12 (30.5%)
Increased	139 (81.3%)	12 (7.0%)	107 (62.6%)	118 (69.0%)	116 (67.8%)
Decreased	32 (18.7%)	135 (79.0%)	36 (21.1%)	16 (9.4%)	22 (12.9%)
Unhanged	–	24 (14.0%)	28 (16.4%)	37 (21.6%)	33 (19.3)

The taVNS significantly increased all HRV parameters: the mean R-R interval in 81%, Flexibility in 63%, Dynamics (RMSSD) in 69%, Tone in 68% of patients, and decreased the HRV age by 9 years. The mean pre-taVNS HRV age was 16 years higher than the chronological age. The taVNS induced change represents the mean difference between pre- and post-taVNS values of all patients. The mean percentage change is also shown. Increases of RR-interval, flexibility, RMSSD and tone and decrease of HRV-age indicate increased parasympathetic or decreased sympathetic tone. Pre = baseline data; post = post-taVNS stimulation data. Wilcoxon's matched pairs signed rank test was used to calculate p-values. The Bonferroni adjustment method for multiple testing produced a rejection p-value of 0.01. All p-values remained statistically significant. Effect sizes (Cohen's d) ranged from small to medium, the largest was observed in Dynamic % (RMSSD).

TABLE 2 | Correlations between HRV parameters and scores of sleep and anxiety between age groups.

	R-R-interval	HRV-age	RMSSD	sleep/anxiety
	pre/post taVNS	pre/post taVNS	pre/post taVNS	
All patients (*n* = 171)	+52 (6.4%)	−9 (13.7%)	+18 (53.2%)	55
<41 year, *n* = 41	+53,6 (6,9%)	−7,76 (−15,5%)	+15,2 (42,6%)	61
>63 year, *n* = 21	+85,0 (10,5%)	−11,7 (−14,7%)	+30,1 (93,3%)	52
p-Values	0,515	0,0718	0,0392	0,456
Cohen's *d*	0,395	0,315	0,574	0,276

Correlations between HRV parameters (R-R interval, HRV age and RMSSD), and scores of questionnaires for sleep and anxiety between Group A (age < 41 years) and Group B (age > 63 years). Test-taVNS increased all three HRV parameters much more often in patients of the older group: R-R interval in 86% in >63, 73% in < 41 groups, RMSSD 81% in >63, 66% in < 41 groups and HRV age 81% in >63, 58% in < 41 group. Shown is the mean taVNS induced change (mean difference between pre- and post-taVNS values) of all patients as well as of the two age groups. The taVNS-induced numerical increases of these three HRV parameters were also greater in the older group. Only the RMSSD changes reached statistical significance using the non-parametric Kolmogorov–Smirnov test (when comparing mean changes between the two groups) That significance was removed by the Bonferroni correction as the adjustment for multiple testing produced a rejection p-value of 0.0125. Effect sizes (Cohen's d) ranged from small to medium, the largest was observed in Dynamic % (RMSSD). A positive effect means that the change of the >63 years group was larger than the change of the <43 years group.

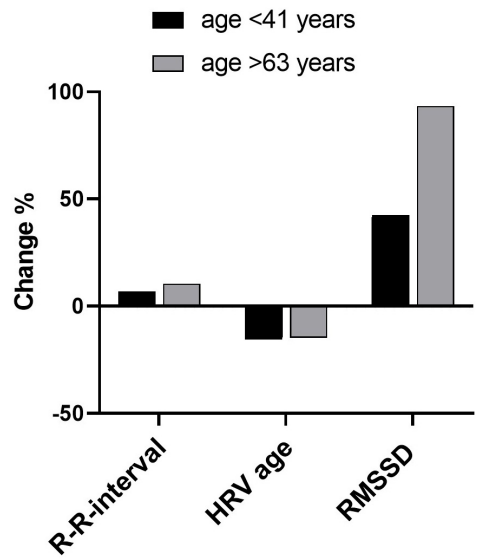


FIGURE 3 | Correlations between HRV parameters between Group A (below 41 years) and Group B (above 63 years) shown in **Table 2**. The taVNS-induced numerical increases of these three HRV parameters were greater in the older group. However, only the RMSSD changes reached statistical significance.

To compare taVNS responses to questionnaire-based clinical data, we selected two patient groups, based on the magnitude of taVNS responses. Group A (21 patients) consisted of super-responders showing a post-taVNS RMSSD increase of 400–2000%. Group B (43 patients) consisted of non-responders showing unchanged or decreased post-taVNS RMSSD. Comparison of the values of THI, sleep disturbance and anxiety showed that group A comprized of more patients with mean THI scores > 60/100 and with sleep disturbance and anxiety scores > 60/100. However, the differences were not statistically significant (**Table 3**, **Figure 4**). Super-responders had also higher average tinnitus pitch (8.4 kHz) than non-responders (6.6 kHz) (**Table 3**, **Figure 4**).

Results of VSEP Testing

There was a strong stimulation artifact (0 ms) after which oscillations were registered at about 3 ms. These have been earlier described to be of brainstem origin (VSEP) (Fallgatter et al., 2003). This response, however, was interpreted and presumed to be of local origin, arising from muscles in the ear region, not in the brainstem, in accordance to Leutzow et al. (2013). Therefore, we have not used VSEP as a biomarker for taVNS.

Results of the 1-Year Follow-Up Outcome Questionnaires

One-year follow-up therapeutic outcome data was possible to receive from 78 out of 113 patients (69%). Both the loudness and annoyance of tinnitus had decreased in about two thirds and, importantly, stress had decreased in more than 80% of patients (**Figure 5A**). About 76% of patients reported that they had benefited from the TCPT (including taVNS) treatment. When asked whether they would recommend similar treatment for a friend or near relative with similar health problems, 90% answered yes or probably yes (**Figure 5B**). When asked what constituent of the TCPT therapy regimen was most efficient in 1 to 5 scale, counseling showed an efficacy of 3.4, followed by taVNS (3.1) and sound therapy (2.8) (**Figure 5C**). Thus, it seems that taVNS is a useful addition to tinnitus treatments. We stress, however, that the most important constituent of tinnitus therapy is directive counseling. That has been our opinion for decades and it was also supported by the results of this follow-up study.

DISCUSSION

The main aim of this study is to share our experience of the usage of taVNS in the treatment of distressing tinnitus. Therefore, we discuss only matters that we feel important in clinical practice. Our study shows that taVNS is safe. Baseline HRV data of 171 patients showed that more than three quarters of TRMS patients had increased sympathetic prevalence (preponderance) before the first test-taVNS. The mean values of different HRV parameters changed toward increased parasympathetic activity by test-taVNS in about 80% of patients. These changes were more pronounced in patients showing greater tinnitus handicap, more severe associated symptoms, higher stress levels and higher age before the test stimulation. No significant adverse effects were reported in follow-up questionnaires. Our conclusion is that our tinnitus treatment program, including taVNS, alleviates stress and handicap caused by tinnitus.

TABLE 3 | Comparison between taVNS responses and questionnaire-based clinical data in super-responders and non-responders.

	THI	Sleep	Anxiety	Loudness	Annoyance	Frequency
A. Super responders (RMSDD increase 400–2000%)	61,05 (*n* = 21)	70,00 (*n* = 20)	68,95 (*n* = 19)	67.35 (*n* = 19)	74.12 (*n* = 19)	8398 Hz (*n* = 20)
B. Non-responders (decrease or no change)	56,67 (*n* = 43)	61,22 (*n* = 37)	58,11 (*n* = 37)	60,63 (*n* = 37)	70,47 (*n* = 37)	6562 Hz (*n* = 40)
p-Values	0,246	0,642	0,198	0,147	0,197	0,122
Cohen's *d*	0,301	0,128	0,358	0,429	0,386	0,414

Comparison between taVNS responses and questionnaire-based clinical data and tinnitus frequency in two patient groups. Group A (n = 21): super-responders (taVNS-induced RMSSD increase of 400–2000%). Group B (n = 43): non-responders (RMSSD decreased or unchanged). The mean values of THI, sleep disturbance, anxiety and tinnitus frequency were all greater in group A, but the differences were not statistically significant (Student's t-test). Bonferroni adjustment method for multiple testing produced a rejection p-value of 0.0083. Effect sizes (Cohen's d) ranged from small to medium. A positive effect indicates that the mean of the super-responder group was larger than the mean of the non-responder group.

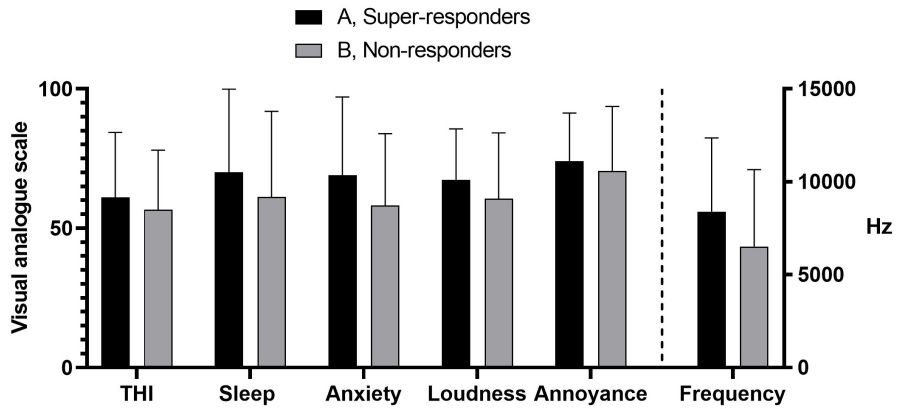


FIGURE 4 | Comparison between taVNS responses and questionnaire-based clinical data and tinnitus frequency in groups A (super-responders) and B (non-responders), shown in **Table 3**. The mean values of THI, sleep disturbance, anxiety and tinnitus frequency were all greater in group A, but the differences were not statistically significant. Error bars represent standard deviations. THI = tinnitus handicap inventory.

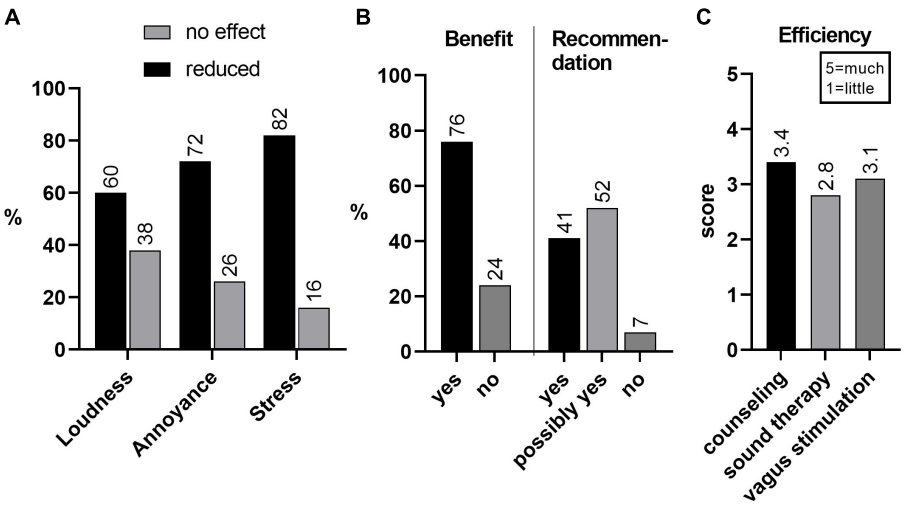


FIGURE 5 | (A) Results of 1-year-follow-up outcome of 113 patients of which 78 (69%) data was received. Tinnitus annoyance had decreased in 72% and tinnitus-triggered stress in 82%. Symptoms had increased in 2%. **(B)** 76% of patients reported that they had benefited from TCPT treatment, including taVNS; 41% would recommend and 52% possibly recommend TCPT plus taVNS t for a friend or relative if suffering from similar health problems. **(C)** Of the components of treatment, counseling was reported most helpful (score 3.4 from the range 1–5), followed by taVNS (3.1) and sound therapy (2.8).

The main problem of patients with disturbing moderate or severe tinnitus is usually TRMS (Andersson and Hesser, 2013) and associated imbalance of the ANS, leading to sympathetic prevalence and correspondingly reduced parasympathetic activity (Thayer et al., 2012; Chalmers et al., 2014). Therefore, the optimal TRMS treatment would be by trophotropic

(parasympathetic activity enhancing) means. This could be achieved by behavioral methods such as cervical vagal massage, Valsalva maneuver or respiratory VNS (Gerritsen and Band, 2018) or by general relaxation generating methods such as yoga, mindfulness, biofeedback and cognitive behavioral therapy. The present study, in agreement with our previous results (Ylikoski et al., 2017), suggests that the therapeutic trophotropic effect can be accentuated by taVNS in TRMS and, thereby, tinnitus handicap can be attenuated. This would be in accordance with recent studies reporting that (implanted) VNS paired with tones as well as taVNS constitute promising novel treatments for tinnitus (Lehtimäki et al., 2013; Tyler et al., 2017; Yakunina et al., 2018). On the other hand, another clinical study did not report improvement of tinnitus with taVNS alone, although the therapy was found to be safe (Kreuzer et al., 2014).

The vagus nerve provides a unique therapeutical entrance to the CNS. Although VNS has become an established intervention therapy for therapy resistant epilepsy and depression, the exact mechanisms remain unsolved. Preclinical studies have shown that VNS therapy results in intermittently increased release of multiple neuromodulators, including norepinephrine, acetylcholine, serotonin and brain-derived neurotrophic factor (BDNF) (Hassert et al., 2004; Dorr and Debonnel, 2006). BDNF is a key player in the CNS neuronal plasticity. Maladaptive plasticity is thought to be involved in many medical entities, particularly in illnesses such as phantom pain, dystonias and tinnitus (Flor et al., 2001; Engineer et al., 2011; Kilgard, 2012). In addition, enhancement of neuronal plasticity through VNS has been shown to improve functional recovery in animal models and patients with stroke-induced upper limb paresis (Dawson et al., 2016; Engineer et al., 2019) and in animal models of spinal cord injury (Ganzer et al., 2018). BDNF, norepinephrine and serotonin have been suggested to play a key role in this enhanced plasticity (Hulsey et al., 2017). BDNF is an activity-dependent neurotrophic factor (Barde et al., 1982). Therefore, therapeutic sessions have consisted of VNS combined with simultaneous activities such as physical therapeutic movements in upper limb paresis or pairing with tones in tinnitus (Engineer et al., 2011; Dawson et al., 2016). In depression, the activity part is thought to consist of psychotherapy.

We emphasize that taVNS should not be applied as a solo but an adjunctive therapy. In our study the treatment of patients with distressing tinnitus was always started with a 1.5 h office visit during which patient's complaints were dealt with our TRT modification, the TCPT program. This consists of careful diagnostics with hearing and tinnitus profiling, counseling and instructions for sound therapy. The taVNS has been the fourth component of TCPT, initiated at the office visit and continued, together with sound therapy (the activity component), as home therapy. Our inquiries (**Figure 5**) indicate that counseling is the most important therapy constituent for this kind of distressed patients. During counseling, which takes about 1 h, we try to demystify tinnitus by explaining what tinnitus is all about and how one should behave in order to diminish its annoyance; not to be afraid that it worsens or never disappears. It has been shown that uncertainties and fears constitute a potent stressor and can easily cause diseases (Peters et al., 2017). Specifically, fear and anxiety can be significant co-factors, possibly modulated by amygdala, in triggering TRMS (Andersson et al., 1999; Cima et al., 2012). This type of counseling should be applied also to the therapeutic regimen of anxious and depressive patients.

Mechanisms of TRMS

The prominent feature of our patients was the SR (arousal) caused by tinnitus. SR is such a personal experience that it is not possible to ascertain – or even speculate- why most of the patients had developed a severe SR. Also, the reliability of our main markers for SR, the questionnaires and HRV tests, are not accurate and often even debatable. However, there are some general clinical and test-based characteristics, which allow to suggest speculative stress pathways at least in the patients in which tinnitus was initially triggered by exposure to excessively loud sounds. These patients had contracted NIHD that is now known to be the most common consequence of noise trauma. Its most common symptoms are tinnitus and hyperacusis, the audiometrically measurable hearing impairment being a much less common feature (Kähäri et al., 2001; Szibor et al., 2018). Usually, the intensity of the exposing sound (mostly music) is relatively low in NIHD and its action on the cochlea is thought to be cellular stress/damage that does not lead to significant hair cell loss. Low-level noise exposure is known to cause synaptopathy, the damage of the synapses between inner hair cells and auditory nerve fibers (Kujawa and Liberman, 2009). It is generally accepted that tinnitus frequency and the predicted location of the lesion along the tonotopic axis of the cochlear are closely related. In the present study, tinnitus frequency tended to be very high, higher than 6 kHz in more than 85% of cases, indicating that the presumable cellular stress response was localized to the extreme basal coil of the cochlea. Most researchers agree that tinnitus can be linked to changes at one or more relays along the peripheral and central auditory pathways including auditory cortex (Jastreboff, 1990; Lockwood et al., 1998; Giraud et al., 1999; Møller, 2003; Eggermont and Roberts, 2004; Rauschecker et al., 2010). Although it is generally accepted that the dysfunction of the auditory system is necessary for tinnitus to occur, it is unclear whether this defect alone is sufficient to cause chronic tinnitus or whether additional mechanisms outside the auditory-sensory regions are involved. Clinically, there is a clear relationship between tinnitus and the emotional state (Sullivan et al., 1988; Dobie, 2003) and it has led to the suggestion that the limbic system plays a role in modulating or perpetuating tinnitus (Jastreboff, 1990; Rauschecker et al., 2010). Indeed, the lifetime incidence of clinical depression in tinnitus patients is estimated to be more than twice of the national average (∼35% vs. 15%, respectively, Folmer et al., 1999). Treatment regimens that include forms of cognitive-behavioral therapy have been shown to be effective for many tinnitus patients (Jastreboff, 2007; Robinson et al., 2008). Although the exact nature of the involvement of the limbic system in chronic tinnitus remains to be shown, there is substantial – mainly neuroimaging – evidence indicating that networks such as the corticostriatal circuit and the amygdala-anterior cingulate cortex axis are involved (Leaver et al., 2011; Chen et al., 2017; Xu et al., 2019). It has been shown that the corticostriatal circuit, which includes the nucleus

accumbens and ventromedial posterior frontal cortex, does indeed differ in the brains of individuals with tinnitus (Leaver et al., 2011). The corticostriatal circuit is part of the general "appraisal network", determining which sensations are important and ultimately affecting how (or whether) those sensations are experienced (Simmons et al., 2020). Our theory of the pathogenetic mechanism of NIHD is schematically summarized in **Figure 6**.

The Inflammatory Reflex or Neuroinflammation in the Pathogenesis of Stress and Tinnitus, and Possible Attenuation by taVNS

Although the common pathways between stress exposure and pathophysiological processes leading to tissue damage are still debatable, several results indicate that stress can activate an inflammatory response in the brain and in the periphery (Calcia et al., 2016, for review). In this damaging process, stress-induced pro-inflammatory factors including C-reactive protein, IL-6, TNFα, IL-1β and NF-κB, have an important role (Miller et al., 2008). In common, over-activated immune system, increased sympathetic nervous system activity and reduced glucocorticoid (GC) responsiveness may work tandemly in the activation of inflammatory responses during stress. GCs, catecholamines, cytokines and other mediators are thought to be the main mediators of the stress-induced pro-inflammatory effect. Correspondingly, when the auditory system in a rodent model was exposed to acoustic overstimulation causing hearing impairment and tinnitus, neuroinflammation in the central auditory system was found to be importantly involved (Wang et al., 2019).

After exposure to acoustic overstimulation from loud noise or music, the resulting tinnitus is high-pitched (Szibor et al., 2018). It can be very difficult to tolerate and habituate this tinnitus and, therefore, it may lead to sleep disturbances, anxiety and finally to SR. Tinnitus can be regarded as the consequence of multisensory interactions between the auditory and limbic systems. This is because extensive functional networks and tinnitus distress strongly correlate with enhanced effective connectivity that is directed from the amygdala to the auditory cortex (Rauschecker et al., 2010; Chen et al., 2017). When the stimulation patterns and dynamics of functional networks during VNS were examined by fMRI, the vagus nerve was found to convey signals to the brain through the polysynaptic neuronal pathways, by projecting to the brainstem nuclei (NTS, locus coeruleus), subcortical areas and lastly to the cortex (Henry, 2002; Ressler and Mayberg, 2007), thus covering the entire CAN. fMRI and a spatially independent component analysis were utilized in a recent experimental study (Cao et al., 2017). That study demonstrated that VNS activated 15 out of 20 brain networks and that the activated regions covered > 75% of the brain volume.

Very soon after the acoustic trauma, which means during ongoing inflammatory response or neuroinflammation, patients usually seek medical assistance because of uncertainty (and fear) with questions regarding possible consequences and management. If no appropriate treatment or even counseling are available and only negative counseling is offered ("nothing can be done"), a complete SR with self-perpetuating cycle develops: distress worsens tinnitus and worsening tinnitus accentuates SR. This is about the clinical picture characterizing most of the patients included in the present study. Therefore, it is not surprising that our TCPT therapeutic regimen, including taVNS as the adjunctive treatment, significantly benefited the great majority of patients. In this type of condition, taVNS may be especially effective, perhaps due to a dual action: it may attenuate the underlying neuroinflammation or inflammatory process in parallel or subsequent to SR.

Of special interest are our findings indicating that aged patients are more responsive to acute taVNS than younger ones, as revealed by HRV tests. Our results are preliminary and appropriate controls are missing, but if age-related differences in HRV responses hold true also in controlled studies, it may open new avenues for the treatment of hearing disorders, particularly the two most common disorders, presbyacusis and NIHD. There is no effective treatment available for them today. Targeting neuroinflammation with taVNS might be a novel therapeutic possibility for NIHD with tinnitus. There is strong preclinical scientific evidence of the beneficial role of VNS in the treatment of immunologic reflex-associated disorders, particularly rheumatoid arthritis [reviewed by Tracey (2018)]. As a method taVNS is safer than VNS, because ABVN has no efferent neurons.

In the pathogenesis of AF, another common medical entity, accumulating evidence indicates that the inflammatory pathways play a significant role (Aviles et al., 2003; Hu et al., 2015). In a recent clinical trial, chronic, intermittent taVNS (with the Salustim device used in this study as well) resulted in lower AF burden in about 50% of patients compared to sham control stimulation. These results support the use of taVNS to treat paroxysmal AF in selected patients (Stavrakis et al., 2020).

How to Improve the Efficacy of taVNS?

The stimulation of ABVN is an easy and non-invasive method to obtain the beneficial effects of vagal system activation. However, there are still uncertainties concerning the modes of stimulation, including the optimal stimulation site and parameters. These can be defined only after the appropriate biomonitoring tests become available. While clinical taVNS applications have been widely noted in the literature, the physiological mechanisms supporting such clinical effects are poorly understood, particularly in humans.

May be the most important proof of the usefulness of taVNS has so far been obtained from clinical studies. taVNS has been employed for patients suffering from various disorders, including epilepsy (Stefan et al., 2012), tinnitus (Lehtimäki et al., 2013; Yakunina et al., 2018), depression (Rong et al., 2012; Hein et al., 2013), pain (Napadow et al., 2012; Laqua et al., 2014; Janner et al., 2018) and migraine (Straube et al., 2015; Garcia et al., 2017). Clinical studies do not, however, directly show that the beneficial effects are due to ABVN stimulation. This is because the outer ear has an innervation not only from the cranial nerve X, but also from the cranial nerves VII and V as well as the cervical plexus.

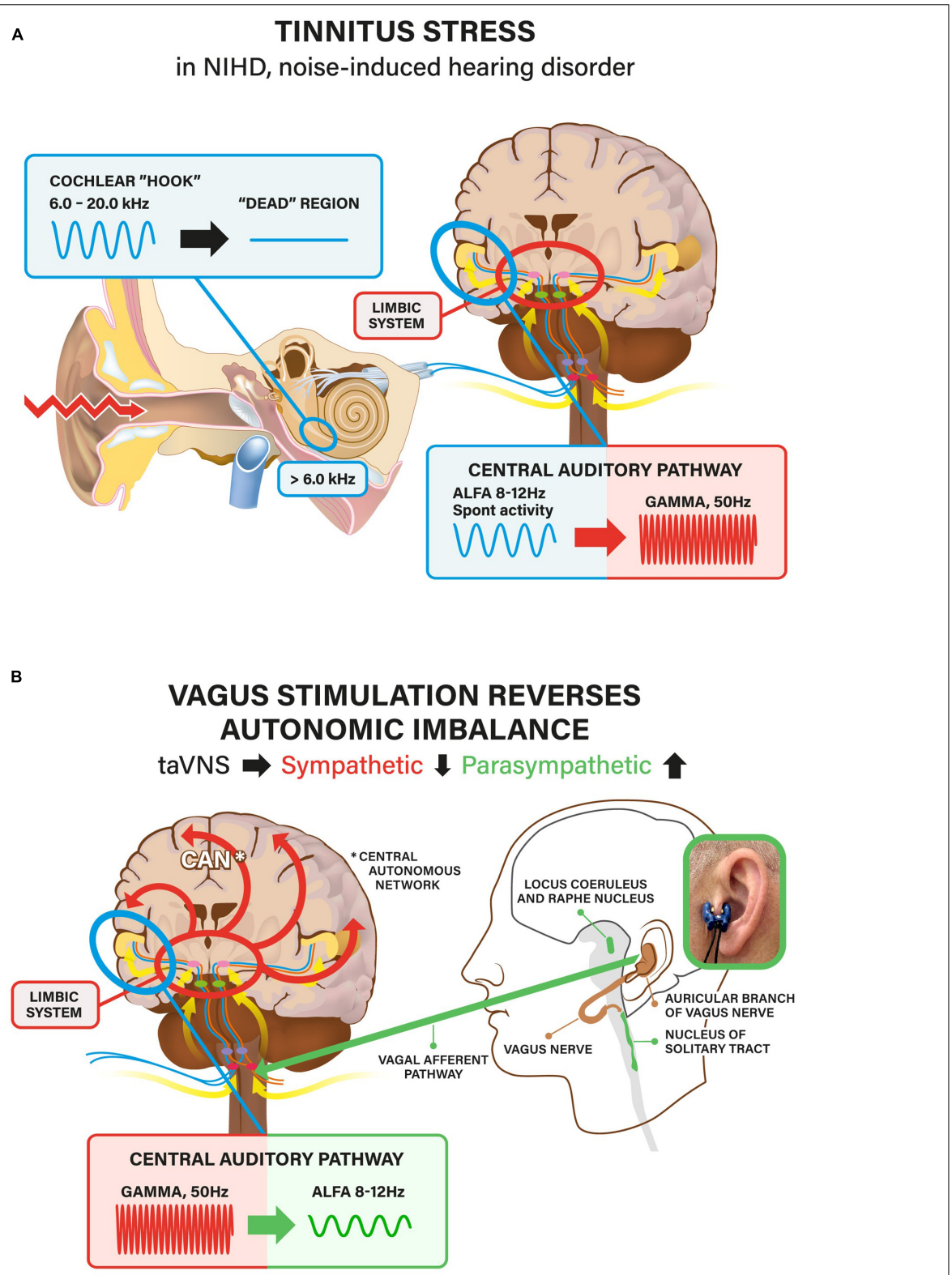


FIGURE 6 | (A) Summary of the hypothesis. Noise exposure (most commonly music) causes dysfunction/damage in the high-frequency (>6.0 kHz) region of the cochlea) in >85% of patients. This leads to high-pitched tinnitus, most commonly at about 8.0 kHz. Bioelectrical impulse flow from the damaged cochlear region toward auditory cortex diminishes or ceases ("dead" region). This leads to reduced (inhibitory) regulation with subsequent neuronal hyperactivity in the central auditory pathway, first in the auditory nuclei of brainstem, later in the auditory cortex. The normal (spontaneous) alpha activity (in EEG) changes to gamma activity.

(Continued)

FIGURE 6 | Continued
The central auditory pathway is intimately connected to the limbic system (that controls emotions). Tinnitus is experienced as an emotionally negative sensation including uncertainties and fears ("what is this all about?"; "does it ever go away?"). Thereby, the perceptive (hearing) network is connected to the distress network (stress). The stressor leads to imbalance of the central autonomous network (CAN) with hyperactivity of the sympathetic nervous system (flight or fight or freeze response) and, correspondingly reduced activity of the parasympathetic nervous system (PNS) (relax, calm down). **(B)** Vagus nerve is the main player of the PNS. Therefore, activation of the vagal system increases PNS activity. For taVNS we have used a specially designed Salustim device that uses an ear-clip electrode inserted to the tragus and electrically stimulates ABVN. The taVNS reverses sympathetic hyperactivity in the limbic system and the CAN imbalance toward parasympathetic direction. Reduction of distress also facilitates the reversal of gamma-hyperactivity back to normal alpha-activity in the auditory central pathway.

Much of our present understanding of the mechanisms and presumed efficacy of taVNS comes from fMRI studies. These studies have shown that taVNS produces significant cortical effects in the vagal afferent pathway. Thus, outer ear stimulation in the regions innervated by ABVN activates afferent vagal networks (Kraus et al., 2007; Dietrich et al., 2008; Frangos et al., 2015; Yakunina et al., 2017; Badran et al., 2018a). These studies have, however, failed to convincingly demonstrate that taVNS activates the crucial brainstem nuclei such as NTS. This has now changed when it was recently demonstrated using a ultrahigh-field (7T) fMRI that taVNS evokes activation in the ipsilateral NTS and upstream monoaminergic source nuclei of the brainstem (Sclocco et al., 2019). This finding supports the idea that the selective stimulation of ABVN is responsible for NTS activation. Corresponding selective NTS activation, comparable to tragal ABVN stimulation, may be possible by using percutaneous ABVN stimulation (Kaniusas et al., 2019). Percutaneous stimulation must, however, be considered as a mini-invasive procedure, because the skin is penetrated. It may be appropriate for the treatment of diseases in medical offices, but not for continuous home-therapy.

Importantly, NTS activity is known to be modulated by respiration, both through the bottom-up afferent pathway from pulmonary stretch receptors and aortic baroreceptors and through the top-down effects from respiratory nuclei in the medulla (Sclocco et al., 2019). Specifically, NTS receives inhibitory influence during inhalation and facilitatory influence during exhalation (Miyazaki et al., 1999; Baekey et al., 2010). Therefore, it has been proposed that NTS targeted by taVNS can be enhanced by gating stimulation to the exhalation phase of the respiratory cycle via respiration-gated auricular vagal afferent nerve stimulation (RAVANS) (Napadow et al., 2012; Garcia et al., 2017). Our taVNS treatment protocol includes instructions for slow breathing ("Respiratory VNS") (Gerritsen and Band, 2018), but electrical stimulation in our device was not synchronized to give electrical stimuli specifically during exhalation.

HRV

Because HRV is a biomonitor for ANS function, we also analyzed HRV changes before and immediately after acute test-taVNS. HRV as well as HR have been found to be useful in monitoring the effects of taVNS (Clancy et al., 2014; Antonino et al., 2017; De Couck et al., 2017; Ylikoski et al., 2017; Badran et al., 2018b; Bretherton et al., 2019). Clancy et al. (2014) demonstrated that 15 min of taVNS administered to the tragus significantly increased HRV, at least partly through reduction of sympathetic nerve activity. In addition, the acute taVNS has been demonstrated to improve spontaneous baroreflex sensitivity (BRS) that may be the most sensitive measure of ANS function and thereby the parasympathetic activity (Antonino et al., 2017; Bretherton et al., 2019). Normal aging is associated with increase in sympathetic prevalence and/or decreases in the vagal tone and overall variability, which is reflected in HRV (Stein et al., 1997; Kuo et al., 1999). There is a general consensus that we all have our own dominant parasympathetic and sympathetic regulation that gradually decreases with advancing age due to a significant reduction of nocturnal parasympathetic activity. Hence, the preservation of parasympathetic function may serve as a biomarker related to the healthy longevity and vitality in late life span (Zulfiqar et al., 2010). In addition to normal aging, a shift toward sympathetic prevalence may contribute to age-related conditions, such as hypertension, heart failure and AF. Evidence suggests that taVNS could play a role in ameliorating these conditions. Bretherton et al. (2019) have suggested that age-related autonomic dysfunction (decrease of HRV and BRS), QoL, mood and sleep changes improve with taVNS administered daily for 2 weeks. This is in line with our previous study (Ylikoski et al., 2017) and also with the observations in the present study showing that the HRV improvement after acute test-taVNS was greater in elderly individuals (with TRMS) than in younger ones. The findings of Bretherton et al. (2019) also point to the influence of initial values in determining the magnitude and direction of change following taVNS: high initial sympathetic prevalence, tension, anger, depression as well as low energy and sleep quality were associated with greater improvements of HRV and BRS. This is also in line with our findings of HRV changes after acute test-taVNS: when the HRV-RMSSD values were correlated to clinical data, patients with high scores in THI, tinnitus annoyance, sleep disturbances and anxiety showed largest changes in RMSSD. Overall, our findings support the idea of Bretherton et al. (2019), when they state: "considering the ease of application and affordability of taVNS, there is significant potential in attenuating symptoms associated with age-related conditions and prolonging the period of healthy ageing."

VSEP

Different physiological and neurophysiological tests have been used to biomonitor the effects of taVNS. According to the literature, VSEP is the most useful online biomonitor. Therefore, we investigated whether VSEP could be used for our biomonitoring purposes. In order to reveal the anatomic site where VSEP arises (hypothetically NTS of the brainstem), we registered VSEP responses using EGI GTEN 100 EEG system with 256 electrodes (Palva and Palva, 2018) and with multiple

stimulation parameters. We found a strong stimulation artifact (0 ms) and thereafter oscillations at about 3 ms, previously described to originate from the brainstem. We interpreted that this response has a local origin, presumably arising from muscles in the ear region, not in the brainstem, in accordance to Leutzow et al. (2013). Therefore, we had to abandon VSEP as a biomarker for taVNS. VSEP seemed to be unrelevant also because of the low numbers (50–100) of epochs reported in prior VSEP studies (Fallgatter et al., 2003; Polak et al., 2007). It is well known that in the most commonly employed brainstem response test, auditory brainstem response (ABR), the minimal number of epochs needed for reliable results vary between 500 and 1000. We are currently investigating whether other features of EEG could be used as online biomonitoring methods for taVNS.

Limitations

The results of the present study should be interpreted with caution because they only represent a retrospective clinical cohort study. However, all our clinical data are based on structured diagnostic forms and questionnaires that were used in the management of the patient population. Furthermore, the consistent improvement of HRV -a seemingly useful marker for mental stress- in 80–90% of our patients suggests that taVNS is a useful (adjunctive) therapeutic means in severe tinnitus. This study encourages future controlled clinical studies on the usefulness of taVNS in tinnitus. The major defect in our retrospective study is the lack of appropriate controls and sham procedures, which are the crucial components of prospective randomized controlled trials (RCTs). Our patient population consists of nonselective series in contrast to that of RTCs in which participants are recruited through various procedures enhancing the selection factor. This aspect is particularly important when the target of investigation is such a common symptom as mental stress.

CONCLUSION

TRMS is an example of a tinnitus-triggered stress condition in which patients may benefit from taVNS. Our clinical data and HRV results before and after test-taVNS suggest that patients with TRMS have ANS imbalance with increased sympathetic activity and, correspondingly, reduced parasympathetic function. Acute test-taVNS increased parasympathetic activity, more in elderly and patients with more severe stress symptoms. Although our follow-up outcome study primarily aimed to study the TCPT therapeutic efficacy in patients with TRMS, showing that this therapeutic program alleviated tinnitus severity, the results can also be interpreted such that the majority of stressed tinnitus patients get additional benefit from taVNS as an adjunct therapy. HRV seems to serve as an easy and rapid method for assessment of SR and thereby ANS balance. Combining clinical data to HRV results may be useful in selecting patients for taVNS. We have now clinical experience on the long-term use of taVNS by several of our patients. They have used taVNS daily for more than 4 years without any adverse effects. They continue to use the device because of subjective benefits. Currently, at the same setting where the present study was performed, we offer taVNS treatment for all our patients who show THI scores of 34 or over. We regard this as an alternative to a possible need for e.g., tranquilizers. However, taVNS should not be used as a solo therapy but as an adjunct to a treatment program in which all the constituents are aimed to restore the sympathovagal imbalance through parasympathetic activation. Generally, this study offers additional support to the idea that taVNS might offer a new, targeted therapeutic tool for patients in whom sympathovagal imbalance is involved. Furthermore, taVNS is patient-friendly and of low-cost. However, as there are not (yet) appropriate online biomarkers available for taVNS, there is still a great need for additional research to find optimal therapeutic regimen as well as better stimulating devices.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

JY contributed to the design of the study, interpretation of data, and writing the manuscript. JY, JL, and MY conducted the clinical work. MM conducted the data analysis and illustrations. UP, ZJ, SS, and AM revised the manuscript. All authors contributed to the article and approved the submitted version.

REFERENCES

Akselrod, S., Gordon, D., Ubel, F. A., Shannon, D. C., Berger, A. C., and Cohen, R. J. (1981). Power spectrum analysis of heart rate fluctuation: a quantitative probe of beat-to-beat cardiovascular control. *Science* 213, 220–222. doi: 10.1126/science.6166045

Andersson, G., and Hesser, H. (2013). Special issue on tinnitus. *Cogn. Behav. Ther.* 42, 81–83. doi: 10.1080/16506073.2013.812369

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

Antonino, D., Teixeira, A. L., Maia-Lopes, P. M., Souza, M. C., Sabino-Carvalho, J. L., Murray, A. R., et al. (2017). Non-invasive vagus nerve stimulation acutely improves spontaneous cardiac baroreflex sensitivity in healthy young men: a randomized placebo-controlled trial. *Brain Stimul.* 10, 875–881. doi: 10.1016/j.brs.2017.05.006

Aviles, R. J., Martin, D. O., Apperson-Hansen, C., Houghtaling, P. L., Rautaharju, P., Kronmal, R. A., et al. (2003). Inflammation as a risk factor for atrial fibrillation. *Circulation* 108, 3006–3010.

Badran, B. W., Dowdle, L. T., Mithoefer, O. J., LaBate, N. T., Coatsworth, J., Brown, J. C., et al. (2018a). Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: a concurrent taVNS/fMRI study and review. *Brain Stimul.* 11, 492–500. doi: 10.1016/j.brs.2017.12.009

Badran, B. W., Mithoefer, O. J., Summer, C. E., LaBate, N. T., Glusman, C. E., and Badran, A. W. (2018b). Short trains of transcutaneous auricular vagus nerve stimulation (taVNS) have parameter-specific effects on heart rate. *Brain Stimul.* 11, 699–708. doi: 10.1016/j.brs.2018.04.004

Baekey, D. M., Molkov, Y. I., Paton, J. F., Rybak, I. A., and Dick, T. E. (2010). Effect of baroreceptor stimulation on the respiratory pattern: insights into respiratory-sympathetic interactions. *Respir. Physiol. Neurobiol.* 174, 135–145. doi: 10.1016/j.resp.2010.09.006

Barde, Y.-A., Edgar, D., and Thoenen, H. (1982). Purification of a new neurotrophic factor from mammalian brain. *EMBO J.* 1, 549–553. doi: 10.1002/j.1460-2075.1982.tb01207.x

Benarroch, E. E. (1993). The central autonomic network: functional organization, dysfunction, and perspective. *Mayo Clin. Proc.* 68, 988–1001. doi: 10.1016/s0025-6196(12)62272-1

Bretherton, B., Atkinson, L., Murray, A., Clancy, J., Deuchars, S., and Deuchars, J. (2019). Effects of transcutaneous vagus nerve stimulation in individuals aged 55 years or above: potential benefits of daily stimulation. *Aging (Albany NY)* 11, 4836–4857. doi: 10.18632/aging.102074

Calcia, M. A., Bonsall, D. R., Bloomfield, P. S., Selvaraj, S., Barichello, T., and Howes, O. D. (2016). Stress and neuroinflammation: a systematic review of the effects of stress on microglia and the implications for mental illness. *Psychopharmacology* 233, 1637–1650. doi: 10.1007/s00213-016-4218-9

Camm, A. J., Malik, M., Bigger, J. T., Breithardt, G., Cerutti, S., Cohen, R. J., et al. (1996). Task force of the European society of cardiology and the North American society of pacing and electrophysiology. Heart rate variability: standards of measurement, physiological interpretation and clinical use. *Circulation* 93, 1043–1065. doi: 10.1161/01.cir.93.5.1043

Cao, J., Lu, K.-H., Powley, T. L., and Liu, Z. (2017). Vagal nerve stimulation triggers widespread responses and alters large-scale functional connectivity in the rat brain. *PLoS One* 12:e0189518. doi: 10.1371/journal.pone.0189518

Chalmers, J. A., Quintana, D. S., Abbott, M. J.-A., and Kemp, A. H. (2014). Anxiety disorders are associated with reduced heart rate variability: a meta-analysis. *Front. Psychiatry* 5:80. doi: 10.3389/fpsyt.2014.00080

Chen, Y. C., Xia, W., Chen, H., Feng, Y., Xu, J. J., Gu, J. P., et al. (2017). Tinnitus distress is linked to enhanced resting-state functional connectivity from the limbic system to the auditory cortex. *Hum. Brain Mapp.* 38, 2384–2397. doi: 10.1002/hbm.23525

Cima, R. F., Maes, I. H., Joore, M. A., Scheyen, D. J., El Refaie, A., and Baguley, D. M. (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

Clancy, J. A., Mary, D. A., Witte, K. K., Greenwood, J. P., Deuchars, S. A., and Deuchars, J. (2014). Non-invasive vagus nerve stimulation in healthy humans reduces sympathetic nerve activity. *Brain Stimul.* 7, 871–877. doi: 10.1016/j.brs.2014.07.031

Cohen, J. (1988). *Statistical Power Analysis for the Behavioral Sciences*, 2nd Edn. Mahwah, NJ: Erlbaum.

Dawson, J., Pierce, D., Dixit, A., Kimberley, T. J., Robertson, M., Tarver, B., et al. (2016). Safety, feasibility, and efficacy of vagus nerve stimulation paired with upper-limb rehabilitation after ischemic stroke. *Stroke* 47, 143–150. doi: 10.1161/strokeaha.115.010477

De Couck, M., Cserjesi, R., Caers, R., Zijlstra, W. P., Widjaja, D., Wolf, N., et al. (2017). Effects of short and prolonged transcutaneous vagus nerve stimulation on heart rate variability in healthy subjects. *Auton. Neurosci.* 203, 88–96. doi: 10.1016/j.autneu.2016.11.003

Dietrich, S., Smith, J., Scherzinger, C., Hofmann-Preiß, K., Freitag, T., Eisenkolb, A., et al. (2008). A novel transcutaneous vagus nerve stimulation leads to brainstem and cerebral activations measured by functional MRI/Funktionelle magnet- resonanztomographie zeigt Aktivierungen des Hirnstamms und weiterer zerebraler Strukturen unter transkutaner vagusnervstimulation. *Biomed. Tech. (Berl.)* 53, 104–111. doi: 10.1515/bmt.2008.022

Dobie, R. A. (2003). Depression and tinnitus. *Otolaryngol. Clin. North Am.* 36, 383–388.

Dorr, A. E., and Debonnel, G. J. (2006). Effect of vagus nerve stimulation on serotonergic and noradrenergic transmission. *Pharmacol. Exp. Ther.* 318, 890–898. doi: 10.1124/jpet.106.104166

Eggermont, J. J., and Roberts, L. E. (2004). The neuroscience of tinnitus. *Trends Neurosci.* 27, 676–682.

Engineer, N. D., Kimberley, T. J., Prudente, C. N., Dawson, J., Tarver, W. B., and Hays, S. A. (2019). Targeted vagus nerve stimulation for rehabilitation after stroke. *Front. Neurosci.* 13:280. doi: 10.3389/fnins.2019.00280

Engineer, N. D., Riley, J. R., Seale, J. D., Vrana, W. A., Shetake, H. A., Sudanagunta, S. P., et al. (2011). Reversing pathological neural activity using targeted plasticity. *Nature* 470, 101–106. doi: 10.1038/nature09656

Faber, T. S., Staunton, A., Hnatkova, K., Camm, A. J., and Malik, M. (1996). Stepwise strategy of using short- and long-term heart rate variability for risk stratification after myocardial infarction. *Pacing Clin. Electrophysiol.* 19, 1845–1851. doi: 10.1111/j.1540-8159.1996.tb03238.x

Fallgatter, A. J., Neuhauser, B., Herrmann, M. J., Ehlis, A. C., Wagener, A., Scheuerpflug, P., et al. (2003). Far field potentials from the brain stem after transcutaneous vagus nerve stimulation. *J. Neural. Trans.* 110, 1437–1443. doi: 10.1007/s00702-003-0087-6

Flor, H., Denke, C., Schaefer, M., and Grüsser, S. (2001). Effect of sensory discrimination training on cortical reorganisation and phantom limb pain. *Lancet* 357, 1763–1764. doi: 10.1016/s0140-6736(00)04890-x

Folmer, R. L., Griest, S. E., Meikle, M. B., and Martin, W. H. (1999). Tinnitus severity, loudness, and depression. *Otolaryngol. Head Neck Surg.* 121, 48–51. doi: 10.1016/s0194-5998(99)70123-3

Frangos, E., Ellrich, J., and Komisaruk, B. R. (2015). Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: fMRI evidence in humans. *Brain Stimul.* 8, 624–636. doi: 10.1016/j.brs.2014.11.018

Ganzer, P. D., Darrow, M. J., Meyers, E. C., Solorzano, B. R., Ruiz, A. D., Robertson, N. M., et al. (2018). Closed-loop neuromodulation restores network connectivity and motor control after spinal cord injury. *eLife* 7, 1–19. doi: 10.1093/acprof:oso/9780199746507.003.0001

Garcia, R. G., Lin, R. L., Lee, J., Kim, J., Barbieri, R., Sclocco, R., et al. (2017). Modulation of brainstem activity and connectivity by respiratory-gated auricular vagal afferent nerve stimulation in migraine patients. *Pain* 158, 1461–1472. doi: 10.1097/j.pain.0000000000000930

Gerritsen, R. J. S., and Band, G. P. H. (2018). Breath of life: the respiratory vagal stimulation model of contemplative activity. *Front. Hum. Neurosci.* 12:397. doi: 10.3389/fnhum.2018.00397

Giraud, A. L., Chery-Croze, S., Fischer, G., Fischer, C., Vighetto, A., Gregoire, M. C., et al. (1999). A selective imaging of tinnitus. *Neuroreport* 10, 1–5.

Hassert, D. L., Miyashita, T., and Williams, C. L. (2004). The effects of peripheral vagal nerve stimulation at a memory-modulating intensity on norepinephrine output in the basolateral amygdala. *Behav. Neurosci.* 118, 79–88. doi: 10.1037/0735-7044.118.1.79

Hein, E., Nowak, M., Kiess, O., Biermann, T., Bayerlein, K., Kornhuber, J., et al. (2013). Auricular transcutaneous electrical nerve stimulation in depressed patients: a randomized controlled pilot study. *J. Neural. Transm.* 120, 821–827. doi: 10.1007/s00702-012-0908-6

Henry, T. R. (2002). Therapeutic mechanisms of vagus nerve stimulation. *Neurology* 59, S3–S14.

Hu, Y. F., Chen, Y. J., Lin, Y. J., and Chen, S. A. (2015). Inflammation and the pathogenesis of atrial fibrillation. *Nat. Rev. Cardiol.* 12, 230–243. doi: 10.1038/nrcardio.2015.2

Hulsey, D. R., Riley, J. R., Loerwald, K. W., Rennaker, R. L., Kilgard, M. P., and Hays, S. A. (2017). Parametric characterization of neural activity in the locus coeruleus in response to vagus nerve stimulation. *Exp. Neurol.* 289, 21–30. doi: 10.1016/j.expneurol.2016.12.005

Janner, H., Klausenitz, C., Gurtler, N., Hahnenkamp, K., and Usichenko, T. I. (2018). Effects of electrical transcutaneous vagus nerve stimulation on the perceived intensity of repetitive painful heat stimuli: a blinded placebo- and sham-controlled randomized crossover investigation. *Anesth Anal.* 126, 2085–2092. doi: 10.1213/ane.0000000000002820

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. (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. (1993). A neurophysiological approach to tinnitus: clinical implications. *Br. J. Audiol.* 27, 7–17. doi: 10.3109/03005369309077884

Kähäri, K. R., Axelsson, A., Hellström, P. A., and Zachau, G. (2001). Hearing assessment of classical orchestral musicians. *Scand. Audiol.* 30, 13–23. doi: 10.1080/010503901750069536

Kaniusas, E., Kampusch, S., Tittgemeyer, M., Panetsos, F., Gines, R. F., and Papa, M. (2019). Current directions in the auricular vagus nerve stimulation I – a physiological perspective. *Front. Neurosci.* 13:854. doi: 10.3389/fnins.2019.00854

Kilgard, M. P. (2012). Harnessing plasticity to understand learning and treat disease. *Trends Neurosci.* 35, 715–722. doi: 10.1016/j.tins.2012.09.002

Kraus, T., Hossl, K., Kiess, O., Schanze, A., Kornhuber, J., and Forster, C. (2007). BOLDfMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. *J. Neural Transm.* 114, 1485–1493. doi: 10.1007/s00702-007-0755-z

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

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

Kuo, T. B., Lin, T., Yang, C. C., Li, C. L., Chen, C. F., and Chou, P. (1999). Effect of aging on gender differences in neural control of heart rate. *Am. J. Physiol.* 277, H2233–H2239.

Laqua, R., Leutzow, B., Wendt, M., and Usichenko, T. (2014). Transcutaneous vagal nerve stimulation may elicit anti-and pro-nociceptive effects under experimentally- induced painda crossover placebo-controlled investigation. *Auton. Neurosci.* 185, 120–122. doi: 10.1016/j.autneu.2014.07.008

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

Lehtimäki, J., Hyvärinen, P., Ylikoski, M., Bergholm, M., Mäkelä, J. P., Aarnisalo, A., et al. (2013). Transcutaneous vagus nerve stimulation in tinnitus: a pilot study. *Acta Otolaryngol.* 133, 378–382. doi: 10.3109/00016489.2012.750736

Leutzow, B., Lange, J., Gibb, A., Schroeder, H., Nowak, A., Wendt, M., et al. (2013). Vagal sensory evoked potentials disappear under the neuromuscular block - an experimental study. *Brain Stimul.* 6, 812–816. doi: 10.1016/j.brs.2013.03.005

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

Malik, M. (1996). Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task force of the European society of cardiology and the North American society of pacing and electrophysiology. *Circulation* 93, 1043–1065. doi: 10.1161/01.cir.93.5.1043

McEwen, B. S. (2000). The neurobiology of stress: from serendipity to clinical relevance. *Brain Res.* 886, 172–189. doi: 10.1016/s0006-8993(00)02950-4

McEwen, B. S., and Akil, H. (2020). Revisiting the stress concept: implications for affective disorders. *J. Neurosci.* 40, 12–21. doi: 10.1523/JNEUROSCI.0733-19.2019

Miller, G. E., Chen, E., Sze, J., Marin, T., Arevalo, J. M. G., Doll, R., et al. (2008). A functional genomic fingerprint of chronic stress in humans: blunted glucocorticoid and increased NF-κB signaling. *Biol. Psychiatry* 64, 266–272. doi: 10.1016/j.biopsych.2008.03.017

Miyazaki, M., Tanaka, I., and Ezure, K. (1999). Excitatory and inhibitory synaptic inputs shape the discharge pattern of pump neurons of the nucleus tractus solitarii in the rat. *Exp. Brain Res.* 129, 191–200. doi: 10.1007/s002210050889

Møller, A. R. (2003). Pathophysiology of tinnitus. *Otolaryngol. Clin. North Am.* 36, 249–266.

Napadow, V., Edwards, R. R., Cahalan, C. M., Mensing, G., Greenbaum, S., Valovska, A., et al. (2012). Evoked pain analgesia in chronic pelvic pain patients using respiratory-gated auricular vagal afferent nerve stimulation. *Pain Med.* 13, 777–789. doi: 10.1111/j.1526-4637.2012.01385.x

Palva, S., and Palva, J. M. (2018). Roles of brain criticality and multiscale oscillations in temporal predictions for sensorimotor processing. *Trends Neurosci.* 41, 729–743. doi: 10.1016/j.tins.2018.08.008

Peters, A., McEwen, B. S., and Friston, K. (2017). Uncertainty and stress: why it causes diseases and how it is mastered by the brain. *Prog. Neurobiol.* 156, 164–188. doi: 10.1016/j.pneurobio.2017.05.004

Polak, T., Ehlis, A. C., Langer, J. B., Plichta, M. M., Metzger, F., Ringel, T. M., et al. (2007). Non-invasive measurement of vagus activity in the brainstem – a methodological progress towards earlier diagnosis of dementias? *J. Neural. Trans.* 114, 613–619. doi: 10.1007/s00702-007-0625-8

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

Ressler, K. J., and Mayberg, H. S. (2007). Targeting abnormal neural circuits in mood and anxiety disorders: from the laboratory to the clinic. *Nature Neurosci.* 10:1116. doi: 10.1038/nn1944

Robinson, S. K., Viirre, E. S., Bailey, K. A., Kindermann, S., Minassian, A. L., Goldin, P. R., et al. (2008). A randomized controlled trial of cognitive-behavior therapy for tinnitus. *Int. Tinnitus J.* 14, 119–126.

Rong, P.-J., Fang, J.-L., Wang, L.-P., Meng, H., Liu, J., Ma, Y.-G., et al. (2012). Transcutaneous vagus nerve stimulation for the treatment of depression: a study protocol for a double blinded randomized clinical trial. *BMC Complement Altern. Med.* 12:255. doi: 10.1186/1472-6882-12-255

Sclocco, R., Garcia, R. G., Kettner, N. W., Isenburg, K., Fisher, H. P., Hubbard, C. S., et al. (2019). The influence of respiration on brainstem and cardiovagal response to auricular vagus nerve stimulation: a multimodal ultrahigh-field (7T) fMRI study. *Brain Stimul.* 12, 911–921. doi: 10.1016/j.brs.2019.02.003

Selye, H. (1950). Stress and the general adaptation syndrome. *Br. Med. J.* 1, 1383–1392. doi: 10.1136/bmj.1.4667.1383

Simmons, J. M., Winsky, L., Zehr, J. L., and Gordon, J. A. (2020). Priorities in stress research: a view from the U.S. National institute of mental health. *Stress* 1, 1–7. doi: 10.1080/10253890.2020.1781084

Stavrakis, S., Stoner, J. A., Humphrey, M. B., Morris, L., Filiberti, A., Reynolds, J. C., et al. (2020). TREAT AF (Transcutaneous electrical vagus nerve stimulation to suppress atrial fibrillation): a randomized clinical trial. *JACC Clin Electrophysiol.* 6, 282–291. doi: 10.1016/j.jacep.2019.11.008

Stefan, H., Kreiselmeyer, G., Kerling, F., Kurzbuch, K., Rauch, C., Heers, M., et al. (2012). Transcutaneous vagus nerve stimulation (t-VNS) in pharmacoresistant epilepsies: a proof of concept trial. *Epilepsia* 53:e00115-18.

Stein, P. K., Freedland, K. E., Skala, J. A., Carney, R. M., Davila-Roman, V., Rich, M. W., et al. (1997). Heart rate variability is independent of age, gender, and race in congestive heart failure with a recent acute exacerbation. *Am. J. Cardiol.* 79, 511–512. doi: 10.1016/s0002-9149(96)00798-9

Straube, A., Ellrich, J., Eren, O., Blum, B., and Ruscheweyh, R. (2015). Treatment of chronic migraine with transcutaneous stimulation of the auricular branch of the vagal nerve (auricular t-VNS): a randomized, monocentric clinical trial. *J. Headache Pain* 16, 543.

Sullivan, M. D., Katon, W., Dobie, R., Sakai, C., Russo, J., Harrop-Griffiths, J. (1988). Disabling tinnitus. Association with affective disorder. *Gen. Hosp. Psychiatry* 10, 285–291. doi: 10.1016/0163-8343(88)90037-0

Szibor, A., Hyvärinen, P., Lehtimäki, J., Pirvola, U., Ylikoski, M., Mäkitie, A., et al. (2018). Hearing disorder from music; a neglected dysfunction. *Acta Otolaryngol.* 138, 21–24.

Tarvainen, M. P., Georgiadis, S., Lipponen, J. A., Hakkarainen, M., and Karjalainen, P. A. (2009). Time-varying spectrum estimation of heart rate variability signals with Kalman smoother algorithm. *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2009, 1–4.

Thayer, J. F., Ahs, F., Fredrikson, M., Sollers, J. J. III, and Wager, T. D. (2012). A meta-analysis of heart rate variability and neuroimaging studies: implications for heart rate variability as a marker of stress and health. *Neurosci. Biobehav. Rev.* 36, 747–756. doi: 10.1016/j.neubiorev.2011.11.009

Tracey, K. J. (2018). Neurons are the inflammatory problem. *Cell* 173, 1066–1068. doi: 10.1016/j.cell.2018.05.005

Tyler, R., Cacace, A., Stocking, C., Tarver, B., Engineer, N., and Martin, J. (2017). Vagus nerve stimulation paired with tones for the treatment of tinnitus: a prospective randomized double-blind controlled pilot study in humans. *Sci. Rep*. 7:11960.

Wang, W., Zhang, L. S., Zinsmaier, A. K., Patterson, G., Leptich, E. J., Shoemaker, S. L., et al. (2019). Neuroinflammation mediates noise-induced synaptic imbalance and tinnitus in rodent models. *PLoS Biol.* 17:e3000307. doi: 10.1371/journal.pbio.3000307

Weinschenk, S. W., Beise, R. D., and Lorenz, J. (2016). Heart rate variability (HRV) in deep breathing tests and 5-min short-term recordings: agreement of ear photoplethysmography with ECG measurements, in 343 subjects. *Eur. J. Appl. Physiol.* 116, 1527–1535. doi: 10.1007/s00421-016-3401-3

Xu, J. J., Cui, J., Feng, Y., Yong, W., Chen, H., Chen, Y. C., et al. (2019). Chronic tinnitus exhibits bidirectional functional dysconnectivity in frontostriatal circuit. *Front. Neurosci.* 13:1299. doi: 10.3389/fnins.2019.01299

Yakunina, N., Kim, S. S., and Nam, E. C. (2017). Optimization of transcutaneous vagus nerve stimulation using functional MRI. *Neuromodulation* 20, 290–300. doi: 10.1111/ner.12541

Yakunina, N., Kim, S. S., and Nam, E. C. (2018). BOLD fMRI effects of transcutaneous vagus nerve stimulation in patients with chronic tinnitus. *PLoS One* 13:e0207281. doi: 10.1371/journal.pone.0207281

Ylikoski, J., Lehtimäki, J., Pirvola, U., Mäkitie, A., Aarnisalo, A., Hyvärinen, P., et al. (2017). Non-invasive vagus nerve stimulation reduces sympathetic preponderance in patients with tinnitus. *Acta Otolaryngol.* 137, 426–431. doi: 10.1080/00016489.2016.1269197

Zulfiqar, U., Jurivich, D. A., Gao, W., and Singer, D. H. (2010). Relation of high heart rate variability to healthy longevity. *Am. J. Cardiol.* 105, 1181–1185. doi: 10.1016/j.amjcard.2009.12.022

Conflict of Interest: JY, JL, and MY are board members of the Helsinki Ear Institute and Salustim Group.

The remaining 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.

frontiers in Human Neuroscience

BRIEF RESEARCH REPORT
published: 27 November 2020
doi: 10.3389/fnhum.2020.600995

tVNS Increases Liking of Orally Sampled Low-Fat Foods: A Pilot Study

Lina Öztürk[1], Pia Elisa Büning[2,3,4†], Eleni Frangos[5], Guillaume de Lartigue[2,6,7,8] and Maria G. Veldhuizen[1,2,3]*

[1] Department of Anatomy, Faculty of Medicine, Mersin University, Mersin, Turkey, [2] The John B. Pierce Laboratory, New Haven, CT, United States, [3] Department of Psychiatry, Yale School of Medicine, New Haven, CT, United States, [4] Department of Psychology, University of Cologne, Cologne, Germany, [5] National Center for Complementary and Integrative Health, National Institutes of Health, Bethesda, MD, United States, [6] Department of Pharmacodynamics, College of Pharmacy, University of Florida, Gainesville, FL, United States, [7] Center for Integrative Cardiovascular and Metabolic Disease, University of Florida, Gainesville, FL, United States, [8] Department of Molecular and Cellular Physiology, Yale School of Medicine, New Haven, CT, United States

Recently a role for the vagus nerve in conditioning food preferences was established in rodents. In a prospective controlled clinical trial in humans, invasive vagus nerve stimulation shifted food choice toward lower fat content. Here we explored whether hedonic aspects of an orally sampled food stimulus can be modulated by non-invasive transcutaneous vagus nerve stimulation (tVNS) in humans. In healthy participants ($n = 10$, five women, 20–32 years old, no obesity) we tested liking and wanting ratings of food samples with varying fat or sugar content with or without tVNS in a sham-controlled within-participants design. To determine effects of tVNS on food intake, we also measured voluntary consumption of milkshake. Spontaneous eye blink rate was measured as a proxy for dopamine tone. Liking of low-fat, but not high-fat puddings, was higher for tVNS relative to sham stimulation. Other outcomes showed no differences. These findings support a role for the vagus nerve promoting post-ingestive reward signals. Our results suggest that tVNS may be used to increase liking of low-calorie foods, which may support healthier food choices.

Keywords: vagus nerve (VN) stimulation, food reward, food preferences, obesity, healthy food choice

OPEN ACCESS

Edited by:
Mathias Weymar, University of Potsdam, Germany

Reviewed by:
Bashar W. Badran, Medical University of South Carolina, United States
Vanessa Teckentrup, University of Tübingen, Germany

****Correspondence:***
Maria G. Veldhuizen
margaveldhuizen@gmail.com

†Present address:
Pia Elisa Büning, Center for Ambulatory Rehab, Nanz Medico GmbH & Co. KG, Oberhausen, Germany

Specialty section:
This article was submitted to Brain Imaging and Stimulation, a section of the journal Frontiers in Human Neuroscience

Received: *01 September 2020*
Accepted: *27 October 2020*
Published: *27 November 2020*

Citation:
Öztürk L, Büning PE, Frangos E, de Lartigue G and Veldhuizen MG (2020) tVNS Increases Liking of Orally Sampled Low-Fat Foods: A Pilot Study. Front. Hum. Neurosci. 14:600995. doi: 10.3389/fnhum.2020.600995

INTRODUCTION

The vagus nerve (VN) carries signals about food from gut to brain (Berthoud, 2008; Lartigue, 2016; Yuan and Silberstein, 2016a). The role of the VN in food consumption is classically thought to mostly entail establishing satiety by transducing signals from mechanoreceptors in the stomach and the release of anorexigenic hormones into the blood (Berthoud, 2008). Recently several pre-clinical studies have dissected a role for vagus nerve signals beyond satiety. Williams et al. (2016) show that vagal sensory neurons are capable of sensing a range of metabolic stimuli from the gut, including macronutrients (Williams et al., 2016). Opto- and chemogenetic experiments showed that nodose vagal sensory neurons are necessary for post-ingestive fat mediated reward (Han et al., 2018). A population of neurons in brainstem that receive sugar signals from the vagus were chemogenetically activated to create preferences to otherwise less-preferred sweet stimuli (Tan et al., 2020). Together, these pre-clinical findings reveal a gut-to-brain post-ingestive fat and sugar-sensing pathway critical for the development of food preference. If these data translate to humans, these findings would support targeting the VN to modulate food preferences.

Vagal nerve stimulation (VNS) involves implanting electrodes on the vagus nerve and using electrical pulses to generate firing potentials (Yuan and Silberstein, 2016b). Interestingly, implantation of a VN stimulation device for treatment of epilepsy or depression is in some retrospective studies accompanied by significant weight loss in humans (Burneo et al., 2002; Ogbonnaya and Kaliaperumal, 2013), and in a prospective study decreased preference of sweet food images (Bodenlos et al., 2007). Non-invasive VNS via the auricular branch (transcutaneous VNS, tVNS) (Ellrich, 2011) is effective in treating depression in clinical studies (Kong et al., 2018), and has enabled experimental studies in healthy human participants (Frangos et al., 2015; Yakunina et al., 2017).

Recent work using tVNS modulation of responses to food stimuli in humans have shown mixed results. tVNS had no effect on electro-encephalogram responses to visual food stimuli relative to other objects, as well as no effect on food intake when tVNS was applied immediately prior to the test session (Obst et al., 2020). However, in an effort allocation task, *concurrent* tVNS increased participants' drive to obtain less-wanted prospective food rewards (Neuser et al., 2020). Two weeks of tVNS concurrent with bottle-feeding improved oral intake in about half of premature or brain injured infants who had failed oral feeding until that time (Badran et al., 2020). These results suggest that concurrent tVNS may affect hedonic responses to orally sampled foods and food intake, which has not been examined to our awareness.

Our first aim was to test the feasibility of using non-invasive VNS via the auricular branch during food consumption. Our second aim was to explore the ability of VNS to change hedonic responses to food. More specifically we asked: does tVNS (relative to sham stimulation) affect liking and wanting of orally sampled fatty and sweet foods, spontaneous eye blink rate (SEBR), and *ad libitum* milkshake consumption? These outcome variables were chosen to cover a range of assays that are thought to reflect food reward responses, including consciously experienced pleasantness (liking ratings) and motivation (wanting ratings), a physiological proxy for dopamine tone (SEBR), and a behavioral measure of motivation (*ad libitum* consumption). If VN responses to food induce dopamine mediated reward responses, we predict that tVNS relative to sham stimulation will increase hedonic responses.

METHODS

Participants

Eleven healthy, non-smoking participants [six women, five men] with a mean (± standard deviation) age of 27.0 (± 4.0) years [range: 20–32], with a mean body mass index (BMI) of 23.2 ± 3.8 kg/m^2 [range: 18.9–28] participated in the study. Participants were recruited through advertisements around Yale University and the city of New Haven. The Yale University School of Medicine Human Investigation Committee approved the informed consent form, which was subsequently obtained from all study participants. All participants reported having no known taste, smell, neurological, psychiatric (including eating disorders), or other pathological disorders. One of the participants was excluded because they reported not feeling any stimulation during one of the two sessions and a loose electrode was observed by the experimenter after the session. The remaining ten participants (five women and five men) were 27.5 ± 4.0 years old with a BMI of 23.1 ± 3.9 kg/m^2.

Design and Procedure

We used a within-participants design in which all participants were exposed to the tVNS and sham conditions on separate days, in a counterbalanced order. Participants were scheduled for the same time of day for each session. Upon arrival to the laboratory, breath alcohol levels (Alcohawk Elite Breathalyzer), urine toxicology for opiates, cocaine, THC, PCP, and barbiturates (Integrated E-Z Split Key Cup II, Innovacon Inc., San Diego, CA) and aurine pregnancy test were measured (no participants excluded). Participants were asked to arrive neither hungry nor full and were asked to rate their hunger level upon arrival using a visual analog scale (VAS; 0 = "I am not hungry at all" and 100 = "I have never been more hungry"). VAS's were also used to rate fullness and thirst. These "internal state" ratings are analyzed and reported in the **Supplementary Table 1** and **Supplementary Figure 1**.

Each session included the same events in the same order (**Figure 1**). First participants were outfitted with the electrodes for electro-oculogram (EOG) measurement and tVNS stimulation. Next, the participant completed a baseline spontaneous eye blink rate (SEBR) measurement. Then we adjusted tVNS stimulation intensity for each participant individually (see tVNS section below for details). Then we started a second SEBR measurement concurrent with tVNS stimulation. We then asked participants to rate their hunger, fullness and thirst a second time. Then the participants completed the fat and sweet food sampling task, and afterward rated hunger, fullness and thirst a third time. Last, they were asked to *ad libitum* consume a milkshake. All tasks were completed concurrent with tVNS stimulation. We used a single-blind design; the participant was not informed of the goal of stimulating the vagus nerve or differential innervation of the ear by the vagus nerve, while the experimenter and data-analyst were not blinded. We did not assess whether participants were aware of the association between ear location and vagus nerve innervation upon debriefing at the end of the study, thus success of the single-blind procedure was not explicitly confirmed.

SEBR Task

We used the EOG Pod (ADInstruments) for eye blink rate measurement. The EOG Pod utilizes the steady corneal-retinal electrical potential to detect eye movement and position. This task has been associated with DA signaling using PET (Groman et al., 2014) and with DA-dependent cognitive functions, including reinforcement learning (Jongkees and Colzato, 2016). To measure electrooculography (EOG), three Ag/AgCl self-adhesive electrodes were placed: (1) one above and another below the eye to record the vertical movements; and (2) one over a neutral point (vertebrae on the back of the neck), which is acting as a reference electrode. Participants were asked to look at a printed black fixation cross on a white poster board at 75 cm

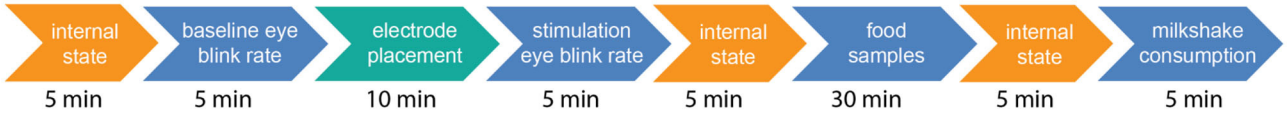


FIGURE 1 | Order of tasks in a test session and approximate timing in minutes. Upon arrival at the laboratory, participants rated their hunger, fullness and thirst ("internal state"). Then a baseline spontaneous eye blink rate (SEBR) was assessed, then the stimulation device was turned on and the intensity for sham or tVNS stimulation was adjusted. Next VNS stimulation perception was assessed. Then the rest of the tasks were performed concurrent with stimulation: another SEBR measurement, internal state ratings, rating of food samples, internal state ratings and last *ad libitum* milkshake consumption. This entire procedure was repeated on two testing days, one session using sham stimulation on the earlobe, and one session using tVNS stimulation on the cymba conchae (order of sessions counterbalanced over participants).

distance in an artificially lit room (blinds closed) with controlled humidity (50%) and room temperature (23°C). Participants were not instructed in any manner about blinking. Eye blink rate was taken as the mean number of blinks per minute during a 5 min measurement period.

tVNS/Sham Stimulation

Mild transcutaneous electrical stimulation was applied counterbalanced to either the cymba conchae of the left ear (tVNS) or the left ear lobe (sham) on separate visits using a commercially available TENS unit (transcutaneous electrical nerve stimulator, Twin Stim® Plus 3rd edition, Roscoe Medical Inc.) attached to a pair of silver electrodes. The electrodes are mounted on a round plastic stabilizer that fits into the cavum with adjustable distance between stabilizer and electrodes (similar to the Cerbomed Nemos® device). For the tVNS condition, the electrodes are positioned into the cymba conchae with electrode gel on each of the electrodes and a piece of medical tape was used to secure the electrode mount. For the sham condition, the entire earpiece was turned 180° to place the electrode on the ear lobe, and again taped into place. Both sham and tVNS stimulation used the following parameters: a biphasic square wave pulse at 25 Hz and a pulse width of 250 μs, with a duty cycle of 30 s on, 30 s off. The total stimulation duration was ∼45 min, the time needed to complete the tasks from second SEBR measurement through *ad libitum* consumption. Stimulation was applied with constant voltage. These parameters are reported in agreement with proposed reporting guidelines (Farmer et al., 2020). The amplitude of stimulation was calibrated for each session (stimulation location) and participant individually with a procedure commonly used (Kaniusas et al., 2019; Farmer et al., 2020), intended to adjust the amplitude to the highest stimulation level that can be reached without causing pain or discomfort. Since there are tissue and innervation differences in the sham and tVNS locations, the resulting stimulation amplitudes may differ between sites, but the calibration ensures that the sensation between sites remains comparable, thus controlling for perception-related placebo effects. While the stimulation was gradually increased, the participant was asked to report when a "pricking, stinging or burning" sensation was felt, which indicated their pain threshold. The stimulus intensity was then immediately decreased gradually until the participant reported an innocuous, comfortable "tingling, vibrating or drumming" sensation. The intensity of the stimulus remained at that selected level for the duration of the session unless the participant reported discomfort, in which case, the stimulus intensity was decreased in the same manner as during calibration to relieve discomfort. For one participant we reduced the intensity of the sham stimulation, which was requested after tasting the puddings and before tasting the Jell-O's. To confirm iso-intense stimulation, participants rated the intensity of the sensation on a General Labeled Magnitude Scale (described in detail in the next section). This type of stimulation is safe and well-tolerated (Redgrave et al., 2018).

Fat and Sweet Food Samples Task

Participant were asked to sample and rate flavor stimuli with varying fat content (puddings) and varying sugar content (Jell-O's). All stimuli were made from commercially available ingredients. Pudding samples were prepared with 0, 3.1, 6.9, and 15.6% fat weight by weight (w/w). The samples were prepared by mixing instant pudding (vanilla or chocolate flavored, Kraft Foods) in varying proportions of milk and heavy cream (Guida's Dairy, Connecticut) to varying fat content. The sugar content was held constant between the four stimuli at 4.6% (w/w). Jell-O samples were prepared by mixing unflavored gelatin powder (Jell-O, Kraft Foods) with Kool-Aid orange or strawberry unsweetened powdered flavor with 0, 0.1, 0.56, and 1 molar (M) sucrose (Sigma-Aldrich) concentration solutions. Each participant was asked which flavors they preferred to receive during scheduling of their appointment. Each stimulus was presented three times. First the puddings were presented in a randomized order within a block of 12 stimuli. Then the Jell-O samples were presented in a randomized order in a second block of 12 stimuli. A trial started with the experimenter cueing the participant to close their eyes (to eliminate color cues signaling content). The participant held out their hand and the experimenter placed a small ice-cream sampling spoon with a volume of about half a teaspoon of the food sample on it. The participant then placed the entire sample in their mouth and swallowed the sample. Then they opened their eyes and made ratings of the following attributes in this order: overall intensity, (dis)liking, sweetness, saltiness, fattiness, creaminess, oiliness and wanting. Each participant had previously been trained to use the General Labeled Magnitude Scale (gLMS) to rate overall intensity, saltiness and sweetness, the Labeled Hedonic Scale (LHS) to rate liking or disliking, and the VAS to rate oiliness, fattiness, creaminess, and wanting of the food samples. The gLMS

is a computerized psychophysical tool that requires subjects to rate the perceived intensity of a stimulus along a vertical axis lined with adjectives that are spaced quasi-logarithmically on the basis of experimentally determined intervals to yield ratio quality data (Green et al., 1996). The LHS was derived using similar methods as the gLMS but asks subjects to rate hedonic liking or disliking (Lim et al., 2009). The wanting VAS was presented with the question "how much do you want to eat this at the end of the experiment?." The left anchor was labeled with "I would never want to eat this" and the right anchor with "I would want to eat this more than anything." Here the hedonic (dis)liking ratings and wanting ratings were of primary interest and the other scales were included to prevent "dumping," bias effects that occur when participants are not asked to rate important attributes that are clearly present in a stimulus. For completeness we visualize the ratings on all other scales in the **Supplementary Figures 2**, **3**, but we include only liking and wanting ratings of the puddings and Jell-Os in the statistical analyses, given our predictions. After making these ratings, the participant rinsed their mouth with demineralized water, expectorated the water into a sink, and then the experimenter initiated a timer for a 30-s interval until the start of the next trial. This task took about 25–30 min.

Ad Libitum Milkshake Consumption

Finally, while the experimenter left the room to retrieve a participation-fee receipt to sign, the participant was given a carton milkshake cup (with lid and straw) with ∼700 g of chocolate milkshake and the instruction "to consume as much as you want". The experimenter returned to the testing room after 5 min. This milkshake was mixed from 1,000 ml of whole (full fat) milk (Guida's Dairy, Connecticut), 200 ml heavy cream (Guida's Dairy, Connecticut) and six tablespoons of "Chocolate Moo-usse" hot chocolate dry powder mix (Silly Cow Farms, Vermont). This mixture was stored in a refrigerator for up to 2 days and served cold (∼4°C). The resulting milkshake had an approximate fat content of 8.27% (w/w) and a caloric density of ∼1.2 kcal/g. The milkshake cup was weighed before and after consumption on a 1,000 g scale (Ohaus) with a precision of 1 g.

Data Analysis

EOG signal was analyzed in LabChart8.1.13 (ADInstruments). We applied a high frequency filter and counted each excursion from a threshold determined per participant per min. Events triggered by this calculation were verified with visual inspection. The frequency of blinks per min was averaged across the duration of the 5 min of the baseline and the stimulation period. The frequency during the stimulation period was normalized per participant to their own baseline and expressed as % baseline.

Ratings for puddings and Jell-Os were averaged across three replications for each variation and then averaged across the two lower concentrations and the two higher fat and sweet concentrations.

For the *ad libitum* consumption task we subtracted the post from the pre-consumption weight and expressed as % of the pre-consumption weight.

We then used JASP 0.12.2 to compare the effect of stimulation condition (tVNS vs. sham) on the dependent variables (stimulation amplitude, perceived stimulation intensity, liking and wanting of low and high fat/sweet food samples, SEBR, and consumption) with Bayesian paired *t*-tests (Rouder et al., 2009) and Student's paired *t*-tests. Since prior information is absent, we used the default Cauchy prior width of 0.707 (Ly et al., 2016). To examine the extent to which our conclusions depend on that prior, we report BF robustness using a wide and ultrawide prior, as well as the prior associated with the maximum BF (Carlsson et al., 2017). We tested the hypothesis that tVNS $\neq$ sham (H1) vs. tVNS $=$ sham (H0) and examined Bayes Factor (BF). A BF below 1 would be interpreted as evidence in favor of H0 relative to H1, while a BF above 1 is interpreted as evidence in favor of H1 relative to H0 (Lee and Wagenmakers, 2014). Further BF interpretations are illustrated in **Figure 2**. and **Table 1**. Here we regard any relative evidence greater than "anecdotal" in favor of H1 (BF $>$ 3) or in favor of H0 (BF $<$ 1/3) as meaningful. These procedures follow the JASP guidelines for conducting and reporting a Bayesian analysis (van Doorn et al., 2020). For Student's paired *t*-tests, we used an alpha of 0.05. The data, JASP analysis files and results files are available online (https://osf.io/njvw5/).

RESULTS

Feasibility

We visually observed head movement throughout the sessions, particularly during expectoration of water in the food sampling task, however, feasibility was confirmed by the ability to complete 21 out of 22 sessions (one session with a loose electrode) without adverse effects. As a result of a loose stimulation electrode during one session, we excluded one participant's data from our data analyses.

During sham stimulation, the stimulation amplitude was lower than during tVNS (very strong evidence in Bayesian paired *t*-test, **Table 1**), however, the perceived intensities were not different (moderate evidence, **Table 1**), as intended by the calibration procedure.

Liking and Wanting of Pudding Samples

Average liking and wanting ratings per participant and descriptive statistics across participants for the pudding samples with varying fat content are given in **Table 1** and **Figures 2A,B**. Under sham stimulation, participants rated low fat puddings on average between the labels "neutral" and "like slightly" (**Figure 2A**). The high fat puddings were rated between the labels "like slightly" and "like moderately" (**Figure 2B**). Under tVNS, the average liking for the low fat puddings increased on average 7.5 points relative to sham and numerically similar to the ratings for the high fat puddings under sham or tVNS stimulation. Under tVNS, the high fat puddings were still rated between the labels "like slightly" and "like moderately." Bayesian paired *t*-test showed moderate evidence in support of liking ratings of low-fat stimuli for tVNS being dissimilar from sham stimulation relative to the hypothesis that they are similar (**Figure 2A** and **Table 1**). Anecdotal evidence was observed for high-fat stimuli being similar in liking relative to the hypothesis that they are dissimilar (**Figure 2A** and **Table 1**).

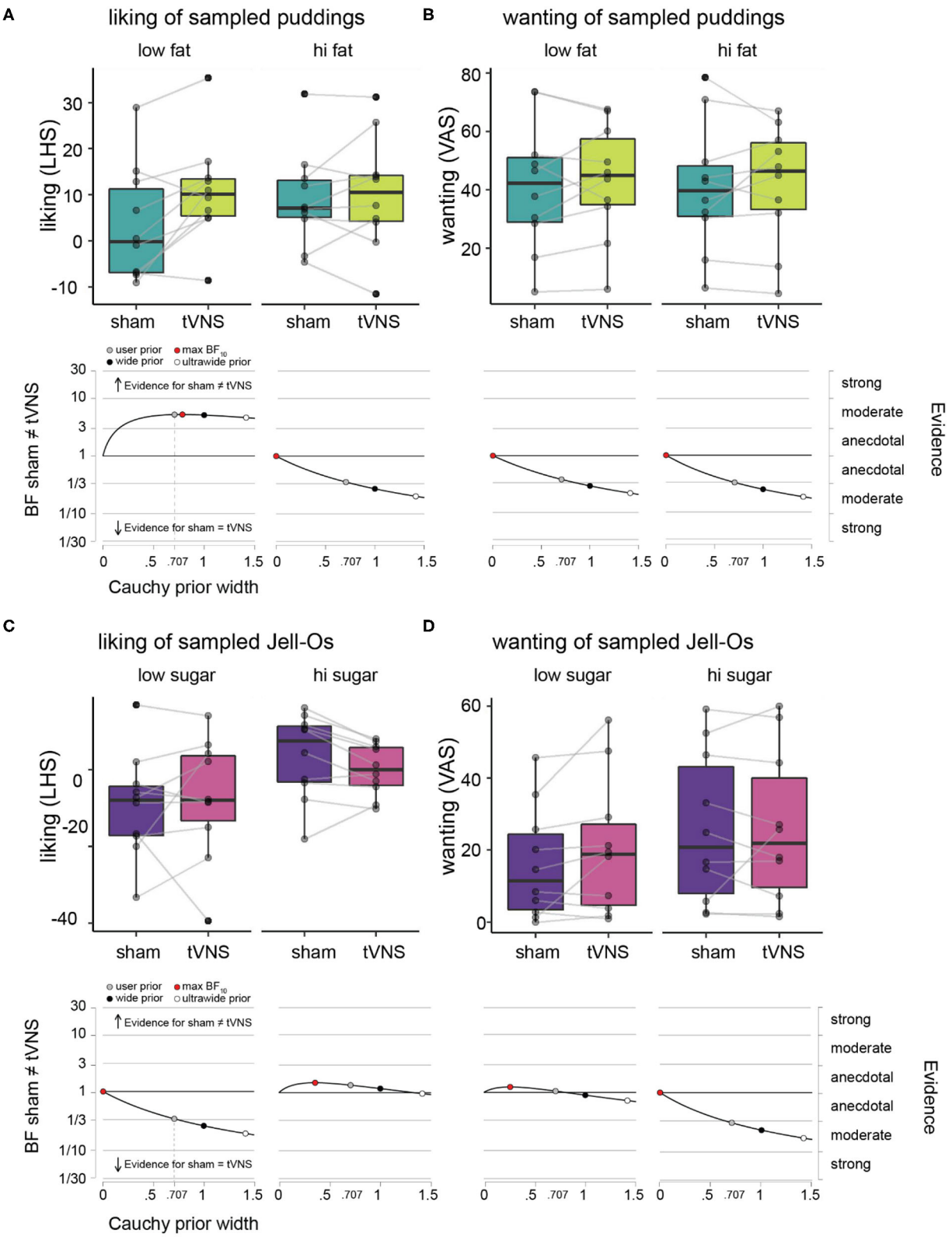


FIGURE 2 | Hedonic ratings of puddings and Jell-Os during sham vs. tVNS. **(A)** Liking ratings on LHS under sham (dark green) vs. tVNS (light green), plotted for the low fat puddings (upper left panel) and high fat puddings (upper right panel) separately. The boxplots indicate central tendencies and spread of the ratings, as follows:

(Continued)

FIGURE 2 | median (middle bar in box), first and third quartiles (lower and upper hinge), 1.5 × the interquartile range (top and bottom whiskers) and outlying points (separate solid black dots outside the whiskers). We overlaid individual data points on the boxplots (transparent gray dots) and connected the dots of an individual participant between the sham and tVNS bars to make it easier to inspect the difference within a single participant. Robustness check illustrating the effects of assigning more conservative Cauchy priors (wide and ultrawide, black and white circles, respectively) relative to the default user prior (gray circle) on Bayes factor values for the effect of sham vs. tVNS for liking ratings, plotted for the low fat puddings (lower left panel) and high fat puddings (lower right panel). **(B)** Wanting ratings for puddings on VAS under sham vs. tVNS and robustness checks for effect of sham vs. tNVS on wanting ratings. Details as in **(A)**. **(C)** Liking ratings on LHS under sham (purple) vs. tVNS (pink), plotted for the low sugar Jell-Os (upper left panel) and high sugar Jell-Os (upper right panel) separately. Details as in **(A)**. **(D)** Wanting ratings for Jell-Os on VAS under sham vs. tVNS and robustness checks for effect of sham vs. tNVS on wanting ratings. Details as in **(A)**.

TABLE 1 | Descriptive statistics, Bayesian statistics, frequentist statistics.

	sham		tVNS		Bayesian statistics		Frequentist statistics	
	ave	sd	ave	sd	BF10	Evidence descriptor*	T-statistic	*p*-value
Stimulation parameters								
Stimulation amplitude (in mA)	5.9	3.1	12.4	5.6	50.31	Very strong evidence for H1 relative to H0	−3.753	0.005
Perceived intensity of stimulation	13.3	4	13.8	5	0.32	moderate evidence for H0 relative to H1	−0.638	0.54
Pudding ratings								
Liking puddings low fat	3.3	12.4	10.8	11.1	5.24	moderate evidence for H1 relative to H0	3.119	0.012
Liking puddings high fat	9.1	10.4	10.3	12.3	0.36	anecdotal evidence for H0 relative to H1	0.609	0.558
Wanting puddings low fat	41.3	22.3	43.3	19.6	0.38	anecdotal evidence for H0 relative to H1	0.711	0.495
Wanting puddings high fat	40.8	22.1	42.0	20.5	0.33	borderline moderate/anecdotal evidence for H0 relative to H1	0.398	0.7
Jell-O ratings								
Liking Jell-O low sugar	−9.4	13.7	−7.5	15.7	0.34	anecdotal evidence for H0 relative to H1	0.499	0.63
Liking Jell-O high sugar	3.6	11.2	0.0	6.7	1.35	anecdotal evidence for H1 relative to H0	−2.043	0.071
Wanting Jell-O low sugar	16.0	15.6	20.6	19.0	1.05	borderline anecdotal evidence for H0 relative to H1/anecdotal evidence for H1 relative to H0	1.832	0.1
Wanting Jell-O high sugar	25.8	21.1	26.0	21.3	0.31	moderate evidence for H0 relative to H1	0.067	0.948
Other measures								
SEBR (% baseline)	12.6	48.7	35.3	50.0	0.54	anecdotal evidence for H0 relative to H1	−0.392	0.704
Ad libitum consumption (% total weight)	36.4	34.7	39.1	35.8	0.33	borderline moderate/anecdotal evidence for H0 relative to H1	−1.177	0.269

**All evidence descriptors are relative, so moderate evidence in favor of H1 is relative to evidence in favor of H0. H1, tVNS ≠ sham; H0, tVNS = sham.*

Under sham stimulation, the average wanting of low-fat puddings was numerically slightly lower than under tVNS (**Figure 2B** and **Table 1**). A similar pattern was observed for the high-fat puddings. Bayesian paired *t*-test showed anecdotal evidence was observed for high-fat stimuli being similar in wanting relative to the hypothesis that they are dissimilar (**Figure 2B** and **Table 1**).

Summarizing, tVNS increased liking ratings of low-fat stimuli by a meaningful amount from close to "neutral" to above "like slightly," which is similar to the liking ratings that high fat puddings received, while tVNS did not affect "wanting" of puddings.

Liking and Wanting of Jell-O Samples

Average liking and wanting ratings per participant and descriptive statistics across participants for the Jell-O samples with varying sugar content are given in **Table 1** and **Figures 2C,D**. Under sham stimulation, low sugar Jell-O samples are rated between "slightly disliked" and "moderately disliked." Under tVNS these ratings increased slightly numerically. Bayesian paired *t*-test showed anecdotal evidence was observed for low sugar stimuli being similar in liking relative to the hypothesis that they are dissimilar under tVNS vs. sham (**Figure 2C** and **Table 1**). The high sugar stimuli were rated slightly above "neutral" in liking under sham, and numerically decreased slightly under tVNS. Bayesian paired *t*-test showed anecdotal evidence was observed for high sugar stimuli being dissimilar in liking relative to the hypothesis that they are similar under tVNS vs. sham.

Wanting ratings for low sugar Jell-O samples numerically slightly increased under tVNS vs. sham (**Figure 2D** and **Table 1**), but with a BF of ∼1 there was no evidence in favor of either hypothesis. The wanting ratings for high sugar Jell-O samples stayed numerically similar, confirmed by a Bayesian paired *t*-test that showed moderate evidence in favor of the high sugar stimuli being similar in wanting relative to the hypothesis that they are dissimilar under tVNS vs. sham.

Summarizing, tVNS did not affect liking or wanting of the low or high sugar Jell-O samples.

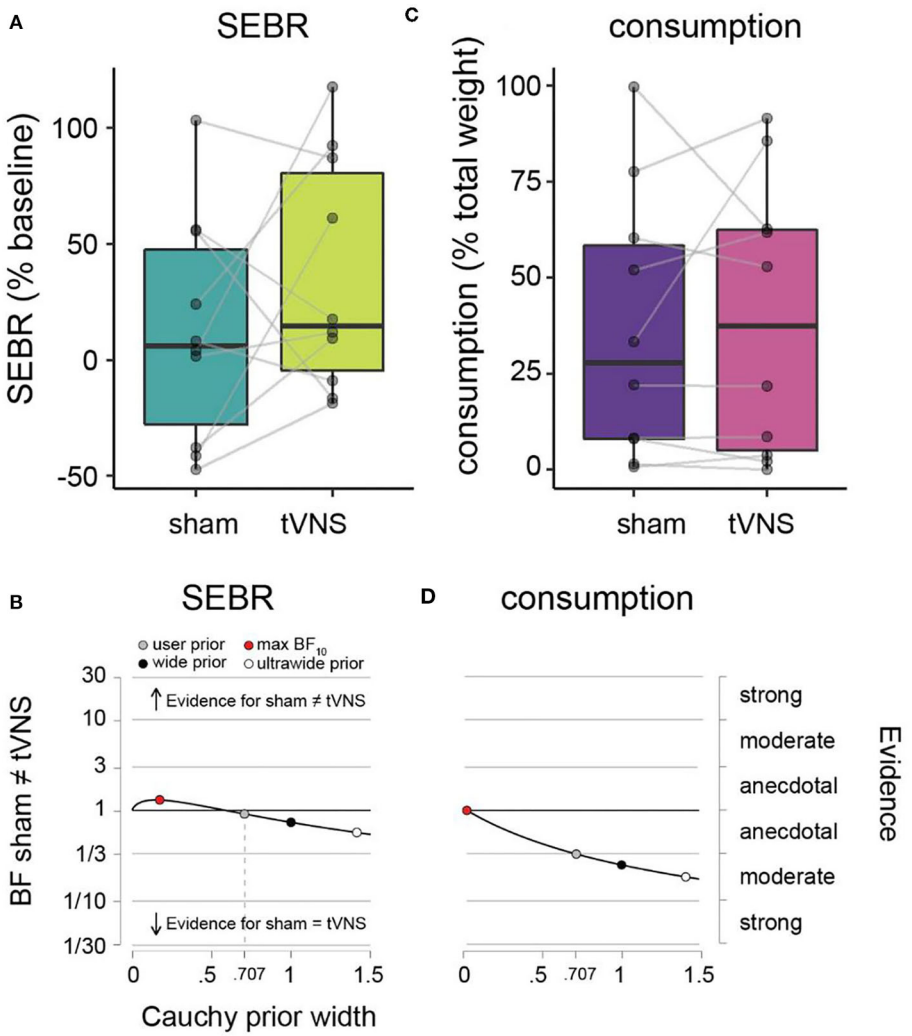


FIGURE 3 | SEBR and *ad libitum* consumption during sham vs. tVNS. **(A)** SEBR (relative to baseline) under sham (dark green) vs. tVNS (light green). **(B)** Robustness checks for effect of sham vs. tNVS on SEBR. **(C)** *Ad libitum* consumption (relative to total weight) under sham (purple) vs. tVNS (pink). **(D)** Robustness checks for effect of sham vs. tVNS on consumption. Details as in **Figure 2**.

Spontaneous Eye Blink Rate

Average SEBR per participant and descriptive statistics across participants are given in **Table 1** and **Figure 3A**. Under sham stimulation, participants on average had a 12.6% (± 48.7%) increase in their SEBR relative to baseline, while under tVNS the average was 35.3% (± 50.0%). No meaningful evidence for differences in favor of H0 or H1 was observed (**Figure 3B** and **Table 1**).

Ad libitum Consumption

Average amount of milkshake consumption per participant and descriptive statistics across participants are given in **Table 1** and **Figure 3C**. Under sham stimulation, participants on average consumed 36.4% (± 34.7%) of the milkshake, while under tVNS the average was 39.1% (± 35.8%). We observed meaningful evidence in favor of H0 relative to H1, such that sham and tVNS have similar effects on milkshake consumption (**Figure 3D** and **Table 1**).

DISCUSSION

The aim of this study was to test the feasibility of using tVNS during food consumption and to test the prediction that tVNS relative to sham stimulation will increase hedonic responses to food. In a small sample of 10 participants we observed an increased liking of low fat, but not high fat foods under tVNS vs. sham stimulation. We observed no effects on wanting of fat foods, and no effects on liking or wanting of foods varying in sugar content.

Concerning feasibility, we observed head movement during food sampling. However, in only 1 out of 22 sessions a loose electrode was observed. Various improvements may prevent

the loss of contact between skin and electrode during eating movements including improved earpieces that mount behind the ear like eye glasses, tacky electrode gel, and more flexible wires.

We observed an increased liking of low fat (but not for high fat foods) under tVNS vs. sham stimulation. This is consistent with observations from pre-clinical and clinical studies with invasive VNS in humans [reviewed by Lartigue (2016)]. In humans dopamine in the dorsal striatum, measured by PET imaging of raclopride binding, positively correlates with meal pleasantness (Small et al., 2003). In animal studies, dopamine release in the dorsal striatum acts as a proxy of caloric value and drives conditioned place and flavor learning and motivated behavior (Sclafani et al., 2011; Tellez et al., 2016). In mice, stimulation of vagal sensory neurons innervating the gut drive the same reward behaviors (Han et al., 2018). Thus, one possible mechanism of action of tVNS in this study includes increased dopaminergic tone, masking the signal of phasic dopamine release to individual post-ingestive stimuli, and normalizing the comparative liking between low and high fat puddings. However, vagal afferent stimulation has been implicated in modulating a range of neurotransmitters (Hulsey et al., 2016, 2019) and affects mood, memory and cognition [reviewed by Frangos et al. (2017)]. Thus, further work is required to determine the mechanisms and downstream brain circuits that are recruited by tVNS.

We also observed that tVNS did not change liking or wanting for stimuli varying in sugar content. This is in line with the observation that vagal deafferentation of the gut results in increased food intake after a fat but not sugar preload (McDougle et al., 2020), and may suggest a more prominent role for the vagus nerve in signaling fat content to the brain. However, it is also possible that the fixed order of non-fatty sweet Jell-O samples presentation after fatty puddings samples may have worked against observing effects on sweetness perception, as a reduction in fat levels may result in a reduction of perceived sweetness (Wiet et al., 1993; Biguzzi et al., 2014). Future studies should consider using for example a factorial design with interleaved trials to manipulate sugar and fat content (Smith et al., 2020).

Dietary fat plays an important role in the development and treatment of obesity, suggesting that tVNS is an interesting avenue for modulating food preferences to shift choices toward healthier, lower fat foods in the treatment of obesity for example. However, as VNS effects may be weight dependent (Pardo et al., 2007; Obst et al., 2020), future studies should examine liking and wanting to consume food in participants with overweight and obesity.

We did not observe differences for SEBR (dopamine tone proxy) or *ad libitum* consumption (satiety and/or motivated behavior). Our specific choice of behavioral assays could not confirm dopamine release as the mechanism of the low-fat food preference shift. However, this does not exclude dopamine release or motivated behavior as a mechanism. For example, recently tVNS was shown to modulate motivated behavior by increasing participants' drive to approach less-wanted rewards (Neuser et al., 2020) and SEBR may not be a good proxy for dopamine function in humans (Dang et al., 2017). We also cannot rule out that the population of neurons that innervates the ear rather than the gut is not the right vagal population to stimulate nor that the choice of ABVNS location in the cymba conchae or stimulation parameters are suboptimal (Badran et al., 2018a,b). Our study has various other limitations, most importantly the small sample size. Independent studies and larger sample sizes will be necessary. Future studies should also assess success of the blinding procedure and prior knowledge of vagal innervation of the ear by the participant, as recently recommended (Farmer et al., 2020).

In conclusion, we observed preliminary evidence in support of tVNS' capability to modulate liking of low fat foods, which may support behavioral choices for healthier foods.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found here: https://osf.io/njvw5/.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Yale University School of Medicine Human Investigation Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MV and GdL conceived the study. MV, EF, and GdL designed the study. MV and PB collected the data. MV performed the statistical analyses. LÖ wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

Modern Diet and Physiology Research Center feasibility grant to MV and GdL. This publication/paper has been produced benefiting from the 2232 International Fellowship for Outstanding Researchers Program of TÜBİTAK (Project No. 118C299) to MV. However, the entire responsibility of the publication/paper belongs to the owner of the publication/paper. The financial support received from TÜBİTAK does not mean that the content of the publication is approved in a scientific sense by TÜBİTAK.

ACKNOWLEDGMENTS

We would like to thank Dr. Dana Small for making laboratory resources and equipment available for data collection in this study.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnhum.2020.600995/full#supplementary-material

REFERENCES

Badran, B. W., Brown, J. C., Dowdle, L. T., Mithoefer, O. J., LaBate, N. T., Coatsworth, J., et al. (2018a). Tragus or cymba conchae? Investigating the anatomical foundation of transcutaneous auricular vagus nerve stimulation (taVNS). *Brain Stimul.* 11, 947–948. doi: 10.1016/j.brs.2018.06.003

Badran, B. W., Jenkins, D. D., Cook, D., Thompson, S., Dancy, M., DeVries, W. H., et al. (2020). Transcutaneous auricular vagus nerve stimulation-paired rehabilitation for oromotor feeding problems in newborns: an open-label pilot study. *Front. Hum. Neurosci.* 14:77. doi: 10.3389/fnhum.2020.00077

Badran, B. W., Mithoefer, O. J., Summer, C. E., LaBate, N. T., Glusman, C. E., Badran, A. W., et al. (2018b). Short trains of transcutaneous auricular vagus nerve stimulation (taVNS) have parameter-specific effects on heart rate. *Brain Stimul.* 11, 699–708. doi: 10.1016/j.brs.2018.04.004

Berthoud, H. -,r. (2008). Vagal and hormonal gut–brain communication: from satiation to satisfaction. *Neurogastroenterol. Motil.* 20, 64–72. doi: 10.1111/j.1365-2982.2008.01104.x

Biguzzi, C., Schlich, P., and Lange, C. (2014). The impact of sugar and fat reduction on perception and liking of biscuits. *Food Qual. Prefer.* 35, 41–47. doi: 10.1016/j.foodqual.2014.02.001

Bodenlos, J. S., Kose, S., Borckardt, J. J., Nahas, Z., Shaw, D., O'Neil, P. M., et al. (2007). Vagus nerve stimulation acutely alters food craving in adults with depression. *Appetite* 48, 145–153. doi: 10.1016/j.appet.2006.07.080

Burneo, J., Faught, E., Knowlton, R., Morawetz, R., and Kuzniecky, R. (2002). Weight loss associated with vagus nerve stimulation. *Neurology* 59, 463–464. doi: 10.1212/WNL.59.3.463

Carlsson, R., Schimmack, U., Williams, D. R., and Bürkner, P.-C. (2017). Bayes factors from pooled data are no substitute for bayesian meta-analysis: commentary on scheibehenne, jamil, and wagenmakers (2016). *Psychol. Sci.* 28, 1694–1697. doi: 10.1177/0956797616684682

Dang, L. C., Samanez-Larkin, G. R., Castrellon, J. J., Perkins, S. F., Cowan, R. L., Newhouse, P. A., et al. (2017). Spontaneous eye blink rate (EBR) is uncorrelated with dopamine D2 receptor availability and unmodulated by dopamine agonism in healthy adults. *ENeuro* 4:ENEURO.0211-17.2017. doi: 10.1523/ENEURO.0211-17.2017

Ellrich, J. (2011). Transcutaneous vagus nerve stimulation. *Eur. Neurol. Rev.* 6, 254–256. doi: 10.17925/ENR.2011.06.04.254

Farmer, A. D., Strzelczyk, A., Finisguerra, A., Gourine, A. V., Gharabaghi, A., Hasan, A., et al. (2020). International consensus based review and recommendations for minimum reporting standards in research on transcutaneous vagus nerve stimulation (version 2020). *Front. Hum. Neurosci.* 14. doi: 10.3389/fnhum.2020.568051

Frangos, E., Ellrich, J., and Komisaruk, B. R. (2015). Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: FMRI evidence in humans. *Brain Stimul.* 8, 624–636. doi: 10.1016/j.brs.2014.11.018

Frangos, E., Richards, E. A., and Bushnell, M. C. (2017). Do the psychological effects of vagus nerve stimulation partially mediate vagal pain modulation? *Neurobiol. Pain* 1, 37–45. doi: 10.1016/j.ynpai.2017.03.002

Green, B. G., Dalton, P., Cowart, B., Shaffer, G., Rankin, K., and Higgins, J. (1996). Evaluating the "labeled magnitude scale" for measuring sensations of taste and smell. *Chem. Senses* 21, 323–334. doi: 10.1093/chemse/21.3.323

Groman, S. M., James, A. S., Seu, E., Tran, S., Clark, T. A., Harpster, S. N., et al. (2014). In the blink of an eye: relating positive-feedback sensitivity to striatal dopamine D2-like receptors through blink rate. *J. Neurosci.* 34, 14443–14454. doi: 10.1523/JNEUROSCI.3037-14.2014

Han, W., Tellez, L. A., Perkins, M. H., Perez, I. O., Qu, T., Ferreira, J., et al. (2018). A neural circuit for gut-induced reward. *Cell* 175, 665–678.e23. doi: 10.1016/j.cell.2018.08.049

Hulsey, D. R., Hays, S. A., Khodaparast, N., Ruiz, A., Das, P., Rennaker, R. L., et al. (2016). Reorganization of motor cortex by vagus nerve stimulation requires cholinergic innervation. *Brain Stimul.* 9, 174–181. doi: 10.1016/j.brs.2015.12.007

Hulsey, D. R., Shedd, C. M., Sarker, S. F., Kilgard, M. P., and Hays, S. A. (2019). Norepinephrine and serotonin are required for vagus nerve stimulation directed cortical plasticity. *Exp. Neurol.* 320:112975. doi: 10.1016/j.expneurol.2019.112975

Jongkees, B. J., and Colzato, L. S. (2016). Spontaneous eye blink rate as predictor of dopamine-related cognitive function—a review. *Neurosci. Biobehav. Rev.* 71, 58–82. doi: 10.1016/j.neubiorev.2016.08.020

Kaniusas, E., Kampusch, S., Tittgemeyer, M., Panetsos, F., Gines, R. F., Papa, M., et al. (2019). Current directions in the auricular vagus nerve stimulation I — a physiological perspective. *Front. Neurosci.* 13:854. doi: 10.3389/fnins.2019.00854

Kong, J., Fang, J., Park, J., Li, S., and Rong, P. (2018). Treating depression with transcutaneous auricular vagus nerve stimulation: state of the art and future perspectives. *Front. Psychiatry* 9:20. doi: 10.3389/fpsyt.2018.00020

Lartigue, G. (2016). Role of the vagus nerve in the development and treatment of diet-induced obesity. *J. Physiol.* 594, 5791–5815. doi: 10.1113/JP271538

Lee, M. D., and Wagenmakers, E.-J. (2014). *Bayesian Cognitive Modeling: A Practical Course*. New York, NY: Cambridge University press.

Lim, J., Wood, A., and Green, B. G. (2009). Derivation and evaluation of a labeled hedonic scale. *Chem. Senses* 34, 739–751. doi: 10.1093/chemse/bjp054

Ly, A., Verhagen, J., and Wagenmakers, E.-J. (2016). Harold Jeffreys's default bayes factor hypothesis tests: explanation, extension, and application in psychology. *J. Math. Psychol.* 72, 19–32. doi: 10.1016/j.jmp.2015.06.004

McDougle, M., Quinn, D., Diepenbroek, C., Singh, A., de la Serre, C., and de Lartigue, G. (2020). Intact vagal gut-brain signalling prevents hyperphagia and excessive weight gain in response to high-fat high-sugar diet. *Acta Physiol.* doi: 10.1111/apha.13530. [Epub ahead of print].

Neuser, M. P., Teckentrup, V., Kühnel, A., Hallschmid, M., Walter, M., and Kroemer, N. B. (2020). Vagus nerve stimulation boosts the drive to work for rewards. *Nat. Commun.* 11:3555. doi: 10.1038/s41467-020-17344-9

Obst, M. A., Heldmann, M., Alicart, H., Tittgemeyer, M., and Münte, T. F. (2020). Effect of short-term transcutaneous vagus nerve stimulation (tVNS) on brain processing of food cues: an electrophysiological study. *Front. Hum. Neurosci.* 14:206. doi: 10.3389/fnhum.2020.00206

Ogbonnaya, S., and Kaliaperumal, C. (2013). Vagal nerve stimulator: evolving trends. *J. Nat. Sci. Biol. Med.* 4, 8–13. doi: 10.4103/0976-9668.107254

Pardo, J. V., Sheikh, S., Kuskowski, M. A., Surerus-Johnson, C., Hagen, M., Lee, J., et al. (2007). Weight loss during chronic, cervical vagus nerve stimulation in depressed patients with obesity: an observation. *Int. J. Obes.* 31, 1756–1759. doi: 10.1038/sj.ijo.0803666

Redgrave, J., Day, D., Leung, H., Laud, P. J., Ali, A., Lindert, R., et al. (2018). Safety and tolerability of transcutaneous vagus nerve stimulation in humans; a systematic review. *Brain Stimul.* 11, 1225–1238. doi: 10.1016/j.brs.2018.08.010

Rouder, J. N., Speckman, P. L., Sun, D., Morey, R. D., and Iverson, G. (2009). Bayesian t tests for accepting and rejecting the null hypothesis. *Psychon. Bull. Rev.* 16, 225–237. doi: 10.3758/PBR.16.2.225

Sclafani, A., Touzani, K., and Bodnar, R. J. (2011). Dopamine and learned food preferences. *Physiol. Behav.* 104, 64–68. doi: 10.1016/j.physbeh.2011.04.039

Small, D. M., Jones-Gotman, M., and Dagher, A. (2003). Feeding-induced dopamine release in dorsal striatum correlates with meal pleasantness ratings in healthy human volunteers. *NeuroImage* 19, 1709–1715. doi: 10.1016/s1053-8119(03)00253-2

Smith, K. R., Papantoni, A., Veldhuizen, M. G., Kamath, V., Harris, C., Moran, T. H., et al. (2020). Taste-related reward is associated with weight loss following bariatric surgery. *J. Clin. Invest.* 130, 4370–4381. doi: 10.1172/JCI137772

Tan, H.-E., Sisti, A. C., Jin, H., Vignovich, M., Villavicencio, M., Tsang, K. S., et al. (2020). The gut–brain axis mediates sugar preference. *Nature* 580, 511–516. doi: 10.1038/s41586-020-2199-7

Tellez, L. A., Han, W., Zhang, X., Ferreira, T. L., Perez, I. O., Shammah-Lagnado, S. J., et al. (2016). Separate circuitries encode the hedonic and nutritional values of sugar. *Nat. Neurosci.* 19, 465–470. doi: 10.1038/nn.4224

van Doorn, J., van den Bergh, D., Böhm, U., Dablander, F., Derks, K., Draws, T., et al. (2020). The JASP guidelines for conducting and

reporting a Bayesian analysis. *Psychon Bull Rev*. doi: 10.3758/s13423-020-01798-5

Wiet, S. G., Ketelsen, S. M., Davis, T. R., and Beyts, P. K. (1993). fat concentration affects sweetness and sensory profiles of sucrose, sucralose, and aspartame. *J. Food Sci*. 58, 599–602. doi: 10.1111/j.1365-2621.1993.tb04333.x

Williams, E. K., Chang, R. B., Strochlic, D. E., Umans, B. D., Lowell, B. B., and Liberles, S. D. (2016). Sensory neurons that detect stretch and nutrients in the digestive system. *Cell* 166, 209–221. doi: 10.1016/j.cell.2016.05.011

Yakunina, N., Kim, S. S., and Nam, E.-C. (2017). Optimization of transcutaneous vagus nerve stimulation using functional MRI. *Neuromodulation* 20, 290–300. doi: 10.1111/ner.12541

Yuan, H., and Silberstein, S. D. (2016a). Vagus nerve and vagus nerve stimulation, a comprehensive review: part I. *Headache* 56, 71–78. doi: 10.1111/head.12647

Yuan, H., and Silberstein, S. D. (2016b). Vagus nerve and vagus nerve stimulation, a comprehensive review: part II. *Headache* 56, 259–266. doi: 10.1111/head.12650

Conflict of Interest: 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.

frontiers in Human Neuroscience

ORIGINAL RESEARCH
published: 08 March 2021
doi: 10.3389/fnhum.2021.648556

Transcutaneous Auricular Neurostimulation (tAN): A Novel Adjuvant Treatment in Neonatal Opioid Withdrawal Syndrome

Dorothea D. Jenkins[1†], Navid Khodaparast[2†], Georgia H. O'Leary[1,3], Stephanie N. Washburn[2], Alejandro Covalin[2] and Bashar W. Badran[3]*

[1]*Department of Pediatrics, Medical University of South Carolina, Charleston, SC, United States,* [2]*Spark Biomedical, Inc., Dallas, TX, United States,* [3]*Department of Psychiatry & Behavioral Sciences, Brain Stimulation Division, Medical University of South Carolina, Charleston, SC, United States*

OPEN ACCESS

Edited by:
Tino Zaehle, Otto von Guericke University Magdeburg, Germany

Reviewed by:
Marius Keute, Tübingen University Hospital, Germany
Beatrice Bretherton, University of Leeds, United Kingdom

***Correspondence:**
Navid Khodaparast
navid.khodaparast@sparkbiomedical.com

[†]*These authors share first authorship*

Specialty section:
This article was submitted to Brain Imaging and Stimulation, a section of the journal Frontiers in Human Neuroscience

Received: *31 December 2020*
Accepted: *15 February 2021*
Published: *08 March 2021*

Citation:
Jenkins DD, Khodaparast N, O'Leary GH, Washburn SN, Covalin A and Badran BW (2021) Transcutaneous Auricular Neurostimulation (tAN): A Novel Adjuvant Treatment in Neonatal Opioid Withdrawal Syndrome. Front. Hum. Neurosci. 15:648556. doi: 10.3389/fnhum.2021.648556

Maternal opioid use during pregnancy is a growing national problem and can lead to newborns developing neonatal opioid withdrawal syndrome (NOWS) soon after birth. Recent data demonstrates that nearly every 15 min a baby is born in the United States suffering from NOWS. The primary treatment for NOWS is opioid replacement therapy, commonly oral morphine, which has neurotoxic effects on the developing brain. There is an urgent need for non-opioid treatments for NOWS. Transcutaneous auricular neurostimulation (tAN), a novel and non-invasive form of electrostimulation, may serve as a promising alternative to morphine. tAN is delivered *via* a multichannel earpiece electrode worn on and around the left ear, targeting two cranial nerves—the vagus and trigeminal nerves. Prior research suggests that auricular neurostimulation exerts an anxiolytic effect on the body by releasing endogenous opioids and reduces withdrawal symptoms in adults actively withdrawing from opioids. In this first-in-human prospective, open-label trial, we investigated tAN as an adjuvant to morphine therapy in eight infants >33 weeks gestational age suffering from NOWS and receiving oral morphine treatment. Infants received tAN for 30 min 1 h before receiving a morphine dose. tAN was delivered at 0.1 mA below perception intensity at two different nerve targets on the ear: Region 1, the auricular branch of the vagus nerve; and Region 2, the auriculotemporal nerve. tAN was delivered up to four times daily for a maximum of 12 days. The primary outcome measures were safety [heart rate monitoring, Neonatal Infant Pain Scale (NIPS), and skin irritation] and morphine length of treatment (LOT). tAN was well-tolerated and resulted in no unanticipated adverse events. Comparing to the national average of 23 days, the average oral morphine LOT was 13.3 days (median 9 days) and the average LOT after tAN initiation was 7 days (median 6 days). These preliminary data suggest that tAN is safe and may serve as a promising alternative adjuvant for treating NOWS and reducing the amount of time an infant receives oral morphine.

Keywords: neonatal opioid withdrawal syndrome (NOWS), non-invasive neuromodulation, opioids, morphine, transcutaneous auricular neurostimulation, tAN, bioelectronic medicine

INTRODUCTION

Neonatal opioid withdrawal syndrome (NOWS) is a condition in which infants experience withdrawal symptoms after *in utero* exposure to prescription or non-prescription opioids such as methadone or heroin (Witt et al., 2017). Due to abrupt termination of opioid delivery to the central nervous system at birth, newborns typically experience withdrawal symptoms within 48–72 h (Kocherlakota, 2014), including tachycardia, tremors, high-pitched crying, tachypnea, vomiting, weight loss, mottling, dysregulation of body temperature, and disrupted sleep (Kraft and van den Anker, 2012; Ko et al., 2017; Patrick et al., 2020).

Treatment of NOWS usually follows a multi-modal regime centered on controlled withdrawal and replacement drug therapy with oral morphine. Although there is no nationwide standard of care, oral morphine or methadone are considered as the first-line therapy. Other drugs, including clonidine and phenobarbital, are used as adjuncts when needed. This approach is not optimal, as it can impart additional stress on the newborn (Hudak and Tan, 2012). Additionally, these pharmacotherapies themselves produce harmful side effects. Administering morphine during early neurodevelopment, specifically during a critical perinatal period, may cause neuronal apoptosis, white matter injury, decreased myelin maturation, and oxidative stress, leading to long-term developmental consequences (Rao et al., 2007; Attarian et al., 2014; Steinhorn et al., 2015; Flannery et al., 2020).

The American Academy of Pediatrics recommends attempting the use of non-pharmacologic treatment, which includes placing the infant in a dark and quiet environment, swaddling, rocking, breastfeeding, and providing high-calorie nutrition in frequent small feedings, among other techniques (Hudak and Tan, 2012; Grossman et al., 2018). When utilized appropriately, such non-pharmacological interventions have resulted in a reduction in length of stay, length of treatment (LOT), and percentage of infants requiring pharmacotherapy (MacMillan et al., 2018). With NOWS babies already under stress from opioid withdrawal, a non-pharmacological treatment may greatly benefit these patients, lowering the need for additional medications and potentially reducing their hospital stay.

Considering the hyperactivation of the sympathetic nervous system in NOWS (Jansson et al., 2010), activation of counterregulatory parasympathetic nerves *via* the release of acetylcholine might be beneficial (Janssen et al., 2011; Hu et al., 2021). Recently, a non-invasive form of vagus nerve stimulation (VNS) known as transcutaneous auricular VNS (taVNS) targets the auricular branch of the vagus nerve (ABVN) and activates vagal afferent and efferent networks (Kraus et al., 2013; Garcia et al., 2017; Yakunina et al., 2017; Badran et al., 2018a, 2019; Kaniusas et al., 2019). In addition to auricular vagal nerve branches, the ear is innervated by other cranial nerve branches such as the auriculotemporal nerve (the branch of the trigeminal nerve located superficial to the temporal mandibular joint). Stimulation of the ABVN at lower frequencies releases CNS endorphins (Sator-Katzenschlager and Michalek-Sauberer, 2007), inhibits the release of pro-inflammatory cytokines that augment pain (Chen et al., 2015), and has been shown to have sustained antinociceptive effects in multiple studies including post-operative studies of opioid intake (Sator-Katzenschlager et al., 2004; Kovacic et al., 2017). Animal and human research suggest that endogenous opioids may supplant the need for exogenous opioids, thus leading to antinociception and mitigation of opioid withdrawal symptoms (Liu et al., 1999; Wu et al., 1999; Han, 2004; Bonnette, 2008; Meade et al., 2010; de Andrade et al., 2011; He et al., 2011; Van Bockstaele and Valentino, 2013; Toubia and Khalife, 2019). In previous studies, auricular neurostimulation decreased symptoms of acute opioid withdrawal in adults (Severson et al., 1977; Wen et al., 1978; Ellison et al., 1987; Miranda and Taca, 2018). In neonates, alternative medicine approaches to auricular neurostimulation have recently been studied as adjunctive therapies for NOWS. Acupuncture (Filippelli et al., 2012; Raith et al., 2015), acupressure stickers, and laser acupuncture have been applied resulting in a reduction in withdrawal symptoms in newborns with NOWS. These results suggest that activating auricular neural pathways may have promising clinical effects and serve as adjunctive therapy for NOWS.

As various methods of auricular neuromodulation demonstrate a reduction in withdrawal symptoms and activate parasympathetic pathways important in autonomic regulation, our scientific premise is that tAN, when delivered before administration of oral morphine, may release endogenous opioids and reduce withdrawal symptoms, resulting in reduced LOT with oral morphine. In this first-in-human trial, we aimed to explore the safety and feasibility of delivering tAN as adjunctive therapy to oral morphine replacement to reduce the signs and symptoms associated with NOWS in neonates.

MATERIALS AND METHODS

Study Overview/Design

This first-in-human prospective, open-label trial was approved by the Medical University of South Carolina (MUSC) Institutional Review Board and was conducted at MUSC Shawn Jenkins Children's Hospital in Charleston South Carolina. Parental consent was obtained by the study team before any study-related activities. Eight infants who were diagnosed with NOWS and receiving oral morphine replacement therapy were enrolled between May and December 2020. All infants received tAN in addition to morphine replacement therapy. tAN stimulation was delivered 1 h before each scheduled morphine dose during the peak withdrawal period (**Figure 1**). Primary outcome measures included safety (heart rate decrease, bradycardia, skin irritation, pain scores) and morphine LOT.

Participants

Infants >33 weeks gestational age at enrollment with opioid dependence receiving oral morphine and on minimal respiratory support were included in this open-label clinical trial. Infants were excluded if they had any of the following: unstable respiratory or cardiovascular status or requiring significant respiratory support, repeated autonomic instability (apnea or bradycardia) which was not self-resolving, major unrepaired

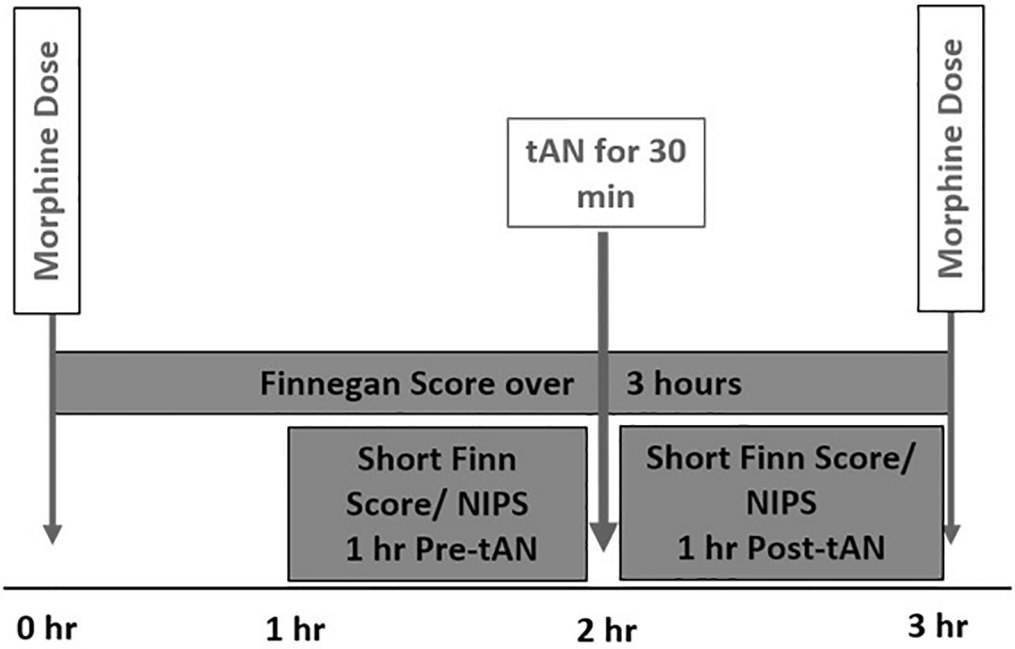


FIGURE 1 | Incorporation of transcutaneous auricular neurostimulation (tAN) therapy with scheduled morphine therapy.

congenital anomalies impacting the respiratory or cardiovascular system, cardiomyopathy, and abnormal ear anatomy not allowing the device to fit.

Opioid Replacement Therapy and Withdrawal Symptom Scoring

NOWS withdrawal symptoms include hyperirritability, hypertonicity, high-pitched crying, tachypnea, gastrointestinal dysfunction, and sleep disturbances. The Finnegan Neonatal Abstinence Scoring Tool (FNAST) is a validated assessment tool designed to measure 21 signs of withdrawal in infants (Finnegan et al., 1975; Devlin et al., 2020). The tool provides a means to rate the severity of withdrawal symptoms every 3 h after feeding using a standard format. In addition to the standard FNAST, a simplified FNAST scale with a high statistical correlation to the standard FNAST was used as part of the study protocol (Gomez Pomar et al., 2017; Devlin et al., 2020). This score was recorded by neonatal nurses 1 h before and after each tAN session to measure the immediate effects of tAN on withdrawal symptom severity.

The oral morphine administration protocol for NOWS at MUSC Shawn Jenkins Children's Hospital initiates treatment with an oral morphine dose of 0.05 mg/kg/dose q 3 h, depending on clinical circumstances. If withdrawal symptoms remain well-controlled, defined as FNAST scores <8 for 72 h on a set dose of morphine, that dose is established as the "control dose," and is the starting point for morphine weaning. In the standard protocol, morphine is weaned by 15% every 24–48 h if FNAST scores remain <8 for 24 h.

Transcutaneous Auricular Neurostimulation (tAN) Adjuvant Protocol

After consent, the study team began administering tAN as an adjuvant therapy to morphine, four times per day, 1 h before morphine dose, for up to 12 days or the discontinuation of morphine (whichever came first). tAN administration began either, within the 72-h control dose period or after starting morphine weaning, for all infants enrolled. We followed an accelerated morphine weaning protocol for this study, weaning morphine every 12 h if FNAST scores remained <8 after tAN treatments were started. If after weaning or discontinuing morphine the infant had two consecutive FNAST scores >12 or three consecutive FNAST scores >8, an increase in dose or a rescue dose of morphine equal to the lowest dose before discontinuing morphine was given.

Transcutaneous Auricular Neurostimulation (tAN) Overview

tAN was administered using a unique wearable earpiece (Roo™ Therapy System, Spark Biomedical, Dallas, TX, USA) that targets the auricular branch of the vagus nerve (ABVN) and auriculotemporal nerve (ATN). This device recently received breakthrough device designation by the FDA. The system includes a disposable, multi-channel earpiece electrode and an External Pulse Generator (EPG) that are connected using a cable (**Figure 2**). The earpiece is worn on and around the left ear. The earpiece electrodes are made of hydrogel that is biocompatible and can remain on the infant's

FIGURE 2 | The Roo™ therapy system. **(A)** The clinician application connects an iOS device to the patient controller using bluetooth low energy. The application allows a clinician to start a communication session with a Patient Controller to adjust therapy parameters, view diagnostic logs, and event history. **(B)** The Patient Controller delivers electrical stimulation to the earpiece *via* a removable cable. Clinicians can modulate therapy intensity by pressing up/down buttons and check therapy status with LED lights. **(C)** The earpiece (skin-facing side) delivers electrical stimulation *via* four metallic electrodes covered in hydrogel and surrounded with adhesive hydrocolloid (black region). **(D)** Hypothesized auricular dermatomes. ATN, auriculotemporal nerve; ABVN, auricular branch of the vagus nerve; GAN, greater auricular nerve. Modified from Butt et al. (2020). **(E)** The Roo system provides stimulation in a bipolar configuration forming stimulation channels/circuits between pairs of electrodes. The system has two channels: Channel 1 is formed between the electrode in Region 1 (ABVN) and the lower electrode in Region 3, and Channel 2 is formed between the electrode in Region 2 (ATN) and the upper electrode in Region 3. **(F)** Roo system on a neonate.

ear for several treatment sessions without the need for replacement. Due to variations in the scalp and/or skull, certain instances required medical tape to hold the earpiece to the head. The addition of medical tape was well-tolerated in all infants.

tAN stimulation was delivered at 0.1 mA below perception intensity at two different areas using two discrete frequencies. Area 1/Channel 1 innervates the cymba concha region of the ear and targets the ABVN whereas Area 2/Channel 2 innervates the temporomandibular joint, anterior to the tragus region and targets the ATN. Stimulation was pre-programmed to a pulse-width of 250 μs. Channel 1 was programmed to a frequency of 5 Hz and Channel 2 was programmed to 100 Hz. The current intensity duty cycle was set to run for 5 min with a 10 s off period. Perception intensity for each area is independent and was determined by starting at 0.1 mA and increased by 0.1 mA until the infant responded to stimulation, usually by eye blinking or head turn. After the infant responded to stimulation, the current intensity was turned down by 0.1 mA and was set and to run for 30 min. When the 30-min tAN stimulation period was complete, the system was turned off and unplugged. The ear electrode may have been left on the infant if still adhering well to the skin.

tAN was administered up to four times per day for up to 12 days total. If the infant was weaned off morphine within 10 days, a 2-day tAN weaning period was implemented. On Day 1 of this weaning period, tAN was administered twice and on Day 2 tAN was administered once. All infants were observed for 48 h after cessation of morphine treatment and 24 h after cessation of tAN adjuvant therapy.

Safety Outcome Monitoring

Safety measures included heart rate monitoring, pain assessment, and adverse event monitoring. Bradycardia (HR <80 bpm) was monitored using bedside neonatal intensive care unit cardiac monitors. The Neonatal Infant Pain Scale (NIPS)

is a validated pain scale utilized in the NICU (Lawrence et al., 1993; Witt et al., 2016) was used to monitor pain levels and was recorded before and after the 30-min tAN stimulation period. There are six components to the NIPS: facial expression, crying, breathing patterns, arm and leg movements, and state of arousal. The NIPS scale scoring ranges from 0 to 7, with scores greater than 3 indicating discomfort. A score of 3 is similar to the pain level associated with a heel stick procedure to obtain blood and the maximum score of 6 is similar to a circumcision procedure without analgesia (Butler-O'Hara et al., 1998; Anand, 2001). Neonatal nurses routinely record NIPS scores throughout the day and infants with NOWS frequently have elevated NIPS scores. Thus, tAN stimulation was only halted or adjusted to a lower intensity if NIPS scores were 3 greater than baseline. The skin on the exterior and just inside the left ear was examined daily before electrode placement for redness and any signs of irritation. Stimulation of the right ear occurred if redness, signs of irritation, or any other issues were observed at the site of the electrode.

Statistical Methodology

As this was a first-in-human prospective, single-arm, open-label trial, no formal statistical hypotheses were tested nor was the sample size derived using statistical principles. The sample size was based on an estimate of the number of infants that could be enrolled within a reasonable timeframe. Continuous data were summarized using descriptive statistics including *N* (number in analysis set), *n* (number of non-missing observations), mean, standard deviation, median, interquartile range (IQR), and range unless otherwise noted. Categorical variables were summarized using frequency counts and percentages. Paired sample *t*-tests were used to assess changes in heart rate.

RESULTS

Demographics

Eight of the nine infants considered for participation were consented and enrolled in the study (parental refusal consent $n = 1$). Relevant infant and maternal demographics and baseline clinical characteristics are provided in **Tables 1**, **2**. These infants had a median gestational age of 38.2 weeks at enrollment and were exposed *in utero* to methadone, tobacco (tAN1); heroin, buprenorphine, cocaine, and tobacco (tAN2); buprenorphine (tAN3); opioids, methamphetamines, and benzodiazepines (tAN4); heroin, cocaine, and methadone (tAN5); heroin, methadone, tobacco, and THC (tAN6); hydromorphone (tAN7); and heroin, methamphetamines, methadone, and tobacco (tAN8). Additionally, tAN4 was on lorazepam and tAN6 was on clonidine at enrollment. The mean (SD) control oral morphine dose was 0.076 (±0.041) mg/kg administered every 3 h. Only three out of the eight infants (37.5%) received maternal breastmilk during the study. Half of the mothers were on a maintenance dose of methadone (daily dose ranging from 40 to 75 mg), 25% were on a maintenance dose of buprenorphine, and the remaining 25% were taking short-acting opioids.

TABLE 1 | Infant and mother demographics.

Infants (*N* = 8)	
Median gestational age (range) in weeks	38.2 (35.1–43.9)
Median birth weight (range) in grams	2,810 (2,190–3,960)
Male sex—no. (%)	5 (62.5%)
Breast-feeding subgroup—no. (%)	3 (37.5%)
Median highest oral morphine dose (range) in mg/kg	0.06 (0.05–0.17)
Region 1 ABVN: Mean mA (SD) current intensity	0.32 (0.04)
Region 2 ATN: Mean mA (SD) current intensity	0.38 (0.14)
Mothers (*N* = 8)	
Use of methadone	
Maintenance therapy—no. (%)	4 (50%)
Average daily dose (range) in mg	58.3 (40–75)
Use of buprenorphine—no. (%)	2 (25%)
Use of short-acting opioid—no. (%)	2 (25%)
Use of tobacco—no. (%)	
Any	4 (50%)
5 *cigarettes a day*	
Drugs identified on meconium or infant drug screen—no. (%)	
Cocaine	1 (12.5%)
Amphetamine	1 (12.5%)
Morphine	1 (12.5%)

tAN Treatment

tAN was administered for an average (SD) of 9.5 (±2.6) days (range: 5–12 days) and the average (SD) number of tAN sessions was 30 (±9.5) (range: 13–43) across all eight infants. On average, infants received 3.1 (±0.4) tAN treatments per day.

Safety

tAN therapy did not result in any unanticipated adverse events (device and non-device related) in any subject. One infant experienced redness at the stimulation site which resolved after 12 h on two separate occasions. Redness occurred after removing the hydrogel with sterile-water-treated gauze in most infants, but there was no irritation evident before the next treatment 3 h later, or after completion of tAN therapy in any infant.

Heart rate was monitored before tAN treatment, during tAN treatment, and after tAN treatment for bradycardia. Mean (SD) heart rate was 161.9 (±24.2) bpm prior to tAN treatment, 157.3 (±23.0) bpm during tAN treatment, 160.6 (±14.6) immediately after tAN treatment and 162.4 (±17.0) 15 min following tAN treatment. There were no observed episodes of bradycardia during or after tAN treatment and the mean heart rate was not significantly changed at any time point from the pre-tAN baseline value (paired samples *t*-test; $p > 0.05$).

The median NIPS score was 0 (IQR: 0.0–3.0) before tAN therapy and 0 (IQR: 0.0–2.0) after tAN treatment, suggesting minimal discomfort during stimulation. There were 26 sessions (12.0%) across the 217 total tAN sessions with concurrent NIPS score collection during which NIPS scores increased by 3 or more points from the pre-stimulation value during the post-tAN assessment.

TABLE 2 | Baseline infant medication use and concomitant medical problems.

Participant	Medications	Concomitant medical problem(s)
tAN 1	Vitamin D	Hypoglycemia, resolved
tAN 2	Vitamin D	Early Latent Syphilis
	Dolutegravir (5 mg)	Herpes simplex virus infection
	Lamivudine (10 mg/ml)	Hepatitis C
	Nystatin	Gonorrhea
	Sulfamethoxazole-Trimethoprim	Chlamydia
	Zidovudine (10 mg/ml)	
tAN 3	Vitamin D	Hyperbilirubinemia
		Abnormal x-ray of humerus
		Hyperosmolality/Hypernatremia
		Respiratory distress syndrome requiring initial CPAP
tAN 4	Ativan (0.05 mg/kg)	Meconium aspiration below vocal cords resolved
tAN 5	Penicillin	Hepatitis B (maternal)
	Vitamin D	Hepatitis C (maternal)
		Ankyloglossia
		Syphilis (maternal)
		Tethered labial frenulum
tAN 6	Clonidine (7 mcg- >10 mcg)	Tobacco use (maternal)
	Simethicone (40 mg/0.6ml oral drop)	
tAN 7	Gentamicin	Respiratory distress syndrome, resolved
	Ampicillin	
	Ativan given once	
tAN 8	Vitamin D	Hyperbilirubinemia
	Acetylcysteine	Failed newborn hearing screen[1]
	Acyclovir	Hepatitis C (maternal)
	Ampicillin	Hepatitis B (maternal)
	Gentamicin	Lactobezoar

[1] *Failed routine newborn hearing screen, 8 days before starting tAN treatment. The infant had a formal hearing screen as an outpatient 9 days after discharge and passed the hearing screen at that time.*

Morphine Administration Outcomes

Figure 3 shows the mean daily morphine dose 2 days before, during, and 2 days post-tAN therapy for individual subjects (**Figure 3A**) and across the entire cohort (**Figure 3B**). The mean (SD) daily dose of morphine was 0.063 (±0.043) mg/kg at the start of tAN therapy, and morphine was consistently weaned every 12 h once tAN started. **Table 3** provides both mean and median for total oral morphine LOT and oral morphine LOT after initiation of tAN treatment. The median oral morphine LOT was 9.0 days (IQR: 6.5–12.8; range: 4–43), and median LOT after tAN initiation was 6.0 days (IQR: 4.8–8; range: 3–16) (**Table 3**). One infant (tAN6) was enrolled after transfer from another facility at 3 weeks of age, after failing oral morphine replacement treatment. This infant skewed the mean (SD) total oral morphine LOT to 13.3 (±12.8) days and oral morphine LOT after initiation of tAN to 7.0 (±4.0) days. The number of days for both total oral morphine LOT and oral morphine LOT after tAN initiation for tAN6 were over two standard deviations above the mean, justifying exclusion from analyses. When tAN6 was excluded, median total oral morphine LOT and oral morphine LOT after tAN treatment stayed the same; however, mean (SD) total oral morphine LOT was similar to the median values at 9.0 (±4.7) days and oral morphine LOT after tAN at 5.7 (±1.9) days.

During tAN therapy, all eight infants achieved average FNAST scores <8, indicating an overall reduction in withdrawal symptoms. All infants, except for one (tAN6), were completely weaned off morphine during tAN treatment. Following 12 days of tAN treatment, the mean daily dose of morphine for tAN6 was 0.018. The infant was completely weaned off morphine 4 days following tAN treatment. In tAN4, lorazepam weaning was initiated 1 day after discontinuing morphine. In total, five rescue morphine doses, resulting from FNAST scores >8, were administered across all subjects.

The median length of hospital stay, calculated as the number of days from date of birth to discharge (including all levels of care), across all eight infants was 17.0 days (IQR: 15.3–28.5; Range: 10–46) and the mean (SD) length of stay was 22.6 (±12.5) days.

DISCUSSION

In this prospective open-label trial, tAN was applied as an adjuvant therapy to oral morphine in neonates diagnosed with NOWS from *in utero* exposure to opioids including methadone, heroin, buprenorphine, and methamphetamines. tAN was administered 1 h before morphine administration, up to four times daily for up to 12 days. tAN as an adjuvant was safe, well-tolerated, and facilitated the successful rapid weaning of oral morphine in these newborns. The median LOT from the start of administering oral morphine (9 days) and the median LOT after tAN initiation (6.0 days) were both significantly lower than previously published data suggesting that the average NICU stay for infants undergoing pharmacotherapy for NOWS is 23 days (Patrick et al., 2015). Given that tAN treatment permitted rapid consistent weaning, our preliminary data suggests that using tAN as an adjuvant to pharmacotherapy could significantly reduce NICU stay. Furthermore, as indicated by the NIPS and adverse event profile, tAN did not produce any additional risks outside those observed with oral morphine. If these early results are confirmed, adjuvant tAN therapy compares favorably with the LOT and with the risk profile of oral opioid treatment as well as with other alternative non-invasive adjuvant NOWS treatments.

TABLE 3 | Effectiveness outcomes (*N* = 8).

Outcome	Median (IQR)	Mean (SD)	Mean (SD) excludes tAN6[1] (*N* = 7)	Range
Total oral morphine length of treatment (LOT) in days	9.0 (6.5–12.8)	13.3 (12.8)	9.0 (4.7)	4–43
Oral morphine length of treatment (LOT) after tAN initiation in days	6.0 (4.8–8)	7.0 (4.0)	5.7 (1.9)	3–16
Length of hospital stay in days	17.0 (15.3–28.5)	22.6 (12.5)	19.3 (8.9)	10–46

[1] *Number of days for both total oral morphine LOT and oral morphine LOT after tAN initiation were over 2 standard deviations above the mean for tAN6, justifying exclusion in analyses.*

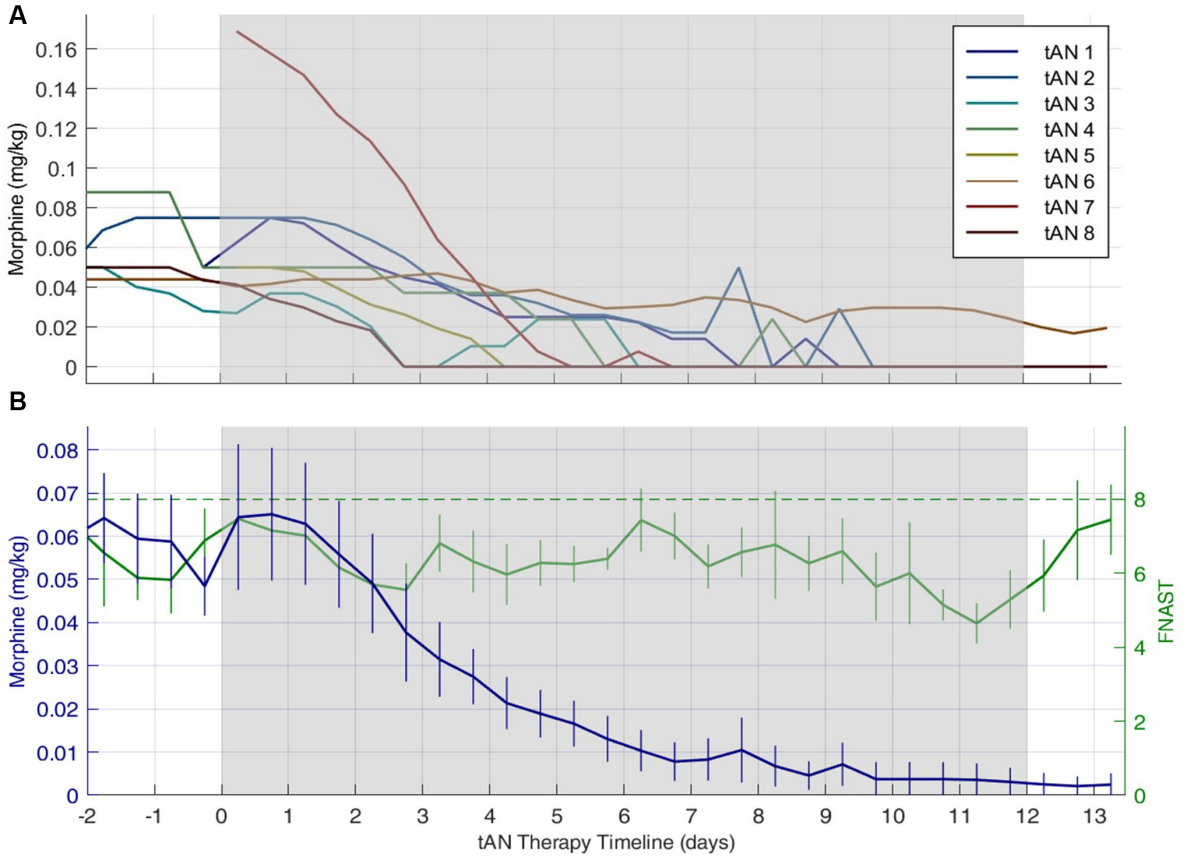


FIGURE 3 | Individual and combined subjects daily finnegan neonatal abstinence scoring tool (FNAST) value and morphine dose plots. Daily morphine dose 2 days before, during, and 2 days post-tAN therapy. Gray shaded region indicates when tAN therapy was administered. Panel **(A)** represents individual subject morphine dose and **(B)** represents mean daily morphine dose across the entire cohort. In **(B)** the green dash line indicates the Finnegan score threshold requiring a change in morphine dose, the solid green line indicates mean FNAST scores, and the solid blue line indicates mean morphine dose. The number of days on oral morphine before the start of tAN therapy varied across all subjects but was accounted for in total morphine LOT. Data are means ± standard error of the means.

Developing an Alternative to Opioid Replacement Therapy

Currently, no nationwide standard of care exists for managing NOWS (Patrick et al., 2014; Barlow et al., 2017). Treatment of NOWS usually follows a multimodal regime centered on controlled withdrawal and replacement drug therapy with oral morphine. However, newer opioid agents are being tested. The recent Blinded Buprenorphine OR Neonatal morphine solution (BBORN) trial reports the median length of treatment for oral morphine and sublingual buprenorphine as 28 and 15 days, respectively (Kraft et al., 2017). Furthermore, a randomized control trial that examined the safety and efficacy of methadone vs. morphine reported a median length of treatment of 15 and 11.5 days (Davis et al., 2018). However, treatments that reduce the need for neurotoxic opioids are a high priority in this vulnerable population (Flannery et al., 2020). The NEOPAIN trial, a large multicenter randomized study of 898 infants demonstrated that although morphine is effective in decreasing clinical signs of pain, it can cause significant acute adverse effects such as changes in the heart and respiratory rate, hypotension, nasogastric feeds, and need for intravenous supplemental nutrition (Anand et al., 2004; Attarian et al., 2014). Based on the vulnerability of the population and the potential for adverse effects, the authors of the NEOPAIN study suggested that morphine administration should be used judiciously and cautiously.

Furthermore, a review published in 2014 suggests that current protocols of morphine replacement in neonates alter critical development periods and can lead to adverse neuropsychological effects (Attarian et al., 2014). Specifically, morphine has been shown to affect apoptotic protein expression in animals and humans, suggesting that opioids alter various neurological pathways other than pain pathways. In support of the NEOPAIN study and the 2001 Consensus Statement for the Prevention and Management of Pain in the Newborn, the authors conclude that non-pharmacologic interventions should be a primary treatment option, and if unsuccessful, the use of opioid analgesics should be thoughtful and cautious. The use of opioid-based medication as the standard of care remains concerning due to the relatively high potential of compounding harm to the infants' development and health.

Multiple studies have assessed the debilitating consequences of NOWS including otitis media and delayed cognitive and/or motor development (Rosen and Johnson, 1982; Kirkwood and Kirkwood, 1983). These consequences have been studied for decades. A study from 1982 comparing 38 neonates with NOWS to 23 healthy neonates found that neonates exposed to methadone prenatally had a significantly higher incidence of otitis media after an 18-month follow-up (Rosen and Johnson, 1982). A subsequent study in 1983 expanded on this finding demonstrating that recurrent otitis media is associated with persistent hearing loss and subsequent impairments in language skills (Kirkwood and Kirkwood, 1983). The authors concluded that children with recurring otitis media have a greater risk of learning disabilities which can have a substantial impact on the day-to-day function of both the child and caregiver. Additionally, NOWS has been shown to cause developmental cognitive delays based on the McCarthy Scales of Children's Abilities, which assesses cognitive ability, including general cognitive index, perception, and memory. Children who had been exposed to heroin prenatally (n = 22) were shown to perform far worse on the McCarthy Scales when compared to their control counterparts (Wilson et al., 1979).

To assess motor development in infants, physicians use the Bayley Scale of Infant Development (BSID) to measure the current developmental functioning in areas of cognition, motor skills, and behavior. The BSID consists of two parts: the Psychomotor Developmental Index (PDI) and the Bayley Mental Developmental Index (MDI; Lennon et al., 2008). Multiple studies have shown that infants with NOWS have significantly lower PDI and MDI scores compared to healthy infants at 12, 18, and 24 months of age (Strauss et al., 1976; Rosen and Johnson, 1982; Johnson et al., 1984). A retrospective study also found that 3-year-old children (n = 28) with NOWS had significantly lower overall BSID composite scores compared to healthy children. A study focused specifically on the effects of buprenorphine found that 28 children (5–6 years of age) exposed to buprenorphine prenatally had significant problems with motor skills, memory, hyperactivity, impulsivity, and attention (Sundelin Wahlsten and Sarman, 2013).

Concerning non-pharmacologic treatment options, a protocol developed by the National Acupuncture Detoxification Association (NADA) has demonstrated effectiveness in reducing withdrawal symptoms in adults and has been recently utilized in newborns as an adjunctive treatment for NOWS (Raith et al., 2015; Weathers et al., 2015; Jackson et al., 2020). Raith et al. (2015) found that using the NADA protocol, handheld laser acupuncture is an effective adjunctive treatment for NOWS. More specifically, handheld laser acupuncture resulted in a significantly reduced duration of oral morphine and a significantly reduced length of hospital stay; the median length of treatment for morphine and morphine + laser was 39 and 28 days. Applying tAN using the Roo System in our cohort, also resulted in a decrease in the duration of oral morphine and overall days of treatment for each neonate. The similarity in these findings is likely due to the treatment location. The five acupoint regions targeted in the NADA protocol are innervated by two specific cranial nerve branches: the ABVN and the ATN (Round et al., 2013; Butt et al., 2020), the same cranial nerve branches targeted by the Roo System (**Figures 2D–F**), which uses hydrogel electrodes to deliver mild electrical stimulation.

Proposed Mechanism of tAN

Functional magnetic resonance imaging studies have examined the effects of ABVN stimulation on brain network activity. In comparison to earlobe stimulation, ABVN stimulation activates the nucleus tractus solitarius, locus coeruleus, spinal trigeminal nucleus, parabrachial area, periaqueductal gray, amygdala, and nucleus accumbens (Frangos et al., 2015; Yakunina et al., 2017; Badran et al., 2018b; Kaniusas et al., 2019; Qureshi et al., 2020). An earlier study delivered direct electrical stimulation to the parabrachial area (PBA) and arcuate horn (ARH) regions which triggered the release of endogenous opioids (endorphins), allowing for analgesic effects (Han et al., 1991). Importantly, this analgesic effect was demonstrated to be dependent on stimulation frequency. Whereas the ARH provided optimal analgesic effect at lower frequencies (16–32 Hz), and PBA at higher frequencies (64–128 Hz), respectively. **Figure 4** illustrates a possible mechanism for tAN as a method to activate the endorphinergic system *via* modulation specific brain regions. As an example, endorphins can bind to the opioid receptors in the ventral tegmental area activating dopaminergic neurons, and potentially leading to amelioration of pain and withdrawal symptoms (Han et al., 1991; Han and Wang, 1992; Oleson, 2002; Sator-Katzenschlager and Michalek-Sauberer, 2007; Meade et al., 2010). Additionally, the locus coeruleus (LC)-norepinephrine system plays a role in dependence/addiction and is a primary target for multiple substances of abuse, including opioids (Valentino and Volkow, 2020). During a state of stress, LC neurons are hyperactive, which leads to hallmark symptoms of withdrawal: hyperarousal and insomnia (Aghajanian, 1978; Ivanov and Aston-Jones, 2001). Endorphins that bind LC receptors can attenuate LC excitation, and ultimately alleviate withdrawal symptoms. Furthermore, α2-adrenergic antagonists (clonidine and lofexidine) suppress LC activation and are frequently used clinically to reduce opioid withdrawal. Thus, the physiological effects we observe support our hypothesis that tAN may be modulating these specific systems. However, further studies are needed to elucidate the therapeutic mechanism.

Limitations and Future Directions

This first-in-human study was open-label with no sham control group, limiting interpretation of the study results in terms of the placebo effect (placebo effect by proxy) and true treatment effect. Although there is still debate as to whether the placebo effect exists in infants and to what degree (Barbier et al., 1994; Paul et al., 2014; Colloca, 2015; Kossowsky and Kaptchuk, 2015), a randomized, sham-controlled study is warranted to further explore treatment effect of adjuvant use of tAN during oral morphine weaning. Although the therapeutic benefits appear to be clinically meaningful, an RCT design would further address

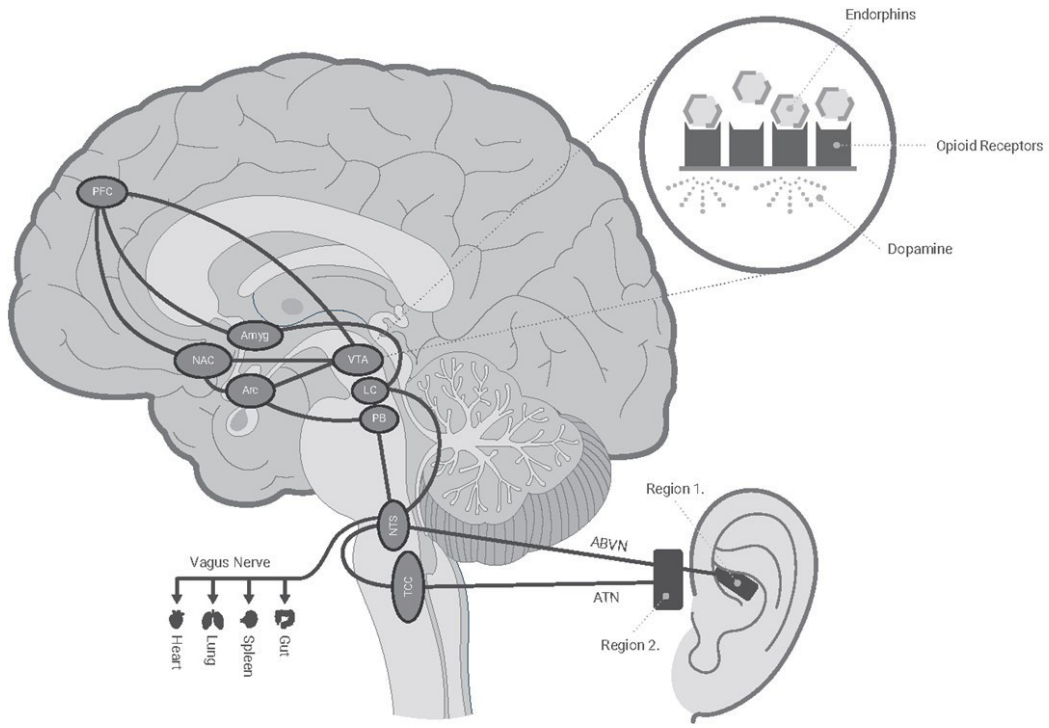


FIGURE 4 | Schematic of hypothesized tAN innervation with central targets. Roo delivers individually tailored electrical stimulation targeting auricular cranial nerve branches of the ABVN (Vagus) and ATN (Trigeminal) nerves. These nerves send afferent signals to the NTS and TCC. The information is processed in the medulla and relayed to higher regions which may lead to the release of endogenous opioids (endorphins) and alleviation of opioid withdrawal symptoms. ABVN, auricular branch of the vagus nerve; Amyg, amygdala; Arc, arcuate nucleus; ATN, auriculotemporal nerve; LC, Locus coeruleus; NAC, nucleus accumbens; NTS, nucleus tractus solitarius; PB, parabrachial nucleus; PFC, prefrontal cortex; TCC, trigeminocervical complex; VTA, ventral tegmental.

additional study limitations, namely the small sample size and conduct at a single center.

The inclusion of infants at different durations of morphine therapy does not allow for interpretation of the potential effect of tAN during early administration of morphine therapy (e.g., different levels of opioid withdrawal). However, the results of this study may be more representative of real-world clinical utilization of tAN therapy given the inclusion of infants at different durations of morphine therapy.

Lastly, our approach to tAN therapy included stimulation of the left AVBN and ATN. Both vagal and trigeminal branches have been targeted for pain therapy (Miller et al., 2016). Interestingly, both vagal and trigeminal afferents synapse on to the periaqueductal gray (Benarroch, 2012), stimulation of which, in humans, has shown to release endogenous opioids (Sims-Williams et al., 2017). Given these findings, we hypothesized that stimulation of vagal as well as trigeminal activation would synergistically mediate endogenous opioid release. However, this clinical trial was not designed to test these two working hypotheses. Further investigation into physiological biomarkers of opioid withdrawal and the mobilization of endogenous opioids may optimize tAN therapy. Although *in vivo* testing in human neonates is limited by ethical constraints (i.e., positron emission tomography scanning with radiotracers for opioid receptors), we may test salivary cortisol as a non-invasive measure of stress and measure oxidative stress in the brain *via* non-invasive magnetic resonance spectroscopy (Moss et al., 2018). While both of these markers are associated with opioid withdrawal (Ward et al., 2020), they are not direct measures of endogenous opioid release by tAN vs. another mechanism. Therefore, precise elucidation of mechanism relies on laboratory models of NOWS or pain models of neuromodulation of these cranial nerves.

CONCLUSION

This is the first study investigating the effects of tAN, as adjunctive therapy to oral morphine, in the reduction of opioid withdrawal signs and symptoms in newborns with NOWS. Across all study participants, tAN was shown to be safe, well-tolerated, and seemed to facilitate the rapid weaning of oral morphine. The results also suggest that tAN may provide alleviation of withdrawal symptoms associated with NOWS. If proven safe and effective in future trials, tAN may expand non-pharmacological treatment options for these infants.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Medical University of South Carolina (MUSC) Institutional Review Board. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

BB, DJ, GO'L, AC, NK, and SW made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data, participated in drafting, editing, and final approval of this manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported by NIH National Institute on Drug Abuse (NIDA) R43 DA050360-01 (Principal Investigators: DJ and NK). Roo device development was supported by FDA P50 Pediatric Device Consortia Grant # 5P50FD006428 (SWPDC).

ACKNOWLEDGMENTS

We would like to thank Caroline Benner for her assistance in editing, formatting, and finalizing the manuscript.

REFERENCES

Aghajanian, G. K. (1978). Tolerance of locus coeruleus neurones to morphine and suppression of withdrawal response by clonidine. *Nature* 276, 186–188. doi: 10.1038/276186a0

Anand, K. J. S. (2001). Consensus statement for the prevention and management of pain in the newborn. *Arch. Pediatr. Adolesc. Med.* 155, 173–180. doi: 10.1001/archpedi.155.2.173

Anand, K. J. S., Hall, R. W., Desai, N., Shephard, B., Bergqvist, L. L., Young, T. E., et al. (2004). Effects of morphine analgesia in ventilated preterm neonates: primary outcomes from the NEOPAIN randomised trial. *Lancet* 363, 1673–1682. doi: 10.1016/S0140-6736(04)16251-X

Attarian, S., Tran, L. C., Moore, A., Stanton, G., Meyer, E., and Moore, R. P. (2014). The neurodevelopmental impact of neonatal morphine administration. *Brain Sci.* 4, 321–334. doi: 10.3390/brainsci4020321

Badran, B. W., Brown, J. C., Dowdle, L. T., Mithoefer, O. J., LaBate, N. T., Coatsworth, J., et al. (2018a). Tragus or cymba conchae? Investigating the anatomical foundation of transcutaneous auricular vagus nerve stimulation (taVNS). *Brain Stimul.* 11, 947–948. doi: 10.1016/j.brs.2018.06.003

Badran, B. W., Dowdle, L. T., Mithoefer, O. J., LaBate, N. T., Coatsworth, J., Brown, J. C., et al. (2018b). Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) *via* electrical stimulation of the tragus: a concurrent taVNS/fMRI study and review. *Brain Stimul.* 11, 492–500. doi: 10.1016/j.brs.2017.12.009

Badran, B. W., Yu, A. B., Adair, D., Mappin, G., DeVries, W. H., Jenkins, D. D., et al. (2019). Laboratory administration of transcutaneous auricular vagus nerve stimulation (taVNS): technique, targeting, and considerations. *J. Vis. Exp.* 143:10.3791/58984. doi: 10.3791/58984

Barbier, P., Lionnet, C., Jonville, A. P., Hamon, B., Autret, E., Laugier, J., et al. (1994). L'effet placebo existe-t-il chez le nouveau-né? [Does the placebo effect exist in newborn infants?]. *Therapie* 49, 113–116.

Barlow, K., Cannon, A., Dankovich, K., James, A., Merritt, M., and Hudson, J. A. (2017). Neonatal abstinence syndrome: an epidemic. *Greenville Health Syst.* 2, 11–15. Available online at: https://mainbabies.wpengine.com/wp-content/uploads/2017/11/GHS-Proc-Neonatal-Abstinence-Syndrome-An-Epidemic-1.pdf.

Benarroch, E. E. (2012). Periaqueductal gray: an interface for behavioral control. *Neurology* 78, 210–217. doi: 10.1212/WNL.0b013e31823fcdee

Bonnette, M. L. (2008). CE FEATURE auricular transcutaneous electrical neurostimulation, addiction education, behavioral training, coaching support and the nicotine addiction treatment process. *J. Addict. Nursing* 19, 130–140. doi: 10.1080/10884600802305976

Butler-O'Hara, M., LeMoine, C., and Guillet, R. (1998). Analgesia for neonatal circumcision: a randomized controlled trial of EMLA cream vs dorsal penile nerve block. *Pediatrics* 4:E5. doi: 10.1542/peds.101.4.e5

Butt, M. F., Albusoda, A., Farmer, A. D., and Aziz, Q. (2020). The anatomical basis for transcutaneous auricular vagus nerve stimulation. *J. Anat.* 236, 588–611. doi: 10.1111/joa.13122

Chen, Y.-W., Tzeng, J.-I., Lin, M.-F., Hung, C.-H., and Wang, J.-J. (2015). Transcutaneous electrical nerve stimulation attenuates postsurgical allodynia and suppresses spinal substance P and proinflammatory cytokine release in rats. *Phys. Ther.* 95, 76–85. doi: 10.2522/ptj.20130306

Colloca, L. (2015). Placebo effects in infants, toddlers, and parents. *JAMA Pediatr.* 169, 504–505. doi: 10.1001/jamapediatrics.2014.3795

Davis, J. M., Shenberger, J., Terrin, N., Breeze, J. L., Hudak, M., Wachman, E. M., et al. (2018). Comparison of safety and efficacy of methadone vs morphine for treatment of neonatal abstinence syndrome: a randomized clinical trial. *JAMA Pediatr.* 172, 741–748. doi: 10.1001/jamapediatrics.2018.1307

de Andrade, D. C., Mhalla, A., Adam, F., Texeira, M. J., and Bouhassira, D. (2011). Neuropharmacological basis of rTMS-induced analgesia: the role of endogenous opioids. *Pain* 152, 320–326. doi: 10.1016/j.pain.2010.10.032

Devlin, L. A., Breeze, J. L., Terrin, N., Pomar, E. G., Bada, H., Finnegan, L. P., et al. (2020). Association of a simplified finnegan neonatal abstinence scoring tool with the need for pharmacologic treatment for neonatal abstinence syndrome. *JAMA Netw. Open* 3:e202275. doi: 10.1001/jamanetworkopen.2020.2275

Ellison, F., Ellison, W., Daulouede, J. P., Daubech, J. F., Pautrizel, B., Bourgeois, M., et al. (1987). Opiate withdrawal and electro-stimulation. Double blind experiments. *Encephale* 10, 225–229.

Filippelli, A. C., White, L. F., Spellman, L. W., Broderick, M., Highfield, E. S., Sommers, E., et al. (2012). Non-insertive acupuncture and neonatal abstinence syndrome: a case series from an inner city safety net hospital. *Glob. Adv. Health Med.* 4, 48–52. doi: 10.7453/gahmj.2012.1.4.007

Finnegan, L. P., Connaughton, J. F., Kron, R. E., and Emich, J. P. (1975). Neonatal abstinence syndrome: assessment and management. *Addict. Dis.* 2, 141–158.

Flannery, T., Davis, J. M., Czynski, A. J., Dansereau, L. M., Oliveira, E. L., Camardo, S. A., et al. (2020). Neonatal abstinence syndrome severity index predicts 18-month neurodevelopmental outcome in neonates randomized to morphine or methadone. *J. Pediatr.* 227, 101–107. doi: 10.1016/j.jpeds.2020.08.034

Frangos, E., Ellrich, J., and Komisaruk, B. R. (2015). Non-invasive access to the vagus nerve central projections *via* electrical stimulation of the external ear: fMRI evidence in humans. *Brain Stimul.* 8, 624–636. doi: 10.1016/j.brs.2014.11.018

Garcia, R. G., Lin, R. L., Lee, J., Kim, J., Barbieri, R., Sclocco, R., et al. (2017). Modulation of brainstem activity and connectivity by respiratory-gated auricular vagal afferent nerve stimulation in migraine patients. *Pain* 158, 1461–1472. doi: 10.1097/j.pain.0000000000000930

Gomez Pomar, E., Finnegan, L. P., Devlin, L., Bada, H., Concina, V. A., Ibonia, K. T., et al. (2017). Simplification of the finnegan neonatal abstinence scoring system: retrospective study of two institutions in the USA. *BMJ Open* 7:9:e016176. doi: 10.1136/bmjopen-2017-016176

Grossman, M. R., Lipshaw, M. J., Osborn, R. R., and Berkwitt, A. K. (2018). A novel approach to assessing infants with neonatal abstinence syndrome. *Hosp. Pediatr.* 8, 1–6. doi: 10.1542/hpeds.2017-0128

Han, J.-S. (2004). Acupuncture and endorphins. *Neurosci. Lett.* 361, 258–261. doi: 10.1016/j.neulet.2003.12.019

Han, J. S., and Wang, Q. (1992). Mobilization of specific neuropeptides by peripheral stimulation of identified frequencies. *Physiology* 7, 176–180. doi: 10.1152/physiologyonline.1992.7.4.176

Han, J. S., Chen, X. H., Sun, S. L., Xu, X. J., Yuan, Y., Yan, S. C., et al. (1991). Effect of low-and high-frequency TENS on Met-enkephalin-Arg-Phe and dynorphin A immunoreactivity in human lumbar CSF. *Pain* 47, 295–298. doi: 10.1016/0304-3959(91)90218-M

He, Y., Wu, F.-X., Miao, X.-R., Xu, X.-W., Sun, Y.-M., Chen, C.-Y., et al. (2011). Suppression of acute morphine withdrawal syndrome by adenovirus-mediated β-endorphin in rats. *Brain Res.* 1422, 13–19. doi: 10.1016/j.brainres.2011.07.063

Hu, B., Akerman, S., and Goadsby, P. J. (2021). Characterization of opioidergic mechanisms related to the anti-migraine effect of vagus nerve stimulation. *Neuropharmacology* 11:108375. doi: 10.1016/j.neuropharm.2020.108375

Hudak, M. L., Tan, R. C., Committee on Drugs, and Committee on Fetus and Newborn and American Academy of Pediatrics (2012). Neonatal drug withdrawal. *Pediatrics* 129, e540 –e560. doi: 10.1542/peds.2011-3212

Ivanov, A., and Aston-Jones, G. (2001). Local opiate withdrawal in locus coeruleus neurons *in vitro*. *J. Neurophysiol.* 85, 2388–2397. doi: 10.1152/jn.2001.85.6.2388

Jackson, H. J., Lopez, C., Miller, S., and Englehardt, B. (2020). Feasibility of auricular acupressure as an adjunct treatment for neonatal opioid withdrawal syndrome (NOWS). *Subst. Abuse* doi: 10.1080/08897077.2020.1784360. [Epub ahead of print].

Jansson, L. M., Dipietro, J. A., Elko, A., and Velez, M. (2010). Infant autonomic functioning and neonatal abstinence syndrome. *Drug Alcohol Depend.* 109, 198–204. doi: 10.1016/j.drugalcdep.2010.01.004

Janssen, P., Pottel, H., Vos, R., and Tack, J. (2011). Endogenously released opioids mediate meal-induced gastric relaxation *via* peripheral mu-opioid receptors. *Aliment. Pharmacol. Ther.* 33, 607–614. doi: 10.1111/j.1365-2036.2010.04557.x

Johnson, H. L., Diano, A., and Rosen, T. S. (1984). 24-month neurobehavioral follow-up of children of methadone-maintained mothers. *Infant Behav. Dev.* 7, 115–123. doi: 10.1016/s0163-6383(84)80027-2

Kaniusas, E., Kampusch, S., Tittgemeyer, M., Panetsos, F., Gines, R. F., Papa, M., et al. (2019). Current directions in the auricular vagus nerve stimulation I—A physiological perspective. *Front. Neurosci.* 13:854. doi: 10.3389/fnins.2019.00854

Kirkwood, C. R., and Kirkwood, M. E. (1983). Otitis media and learning disabilities: the case for a causal relationship. *J. Fam. Pract.* 17, 219–227.

Ko, J. Y., Wolicki, S., Barfield, W. D., Patrick, S. W., Broussard, C. S., Yonkers, K. A., et al. (2017). CDC Grand Rounds: public health strategies to prevent neonatal abstinence syndrome. *Morb. Mortal. Wkly. Rep.* 66, 242–245. doi: 10.15585/mmwr.mm6609a2

Kocherlakota, P. (2014). Neonatal abstinence syndrome. *Pediatrics* 134, e547–e561. doi: 10.1542/peds.2013-3524

Kossowsky, J., and Kaptchuk, T. J. (2015). Placebo effects in infants, toddlers, and parents. *JAMA Pediatr.* 169:505. doi: 10.1001/jamapediatrics.2014.3798

Kovacic, K., Hainsworth, K., Sood, M., Chelimsky, G., Unteutsch, R., Nugent, M., et al. (2017). Neurostimulation for abdominal pain-related functional gastrointestinal disorders in adolescents: a randomised, double-blind, sham-controlled trial. *Lancet Gastroenterol. Hepatol.* 10, 727–737. doi: 10.1016/S2468-1253(17)30253-4

Kraft, W. K., Adeniyi-Jones, S. C., Chervoneva, I., Greenspan, J. S., Abatemarco, D., Kaltenbach, K., et al. (2017). Buprenorphine for the treatment of the neonatal abstinence syndrome. *N. Engl. J. Med.* 376, 2341–2348. doi: 10.1056/NEJMoa1614835

Kraft, W. K., and van den Anker, J. N. (2012). Pharmacologic management of the opioid neonatal abstinence syndrome. *Pediatr. Clin. North Am.* 59, 1147–1165. doi: 10.1016/j.pcl.2012.07.006

Kraus, T., Kiess, O., Hösl, K., Terekhin, P., Kornhuber, J., and Forster, C. (2013). CNS BOLD fMRI effects of sham-controlled transcutaneous electrical nerve stimulation in the left outer auditory canal—a pilot study. *Brain Stimul.* 6, 798–804. doi: 10.1016/j.brs.2013.01.011

Lawrence, J., Alcock, D., McGrath, P., Kay, J., MacMurray, S. B., and Dulberg, C. (1993). The development of a tool to assess neonatal pain. *Neonatal Netw.* 12, 59–66.

Lennon, E. M., Gardner, J. M., Karmel, B. Z., and Flory, M. J. (2008). "Bayley scales of infant development," in *Encyclopedia of Infant and Early Childhood Development*, eds M. M. Haith and J. B. Benson (Oxford: Academic Press), 145–156.

Liu, N., Rockhold, R. W., and Ho, K. (1999). Electrical stimulation of nucleus paragigantocellularis induces opioid withdrawal-like behaviors in the rat. *Pharmacol. Biochem. Behav.* 62, 263–271. doi: 10.1016/s0091-3057(98)00164-6

MacMillan, K., Rendon, C. P., Verma, K., Riblet, N., Washer, D. B., and Volpe Holmes, A. (2018). Association of rooming-in with outcomes for neonatal abstinence syndrome: a systematic review and meta-analysis. *JAMA Pediatr.* 172, 345–351. doi: 10.1001/jamapediatrics.2017.5195

Meade, C. S., Lukas, S. E., McDonald, L. J., Fitzmaurice, G. M., Eldridge, J. A., Merrill, N., et al. (2010). A randomized trial of transcutaneous electric acupoint stimulation as adjunctive treatment for opioid detoxification. *J. Subst. Abuse Treat.* 38, 12–21. doi: 10.1016/j.jsat.2009.05.010

Miller, S., Sinclair, A. J., Davies, B., and Matharu, M. (2016). Neurostimulation in the treatment of primary headaches. *Pract. Neurol.* 16, 362–375. doi: 10.1136/practneurol-2015-001298

Miranda, A., and Taca, A. (2018). Neuromodulation with percutaneous electrical nerve field stimulation is associated with reduction in signs and symptoms of opioid withdrawal: a multisite, retrospective assessment. *Am. J. Drug Alcohol Abuse* 44, 56–63. doi: 10.1080/00952990.2017.1295459

Moss, H. G., Brown, T. R., Wiest, D. B., and Jenkins, D. D. (2018). N-Acetylcysteine rapidly replenishes central nervous system glutathione measured *via* magnetic resonance spectroscopy in human neonates with hypoxic-ischemic encephalopathy. *J. Cereb. Blood Flow Metab.* 38, 950–958. doi: 10.1177/0271678X18765828

Oleson, T. (2002). Auriculotherapy stimulation for neuro-rehabilitation. *NeuroRehabilitation* 17, 49–62. doi: 10.3233/NRE-2002-17107

Patrick, S. W., Barfield, W. D., Poindexter, B. B., Committee on Fetus and Newborn, and Committee on Substance Use and Prevention (2020). Neonatal opioid withdrawal syndrome. *Pediatrics* 146:e2020029074. doi: 10.1542/peds.2020-029074

Patrick, S. W., Davis, M. M., Lehman, C. U., and Cooper, W. O. (2015). Increasing incidence and geographic distribution of neonatal abstinence syndrome: United States 2009–2012. *J. Perinatol.* 35, 650–655. doi: 10.1038/jp.2015.36

Patrick, S. W., Kaplan, H. C., Passarella, M., Davis, M. M., and Lorch, S. A. (2014). Variation in treatment of neonatal abstinence syndrome in US children's hospitals, 2004–2011. *J. Perinatol.* 34, 867–872. doi: 10.1038/jp.2014.114

Paul, I. M., Beiler, J. S., Vallati, J. R., Duda, L. M., and King, T. S. (2014). Placebo effect in the treatment of acute cough in infants and toddlers: a randomized clinical trial. *JAMA Pediatr.* 168, 1107–1113. doi: 10.1001/jamapediatrics.2014.1609

Qureshi, I. S., Datta-Chaudhuri, T., Tracey, K. J., Pavlov, V. A., and Chen, A. C. H. (2020). Auricular neural stimulation as a new non-invasive treatment for opioid detoxification. *Bioelectron. Med.* 6:7. doi: 10.1186/s42234-020-00044-6

Raith, W., Schmölzer, G. M., Resch, B., Reiterner, F., Avian, A., Koestenberger, M., et al. (2015). Laser acupuncture for neonatal abstinence syndrome: a randomized controlled trial. *Pediatrics* 136, 876–884. doi: 10.1542/peds.2015-0676

Rao, R., Sampers, J. S., Kronsberg, S. S., Brown, J. V., Desai, N. S., and Anand, K. J. (2007). Neurobehavior of preterm infants at 36 weeks postconception as a function of morphine analgesia. *Am. J. Perinatol.* 24, 511–517. doi: 10.1055/s-2007-986675

Rosen, T. S., and Johnson, H. L. (1982). Children of methadone-maintained mothers: follow-up to 18 months of age. *J. Pediatr.* 101, 192–196. doi: 10.1016/s0022-3476(82)80115-7

Round, R., Litscher, G., and Bahr, F. (2013). Auricular acupuncture with laser. *Evid. Based Complement. Alternat. Med.* 2013:984763. doi: 10.1155/2013/984763

Sator-Katzenschlager, S. M., Scharbert, G., Kozek-Langenecker, S. A., Szeles, J. C., Finster, G., Schiesser, A. W., et al. (2004). The short- and long-term benefit in chronic low back pain through adjuvant electrical versus manual auricular acupuncture. *Anesth. Analg.* 98, 1359–1364. doi: 10.1213/01.ane.0000107941.16173.f7

Sator-Katzenschlager, S. M., and Michalek-Sauberer, A. (2007). P-Stim auricular electroacupuncture stimulation device for pain relief. *Expert Rev. Med. Devices* 4, 23–32. doi: 10.1586/17434440.4.1.23

Severson, L., Markoff, R. A., and Chun-Hoon, A. (1977). Heroin detoxification with acupuncture and electrical stimulation. *Int. J. Addict.* 12, 911–922. doi: 10.3109/10826087709027260

Sims-Williams, H., Matthews, J. C., Talbot, P. S., Love-Jones, S., Brooks, J. C., Patel, N. K., et al. (2017). Deep brain stimulation of the periaqueductal gray releases endogenous opioids in humans. *NeuroImage* 146, 833–842. doi: 10.1016/j.neuroimage.2016.08.038

Steinhorn, R., McPherson, C., Anderson, P. J., Neil, J., Doyle, L. W., and Inder, T. (2015). Neonatal morphine exposure in very preterm infants—cerebral development and outcomes. *J. Pediatr.* 166, 1200–1207. doi: 10.1016/j.jpeds.2015.02.012

Strauss, M. E., Starr, R. H., Ostrea, E. M., Chavez, C. J., and Stryker, J. C. (1976). Behavioural concomitants of prenatal addiction to narcotics. *J. Pediatr.* 89, 842–846. doi: 10.1016/s0022-3476(76)80822-0

Sundelin Wahlsten, V., and Sarman, I. (2013). Neurobehavioural development of preschool-age children born to addicted mothers given opiate maintenance treatment with buprenorphine during pregnancy. *Acta Paediatr.* 102, 544–549. doi: 10.1111/apa.12210

Toubia, T., and Khalife, T. (2019). The endogenous opioid system: role and dysfunction caused by opioid therapy. *Clin. Obstet. Gynecol.* 62, 3–10. doi: 10.1097/GRF.0000000000000409

Valentino, R. J., and Volkow, N. D. (2020). Drugs, sleep, and the addicted brain. *Neuropsychopharmacology* 45, 3–5. doi: 10.1038/s41386-019-0465-x

Van Bockstaele, E. J., and Valentino, R. J. (2013). Neuropeptide regulation of the locus coeruleus and opiate-induced plasticity of stress responses. *Adv. Pharmacol.* 68, 405–420. doi: 10.1016/B978-0-12-411512-5.00019-1

Ward, P., Moss, H. G., Brown, T. R., Kalivas, P., and Jenkins, D. D. (2020). N-acetylcysteine mitigates acute opioid withdrawal behaviors and CNS oxidative stress in neonatal rats. *Pediatr. Res.* 88, 77–84. doi: 10.1038/s41390-019-0728-6

Weathers, L., Driver, K., Zaritt, J., Kneusel, M., Reinhart, R., Roberts, S., et al. (2015). Safety, acceptability and feasibility of auricular acupuncture in neonatal abstinence syndrome: a pilot study. *Med. Acupunct.* 27, 453–460. doi: 10.1089/acu.2015.1133

Wen, H. L., Ho, W. K., Wong, H. K., Mehal, Z. D., Ng, Y. H., and Ma, L. (1978). Reduction of adrenocorticotropic hormone (ACTH) and cortisol in drug addicts treated by acupuncture and electrical stimulation (AES). *Comp. Med. East West* 6, 61–66. doi: 10.1142/s0147291778000095

Wilson, G. S., McCreary, R., Kean, J., and Baxter, J. C. (1979). The development of preschool children of heroin-addicted mothers: a controlled study. *Pediatrics* 63, 135–141. doi: 10.1016/S0022-3476(79)80107-9

Witt, C. E., Rudd, K. E., Bhatraju, P., Rivara, F. P., Hawes, S. E., and Weiss, N. S. (2017). Neonatal abstinence syndrome and early childhood morbidity and mortality in Washington state: a retrospective cohort study. *J. Perinatol.* 37, 1124–1129. doi: 10.1038/jp.2017.106

Witt, N., Coynor, S., Edwards, C., and Bradshaw, H. (2016). A guide to pain assessment and management in the neonate. *Curr. Emerg. Hosp. Med. Rep.* 4, 1–10. doi: 10.1007/s40138-016-0089-y

Wu, L. Z., Cui, C. L., and Han, J. S. (1999). Treatment of heroin addicts by 4-channel Han's acupoint nerve stimulator (HANS). *J. BeiJ. Med. Univ.* 31, 239–242.

Yakunina, N., Kim, S. S., and Nam, E. C. (2017). Optimization of transcutaneous vagus nerve stimulation using functional MRI. *Neuromodulation* 20, 290–300. doi: 10.1111/ner.12541

Conflict of Interest: BB and DJ are named inventors on brain stimulation patents/devices assigned to the Medical University of South Carolina. BB has equity in Bodhi NeuroTech, Inc. NK, SW, and AC are employees and shareholders of Spark Biomedical, Inc.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Human Neuroscience

REVIEW
published: 23 March 2021
doi: 10.3389/fnhum.2020.568051

International Consensus Based Review and Recommendations for Minimum Reporting Standards in Research on Transcutaneous Vagus Nerve Stimulation (Version 2020)

Adam D. Farmer[1], Adam Strzelczyk[2], Alessandra Finisguerra[3], Alexander V. Gourine[4], Alireza Gharabaghi[5], Alkomiet Hasan[6,7], Andreas M. Burger[8], Andrés M. Jaramillo[9], Ann Mertens[10], Arshad Majid[11], Bart Verkuil[12], Bashar W. Badran[13], Carlos Ventura-Bort[14], Charly Gaul[15], Christian Beste[16], Christopher M. Warren[17], Daniel S. Quintana[18,19,20], Dorothea Hämmerer[21,22,23], Elena Freri[24], Eleni Frangos[25], Eleonora Tobaldini[26,27], Eugenijus Kaniusas[28,29], Felix Rosenow[2], Fioravante Capone[30], Fivos Panetsos[31], Gareth L. Ackland[32], Gaurav Kaithwas[33], Georgia H. O'Leary[13], Hannah Genheimer[34], Heidi I. L. Jacobs[35,36], Ilse Van Diest[37], Jean Schoenen[38], Jessica Redgrave[11], Jiliang Fang[39], Jim Deuchars[40], Jozsef C. Széles[41], Julian F. Thayer[42], Kaushik More[43,44], Kristl Vonck[10], Laura Steenbergen[45], Lauro C. Vianna[46], Lisa M. McTeague[13], Mareike Ludwig[47], Maria G. Veldhuizen[48], Marijke De Couck[49,50], Marina Casazza[51], Marius Keute[5], Marom Bikson[52], Marta Andreatta[34,53], Martina D'Agostini[37], Mathias Weymar[14,54], Matthew Betts[47,55,56], Matthias Prigge[44], Michael Kaess[57,58], Michael Roden[59,60,61], Michelle Thai[62], Nathaniel M. Schuster[63], Nicola Montano[26,27], Niels Hansen[64,65], Nils B. Kroemer[66], Peijing Rong[67], Rico Fischer[68], Robert H. Howland[69], Roberta Sclocco[70,71], Roberta Sellaro[72,73,74], Ronald G. Garcia[75,76], Sebastian Bauer[2], Sofiya Gancheva[59,60,77], Stavros Stavrakis[78], Stefan Kampusch[28,29], Susan A. Deuchars[40], Sven Wehner[79], Sylvain Laborde[80], Taras Usichenko[81,82], Thomas Polak[83], Tino Zaehle[84], Uirassu Borges[80,85], Vanessa Teckentrup[66], Vera K. Jandackova[86,87], Vitaly Napadow[70,71] and Julian Koenig[57,88] on behalf of tVNS-ConsensusGroup*

[1] Department of Gastroenterology, University Hospitals of North Midlands NHS Trust, Stoke on Trent, United Kingdom, [2] Department of Neurology, Epilepsy Center Frankfurt Rhine-Main, Goethe-University Frankfurt, Frankfurt am Main, Germany, [3] Scientific Institute, IRCCS E. Medea, Pasian di Prato, Italy, [4] Department of Neuroscience, Physiology and Pharmacology, Centre for Cardiovascular and Metabolic Neuroscience, University College London, London, United Kingdom, [5] Institute for Neuromodulation and Neurotechnology, University Hospital and University of Tuebingen, Tuebingen, Germany, [6] Department of Psychiatry, Psychotherapy and Psychosomatics, Medical Faculty, University of Augsburg, Augsburg, Germany, [7] Department of Psychiatry and Psychotherapy, University Hospital, LMU Munich, Munich, Germany, [8] Laboratory for Biological Psychology, Faculty of Psychology and Educational Sciences, University of Leuven, Leuven, Belgium, [9] Leibniz Institute for Neurobiology, Magdeburg, Germany, [10] Department of Neurology, Institute for Neuroscience, 4Brain, Ghent University Hospital, Gent, Belgium, [11] Sheffield Institute for Translational Neuroscience (SITraN), University of Sheffield, Sheffield, United Kingdom, [12] Clinical Psychology and the Leiden Institute of Brain and Cognition, Leiden University, Leiden, Netherlands, [13] Department of Psychiatry, Medical University of South Carolina, Charleston, SC, United States, [14] Department of Biological Psychology and Affective Science, Faculty of Human Sciences, University of Potsdam, Potsdam, Germany, [15] Migraine and Headache Clinic Koenigstein, Königstein im Taunus, Germany, [16] Cognitive Neurophysiology, Department of Child and Adolescent Psychiatry, Faculty of Medicine, TU Dresden, Dresden, Germany, [17] Utah State University, Logan, UT, United States, [18] NORMENT, Division of Mental Health and Addiction, University of Oslo and Oslo University Hospital, Oslo, Norway, [19] Department of Psychology, University of Oslo, Oslo, Norway, [20] KG Jebsen Centre for Neurodevelopmental Disorders, University of Oslo, Oslo, Norway, [21] Medical Faculty, Institute of Cognitive Neurology and Dementia Research, Otto-von-Guericke University, Magdeburg, Germany

OPEN ACCESS

Edited by:
Yusuf Ozgur Cakmak, University of Otago, New Zealand

Reviewed by:
Chunhong Liu, Capital Medical University, China
Teresa Schuhmann, Maastricht University, Netherlands

***Correspondence:**
Julian Koenig
julian.koenig@med.uni-heidelberg.de

Specialty section:
This article was submitted to Brain Imaging and Stimulation, a section of the journal Frontiers in Human Neuroscience

Received: *31 May 2020*
Accepted: *01 September 2020*
Published: *23 March 2021*

[22] Institute of Cognitive Neuroscience, University College London, London, United Kingdom, [23] Center for Behavioral Brain Sciences Magdeburg (CBBS), Otto-von-Guericke University, Magdeburg, Germany, [24] Department of Pediatric Neuroscience, Fondazione IRCCS Istituto Neurologico Carlo Besta, Milan, Italy, [25] Pain and Integrative Neuroscience Branch, National Center for Complementary and Integrative Health, NIH, Bethesda, MD, United States, [26] Department of Internal Medicine, Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico, Milan, Italy, [27] Department of Clinical Sciences and Community Health, University of Milan, Milan, Italy, [28] Institute of Electrodynamics, Microwave and Circuit Engineering, TU Wien, Vienna, Austria, [29] SzeleSTIM GmbH, Vienna, Austria, [30] Unit of Neurology, Neurophysiology, Neurobiology, Department of Medicine, Università Campus Bio-Medico di Roma, Rome, Italy, [31] Faculty of Biology and Faculty of Optics, Complutense University of Madrid and Institute for Health Research, San Carlos Clinical Hospital (IdISSC), Madrid, Spain, [32] Translational Medicine and Therapeutics, Barts and The London School of Medicine and Dentistry, William Harvey Research Institute, Queen Mary University of London, London, United Kingdom, [33] Department of Pharmaceutical Sciences, School of Biosciences and Biotechnology, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, India, [34] Department of Biological Psychology, Clinical Psychology and Psychotherapy, University of Würzburg, Würzburg, Germany, [35] Division of Nuclear Medicine and Molecular Imaging, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA, United States, [36] Faculty of Health, Medicine and Life Sciences, School for Mental Health and Neuroscience, Alzheimer Centre Limburg, Maastricht University, Maastricht, Netherlands, [37] Research Group Health Psychology, Faculty of Psychology and Educational Sciences, University of Leuven, Leuven, Belgium, [38] Headache Research Unit, Department of Neurology-Citadelle Hospital, University of Liège, Liège, Belgium, [39] Functional Imaging Lab, Department of Radiology, Guang An Men Hospital, China Academy of Chinese Medical Sciences, Beijing, China, [40] School of Biomedical Science, Faculty of Biological Science, University of Leeds, Leeds, United Kingdom, [41] Division for Vascular Surgery, Department of Surgery, Medical University of Vienna, Vienna, Austria, [42] Department of Psychological Science, University of California, Irvine, Irvine, CA, United States, [43] Institute for Cognitive Neurology and Dementia Research, Otto-von-Guericke-University Magdeburg, Magdeburg, Germany, [44] Neuromodulatory Networks, Leibniz Institute for Neurobiology, Magdeburg, Germany, [45] Clinical and Cognitive Psychology and the Leiden Institute of Brain and Cognition, Leiden University, Leiden, Netherlands, [46] NeuroV̇ASQ̇ - Integrative Physiology Laboratory, Faculty of Physical Education, University of Brasilia, Brasilia, Brazil, [47] Department of Anatomy, Faculty of Medicine, Mersin University, Mersin, Turkey, [48] Mental Health and Wellbeing Research Group, Vrije Universiteit Brussel, Brussels, Belgium, [49] Faculty of Health Care, University College Odisee, Aalst, Belgium, [50] Division of Epileptology, Fondazione IRCCS Istituto Neurologico C. Besta, Milan, Italy, [51] Department of Neurosurgery, University of Tübingen, Tübingen, Germany, [52] Department of Biomedical Engineering, City College of New York, New York, NY, United States, [53] Department of Psychology, Education and Child Studies, Erasmus University Rotterdam, Rotterdam, Netherlands, [54] Faculty of Health Sciences Brandenburg, University of Potsdam, Potsdam, Germany, [55] Deutsches Zentrum für Neurodegenerative Erkrankungen (DZNE), Magdeburg, Germany, [56] Center for Behavioral Brain Sciences, Otto-von-Guericke University, Magdeburg, Germany, [57] University Hospital of Child and Adolescent Psychiatry and Psychotherapy, University of Bern, Bern, Switzerland, [58] Section for Translational Psychobiology in Child and Adolescent Psychiatry, Department of Child and Adolescent Psychiatry, Centre for Psychosocial Medicine, University of Heidelberg, Heidelberg, Germany, [59] Division of Endocrinology and Diabetology, Medical Faculty, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany, [60] Institute for Clinical Diabetology, German Diabetes Center, Leibniz Center for Diabetes Research at Heinrich Heine University, Düsseldorf, Germany, [61] German Center for Diabetes Research, Munich, Germany, [62] Department of Psychology, College of Liberal Arts, University of Minnesota, Minneapolis, MN, United States, [63] Department of Anesthesiology, Center for Pain Medicine, University of California, San Diego Health System, La Jolla, CA, United States, [64] Department of Psychiatry and Psychotherapy, University of Göttingen, Göttingen, Germany, [65] Laboratory of Systems Neuroscience and Imaging in Psychiatry (SNIPLab), University of Göttingen, Göttingen, Germany, [66] Department of Psychiatry and Psychotherapy, University of Tübingen, Tübingen, Germany, [67] Institute of Acupuncture and Moxibustion, China Academy of Chinese Medical Sciences, Beijing, China, [68] Department of Psychology, University of Greifswald, Greifswald, Germany, [69] Department of Psychiatry, University of Pittsburgh School of Medicine, UPMC Western Psychiatric Hospital, Pittsburgh, PA, United States, [70] Department of Radiology, Athinoula A. Martinos Center for Biomedical Imaging, Massachusetts General Hospital, Charlestown, MA, United States, [71] Department of Radiology, Logan University, Chesterfield, MO, United States, [72] Cognitive Psychology Unit, Institute of Psychology, Leiden University, Leiden, Netherlands, [73] Leiden Institute for Brain and Cognition, Leiden, Netherlands, [74] Department of Developmental Psychology and Socialisation, University of Padova, Padova, Italy, [75] Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown, MA, United States, [76] Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School, Boston, MA, United States, [77] Heart Rhythm Institute, University of Oklahoma Health Sciences Center, Oklahoma City, OK, United States, [78] Faculty of Biological Science, School of Biomedical Science, University of Leeds, Leeds, United Kingdom, [79] Department of Surgery, University Hospital Bonn, Bonn, Germany, [80] Department of Performance Psychology, Institute of Psychology, Deutsche Sporthochschule, Köln, Germany, [81] Department of Anesthesiology, University Medicine Greifswald, Greifswald, Germany, [82] Department of Anesthesia, McMaster University, Hamilton, ON, Canada, [83] Laboratory of Functional Neurovascular Diagnostics, AG Early Diagnosis of Dementia, Department of Psychiatry, Psychosomatics and Psychotherapy, University Clinic Würzburg, Würzburg, Germany, [84] Department of Neurology, Otto-von-Guericke University, Magdeburg, Germany, [85] Department of Social and Health Psychology, Institute of Psychology, Deutsche Sporthochschule, Köln, Germany, [86] Department of Epidemiology and Public Health, Faculty of Medicine, University of Ostrava, Ostrava, Czechia, [87] Department of Human Movement Studies, Faculty of Education, University of Ostrava, Ostrava, Czechia, [88] Section for Experimental Child and Adolescent Psychiatry, Department of Child and Adolescent Psychiatry, Centre for Psychosocial Medicine, University of Heidelberg, Heidelberg, Germany

Given its non-invasive nature, there is increasing interest in the use of transcutaneous vagus nerve stimulation (tVNS) across basic, translational and clinical research. Contemporaneously, tVNS can be achieved by stimulating either the auricular branch or the cervical bundle of the vagus nerve, referred to as transcutaneous auricular vagus nerve stimulation(VNS) and transcutaneous cervical VNS, respectively. In order to advance the field in a systematic manner, studies using these technologies need to adequately report sufficient methodological detail to enable comparison of results between studies, replication of studies, as well as enhancing study participant safety. We systematically reviewed the existing tVNS literature to evaluate current reporting practices. Based on this review, and consensus among participating authors, we propose a set of minimal reporting items to guide future tVNS studies. The suggested items address specific technical aspects of the device and stimulation parameters. We also cover general recommendations including inclusion and exclusion criteria for participants, outcome parameters and the detailed reporting of side effects. Furthermore, we review strategies used to identify the optimal stimulation parameters for a given research setting and summarize ongoing developments in animal research with potential implications for the application of tVNS in humans. Finally, we discuss the potential of tVNS in future research as well as the associated challenges across several disciplines in research and clinical practice.

Keywords: transcutaneous vagus nerve stimulation, minimum reporting standards, guidelines & recommendations, transcutaneous auricular vagus nerve stimulation, transcutaneous cervical vagus nerve stimulation

INTRODUCTION

Brief History of Transcutaneous Vagus Nerve Stimulation

The vagus nerve (VN) is the Xth cranial nerve and the longest nerve, which courses from the brainstem to the distal third of the colon. It is the main neural substrate of the parasympathetic nervous system and is composed of afferent and efferent pathways, although the former predominate (80%) (Butt et al., 2020). As part of a complex network of neural structures that serves to maintain psychophysiological balance in the organism, its importance cannot be underestimated. The vagus "nerve" is actually two nerves, a left vagus and a right vagus, with slightly different neural origins and targets. It is composed of different types of fibers that vary in myelination, size, and conduction speed (e.g., *for an excellent review on vagus nerve physiology see* Yuan and Silberstein, 2016a,b). Three types of fibers have been identified, each with distinct physiological properties. In general, the larger the fiber, the faster the conduction speed. Myelinated A-fibers are composed of small and large fibers. The small fibers are visceral afferent fibers and the large are both afferent and efferent somatic fibers. Afferent and efferent preganglionic fibers are called B-fibers. Finally, ∼70% of all vagal fibers are unmyelinated C-fibers and convey visceral information from the vast array of visceral organs. Acetylcholine is the primary neurotransmitter of the vagus nerve. It activates cholinergic receptors that are subdivided into nicotinic and muscarinic receptors. However, there is evidence of cross-talk between the vagus and sympathetic nerve fibers as evidenced by tyrosine hydroxylase in the thoracic and cervical trunks of the vagus. There are four vagal nuclei in the medulla, each with distinct but often overlapping targets. The nucleus ambiguus is the source of most cardiovagal motor neurons. The dorsal motor nucleus also contains some cardiovagal motor neurons but primarily innervates the subdiaphragmatic visceral organs. The nucleus of the solitary tract (NTS) is the major hub for afferent information. Finally, the spinal nucleus of the trigeminal nerve, via the superior jugular ganglion, transmits afferent and efferent impulses primarily from the head and vocal structures and has several branches including the auditory branch (Yuan and Silberstein, 2016a). Furthermore, the vagus nerve has projections to higher brain centers including the prefrontal cortex primarily via synaptic connections in the NTS (Thayer and Lane, 2009). In addition, there may be variation among species in the anatomy and physiology of the vagus requiring comparisons of studies across species to be done mindfully. An understanding of the complex anatomy and physiology of the vagus nerve is essential to an understanding of vagus nerve stimulation.

According to the reports of historians and archaeologists, clinical applications of auricular stimulation (broadly defined) were used across many ancient cultures. For instance, tactile stimulation of the auditory meatus was mentioned in some of the earliest known texts on Chinese medicine and acupuncture (Hou

et al., 2015). Interestingly, therapeutic auricular stimulation was not confined to China, and was prevalent across many cultures. Thousands of years ago, the practice of cauterizing a portion of the auricle was common amongst certain tribes in Arabia, while in ancient Egypt, women pricked or cauterized the external auricle for contraceptive purposes and physicians in ancient Persia treated sciatic pain and sexually-related diseases by auricular cauterization (Hou et al., 2015). The Italian anatomist and surgeon Antonio Valsalva published his famous Tractatus de Aure Humana, where he described the treatment of toothache by scarification of the antitragus (Valsalva, 1704). In the last half of the twentieth century, the auricular acupuncture (i.e., needling of specific areas of external auricle) became popular in clinical medicine (Nogier, 1957). Based on Nogier's work in the German Journal of Acupuncture 1957, the Nanjing Army Ear Acupuncture Research Group from China further evaluated auricular somatotopy (Huang, 1974), and auricular acupuncture developed as a unique "microsystem" for acupuncture therapy. Currently, auricular acupuncture, which can mimic transcutaneous vagus nerve stimulation (tVNS), is reported in numerous systematic reviews to be effective in treatment of insomnia and relief of acute and chronic pain (Vieira et al., 2018). Ultimately, there is a great deal of overlap between acupuncture, particularly electroacupuncture, and neuromodulation therapies such as tVNS (Usichenko et al., 2017b), and the rich evidence base supporting auricular acupuncture should be better integrated to help inform further development of tVNS therapy (Napadow, 2019).

The origins of VN stimulation (VNS) date back in excess of 100 years. In the late eighteenth and early nineteenth century, it was believed that epilepsy was caused by excessive blood flow to the brain, termed venous hyperaemia, with patients frequently being treated by manual compression of the carotid arteries in the neck to suppress blood flow. In the late nineteenth century, American neurologist *James L. Corning* developed a "carotid fork"—a device to facilitate carotid compression, which was later augmented by stimulation electrodes. Corning intended to stimulate cervical branches of the VN, which course in close proximity to the carotid artery, in order to decrease heart rate (HR) and, subsequently, blood flow to the brain. Even though Corning reported treatment success, the method was not widely accepted at the time due to safety concerns and a lack of reproducibility of therapeutic response (Lanska, 2002).

Implantable VNS (iVNS) was developed by Jake Zabara in the 1980s as it was found to have promising antiepileptic effects in canine models (Zabara, 1985, 1992) and proceeded to become one of the earliest forms of neuromodulation in humans (Yuan and Silberstein, 2016a). Globally, by 2014 over 100,000 patients have had iVNS implanted (Johnson and Wilson, 2018; *also see* Chakravarthy et al., 2015). The first controlled clinical trials of iVNS as a treatment for refractory epilepsy were conducted in the early 1990s (Penry and Dean, 1990; Uthman et al., 1993) and reported substantial reductions in seizure frequency, even though a significant proportion of patients did not display a symptomatic improvement. Following a number of further clinical trials, iVNS, applied to the left cervical VN, was approved by the US Food and Drug Administration (FDA) for management of pharmacoresistant epilepsy in 1997 (Morris et al., 2013). In subsequent observational studies of patients with epilepsy, it was reported that patients' mood improved following iVNS treatment (Harden et al., 2000). These results spawned a series of studies in patients with depression which led, in 2005, to FDA approval of iVNS for the treatment of pharmacoresistant depression (Cristancho et al., 2011; Desbeaumes Jodoin et al., 2018). More recently, iVNS has been evaluated as treatment for a diverse array of disorders including heart failure (Ferrari et al., 2011; Wang et al., 2015a), rheumatoid arthritis (Koopman et al., 2016), inflammatory bowel disease (Levine et al., 2014), sepsis (Wang et al., 2016; Yang et al., 2017), and chronic pain (Lange et al., 2011).

iVNS necessitates a costly, invasive surgical procedure involving the implantation of a bipolar helical electrode to the left cervical VN which is subsequently attached to a pulse generator, most frequently positioned in a left infraclavicular subcutaneous pocket. Non-invasive tVNS approaches have been developed as a less expensive, patient friendly and rapidly deployable alternative. Transcutaneous cervical VNS (tcVNS) is conceptually similar to Corning's initial approach of transcutaneously stimulating the VN in the neck, adjacent to the carotid artery. tcVNS is FDA-approved for the treatment of migraine and cluster headache management and has been subjected to intensive research effort (Goadsby et al., 2014; Nesbitt et al., 2015).

The most widely commercially available tcVNS device (gammaCore®, electroCore, Inc.) is hand-held and delivers sinusoidal alternating current with a broadband amplitude-modulated frequency spectrum (Nesbitt et al., 2015). In the USA, the gammaCore® device received FDA approval for the treatment of acute cluster headache treatment in 2017, and for acute migraine treatment and adjunctive cluster headache prevention in 2018. Transcutaneous auricular VNS (taVNS) is under investigation for a wide range of clinical applications, however, is not FDA-cleared for the treatment of any disorder. The most widely used commercially available taVNS device (NEMOS®, tVNS technologies) delivers current in rhythmic square pulses (Yuan and Silberstein, 2016b). The NEMOS® device received European certification (CE certification, which indicates legal conformity and safety, but not necessarily clinical efficacy) as a treatment for epilepsy and depression in 2010, for chronic pain in 2012 and for anxiety in 2019. Importantly, given their non-invasiveness, tcVNS and taVNS are widely used not only for clinical purposes, but also in healthy populations for basic research in cognitive neuroscience and related fields (Yuan and Silberstein, 2016b). The increased availability of these devices, coupled with their user friendliness, has resulted in an increase in research publications on tVNS, see **Figure 1**.

An array of stimulation parameters needs to be considered when it comes to using tVNS in both research and clinical settings. Stimulation parameters of tVNS can vary in terms

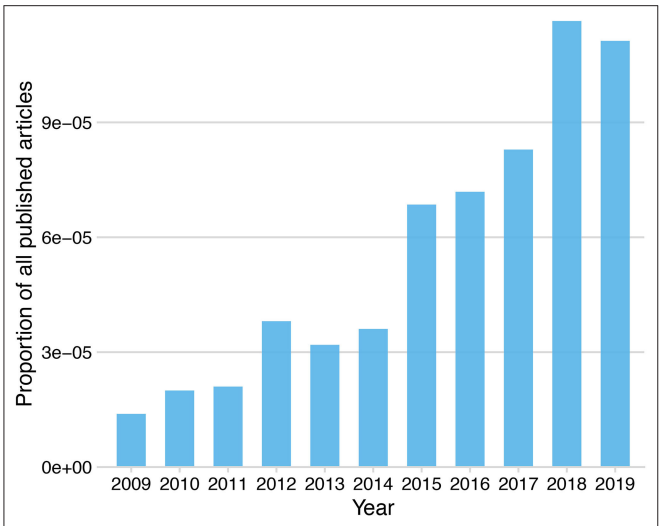


FIGURE 1 | Proportion of published research articles including the keyword "transcutaneous vagus nerve stimulation" listed on PubMed by year.

of its current intensity (mA), pulse width (μs), frequency (Hz), duty cycle (s), and session duration (min) (Badran et al., 2019). Furthermore, side effects of stimulation, type of sham or control stimulation, location of the stimulation and sham electrode placement may influence the outcomes of tVNS. The impact of each of these stimulation parameters on psychophysiology and on clinical outcomes is incompletely understood. Despite the increasing number of studies, there is no clear consensus regarding the optimal parameters that need to be adopted for tVNS research. Moreover, there is no clear consensus regarding the minimal standard reporting items within the tVNS literature. Recently, calls for full disclosure of tVNS stimulation parameters have been made (Redgrave et al., 2018; Burger et al., 2020a). Herein, we aim to provide multidisciplinary recommendations regarding standard reporting items for future tVNS research. These recommendations are based on a systematic review of existing tVNS studies, evaluation of current reporting practices and finally on a broad consensus among research groups studying tVNS.

VNS Nomenclature: Techniques and Targets

The following section reviews four currently accepted VNS modalities—(1) cervically implanted VNS (iVNS), (2) transcutaneous cervical VNS (tcVNS), (3) transcutaneous auricular VNS (taVNS) (4) percutaneous auricular VNS (paVNS). For simplicity, hereinafter, we will refer to both transcutaneous forms of VNS (taVNS and tcVNS) as tVNS.

Cervically Implanted VNS (iVNS)

Classically, the VN is stimulated via implanted electrodes targeting (mostly) the left cervical branch of the VN (Mertens et al., 2018; Kaniusas et al., 2019a). iVNS commonly uses a bipolar cuff electrode (e.g., VNS Therapy, LivaNova). Despite being well-established, this method remains expensive and is associated with peri and post implantation risks. Furthermore, the electrode implant is irreversible. Moreover, stimulation is not restricted to afferent fibers of the cervical VN—as usually targeted by the therapy—but extends to (visceral) efferent fibers of the VN as well (Howland, 2014). Consequently, several adverse effects such as cough, voice alteration, swallowing difficulties, or bradycardia have been reported (Liporace et al., 2001).

Transcutaneous Cervical VNS (tcVNS)

The cervical VN can also be stimulated transcutaneously by using two skin electrodes, e.g., by a hand-held device (e.g., GammaCore, electroCore, Inc.), which are applied at the neck (Barbanti et al., 2015; Gaul et al., 2016; Silberstein et al., 2016a; Frangos and Komisaruk, 2017). This form of transcutaneous stimulation is now FDA approved for the acute treatment of migraine and for the acute treatment and prevention of episodic cluster headache. However, despite its relative convenience, this method is not devoid of adverse effects. Given that tVNS requires the stimulation to pass through the skin barrier, relatively strong currents are needed. The resulting stimulation fields in the neck are diffuse, so that cervical non-vagal nerves can be co-stimulated, as well as efferent cervical fibers. Commonly observed adverse effects for the cervical tVNS approach include prickling at the stimulation site, neck pain, dizziness, headache, nasopharyngitis, oropharyngeal pain and sensitivity to the conducting gel (Gaul et al., 2016; Redgrave et al., 2018). An MRI-derived *Finite Element Method* model was developed to analyze the cellular components activated with tcVNS. Due to the different types of tissue between the surface electrodes on the skin and the VN, both macroscopic (skin, muscle, fat) and mesoscopic (nerve sheath, cerebrospinal fluid) components were used to predict activation thresholds and electric field changes. It was demonstrated that the overall current requirement to achieve adequate stimulation is influenced by deeper tissues and that tissue conductivity has a direct effect on axon membrane polarization. This model predicts that tcVNS will activate A and B axon fibers, but not C fibers (Mourdoukoutas et al., 2018).

Transcutaneous Auricular VNS (taVNS)

The auricular branch of the VN is primarily an afferent fiber which innervates the ear and joins the main bundle of the VN projecting to the nucleus tractus solitarius (NTS). taVNS is achieved via surface skin electrodes applied in the vagally-innervated ear regions (Ellrich, 2011) on the outer ear (Ellrich, 2011; Frangos et al., 2015; Straube et al., 2015; Badran et al., 2018a,b). Typically, taVNS uses two surface electrodes (e.g., NEMOS, tVNS Technologies GmbH). This method is CE marked (but not FDA approved) for epilepsy, depression, anxiety, pain, and migraine. A relatively large surface of electrodes yields diffuse stimulation fields, so that not only vagal but also non-vagal auricular nerves can be recruited, the implications of which remain controversial (Kaniusas et al., 2019a). The stimulation is considered safe (Badran et al., 2018c). As in

the case of the percutaneous tcVNS, the expected side effects are mostly minor and may include headache, pain and skin irritation at the stimulation site, and dizziness (Mertens et al., 2018). Researchers are still working to determine optimal ear targeting approaches as there is paucity of data comprehensively describing the innervation of the ear. An anatomical dissection of the human auricle describes how the auricular branch of the VN diffusely innervates the ear (Peuker and Filler, 2002), with the cymba concha region being exclusively innervated by the auricular branch of the VN, along with other areas such as the posterior and inferior walls of the ear canal. Many of these targets are hypothesized to be regions for engagement of vagal afferents (Badran et al., 2018a; Burger and Verkuil, 2018). Various ear targets, practical procedures and electrode placement techniques for taVNS in the laboratory or clinical setting have been outlined along with stimulation parameter considerations (Badran et al., 2019; Sclocco et al., 2019, 2020).

Percutaneous Auricular VNS (paVNS)

A minimally invasive form of paVNS (Kampusch et al., 2013) can be performed with miniature needle electrodes penetrating the skin in the targeted outer ear regions innervated mainly by the auricular branch of the VN (Sator-Katzenschlager et al., 2004; Kaniusas et al., 2019b). This so-called paVNS typically uses 2–3 needle electrodes (e.g., AuriStim, DyAnsys). The small size of needle electrodes and the resulting spatially focused stimulating fields favors precise and specific stimulation of the local afferent auricular branch VN endings. Minor side effects of paVNS are local skin irritation (dermatitis), local bleeding, pain at the stimulation site, and dizziness.

Thus, the term tVNS is a broadly encompassing term and is not location-specific, i.e., neck or ear, as both tcVNS and taVNS may have similar biological effects. It is important to note there is limited data on the head-to-head testing of tcVNS with taVNS and these studies should be explored.

In the following sections, we will focus on tVNS, given that these techniques have been subject to intensive study. The key difference between taVNS and tcVNS is the branch of the VN which is putatively targeted. taVNS targets the auricular branch of the VN, a subsidiary from both left and right VN main bundles that innervates the ear on the same side (Peuker and Filler, 2002). In contrast, tcVNS targets the cervical branch of the VN (Barbanti et al., 2015; Gaul et al., 2016; Silberstein et al., 2016b; Frangos and Komisaruk, 2017). It remains unclear whether neck and ear stimulation produce similar biological and end organ effects.

tVNS can also be associated with indirect sporadic effects, due to rare afferent-efferent vagal reflexes via the NTS. For instance, tVNS may sometimes cause a reflexive cough, colloquially known as Arnold's ear-cough reflex. Other vegetative reflexes, such as the ear-gag reflex, ear-lacrimation reflex, ear-syncope reflex, and vaso-vagal reflex can also be observed, albeit relatively rarely (Tekdemir et al., 1998; Ellrich, 2011; Napadow et al., 2012). Overall, tVNS is associated with fewer side effects in comparison to iVNS, which has the potential to be associated with increased tolerability. In addition, portable devices are relatively easy to handle and are more cost effective than implantable devices (Morris et al., 2016).

MODES OF APPLICATION

Long-Term Stimulation in Clinical Trials and Intervention Studies

Epilepsy

The effect of tVNS on pharmacoresistant epilepsy has been investigated in several studies. An early pilot study (Stefan et al., 2012) demonstrated that seizure frequency was reduced in five out of seven patients after 9 months of tVNS therapy, and that tVNS was well-tolerated. This reduction was also observable in a larger sample size over a 12 months period (Aihua et al., 2014). Another 6 months pilot study (He et al., 2013a) demonstrated seizure frequency reductions in 9 out of 14 children. A more recent 20-week placebo-controlled clinical trial of 76 patients with epilepsy (Bauer et al., 2016) reported that tVNS decreased seizure frequency. However, only about half of the patients were classified as responders—defined as seizure frequency reduction >25%. In a more recent prospective study of 20 patients (Barbella et al., 2018), only one third derived clinical benefit from tVNS. In a randomized clinical trial of 47 patients with epilepsy, Rong et al. reported that after 24 weeks of daily treatment 16% were seizure free and 38% had reduced seizure frequency (Rong et al., 2014). A larger-scale clinical trial of tVNS in epilepsy is pending, as the evidence regarding efficacy is currently insufficient for routine clinical care (Boon et al., 2018). Although the mechanisms of VNS in epilepsy are not fully understood, it is suggested that the nuclei of the brainstem are involved. The NTS has direct or indirect projections toward the locus coeruleus (LC) and raphe nuclei, which are suggested to be associated with seizures through noradrenergic and serotonergic neurons, exerting an antiepileptic effect. In particular, antiepileptic effects have been associated with an increase in norepinephrine (Krahl and Clark, 2012; Panebianco et al., 2016). Another theory suggests that VNS can activate inhibitory structures in the brain, with an increase in free gamma-aminobutyric acid (GABA) levels in cerebrospinal fluid and GABA-A receptor density in the hippocampus of patients who responded favorably (Marrosu et al., 2003). In recent years, the idea has grown that inflammation is involved in the development of seizures and epilepsy and, therefore, activation of anti-inflammatory pathways through VNS could explain antiepileptic effects (Krahl and Clark, 2012; Bonaz et al., 2013; Panebianco et al., 2016). Early work in rats indicated that the recruitment of vagal C-fibers is necessary for the suppression of seizures, by activating the C-fibers of the Vagal nerve (Woodbury and Woodbury, 1990), mediating GABA and glycine levels.

Depression

A placebo-controlled pilot study of patients with depression (Hein et al., 2013) found that 2 weeks of tVNS decreased depression severity using validated measures. This finding was replicated later in a larger patient sample, although this non-randomized study identified only about one third of the patients enrolled as tVNS responders (Rong et al.,

2016). Neuroimaging studies in mild to moderately depressed patients have demonstrated that tVNS altered functional brain connectivity in the default mode network (Fang et al., 2016; Liu et al., 2016) and led to insula activation that was correlated with the clinical effectiveness of tVNS treatment (Fang et al., 2017). Furthermore, a decrease in functional connectivity between the bilateral medial hypothalamus and rostral anterior cingulate cortex (rACC) (Tu et al., 2018), as well as an increase of functional connectivity between the left nucleus accumbens and bilateral rACC (Wang et al., 2018) during 4 weeks of tVNS treatment were reported. Another potential mechanism, by which tVNS may exert an antidepressant action, is the modulation of inflammatory processes that are currently discussed (Rawat et al., 2019; *also see* Pavlov and Tracey, 2012). A previous review has summarized existing research on tVNS in depression in greater detail (Kong et al., 2018 also see Lv et al., 2019).

Tinnitus

A third clinical field in which several tVNS studies exist is tinnitus. One pilot study (Lehtimäki et al., 2013) found that 10 days of tVNS, combined with sound therapy, ameliorated patient-reported tinnitus severity and attenuated their auditory event-related field signal on magnetoencephalography (MEG). Another pilot study similarly observed a clinically meaningful amelioration of patient-reported tinnitus severity in four out of 10 patients after 20 days of combined tVNS and sound therapy (De Ridder et al., 2014). This has been replicated in a larger sample (30 patients), 15 of which were classified as responders to combined tVNS and sound therapy (Shim et al., 2015). However, a further pilot study administering tVNS (without sound therapy) for 6 months did not show any clinically meaningful effect (Kreuzer et al., 2014). It appears that the VNS generates improvements in patients with tinnitus due to the suppression of auditory, limbic and other areas of the brain involved in the generation / perception of tinnitus through the ascending auditory and vagal pathways (Yakunina et al., 2018). The rationale for the treatment of tinnitus using tVNS is build around the idea, that tVNS together with the presentation of tones can boost neuronal plasticity: the joint use of VNS and tones produces a reduction in the activity of the gamma band in the left auditory cortex, as well as the phase coherence between the cortex. Auditory and areas associated with tinnitus distress, including the cingulate cortex (Vanneste et al., 2017).

Other Clinical Conditions

The effect of tVNS on a variety of other diseases has been explored. A pilot study of tVNS in schizophrenia found no effect on symptom severity (Hasan et al., 2015). Moreover, many potential targets for treatment via tVNS have been suggested, including attention deficit hyperactivity disorder (ADHD) (Beste et al., 2016), autism spectrum disorders (Jin and Kong, 2016), Alzheimer's dementia (Jacobs et al., 2015), post-operative cognitive dysfunction (Xiong et al., 2009), increased risk of type II diabetes (Huang et al., 2014), preterm infants with oromotor dysfunction (Badran et al., 2020), chronic stroke patients (Capone et al., 2017), coronary insufficiency (Afanasiev et al., 2016) and chronic migraine (Straube et al., 2015). The idea that tVNS might be a promising treatment in Alzheimer's dementia has received support through recent evidence that tVNS can recover impaired microglia function in a mouse model of Alzheimer's dementia (Kaczmarczyk et al., 2017; Huffman et al., 2019), and there is an ongoing clinical trial of tVNS as a treatment for mild cognitive impairment (NCT03359902). For ADHD, trigeminal nerve stimulation (TNS) has been suggested as a complementary treatment to tVNS, and a recent study found promising clinical improvements (McGough et al., 2019). A study in patients with chronic pelvic pain (Napadow et al., 2012) found that tVNS ameliorated patient-reported pain intensity and anxiety. Antinociceptive effects of tVNS have been replicated in some studies but not in others, and its effect has remained inconsistent between studies and individuals (Laqua et al., 2014; Usichenko et al., 2017a,b; De Icco et al., 2018; Janner et al., 2018).

Whilst the above studies assume that the effects of tVNS are primarily mediated by central neuromodulation, i.e., effects on neurotransmission and neuroplasticity in the brain, tVNS-induced cardiovagal and cardiosympathetic effects have also been reported, and a number of studies have focused on the clinical potential of these effects. For example, a number of studies have found tVNS to reduce sympathetic nerve activity, indexed through resting muscle sympathetic activity (Clancy et al., 2014; Murray et al., 2016b; Ylikoski et al., 2017). However, cardiac effects of tVNS may be related to stimulation parameters, such as pulse width and stimulation frequency (Badran et al., 2018c) and there remains to date unexplained inter-individual variations in the clinical response to these parameters in the treatment of cardiovascular disease (Murray et al., 2016a).

Taken together, these studies indicate that tVNS has the potential to treat a wide range of clinical conditions. One of the key challenges for its further development appears to be the lack of inter-individual consistency in treatment success. Those differences are currently not well-understood, and may relate to anatomical differences, physiological state, and stimulation parameters. **Table 1** provides an overview of the stimulation parameters that have been deployed in various studies along with other characteristics of those reports[1].

Acute/Short-Term Stimulation in Experimental Trials

Alongside clinical trials and intervention studies, tVNS has gained increasing interest as a tool for neuromodulation in experimental studies. Based on evidence that vagal activity is related to a host of psychological and physiological processes, tVNS promises deeper insights by enabling active manipulation of VN activity. Predominantly, these studies are characterized by short stimulation periods, addressing the immediate effects. Psychological targets have been broad (though not all of them are sensitive to tVNS), including: experimentally induced worry (Burger et al., 2019a); post-error slowing (Sellaro et al., 2015b); attention to fearful faces (Verkuil and Burger, 2019); associative memory (Jacobs et al., 2015) or single-item word memory

[1]The review of studies is based on a PubMed search using the keywords "transcutaneous vagus nerve stimulation" OR "tVNS"

TABLE 1 | Reported stimulation parameters in studies on long-term tVNS.

References	Clinical entity	N/ clinical trial	Device	Electrode(s)	Electrode placement	Stimulation length	Alternating stimulation	Stimulus intensity	Pulse width	Hz	Results
Hein et al. (2013)	Depression	37 MDD patients **Study 1**: 22 (11 sham vs. 11 auricular) **Study 2**: 15 (6 once vs. 9 twice a day of stimulation)	**Study 1**: TENS microstimulator unit NET-2000 made by Auri-Stim Medical, Inc., 11172 Huron St. Suite 22, Denver, CO, USA **Study 2**: NET-1000 (self-application by the patients) also made by Auri-Stim Medical		**Study 1**: On both sides, four electrodes were placed crosswise, each with a diameter of about 3 mm Sham: no current **Study 2**: on both sides Sham: manipulated clamp, no current	**Study 1**: 15 min once for 2 weeks on 5 days each week **Study 2**: 15 min twice a day for 2 weeks on 5 days each week		**Study 1**: 0–max. 600 μA **Study 2**: 130 μA		**Study 1**: 1.5 Hz unipolar rectangle waves **Study 2**: 1.5 Hz	2 weeks tVNS resulted in decreased depression severity
Fang et al. (2016) Fang et al. (2017)	Depression	49 MDD patients single-blinded clinical trial	Ear vagus nerve stimulator Institute of Acupuncture and Moxibustion, China Academy to Chinese Medicine Science (Beijing, China) and Suzhou Medical Appliance Factory (Jiangsu Province, China)	Special ear clips (electrodes) (Huang et al., 2014; Rong et al., 2014)	tVNS Auricular conchae Sham Superior scapha	30 min, twice a day, at least 5 days per week for 4 weeks		4–6 mA	2016: <1 ms 2017: 0.2 ms	20 Hz continuous sinusoidal wave	4 weeks tVNS resulted in decreased depression severity tVNS modulates DMN FC
Liu et al. (2016)	Depression	49 MDD patients single-blinded clinical trial	[…] full details of the study are reported elsewhere (Fang et al., 2016; Rong et al., 2016)	[…] full details of the study are reported elsewhere (Fang et al., 2016; Rong et al., 2016)	Applied on both ears simultaneously during treatment tVNS Auricular conchae Sham Superior scapha	30 min, twice a day (morning, evening), at least 5 days per week for 4 weeks **First cohort:** 12 weeks tVNS **Second cohort:** 4 weeks sham and 8 weeks real tVNS		4–6 mA	<1 ms	20 Hz	4 weeks tVNS resulted in decreased depression severity tVNS modulates amygdala-lateral prefrontal rsFC

(Continued)

TABLE 1 | Continued

References	Clinical entity	N/ clinical trial	Device	Electrode(s)	Electrode placement	Stimulation length	Alternating stimulation	Stimulus intensity	Pulse width	Hz	Results
Rong et al. (2016)	Depression	MDD patients non-randomized, controlled study **First cohort:** $N = 91$ **Second cohort:** $N = 69$	Ear vagus nerve stimulator Institute of Acupuncture and Moxibustion, China Academy to Chinese Medicine Science (Beijing, China) and Suzhou Medical Appliance Factory (Jiangsu Province, China)	Special ear clips (electrodes) (Huang et al., 2014; Rong et al., 2014)	tVNS Auricular conchae Sham Superior scapha	**First cohort:** 12 weeks tVNS **Second cohort:** 4 weeks sham tVNS and 8 weeks real tVNS		4–6 mA	0.2 ms	20 Hz continuous sinusoidal wave	Greater symptom reductions during tVNS for the first 4 weeks
Tu et al. (2018)	Depression	41 MDD patients Non-RCT, single-blinded clinical trial	See Ack "[…] supported by […] Chinese Medicine […] Beijing Natural Science […]"	See Ack "[…] supported by […] Chinese Medicine […] Beijing Natural Science […]"	tVNS Auricular conchae Sham Superior scapha	30 min, twice a day (morning, evening) at least 5 days per week, for 4 weeks		4–6 mA	<1 ms	20 Hz	4 weeks tVNS resulted in decreased depression severity During tVNS decreased FC between MH and rACC
Wang et al. (2018)	Depression	41 MDD patients single-blinded, non-randomized clinical study	Ear vagus nerve stimulator Institute of Acupuncture and Moxibustion, China Academy to Chinese Medicine Science (Beijing, China) and Suzhou Medical Appliance Factory (Jiangsu Province, China)	Special ear clips (electrodes) (Huang et al., 2014; Rong et al., 2014)	tVNS Both ears during treatment (during MRI right ear) Suricular conchae Sham Superior scapha	**First cohort:** 12 weeks tVNS **Second cohort:** 4 weeks sham tVNS and 8 weeks real tVNS		4–6 mA	<1 ms	20 Hz continuous sinusoidal wave	During tVNS increased FC between left NAc and MPFC/rACC and negative correlation with changes in symptom severity
Aihua et al. (2014)	Epilepsy	60 pharmacoresistant epilepsy patients (50% sham) Randomized controlled trial	TENS-200, Hua Tuo brand		Bilateral tVNS ($N = 26$) Ramsay-Hunt Zone Sham ($N = 21$) Earlobe	Three times per day, continuous stimulation for 20 min, for 12 months		Median (IQR) 6 mA	0.2 s	30 Hz	Reduced seizure frequency after 12 months of daily tVNS

(Continued)

TABLE 1 | Continued

References	Clinical entity	N/ clinical trial	Device	Electrode(s)	Electrode placement	Stimulation length	Alternating stimulation	Stimulus intensity	Pulse width	Hz	Results
Barbella et al. (2018)	Epilepsy	20 patients with refractory focal epilepsy, drug resistant	NEMOS (cerbomed GmbH, Erlangen, Germany) not cited because the only device used at the time in Italy for tVNS		Left auricolar concha, not cited, but considered by default the site of electrodes!	4 h per day, divided into two-three sessions of at least 1 h each, for 6 months	20 s on/ 5 min off	0.6–0.8 mA			Reduced seizure frequency in about one third of the patients after 6 months of daily tVNS
Bauer et al. (2016) Hamer et al. (2019)	Epilepsy	76 drug-resistant epilepsy, randomized, double-blind clinical trial Low level: 1 Hz $N = 39$ High level: 25 Hz $N = 37$	NEMOS (cerbomed GmbH, Erlangen, Germany) CE certified tVNS device		Left auricular branch of the vagus nerve at the ear conch	4 h daily, for a period of 20 weeks +8-weeks baseline period	30 s on/30 s off	High level: 0.50–0.47 mA Low level: 1.02–0.83 mA	High level: 250 us Low level: 250 us	High level: 25 Hz Low level: 1 Hz	Reduced seizure frequency after 20 weeks of daily tVNS
He et al. (2013a)	Epilepsy	14 pediatric patients with intractable epilepsy	TENS-200, Suzhou, China	Two pairs of electrode clips, made of conductive rubber, 5 mm in diameter	ta-VNS 1× concha cavity 1× concha cymba	Three times a day, 30 min per session, 24 weeks		0.4–1.0 mA		20 Hz	Reduced seizure frequency in nine out of 14 patients during 6 months of tVNS therapy
Rong et al. (2014)	Epilepsy	50 patients with drug-resistant epilepsy, random clinical trial	TENS, Suzhou Medical Appliance Co. Ltd., Suzhou, China	Electrode clamp with two carbon-impregnated silicone electrode tips connected to the TENS by metal wires for electrical stimulations		Twice times a day, 30 min per session, 24 weeks		1 mA	<1 ms	20–30 Hz	Reduced seizure frequency and seizure free patients after 6 months
Stefan et al. (2012)	Epilepsy	10 patients with pharmacoresistant epilepsy, pilot study		Stimulus area ~2 cm^2	tVNS Left ear	Three times per day (1 h in morning, noon, evening), for 9 months		Mean 25 V	300 μs	10 Hz biphasic	Reduced seizure frequency in five out of seven patients after 9 months of tVNS therapy

(Continued)

TABLE 1 | Continued

References	Clinical entity	N/ clinical trial	Device	Electrode(s)	Electrode placement	Stimulation length	Alternating stimulation	Stimulus intensity	Pulse width	Hz	Results
Bretherton et al. (2019) Study 3	HRV	26 older participants	TENS machine (EMS7500 Roscoe Medical)	Customized auricular electrode clips	Inner and outer surface of the tragus of the ear (Auricular Clips, Body Clock Health Care Ltd, UK)	15 min once daily for 2 weeks		2–4 mA	200 μs	30 Hz	Improvement of autonomic function, health-related QoL, mood, sleep after 2 weeks of daily tVNS
Huang et al. (2014)	Impaired glucose tolerance	72 participants with IGT pilot randomized clinical trial	TENS-200 (developed by Suzhou manufacture of Medical Device and Material)		tVNS Auricular conchae Sham Superior scapha	Twice a day, post-prandial treatment lasted 20 min, half an hour after eating, for 12 weeks		1 mA	≤1 Hz	20 Hz	Reduced systolic blood pressure after 12 weeks of daily tVNS
Straube et al. (2015)	Migraine	46 chronic migraine patients Monocentric, randomized, controlled, double-blind study **1 Hz** $N = 22$ **25 Hz** $N = 24$	NEMOS® taVNS device (Cerbomed, Erlangen, Germany)		Concha of outer ear	4 h per day (free to stimulate for additional hour) for 12 weeks	30 s on 30 s off	Individually fitted, adjustment by patient if it was needed	250 μs	1 Hz 25 Hz	1 Hz group had a significantly larger reduction in headache days per 28 days than patients in the 25 Hz group
Hasan et al. (2015)	Schizophrenia	25 schizophrenia patients, bicentric randomized, sham-controlled, double blind pilot study **Group 1**: active tVNS **Group 2**: sham tVNS	CM02, Cerbomed, Erlangen, Germany	Two titan electrodes	Left auricle branch of vagus nerve	Daily stimulation for 26 weeks 1. settling-in phase (3× 1 h/day) 2. adaption phase 1 (3× 2 h/day) 3. adaption phase 2 (3× 3 h/day) Advised to use stimulator whole day **Group 1**: 12 weeks active 14 weeks sham	30 s on 180 s off Duty cycle 14%	0.1–10 mA	250 μs	25 Hz	No improvement of schizophrenia symptoms in 26-weeks tVNS trial

(Continued)

TABLE 1 | Continued

References	Clinical entity	N/ clinical trial	Device	Electrode(s)	Electrode placement	Stimulation length	Alternating stimulation	Stimulus intensity	Pulse width	Hz	Results
						Group 2: 12 weeks sham 14 weeks active					
Capone et al. (2017)	Stroke	14 patients with ischemic or hemorrhagic chronic stroke, randomized (tVNS vs. sham)	Electric stimulator (Twister—EBM)	2 Ag-AgCl electrodes (5 mm in diameter)	**tVNS** left external acoustic meatus at the inner side of the tragus **Sham** earlobe(left)	Stimulation repeated every 5 min for 60 min, for 10 days	30 s	**tVNS** Mean = 2.0–4.5 **Sham** Mean = 2.8–7.2	0.3 ms	20 Hz	tVNS and robotic rehabilitation can improve arm functionality in chronic stroke patients
Kreuzer et al. (2014)	Tinnitus	50 patients with chronic tinnitus, open single-armed pilot study **Phase 1** $N = 24$ **Phase 2** $N = 26$ (new)	**Phase 1** Cerbomed CM02 (Erlangen, Germany) **Phase 2** NEMOS (Erlangen, Germany)			24 weeks **Phase 1** For at least 6 h per day **Phase 2** 4 h per day	**Phase 1** 30 s on 180 s off **Phase 2** 30 s on 30 s off	0.1–10 mA		25 Hz	No clinically meaningful effect after 6 months pf tVNS
Lähtimäki et al. (2013)	Tinnitus	10 patients with tinnitus, pilot study, short-term tVNS and sound therapy	Tinnoff pulse generator (Jarmo Lehtimäki is an employee and Matti Ylikoski and Jukka Ylikoski are board members of Tinnoff Inc.)	Clip electrode	Auricular branch of vagus nerve, clip at left tragus	Seven sessions, each 45–60 min, for 10 days		>0.8 mA		25 Hz	10 days of tVNS ameliorated patient-reported tinnitus severity
Shim et al. (2015)	Tinnitus	30 patients with refractory chronic tinnitus	TENS eco2 (Schwa-medico, Ehringshausen, Germany)	Silicon electrical pad (2 cm in diameter)	Auricular concha of the patient's left ear	10 sessions intervals of 1–4 days		1–10 mA	200 μs	25 Hz	50% reported symptom relief after 10 tVNS sessions

(Giraudier et al., 2020; Mertens et al., 2020); extinction of fear responses or fear conditioning (Burger et al., 2016, 2017, 2018, 2019b; Genheimer et al., 2017; Szeska et al., 2020); implicit spiritual self-representations (Finisguerra et al., 2019); flow experience (Colzato et al., 2018b); response selection during sequential action (Jongkees et al., 2018) or during action cascading processes (Steenbergen et al., 2015); the recognition of emotions in faces or bodies (Colzato et al., 2017; Sellaro et al., 2018; Koenig et al., 2019); divergent thinking (Colzato et al., 2018a); conflict-triggered adjustment of cognitive control (Fischer et al., 2018); auditory selective attention (Rufener et al., 2018) or visual selective attention (Ventura-Bort et al., 2018); inhibitory control (Beste et al., 2016; Borges et al., 2020); automatic motor inhibition (Keute et al., 2018); cognitive flexibility (Borges et al., 2020; Tona et al., 2020); prosocial behavior (Sellaro et al., 2015a) and reward sensitivity (Neuser et al., 2019).

Other more physiologically oriented studies have investigated the influence of tVNS on cardiac activity (Brock et al., 2017; De Couck et al., 2017; Lamb et al., 2017; Gancheva et al., 2018; Borges et al., 2019; Bretherton et al., 2019; Koenig et al., 2019; Paleczny et al., 2019; Tobaldini et al., 2019; Tran et al., 2019); autonomic outflow (Sclocco et al., 2017); sympathetic nerve activity (Clancy et al., 2014) or cardiac baroreflex sensitivity (Antonino et al., 2017); atrial fibrillation (Stavrakis et al., 2015); cardiac mechanical function (Tran et al., 2019); vagal sensory evoked potentials (Fallgatter et al., 2003, 2005; Polak et al., 2009; Leutzow et al., 2013); persistent hiccups (Schulz-Stübner and Kehl, 2011); visual bistable perception (Keute et al., 2019a); nociceptive neuromodulation (Napadow et al., 2012; Busch et al., 2013; Laqua et al., 2014; Usichenko et al., 2017b; Janner et al., 2018); tumor necrosis factor-alpha (Brock et al., 2017); hepatic energy metabolism (Gancheva et al., 2018); whole blood culture-derived cytokines and chemokines (Lerman et al., 2016); salivary hormones (Ventura-Bort et al., 2018; Koenig et al., 2019; Warren et al., 2019); pupil diameter (Warren et al., 2019); gastroduodenal or gastrointestinal motility (Frøkjaer et al., 2016; Juel et al., 2017); muscle activity in the gastrointestinal tract (Hong et al., 2019), gastric frequency (Teckentrup et al., 2020); electroencephalography (Hyvärinen et al., 2015; Keute et al., 2018; Lewine et al., 2019) and event-related potentials (Lewine et al., 2019), specifically the P3/P300 event-related potential (Ventura-Bort et al., 2018; Warren et al., 2019); cortical excitability (Capone et al., 2015) and changes in blood-oxygen-level-dependent (BOLD) functional Magnetic Resonance Imaging (fMRI) (Kraus et al., 2007, 2013; Dietrich et al., 2008; Frangos et al., 2015; Frangos and Komisaruk, 2017; Garcia et al., 2017; Yakunina et al., 2017, 2018; Badran et al., 2018b; Peng et al., 2018; Sclocco et al., 2019, 2020). Ultrahigh field (7T) fMRI studies with enhanced spatiotemporal resolution have clearly demonstrated tVNS stimulus-evoked activation of the ipsilateral NTS, the primary synapse for vagus nerve traffic to the brain (Garcia et al., 2017; Sclocco et al., 2019, 2020). Cases reports illustrate the use of tVNS in the treatment of a patient with persistent geotropic direction-changing positional nystagmus (Cha et al., 2016) or insomnia (Yu et al., 2017). **Table 2** summarizes the characteristics of these studies on acute/short-term tVNS.

PROPOSED CHECKLIST FOR MINIMUM REPORTING ITEMS

Based on the review of the existing literature, we propose a set of minimum reporting items for tVNS publications in **Table 3**. Important to note, these are not suggested to replace existing standards or guidelines when reporting observational studies (von Elm et al., 2008) or clinical trials (Moher et al., 2001). **Figure 2** provides a graphical overview of the specific tVNS reporting items.

In regards to stimulation level reporting, our general guidance (consistent with recommended reporting practices for other techniques, e.g., Woods et al., 2016; Bikson et al., 2019) is to fully describe the dose and any further details of electrode design that may impact tolerability. As with other reporting items, how and what details should be reported is guided by the principle of reproducibility. Dose is defined as all parameters of the device (hardware and programming) that govern the pattern of current flow through the body including to the nominal nerve target (Peterchev et al., 2012). For electrical stimulation dose encompasses: (1) all aspects of the stimulation waveform (e.g., pulse shape such as square, frequency); (2) details of electrode contact with the skin (e.g., size, shape, location). Factors that go into selecting dose, on a trial or subject basis (such as titration to sensation) are critical to report, but the actual dose applied should also be reported (Peterchev et al., 2012). It is important that complete details of dose be reported, not simply those aspects of dose the investigators think are important to outcomes (or important to mention). It is also important to recognize that referencing a technique by a name of classification does not fully describe dose since the same name may be used to describe different protocols (Guleyupoglu et al., 2013; Bikson et al., 2019). Nor is it sufficient to describe dose by referring to prior publications when those publications did not fully describe dose, when those prior works described a range of approaches broader than tested in the present study, or when any modifications (even incremental) were made. Finally, careful attention should be paid to the use of nomenclature (Bikson et al., 2019) that is not definitive in describing the dose (e.g., unipolar, anodal), may apply to different aspects of the dose (e.g., pulse duty cycle or train duty cycle) or mis-applying terminology (biphasic vs. bipolar).

Details of electrode design, preparation and application that are no genuine part of dose are likewise critical to allow consistent dosing. For example, the critical interface is the contact *surface* between the tissue and electrolyte e.g., hydrogel, paste (for a non-invasive electrode), or metal (for a percutaneous electrode). This needs to be described for every electrode, including electrodes that are considered less important for outcomes (e.g., so called "return" or "reference" electrodes). Other aspects of the electrode, such as materials and thickness, are equally important for reproducibility, including, for example, electrochemical stability

TABLE 2 | Reported stimulation parameters in studies on acute/short-term tVNS.

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
Sclocco et al. (2019)	Model S88x, Grass Instruments, Astro-Med, Inc, West Warwick, RI, USA	Custom ergonomic electrodes, Bionik Medical Devices, Bucaramanga, Colombia	eRAVANS: 1.6 ± 2.3 mA; iRAVANS: 1.7 ± 2.4 mA; GANctrl: 1.4 ± 1.1 mA	450 μs	25	Gated to respiratory cycle (1 s per cycle)	Biphasic rectangular pulse trains	Left cymba concha; Left earlobe	8 min per condition	Brainstem fMRI (no task)	None
Sclocco et al. (2020)	UROstim, schwa-medico GmbH, Ehringshausen, Germany	Custom ergonomic electrodes, Bionik Medical Devices, Bucaramanga, Colombia	2 Hz: 7.18 ± 0.95 mA; 10 Hz: 6.46 ± 1.30 mA; 25 Hz: 5.93 ± 1.21 mA; 100 Hz: 5.57 ± 1.18 mA	300 μs	2, 10, 25, 100	Gated to exhalation (1.5 s per respirator cycle)	Monophasic rectangular pulse trains	Left cymba concha	8.5 min per condition	Brainstem fMRI (no task)	None
Borges et al. (2019)	Nemos®, Cerbomed, Erlangen, Germany	Two titanium electrodes, positioned on top of a silicon earplug	$M = 2.3$ mA ($SD = 0.08$ mA)	200–300 μs	25	30-s waves of electrical stimulation alternated by 30-s breaks		Cymba conchae of the left ear	10 min	None	None
Borges et al. (2020)	Nemos®, Cerbomed, Erlangen, Germany	Two titanium electrodes, positioned on top of a silicon earplug	$M = 2.19$ mA ($SD = 0.93$)	200–300 μs	25	30-s waves of electrical stimulation alternated by 30-s breaks		Cymba conchae of the left ear	9–17 min, depending on the task	Modified Flanker task, Spatial Stroop task, Number Letter task, and Dimension Change Card Sorting task	4 min before each task
Leutzow et al. (2013)	Nihon Kohden MEB 9200	Custom[a]	8 mA	0.1 ms duration	0.5		Electrical square impulses	Right tragus		VSEP simultaneously measured before and after Total Intravenous Anesthesia (TIVA)	
Schulz-Stübner and Kehl (2011)	NMS 300; Xavant Technology, Pretoria, South Africa		6 mA		1		Stimulated at a frequency of 1 Hz for 30 s and then a brief tetanic stimulus was applied	Left interscalene groove	30 s		

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
Kox et al. (2015)	Medtronic model 37022	Stimulation catheter with eight electrodes on a circular distal loop (Achieve Medtronic model 990063-20, Medtronic, Heerlen, The Netherlands)	2–10 V	1 ms	20	Continuous		C5–C7 spinal level	30 min	Continuous physio up to 8 h following stim onset and 2 days post; 10 min before LPS administration and assess temp/symptoms every 30 min for 8 h and 2 days post	
Burger et al. (2019a)	Nemos®, Cerbomed, Erlangen, Germany	Two titanium electrodes, positioned on top of a silicon earplug	0.5 mA	250 μs	25	30-s waves of electrical stimulation alternated by 30-s breaks		Cymba concha of left ear	Across 3 tasks, this current task is 15 min (30 min + more for other tasks)	Breathing Focus Task	15 min
Hyvärinen et al. (2015)	Tinnoff Inc.	Clip electrode	0.5 mA	500 μs	25		Biphasic rectangular pulse	Left tragus	6 min	MEG	1 min
Sellaro et al. (2015b)	CM02, Cerbomed, Erlangen, Germany	Two titan electrodes fastened on a gel frame	0.5 mA	200–300 μs	25	Alternated between on and off periods every 30 s		Outer auditory canal of the left ear	75 min	Flanker and CRT, mood and physio assessed 45 and 75 min post	15 min
Verkuil and Burger (2019)	Nemos®, Cerbomed, Erlangen, Germany	Two titanium electrodes, positioned on top of a silicon earplug	0.5 mA	250 μs	25	30-s waves of electrical stimulation alternated by 30-s breaks		Cymba conchae of the left ear	Across 3 tasks + 15 min	Exogenous cuing task	15 min to first task
Jacobs et al. (2015)	TENSTem dental; Schwa-medico BV, Woudenberg, The Netherlands	Ear clip using a circular electrode of 10 mm diameter connected as an anode	5.0 mA	200 μs	8			Left external acoustic meatus on the inner side of the tragus	17 min	Continuous physio data and retrieval task post-stim	Continuous, 17 min
Burger et al. (2018)	Nemos®, Cerbomed, Erlangen, Germany	Two titanium electrodes, positioned on top of a silicon earplug	0.5 mA	250 μs	25	30-s waves of electrical stimulation alternated by 30-s breaks		Concha of the left outer ear	26 min	Extinction	12 min

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
Finisguerra et al. (2019)	NEMOS® device (CM02 Cerbomed, Erlangen, Germany)	Two titan ear electrodes that are mounted on a gel frame	0.5 mA	200–300 μs	25	Alternated between On/Off periods of 30 s each		Cymba conchae of the left ear	60 min	IATS	15–20 min
Colzato et al. (2018b)	NEMOS®		0.5 mA	200–300 μs	25	Alternated between on and off periods every 30 s		Outer auditory canal of the left ear	50 min	Emotion Regulation Task and assessed flow at the end	20 and 50 min
Jongkees et al. (2018)	CMO2, Cerbomed, Erlangen, Germany	Titan electrodes mounted on a gel frame	0.5 mA	200–300 μs	25	Active stimulation for 30 s, followed by a break of 30 s		Outer auditory canal of left ear	45 min	SRT	15 min
Steenbergen et al. (2015)	CM02, Cerbomed, Erlangen, Germany	Two titan electrodes mounted on a gel frame	0.5 mA	200–300 μs	25	30 s, followed by a break of 30 s		Outer auditory canal of left ear	45 min	Task test phase 25 minutes into stim and HR post-stim	25 and 45 min
Sellaro et al. (2018)	NEMOS®	Two titan electrodes mounted on a gel frame	0.5 mA	200–300 μs	25	On and off periods of stimulation alternated every 30 s		Auricle with the titan electrodes placed either in contact with the concha of left ear	35 min	Emotion regulation	20 min
Colzato et al. (2018a)	NEMOS		0.5 mA	200–300 μs	25	Alternated between on and off periods every 30 s		Concha in the left ear	40 min	Creativity tasks and personality and HRV	15 min
Fischer et al. (2018)	CMO2, Cerbomed, Erlangen, Germany	Two titan electrodes mounted on a gel frame	$M = 1.3$ mA (0.4–3.3 mA)	200–300 μs	25	Continuous		Left cymba conchae	36 min	Oddball then Simon task, physio/mood/ EEG/saliva post	Simultaneous with 2 tasks, 28 min before task of interest, 36 min
Keute et al. (2019a)	Digitimer DS7 and Arduino Uno circuit board	Medical Ag/AgCl electrodes (Ambu Neuroline3), cut to a size of 4 × 4 mm and mounted on a piece of silicone at a center-to-center	3 mA	200 μs	25	Stimulation cycle of 30 s stimulation at 25 Hz, followed by a 30 s break		Cymba conchae of the left ear; anode was placed more rostral	40 min	10-min online task	30 min

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
		distance of 1 cm were used									
Laqua et al. (2014)	TNS SM 2 MF device (Schwa-Medico GmbH, Germany	Anode: silver disk EEG electrode 5 mm in diameter, Schuler Medizintechnik Freiburg, Germany Cathode: (Blue Sensor PECG electrode, Ambu, Germany)	Intensity of stimulation was set individually to maximal but non-painful	0.2 ms impulse duration	Changing frequency between 2 and 100 Hz		Burst-stimulation mode	Anode: bilateral cavum conchae Cathode: mastoid area of the ear	35 min (5 min adaptation + 30 min constant)		Physio measures 15, 30, 40, and 60 min after onset
Brock et al. (2017)	GammaCore; electroCore LLC; Basking Ridge, NJ, USA	Two steel contact electrodes						(1) left cervical vagal nerve and (2) to the right cervical vagus nerve	120 s to each site		Measured 90 min and 24 h after tVNS
Peng et al. (2018)	TENS200, HUATUO GmbH, Hangzhou, China	Silver plate (5 mm in diameter) and an elongated cylindrical silver stimulation electrode (8 mm in length, 3 mm in diameter)	Around 5 mA on the acupuncture points (varied individually between 4 and 8 mA)	250 μs	20		Monophasic-modified rectangle impulse	Area of the acupuncture points CO10-12 and TF4[b] OR anterior wall of the auditory canal[c]	First stimulation period of 30 s and a break/baseline of 60 s. Four alternating stimulation and baseline sequences were performed in total		Simultaneous MRI
Warren et al. (2019)	NEMOS®, Cerbomed, Germany		0.5 mA	200–300 μs	25	Alternating between on and off periods every 30 s		Cymba conchae region	80 min	Saliva 45 min and post-tVNS, and pupilometry 20 and post-tVNS, EEG simultaneous, stimulus discrimination 20 min after tVNS onset	20 min
Burger et al. (2016)	Nemos_, Cerbomed, Erlangen, Germany	Two titanium electrodes, positioned on top of a silicon earplug	0.5 mA		25	30-s waves of electrical stimulation alternated by 30-s breaks		Concha of the left outer ear	∼3 min (8 s*20)	During extinction	10 min

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
Busch et al. (2013)	STV02, Cerbomed, Erlangen, Germany	Bipolar stimulation electrode	0.25 and 10 mA	250 μS	25	Continuous	Modified monophasic rectangle impulse	Left concha at the inner side of the tragus	1 h	Continuous ANS, pain assessment	20 min
Burger et al. (2019b)	CM02, Cerbomed, Erlangen, Germany	Two titan electrodes mounted on a gel frame	0.5 mA	250 μs	25	Active for 30 s, followed by a break of 30 s		Cymba concha of the left outer ear	30 min	Fear generalization and extinction	10 min
Capone et al. (2015)	Twister—EBM	Two Ag–AgCl electrodes (5 mm in diameter)	8 mA	Pulse duration = 0.3 ms	20	Trains lasting 30 s and repeated every 5 min for 60 min	Trains composed by 600 pulses (intra-train pulse frequency = 20 Hz; pulse duration = 0.3 ms)	Left external acoustic meatus at the inner side of the tragus	60 min	Cortical excitabity TMS post-tVNS	60
Sclocco et al. (2017)		Ergonomically-shaped Ag/AgCl electrodes	Low and medium 0.10 ± 0.08 mA and 0.26 ± 0.15 mA	15 ms pulse width	25	Duration of 1 s, delivered at 25 Hz during each exhalation phase of respiration.	Rectangular pulses	Left ear	2 min		Simultaneous with paced breathing and ECG
Genheimer et al. (2017)	NEMOS cerbomed GmbH (Erlangen, Germany)		1.2 (1.1) MA	250 μS	25	30 s on and 30 s off phases		Cymba concha left ear	60 min (20 min + 40 min during task)	During extinction and during entering office for extinction; assess reinstatement next day	20 min
Usichenko et al. (2017b)	DoloBravo Dual Channel Neurostimulator (MTR GmbH, Germany)	Self-manufactured electrode, sized 9 ×9 × 2.1 mm; contact surfaces of each electrode, containing the silver wires wrapped in wool, were moistened with 0.9% NaCl solution	7.6 mA (range 5.0–11.5)	Impulse duration of 200 μs	8	Continuous	Square impulses	Concha of the auricle bilateral	∼6 min		Simultaneous with heat pain during fMRI and threshold assessed post-tDCS

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
Cha et al. (2016)	ES-420, Ito Co., Ltd., Tokyo, Japan	Ball-type electrode	2–7 mA across sites/2 days	200 μs	30			Cymba, the cavum, and the outer surface of the tragus (Right and Left)	4 min per site	N	Assessed dizziness after tVNS
Yakunina et al. (2017)	Custom-made stimulator connected with silver wires to six electrodes	99.99% pure silver (four stimulation and two reference electrodes)	The stimulation intensities at electrodes A, B, C, and D ranged from 0.2–1.8 mA with means ± SD of 0.77 ± 0.42, 0.81 ± 0.48, 0.91 ± 0.47, and 0.81 ± 0.38 mA, respectively	500 μs	25		Monophasic rectangular impulse	4 locations in the left ear: (A) inner surface of the tragus, (B) inferoposterior wall (cartilaginous part) of the ear canal, (C) cymba conchae, and (D) earlobe. The reference electrode for electrodes A, B, and C were placed at the outer surface of the tragus, whereas the reference electrode for electrode D (sham) was placed at the backside of the earlobe	Each location was stimulated in two runs with 30 s of stimulation followed by 1 min of rest; this cycle was repeated four times in a run. Each subject underwent eight 6-min fMRI runs total, with up to 90 s of rest in between run		Simultaneous MRI
Clancy et al. (2014)	Transcutaneous Electrical Nerve Stimulation (TENS) device (V-TENS Plus, Body Clock Health Care Ltd, UK)	Modified surface electrodes	Level of sensory threshold (10–50 mA)	200 μs	30	Continuous		Inner and outer surface of the tragus of the ear	15 min		Simultaneous physio and over 15 min following recording
Antonino et al. (2017)	(TENS) device consisted of a small stimulation unit (V-TENS Plus, Body	Surface electrodes bilaterally placed	45 ± 1 mA	200 μs	30	Continuous		Inner and outer surface of the tragus	15 min		Simultaneous physio and during 10 min period post-tVNS

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
	Clock Health Care Ltd, UK)										
Lamb et al. (2017)		Ag/AgCl disk electrode	80% of comfort threshold of 5.6 mA (range 3–11.3 mA)	100 μS	20		Alternating polarity pulse	Left external auditory meatus and the posterior face of the left tragus		Startle blink and ANS tests	Simultaneous
Lerman et al. (2016)		Two stainless steel contact surfaces and conductive gel	M intensity ranged from 21.3 to 22.59 across the 6 stimulations		25		5-kHz sine wave series that occurred for 1 ms and repeated every 40 ms	Under the angle of the mandible, lateral to the trachea and medial to the sternocleidomastoid (right then left ear 3 times each)	2 min (90 s with 30 s ramp up	*N*	Blood draw 90 min after first stim and next day
Badran et al. (2018b)	Digitimer DS7a	Custom developed round, unipolar stimulation Ag/AgCl electrodes 1 cm in diameter, affixed to the 3D-printed clamps using cyanoacrylate	Mean ± SD: 3.14 ± 0.99 mA	500 μs	25	Three stimulation "on" periods were modeled (onset times: 30, 150, 270 s; duration 60 s)	Monophasic square waves	Left tragus	5.5 min (270 s onset time + 60 s)		Simultaneous MRI
Frøkjaer et al. (2016)	NEMOS, Cerbomed, Erlangen, Germany	Bipolar stimulation electrode	0.1–10 mA readjusted throughout experiment; mean ranged from 1.07 mA to 1.46 across time points	250 μs	30			Left concha	60 min	Cardiac derived parameters obtained at baseline and after 10, 20, and 30 min of tVNS; Quantitative sensory testing assessed at baseline and after 10 and 25 min of tVNS; Conditioned pain modulation Assessment was performed after 40 min of tVNS; Drink test for assessment of	15 min (deep breathing)

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
										gastroduodenal motility performed after 50 min of tVNS	
Garcia et al. (2017)	S88X GRASS stimulator, Astro-Med, Inc, West Warwick, RI	8 mm diameter, Astro-Med, Inc, West Warwick, RI	Exhalatory-gated taVNS (eRAVANS) mean ± SD: 1.22 ± 1.33 mA Inhalatory-gated taVNS (iRAVANS) mean ± SD: 0.85 ± 1.07 mA	450 μs	30	Pulse train duration of 0.5 s gated, with 0.5-s delay, after peak inhalation (i.e., during exhalation, for eRAVANS) or after peak exhalation (i.e. during inhalation, for iRAVANS)	Biphasic rectangular pulses	Auricle of the left ear [(1) the cymba concha and (2) the slope between the antihelix and cavum concha]	360 s		Airpuff stimulation was applied over the right supraorbital region of the forehead in fMRI scans pre- and post-stimulation
Rufener et al. (2018)	NEMOS tVNS device (Cerbomed, Erlangen, Germany)		0.5 mA	250 μs	25	Alternating on/off phases of 30 s		Concha cymbae of the left ear	100.5 min	Oddball task	90 min
Burger et al. (2017)	NEMOS® stimulator unit (Cerbomed, Erlangen, Germany)		0.5 mA	250 μs	25	Each CS was presented for 30 s, followed by a 40 s inter trial interval (ITI). Stimulation (sham) with the tVNS device occurred concurrently with each CS for 30 s	Monophasic square wave pulses	Concha of the left ear	Twenty unreinforced presentations of the CS+ and CS–	Fear extinction	Simultaneous with extinction task; Assessed retention 24 h later
Stavrakis et al. (2015)	Grass S88 stimulator	Flat metal clip	50% below threshold for slowing sinus rate of 39.8 ± 25.7 V	1-ms duration	20	Continuous	Square wave	Right ear, tragus cathode, earlobe anode	1 h		Post-tVNS blood draw and atrial fibrillation induction
Yu et al. (2017)	S20, Jinjiang, Chengdu City, China		50% below threshold for slowing sinus rate of 5.8 ± 3.1 mA	1-ms duration	20	Duty cycle of 5 s on and 5 s off		Right Tragus	155 ± 6 min	Holter Recording	24 h recording post tDCS and daily follow ups for 1 week

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
Paleczny et al. (2019)	IMER Systems, Wroclaw, Poland	Custom-made electrode	Mean amplitude= 722 ± 92 μA	1,000 μs/phase	25	Continuous or Synchronizing the stimulation with the inspiratory or expiratory phase	Rectangular, biphasic, symmetrical pulses (1,000 μs/phase, interphase interval 30 μs)	Medial of the tragus at the entry of the acoustic meatus	2 min each		Simultaneous physio
Fallgatter et al. (2003)	Two conventional bipolar electrode wires were soldered to single-sided copper claddings upon epoxy resin; At the output side two very flexible fine copper stranded wires ∼10 cm in length and with a diameter of 0.05 mm, cut from a radio coil, were soldered to the copper claddings	Epoxy resin (dimension about 1 × 1 cm)	8 mA	0.1 ms duration		Interstimulus interval of 2 s	Electrical square impulses	Cathode of this bipolar stimulation electrode was placed at the inner side of the tragus at the outer ventral edge of the meatus acusticus externus. The anode was also placed at the inner side of the tragus 5 mm more distal. Alternative stimulation sites at the right ear outside the innervation of the auricular branch were tested in the single subject (lobulus auriculae, the scapha, the crus antihelices superior and the top of the helix). The distance between cathode and anode was always kept at 5 mm			Simultaneous VSEP

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
Polak et al. (2009)	Two conventional bipolar electrode wires soldered to single-sided copper claddings at the input side. At the output side, two very flexible fine copper stranded wires were soldered to the copper claddings. The other end of these wires was fixed to the skin with GRASS paste	Stimulation electrode was a piece of epoxy resin with two conventional bipolar electrode wires soldered to single-sided copper claddings at the input side	5, 8, and 10 mA in randomized sequence	0.1 ms duration		Interstimulus interval of 2 s	Electrical square impulses	Cathode of the bipolar stimulation electrode was placed at the inner side of the tragus at the outer ventral edge of the internal auditory meatus, the anode 5 mm away right and left ear			Simultaneous VSEP
Lewine et al. (2019)	gammaCore device		12–20 V		25	Two 120-s long bursts of stimulation applied over a 5 min period	5 kHz sine-wave stimulus for 1 ms	Left carotid sheath	5 min	EEG	15 min after active or sham tcVNS, (3) 120 min after stimulation, and (4) 240 min after stimulation
Napadow et al. (2012)	Cefar Acus II (Cefar Medical, Lund, Sweden)	0.20 × 1.5 mm modified press-tack electrodes (DBC, Korea and Vinco, China)	RAVANS [*M* (*SD*) = 0.43 (0.25 mA)]	450 μS	30	0.5 s and was gated to the exhalation phase of respiration	Rectangular pulses	(1) the cymba concha (+) and (2) the slope between the antihelix and cavum concha (–)	30 min	Deep-tissue pain intensity	15 and 30 min and 14 min after tVNS onset

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
Ventura-Bort et al. (2018)	CMO2, Cerbomed, Erlangen, Germany	Two titan electrodes mounted on a gel frame	$M = 1.3$ mA (0.4–3.3 mA)	200–300 μs	25	Continuous		Left cymba conchae	35 min	Oddball and Simon task	0 min; simultaneous
De Couck et al. (2017)	Cerbomed, Germany	Double ball point electrodes	Mean intensity of 0.7 mA in study 1 and 1 mA in study 2	250 μs	25	Alternating pulse series of 30 s duration followed by 30 s stimulation pause	Rectangular pulses	Cymba conchae area of the outer ear; Left ear and right ear in Study 1 and Right ear in Study 2	10 min each in Study 1 and 1 h in Study 2	HRV	Study 1: simultaneous HRV Study 2: HRV was measured during the first 5 min, between minutes 30–35 and between minutes 55–60 of the 1 h stimulation
Janner et al. (2018)	Transcutaneous electrical nerve stimulation device PuntoBravo (Medizintechnik Rostock GmbH, Rostock, Germany)	Self-manufactured electrodes; electrodes' contact surfaces, wrapped in wool, were moistened with 0.9% sodium chloride solution	M (SD) = 6.8 mA (1.3) for left ear and M (SD) = 8.3 mA (3.9) for right ear	200 μs	Mixed frequency pattern of 100/2 Hz	9 impulses with a frequency of 100 Hz emitted twice per second	Electrical square impulses	Bilateral cymbas conchae	25 min	Heat stimulation	20 min heat stimulation starts, physio and anxiety assessed 20 and 25 min after tVNS onset
Beste et al. (2016)	CM02, Cerbomed, Erlangen, Germany).	Two titan electrodes mounted on a gel frame	0.5 mA	200–300 μs	25	Active for 30 s, followed by a break of 30 s		Left inner ear	60 min	Backward Inhibition Task	Simultaneous
Hong et al. (2019)	Transcutaneous, bipolar stimulation probe (Stimulationssonde 522,015, Inomed)	Bipolar stimulation probe	10 mA	250 μs	25			Cymba conchae of the right ear	10 min		Simultaneous physio and blood draw 1 and 3 h post-tVNS

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
Colzato et al. (2017)	NEMOS® tVNS	Two titan electrodes mounted on a gel frame	0.5 mA	200–300 μs	25	Alternated between on and off periods every 30 sec		Outer auditory canal of left ear	35 min	Reading the Mind in the Eyes Test and physio	20 min start Reading the Mind in the Eyes Test and physio 20 and 35 min after tVNS onset
Gancheva et al. (2018)	Cerbomed NEMOS® (Cerbomed, GmbH, Erlangen, Germany)		0.6–1.4 mA for the taVNS cymba conchae condition (0.9 ± 0.1 mA, mean ± SEM)	0.25 ms duration	25	Continuous	Continuous biphasic square pulses	Cymba conchae of the left external ear	14 min	Physio	Simultaneous physio and up to 2 h post-tVNS
Kraus et al. (2013)	Voltage source (Digitimer Type DS7A, serial D127A)	MRI compatible silver plate (5 mm in diameter)	Mean stimulation intensity in the active group was 32.6 V (min 14 V, max 57 V, *SD* 13.4)	20 ms	8	Constant		Left external acoustic meatus on the inner side of the tragus	Four stimulation periods of 30 s were applied, each followed by a resting period of 1 min	*N*	Simultaneous MRI
Tobaldini et al. (2019)	TENS device (NEMOS®; Cerbomed, Erlangen, Germany)	Surface electrodes	1–6 mA	200 ms	25	Continuous		Left cymba conchae of the external ear	25 min	Physio	Simultaneous physio
Yakunina et al. (2018)	Custom-made stimulator (Yakunina et al., 2017)		0.1 mA weaker than the intensity corresponding to the pain threshold means (*SD*)= 0.71 (0.43) for tragus and means (*SD*)= 0.80 (0.47) for the concha	500 μs	25	Each location was stimulated in two runs with 30 s of stimulation followed by 30 s of rest; this cycle was repeated five times in a run. Each subject underwent a total of six 5-min fMRI runs, with up to 90 s of rest between run	Monophasic rectangular impulse	Inner tragus and cymba conchae of the left ear	25 min runs per location	MRI	Simultaneous MRI

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
Kraus et al. (2007)	EMP2 Expert, schwa-medico GmbH, Ehringshausen, Germany	Silver plate (5 mm in diameter)	Mean intensity for LOW was 4.0 mA (SD 1.0) while for HIGH it was 5.0 mA (*SD* 1.0)	20 ms	8	Three stimulation sequences were applied, each of which consisted of a stimulation period of 30 s, followed by a resting period of 2 min. The first two sequences were performed with low stimulation intensity (LOW) and the last one with high intensity (HIGH)		Left external acoustic meatus on the inner side of the tragus	30 s	MRI	Simultaneous MRI
Keute et al. (2018)	Medical stimulation device (Digitimer DS7, UK)	Two conventional neurostimulation electrodes Ambu Neuroline, DK 4 × 4 mm	8 mA, if tolerable for the subject, and else individually adjusted below pain threshold 5.9 ± 1.6 mA	200 μs	25	Trains of 30 s, each followed by 30 s without stimulation	Monophasic square pulses	Cymba conchae of the left ear; anode being more rostral	25 min	Negative compatibility effect (NCE) task and EEG	25 min; not simultaneous
Sellaro et al. (2015a)	CM02, Cerbomed, Erlangen, Germany	Two titan electrodes fastened on a gel frame	0.5 mA	200–300 μs	25	On/Off periods every 30 s		Outer auditory canal of the left ear	30 min	Measured mood/physio data and started cyberball 20 min into stimulation and at the end of stim	∼20 and 30 min
Dietrich et al. (2008)	Stand-alone electrical nerve stimulator connected with carbon fiber wires to an acrylic electrode array housing a sterling silver stimulation electrode and a reference electrode	Sterling silver stimulation electrode and a reference electrode; The array was attached to the skin with an adhesive tape	Varied individually between 4 and 8 mA	250 μs.	25	The experiment lasted 700 s and was started with a baseline lasting 100 s. This was followed by a first stimulation period of 50 s and a break/baseline of 100 s. Four alternating	Monophasic-modified rectangle impulse	Inner side of the left tragus	700 s	Simultaneous MRI	Simultaneous MRI

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
						stimulation and baseline sequences were performed					
Frangos and Komisaruk (2017)	Hand-held battery-operated stimulation device	A pair of non-ferromagnetic stainless steel surface electrodes (1 cm diameter)	23.9 ± 12.3 V	200 μs (1/5,000 Hz = pw in s)	25	1-ms duration bursts of 5 sinusoidal wave pulses; continuous		Right antero-lateral surface of the neck	2 min	MRI	Simultaneous MRI and rest up to 15 min after tVNS offset
Frangos et al. (2015)	Cerbomed NEMOS	Two hemispheric titanium electrodes	Earlobe: 0.3–0.9 mA Cymba conchae: 0.3–0.8 mA		25	Continuous	0.25 m-duration monophasic square wave pulse	Left cymba conchae, left earlobe	7 min		
Juel et al. (2017)	Nemos®; cerbomed GmbH, Erlangen, Germany	Bipolar stimulation electrode	Ranged from 0.1 to 10 mA	250 μs	30	Continuous		Left concha	60 min	Quantitative sensory testing (QST) Conditioned pain modulation (CPM) paradigm Gastroduodenal motility parameters Vagal Tone Deep Slow Breathing	15- and 30-min after tVNS onset-start DSB for 10 min; Vagal and QST 10 and 25 min after tVNS onset and CPM 40 min post tVNS onset and motility 50 min after tVNS onset
Fallgatter et al. (2005) based on Fallgatter et al. (2003)	Two conventional bipolar electrode wires soldered to single-sided copper claddings at the input side. At the output side, two very flexible fine copper stranded wires (length	Stimulation electrode was a piece of epoxy resin (about 1 × 1 cm)	8 mA	0.1 ms duration		0.1 ms duration, the interstimulus interval was 2 s	Electrical square impulses	Cathode of this bipolar stimulation electrode was placed at the inner side of the tragus at the outer ventral edge of the internal auditory meatus. The anode was placed 5 mm		Vagus sensory evoked potential (VSEP)	Concurrent VSEP

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
	∼10 cm, diameter (0.05 mm) were soldered to the copper claddings. The other end of these wires was fixed to the skin with a very small amount of Grass paste							more distal at the inner side of the tragus			
Tran et al. (2019)	Transcutaneous electrical nerve stimulation (TENS) unit	Ear clip electrode (Parasym device, Parasym Health, Inc., London, UK)	1 mA below the discomfort threshold	200 μs	20			Tragus of the ear	1 h	ECG	HRV after 55 min of tVNS and Echocardio-graphy 40 min after tVNS onset
Szeska et al. (2020)	CMO2, Cerbomed, Erlangen, Germany	Two titan electrodes mounted on a gel frame	Active tVNS: average 2.28 mA Sham: average 2.53 mA	200–300 μs	25 Hz	30 s on, 30 s off		Active tVNS: cymba conchae Sham: center of earlobe	8 min	Multiple-day single-cue fear conditioning and extinction paradigm	3 min
Giraudier et al. (2020)	CMO2, Cerbomed, Erlangen, Germany	Two titan electrodes mounted on a gel frame	Active tVNS: 0.5–3.5 mA, average 1.48 Sham: 0.5–2.5 mA, average 1.31	200–300 μs	25 Hz	30 s on, 30 s off		Left cymba conchae	23 min	Lexical decision task and recognition memory task	5 min; simultaneous; 5 min. (post)
Neuser et al. (2019)	NEMOS, Cerbomed GmbH, Erlangen, Germany	Titanum electrode	Active tVNS: 0.2–3.1 mA Sham: 0.5–3.1 mA $N = 81$ $N = 41$ completed task during left side taVNS $N = 40$ completed task during right-sided taVNS		25 Hz	30 s on, 30 s off	Biphasic impulse frequency	Active tVNS: cymba conchae Sham: earlobe			

(Continued)

TABLE 2 | Continued

References	Device	Electrode(s)	Stimulus intensity	Pulse width	Hz	Alternating stimulation	Stimulus	Electrode placement	Stimulation length	Task	Pre-task stimulation period
Bretherton et al. (2019) Study 1, 2	TENS machine (V-TENS Plus, Body Clock Health Care Ltd, United Kingdom)	Auricular electrode clips attached on the inner and outer surface of the tragus of the ear (Auricular Clips, Body Clock Health Care Ltd, UK)	2–4 mA	200 μs	30 Hz			Inner and outer surface of the tragus of the ear (Auricular Clips, Body Clock Health Care Ltd, UK)	15 min		
(Teckentrup et al., 2020)	NEMOS, Cerbomed, Erlangen, Germany		Individually adapted stimulus intensity (see Frangos et al., 2015)		25 Hz	30 s on, 30 s off	Biphasic impulse frequency	taVNS: left cymba conchae Sham: left earlobe	30 min		
Zhang et al. (2019)	MRI compatible Electronic Acupuncture Treatment Instrument (SDZII, Huatuo, Suzhou, China)		1.5–3 mA	0.2 ms	1 Hz	Continuous wave 20 s on vs. 30 or 20 s off block design of intermittent taVNS		taVNS: left cymba conchae Sham: left tail of the helix	13 min		

[a] *Electrode consisting of two stainless steel straps, wrapped with wool fiber and stapled to a 9 × 9 mm piece of silicon rubber.*

[b] *Silver plate was placed in the left ear triangular fossa; the cylindrical electrode was placed in the left cymba concha.*

[c] *Plate electrode was placed in the left external acoustic meatus on the inner side of the tragus; the cylindrical electrode was placed on the left lower limb (left middle shank).*

TABLE 3 | Minimum reporting standards.

Acute/short-term stimulation	Long-term stimulation
Device level	
– Manufacturer/name/version/edition (if applicable)	
– Regulatory aspects (CE certification, FDA compliance etc.)	
Design level	
– General study design (e.g., randomized controlled)	– General study design (e.g., randomized controlled)
– Between- vs. within-subject design (if applicable)	– Between- vs. within-subject design (if applicable)
– Blinding of subjects, assessors, and statisticians	– Blinding of subjects, assessors, and statisticians
– Intended and actual session duration (min)	– Intended and actual daily dose/total duration of intervention
– Pre-stimulation period (i.e., time before task/segment of interest)	– Time of day of stimulation (i.e., free vs. instructed)
– Time of day (circadian influence)	– Protocol compliance monitoring and completer definition
– Manipulation check (in sham-controlled designs)	
– Type of sham control (if applicable)	
Stimulation level (for active and sham stimulation, if applicable)	
– Stimulation site (specify anatomic location and steps in preparation) (e.g., using an alcohol wipe)	
– Electrode composition and set-up	
– Current intensity (mA)	
– Pulse width (μs)	
– Frequency (Hz)	
– Duty cycle (s)	
– Parameter descriptions: Constant current or voltage, current or voltage intensity (mA or V), pulse width, frequency, duty cycle (ON/OFF time)	
– Waveform descriptions: uni- or bi-directional, anode/cathode placement	
– Pulse shape and burst/non-burst stimulation	
– Voltage (mV) in case of voltage-controlled stimulation	
Subject level	
– Inclusion/exclusion criteria	
– Mean age and age range of sample	
– Sex distribution/ethnicity	
– Assessment of confounding variables	
– Prior knowledge of vagal innervation of the ear by the participant	
Adverse events	
– Detailed reporting on methods to assess adverse events	
– Transparent reporting on any (serious) adverse events	

(Merrill et al., 2005) and tolerability (Minhas et al., 2010; Khadka et al., 2018).

DISCUSSION

Having proposed a set of reporting standards, we will now address some of the outstanding issues, which in our view, future tVNS studies have to objectively and systematically address. These issues have all been examined in previous studies to a greater or lesser extent, but given the lack of reporting standards, no definite conclusions can yet be drawn. It is our hope that having provided these standards, clear answers will become apparent in the years to come. Here we will subsequently discuss issues related to safety, confounding, stimulation parameters, underlying physiology including studies on biomarkers and translational studies.

Safety and Tolerability

In line with our recommendations of providing standardized information on stimulation parameters etc., we encourage the standardized reporting of adverse events as suggested by Redgrave et al. (2018). A systematic literature review on the safety and tolerability of tVNS has evaluated 51 studies, independent of the area of application (Redgrave et al., 2018). The authors report that the most prevalent side effect was local skin irritation from electrode placement, occurring in about 18% of included subjects following long-term stimulation. Nevertheless, it is important to note that 89 studies were not included in this review as these studies had not reported safety or tolerability data and when approached the authors didn't respond to a formal request to provide data.

Potential Confounding Variables

Alongside transparent reporting of stimulation parameters and adverse events, important confounding variables need to be considered and reported. The inter-individual variability in the neurophysiological and behavioral response to tVNS is high and the reasons for this are poorly understood. A diverse array of factors including, but not limited to age and comorbidities, subjects' ear and tissue morphology and innervation, neurotransmitter balances and brain state, may contribute to inter-individual differences in tVNS response. Based on studies using tVNS, iVNS, and other electrical stimulation techniques, we suggest that investigators consider the following variables that can influence the responsiveness to tVNS and can confound the results in their studies.

Age

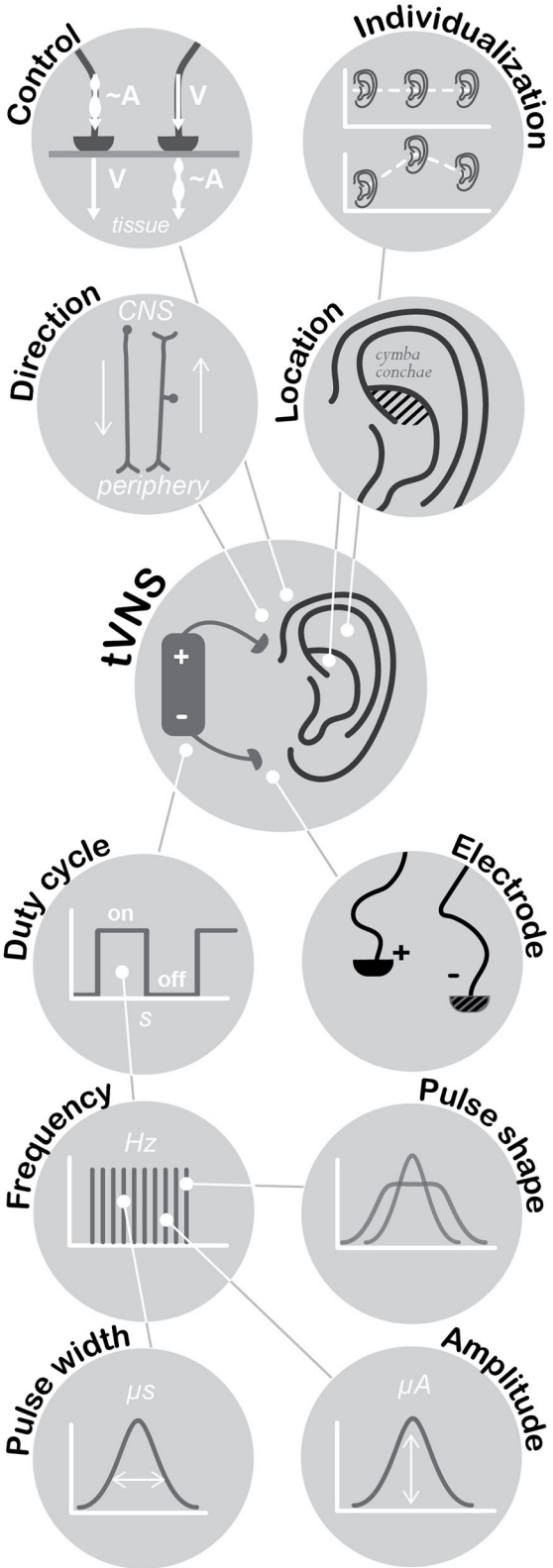
Increasing age affects both parasympathetic and sympathetic activity (e.g., Kuo et al., 1999). For example, age is associated with marked changes at hormonal level, which in turn affect acetylcholine-mediated parasympathetic autonomic activity, which is affected by tVNS (Moodithaya and Avadhany, 2012; Krause and Cohen Kadosh, 2014). Furthermore, sensitivity to electrical transcutaneous stimulation is lower in older age-groups (Kemp et al., 2014).

Sex

In animal studies VNS has greater effects in females, probably because of the effect of oestrogens to the muscarinic acetylcholine in the central nervous system (Du et al., 1994). Similar effects should be expected in human subjects due to both hormonal levels and the gender- and age-dependent differences in the functions of the autonomic nervous system (Koenig and Thayer, 2016; Koenig et al., 2017). Differences in the neuronal pathways and neuronal sensitivity may exist and therefore affect response to tVNS (De Couck et al., 2017; Janner et al., 2018).

Medical Conditions

Neurotransmitter levels may differ between individuals according to specific medication intake and medical condition.



Reporting standard tVNS stimulation parameters

Control

- ☐ Variable current (constant voltage in tissue) or constant voltage (variable current in tissue)

Individualization

- ☐ Uniform or individualized intensity
- ☐ Detection threshold, pain threshold

Direction

- ☐ Unidirectional (afferent or efferent) vs bidirectional (afferent and efferent)

Location

- ☐ Left and/or right
- ☐ Anterior/posterior surface
- ☐ Anatomical label

Electrode

- ☐ Composition
- ☐ Number of electrodes
- ☐ Attachment method (clip, paste, tape, percutaneous)

Duty cycle

- ☐ On period
- ☐ Off period

- ☐ **Frequency**
- ☐ **Pulse shape**
- ☐ **Pulse width**
- ☐ **Amplitude**

FIGURE 2 | Minimum Reporting Standards for Research on Transcutaneous Vagus Nerve Stimulation (Version 2020).

This was shown to cause research subjects to respond differently to stimulation due to a certain dose-response relationship that interacts with initial neurotransmitter levels (Ziemann et al., 2002; Falkenberg et al., 2012). Therefore, to avoid confounds in experiments, we recommend to control for (or exclude) individuals with psychological or psychiatric conditions (e.g., Homma et al., 1993; Salman, 2015) and medication use that affects neurotransmitter systems (unless those study populations are directly relevant to the research question).

Ear and Tissue Anatomy

Different ear sizes and skin properties, such as impedance, water content, structure, and subcutaneous fat thickness as well as auricular anatomy of the vagus innervation may cause different current distributions and require different current strengths to achieve the same current flow (Maffiuletti et al., 2008; Cakmak, 2019). Consequently, physiological and behavioral effects may vary.

Time of the Day /Different State

The brain does not always respond stereotypically to stimulation, as response may depend on the current state of activity (Silvanto et al., 2008), level of fatigue, wakefulness, attention, or mood (Sztajzel et al., 2008; Steenbergen et al., 2020). Controlling for brain state, for instance, by employing a focused behavioral task, or applying stimulation only during a particular brain state, e.g., based on patterns of electroencephalographic (EEG) activity (Brázdil et al., 2019), may potentially improve responsiveness. This may also extend to physiological states in general, such as respiratory phase (Napadow et al., 2012; Garcia et al., 2017; Sclocco et al., 2019).

Adherence

Especially in neuropsychiatric populations, adherence must be controlled. Dependent on the population non-adherence rates up to 50% (Perkins, 2002) have been reported from pharmaceutical trials and it must be assumed that the same numbers will occur. Such non-adherence rates have e.g., reported for the tVNS schizophrenia trial (Hasan et al., 2015) and the adherence should be recorded and analyzed in all future tVNS trials.

Control Condition

Control condition is tVNS tested against sham stimulation (actual stimulation of the earlobe, for example), or no stimulation. Further, authors should report on placebo/expectations effects and which attempts were made to control for this influence. Very recently, problems with the wrongful placement of electrodes in sham-stimulation, in particular the possibility to stimulate muscle zones with potential effects, have been discussed (Cakmak et al., 2017; Liugan et al., 2018).

In future, it is important that researchers are aware of sources of variability that may affect tVNS response, especially in studies using heterogeneous populations, and that they select their desired research population with caution. Furthermore, tracking potential confounds may allow the investigators to control for them in the analysis and to understand outliers within the data. This approach may help to understand factors explaining heterogeneity in the efficacy and response to tVNS.

Left or Right? A Question of Laterality in VNS Targeting

Anecdotally during the development of iVNS, theoretical concerns emerged regarding cardiac safety when implanting electrodes on the right cervical VN in comparison to the left. This theory was only explored in one iVNS trial exploring both left and right iVNS for chronic heart failure which demonstrated equal safety profiles (Premchand et al., 2014). Animal studies suggest that right sided iVNS has stronger cardiac effects (Ng et al., 2001; Yoo et al., 2016). Due to this uncertainty, an important constraint when applying taVNS is the choice of the ear side during stimulation. Individual stimuli delivered to the right cervical VN have two-fold inhibition effects on heart beating cycle, compared to identical stimuli delivered to the left nerve (Brown and Eccles, 1934). The reason is that efferent vagal fibers affecting the sinoatrial node of the heart are thought to be right-lateralized (Nemeroff et al., 2006). Studies in rats have shown that vagal fibers originating in the right dorsal nucleus and the right ambiguous nucleus further inert the region of the syno-atrial nodule, while the fibers of the left dorsal motor nucleus and the projected ambiguous further inert into the atrioventricular nodule region (Brack et al., 2004). Despite the possible side effects of right sided vagal stimulation, a possible treatment for heart failure has been developed using a tcVNS device, measuring the heart rate, that shuts down when bradycardia is detected (De Ferrari and Schwartz, 2011). However, for reasons outlined above, and possibly because a clinical trial showed no arrhythmic effects of tVNS when stimulating the left VN (Kreuzer et al., 2012), taVNS is almost exclusively applied to the left ear. Yet, these concerns have been challenged (Chen et al., 2015). For instance, studies in rodent models have not shown deleterious cardiac side effects (Krahl et al., 2003, also see Ay et al., 2011; He et al., 2013b). A study in healthy human participants has shown that taVNS can be applied to the right ear without associated cardiac side effects (De Couck et al., 2017). Similarly, studies in patients with chronic heart failure (Premchand et al., 2014; Wang et al., 2015b) did not report cardiac side effects suggesting that bilateral or right-lateralized taVNS is not associated with an excess rate of adverse effects. Furthermore, varying the intensity of taVNS has been shown not to impact on cardiac vagal activity in healthy adults (Borges et al., 2019). Critically, to the best of our knowledge, no systematic safety studies to date have directly compared stimulation sites and duration of stimulation to examine possible cardiac adverse effects.

The possibility of safely stimulating both, the left and right VN simultaneously, is of interest. In terms of using tVNS to increase noradrenaline release, it is plausible to suggest that bilateral stimulation may improve efficacy. Animal experimental data suggest a very wide spectrum of effects, critically dependent on stimulation parameters as well as on the duration of stimuli trains (Levy et al., 1969; Slenter et al., 1984) phase-locking the heart beat to the vagal stimuli (Jalife et al., 1983) through the

interaction of neural and muscular reflexes (Brooks and Lange, 1977). It has been shown that tVNS activates brain regions with ipsi and against lateral differences—such as the nucleus of the solitary tract, the amygdala or the nucleus accumbens (Frangos et al., 2015). LC projections to the cortex are mainly ipsilateral (Aston-Jones and Waterhouse, 2016), and noradrenaline levels are increased in both hemispheres after iVNS in rats. It has also been shown that depending on the currents applied (iVNS), different neuronal populations are recruited, and moreover that noradrenaline release in different target areas is also current-dependent (Roosevelt et al., 2006). Thus, hypothetical by stimulating both ears simultaneously, a summation effect could potentially be attained to reach the desired effects (also see Clancy et al., 2014). This idea should be objectively evaluated in the future, since pain threshold in some patients can be as low as 0.5 mA when auricular stimulation is carried out, and therapeutic effects could require higher stimulation currents (>1.0 mA) (Yakunina et al., 2017).

Current-Controlled vs. Voltage-Controlled Stimulation

In this iteration of the consensus, we would like to particularly focus on one particular technical aspect, namely the proper reporting on whether current-controlled or voltage-controlled stimulation is used. In principle current or voltage control settings can be used for tVNS; however, effects of and on the electrode/tissue boundary have to be accounted for (Merrill et al., 2005; Kaniusas et al., 2019a). As is generally the case in neuromodulation (Butson and McIntyre, 2005; Merrill et al., 2005; Vargas Luna et al., 2013), the current-controlled reliably defines the current in the body (e.g., excitable auricular tissue) independent of the highly variable electrode/tissue boundary. However, in the case of voltage-controlled tVNS, the resulting current in the tissue depends strongly on the electrode-skin boundary properties which then influence the resulting stimulation efficiency. The impact of current-controlled vs. voltage-control on the effectiveness will depend on multiple factors including electrode design. For instance, needle electrodes (for example in percutaneous tVNS) act typically as polarizable electrodes so that the boundary is predominantly capacitive, whereas surface electrodes (for example in taVNS) can act as non-polarizable electrodes with a predominantly resistive boundary. One theoretical concern with current-controlled stimulation is that conditions of unexpected high impedance at the electrode-skin interface will result in an associated increase in stimulator output voltage (i.e., needed to overcome this resistance in providing a prescribed current). The maximum voltage is limited by stimulator output compliance voltage. In a situation where the impedance suddenly changes, which can result from the electrode becoming displaced or (partially) detached and then reattached to the skin, a current controlled device may transiently produce a current above the target level (as its internal circuit adjust to the lower impedance load), which in turn can result in an unpleasant shock. This can be readily addressed with robust and motion-free application of current electrodes (e.g., ear clip electrodes, reliable adhesive electrodes), protocols that are cognisant of factors such as when stimulators are powered (Badran et al., 2019), or stimulators that are designed with a rapid accommodation time.

In the case of the voltage application, the polarization voltage is limited by the applied voltage. In addition, any potential detachment of the voltage electrode leads to an even reduced polarization voltage and thus reduced risks of unwanted transients. Consequently, voltage-controlled stimulation may be limited by current changes in situations where electrode-skin contact is not reliable. In addition to adverse events that can result from current flow through the body (e.g., pricking/itching), as with any electrical stimulation, adverse events may result from excessive electrochemical reactions at the electrode-electrolyte interface (Kaniusas, 2019). Specifically, if electrochemical products at the electrode-electrolyte interface reach the skin, skin irritation may ensue. Protocols to limit this include using charge-balanced waveforms (Sooksood et al., 2009, 2010), judicious selection of metal and electrolyte materials (Merrill et al., 2005; Khadka et al., 2018), minimizing total stimulation time at a given location, or ensuring the electrolyte provides sufficient separation between the metal and skin (Minhas et al., 2010).

Empirical Evidence for the Use of Certain Stimulation Parameters

Currently, the popularity of one tVNS device, tVNS Technologies GmbH (Erlangen, Germany), led to a common yet poorly argued parametric setting. Given the lack of flexibility of this device regarding changing the parameters, a signal with a pulse width between 200 and 300 μs at 25 Hz, and a duty cycle of 30-s on, 30-s off has frequently been adopted in studies. However, other parameters have been used in research with tVNS as well, which may explain in part the heterogeneity observed in findings from studies using tVNS (Borges et al., 2019). Consequently, the lack of knowledge regarding optimal stimulation parameters can be seen as a general limitation in this research field (Borges et al., 2019; Butt et al., 2020). Despite an understanding of the importance of the various stimulation parameters in optimizing the efficacy of tVNS, dose-response studies remain scarce. Recently, Badran et al. (2018c) systematically tested the effect of three variations in pulse width and frequency, respectively, on HR and found that a pulse width of 500 μs, if combined with a frequency of 10 Hz, produced the strongest decrease in HR compared to other parameter combinations. However, as HR is the result of mixed inputs from the sympathetic and parasympathetic (vagus) nerves, the effect of tVNS on HR may not necessarily correlate with the outcome of interest (Goldberger et al., 2019). Therefore, we advocate caution when interpreting these results. Some efforts have been made to understand how changing specific stimulation parameters influences the physiological effects of tVNS. Borges et al. (2019) tested the effect of different intensities on cardiac VN activity (Malik, 1996) in three experiments. They also compared different methods to define current intensity regarding cardiac vagal activity, namely presetting the same current intensity for all study participants throughout the experiment (set method) and instructing the study participants to freely choose an intensity

(free stimulation method). Cardiac vagal activity increased during tVNS when compared to resting measurement. However, this increase was not related to stimulation intensity, the method of stimulation, or whether the stimulation was active or sham. De Couck et al. (2017) investigated the effect of stimulation side (*right*, *left ear,* or *sham*), and session duration (10 min or 1 h) on heart rate variability (HRV). They found very specific effects related to heart rate variability components such as standard deviation of the RR intervals (SDNN) as well as low frequency (LF) and LF/high frequency (HF) ratio. However, tVNS had no effects on parameters that serve as an index of cardiac vagal activity, such as root mean square of successive differences in RR intervals (RMSSD) (Malik, 1996). Changes in the frequency domain components of HRV (LH, HF, and LF/HF ratio) were also observed with 1 h of tVNS at the right tragus (Tran et al., 2019). It was also reported that the magnitude and direction of tVNS-induced changes in LF/HF ratio is dependent on resting LF/HF ratio (Bretherton et al., 2019). The greatest effects of tVNS were observed in individuals with the lowest cardiac vagal activity at rest. Regarding stimulation location, Yakunina et al. (2017) compared the effects on brain activation of stimulation carried out at the inner tragus, inferoposterior wall of the ear canal, cymba conchae, and earlobe (sham). Among these areas, only tragus and cymba conchae stimulation activated areas thought to be part of the vagal pathway, such as the NTS. Importantly, the strongest activation of vagally innervated areas was seen during cymba conchae stimulation. These results are consistent with anatomical studies suggesting that the auricular branch of the VN innervates primarily the cymba conchae and the tragus (Peuker and Filler, 2002). Interestingly, a recent study by Sclocco et al. found that stimulation frequency also significantly modulates BOLD fMRI response in NTS, as well as other brainstem nuclei such as LC and raphe nucleus (Sclocco et al., 2020), with 100 Hz stimulation demonstrating enhanced activation in healthy adult volunteers. As anatomy is fundamental to providing effective tVNS (Badran et al., 2018a, also see Burger and Verkuil, 2018), further studies are warranted to delineate the exact anatomical basis of tVNS, in order to better guide future trials.

To summarize, the choice of stimulation parameters, mainly linked to pulse width, frequency, side and location of the stimulation, may influence effects of tVNS on both autonomic and cognitive processes. However, attempts to investigate the effects of individual tVNS stimulation parameters have primarily focussed on presumed physiological effects of tVNS rather than cognitive processes. Furthermore, despite first attempts to address the effects of parametrization, it is not clear what cognitive or autonomic processes have a parametric-specific effect, and this could explain the high heterogeneity of findings in studies using tVNS. Thus, it is time to carry out further studies that aim at understanding the parametric-specific effects of tVNS in order to optimize this tool for different applications.

Potential Biomarkers of Effective Stimulation

The neural mechanisms mediating the effects of tVNS are still poorly understood and, consequently, no clear consensus exists about potential biomarkers that could shed light on the efficacy of tVNS in general, or those guiding a choice in stimulation parameters. In this section, we briefly summarize findings concerning potential biomarkers related to vagal activity (for a detailed review about biomarkers of tVNS, see (Burger et al., 2020a) and finish with some remarks on methodological aspects that may be relevant when assessing biomarkers in tVNS research.

Heart Rate Variability

Some authors have proposed that the beneficial effects of tVNS may rely on increased activity of the VN *per se* (Gidron et al., 2018). Therefore, tVNS-related changes in vagal activity—measured by vagally-mediated HRV measures (vm-HRV) (Thayer and Lane, 2000; Kuo et al., 2005) may be informative to its efficacy. Animal research has consistently found that VNS, particularly to the right VN, increases vm-HRV measures (e.g., Huang et al., 2010; Sun et al., 2013). However, the relation between iVNS and HRV measures is less clear in humans (see Burger et al., 2020a for further details). Similar to reports using iVNS, findings on the modulatory effects of tVNS on vm-HRV measures are heterogeneous. Some studies showed an increase of vm-HRV measures after tVNS (Lamb et al., 2017; Bretherton et al., 2019; Sclocco et al., 2019; Tran et al., 2019), but others showed no effects (Weise et al., 2015; Antonino et al., 2017; De Couck et al., 2017; Burger et al., 2019a,b), or showed a decrease of vm-HRV parameters in individuals with high resting vagal activity (Bretherton et al., 2019) and two other studies during both, active and sham stimulation (Borges et al., 2019, 2020). A potential limitation of vm-HRV measures as a biomarker for tVNS is that the mechanism influencing vm-HRV (i.e., efferent vagal activation) may differ from the mechanistic target of tVNS (i.e., afferent vagal activation), and little is known about the interrelation of these two vagal pathways, i.e., much is known about cervical vagal feedback loops, but not much is known regarding auricular to cervical loops.

Metabolic Markers of Vagal Stimulation

The VN is a key part of the autonomic nervous system and transmits information between the peripheral organs and the brain to support homeostasis (de Lartigue, 2016). Although vagal stimulation primarily targets afferent fibers, preclinical and human work points to efferent effects as well that are mediated via the brain. In animal studies, there is conclusive evidence for reduced food intake and weight loss following iVNS (Roslin and Kurian, 2001; Val-Laillet et al., 2010; Gil et al., 2011; Banni et al., 2012). In rodents, a closed-loop VNS system implanted on the stomach wall substantially reduced food intake and delayed weight gain (Yao et al., 2018) demonstrating the modulatory role of negative feedback signals. In human studies, the vital role of the VN in modulating food intake, energy metabolism, and glycemic control has been demonstrated more recently (Burneo et al., 2002; Pardo et al., 2007; Shikora et al., 2013; Ikramuddin et al., 2014; Cork, 2018). Notably, taVNS has been shown to decrease the frequency of action potentials in human

gastric muscle cells (Hong et al., 2019; Teckentrup et al., 2020) suggesting that an electrogastrogram could be used to non-invasively track successful vagal stimulation. Taken together, these results highlight that stimulating vagal afferents may elicit efferent effects on key markers of energy homeostasis that could be used as a positive control outcome.

Noradrenergic-Related Processes and Markers

One potential mechanism by which tVNS may exert its effect is through the activation of the LC norepinephrine (LC-NE) system (Van Leusden et al., 2015; Hansen, 2019). Evidence pointing to a modulatory role of VN activity on LC-NE system activity comes from neuroimaging studies (Dietrich et al., 2008; Kraus et al., 2013; Frangos et al., 2015; Yakunina et al., 2017) and from studies relating vagal activity with physiological markers of LC-NE system activity, such as the P300 amplitude of event-related potentials (ERPs) (Murphy et al., 2011) see for review (Nieuwenhuis et al., 2005), salivary alpha amylase(sAA; Ehlert et al., 2006; Warren et al., 2017), and pupil dilation (Rajkowski, 1993; Joshi et al., 2016; Warren et al., 2017). For instance, De Taeye et al. observed that epileptic patients that responded favorably to iVNS therapy showed an increase in the P300 amplitude during VNS (De Taeye et al., 2014) see also (Neuhaus et al., 2007; Schevernels et al., 2016; Wostyn et al., 2017). In healthy participants, however, evidence for the modulatory effects of tVNS on the P300 amplitude has been mixed. Some studies found enhancing effects (Rufener et al., 2018; Ventura-Bort et al., 2018; Lewine et al., 2019), but others found no modulation of the P300 (Warren et al., 2017; Fischer et al., 2018). In terms of pupil dilation, although evidence about the relation between VNS and pupil dilatation is rather scarce, findings in animals (Bianca and Komisaruk, 2007; Mridha et al., 2019) and humans (but see Schevernels et al., 2016; Jodoin et al., 2018) seem to point to increased dilation of the pupil under active iVNS compared to no stimulation. By contrast, in four recent tVNS studies, no modulation of pupil dilation in response to the stimulation was found (Keute et al., 2019b; Warren et al., 2019; Burger et al., 2020b). Finally, recent studies have investigated the effects of tVNS on sAA levels as a potential marker of central NE release (Ehlert et al., 2006; Warren et al., 2017). Similar to P300 and pupil dilation, studies exploring tVNS effects on sAA level changes have shown inconsistent results. Some showed increased sAA levels following tVNS, but not after sham stimulation (Fischer et al., 2018; Ventura-Bort et al., 2018; Warren et al., 2019). Three recent studies did not show any sAA changes in response to tVNS (Koenig et al., 2019; Giraudier et al., 2020; D'Agostini et al. under review), Also, documented improvements of sleep quality with tVNS (Bretherton et al., 2019) are inconsistent with LC activation, which is the main brainstem nucleus that promotes arousal.

Taken together, there is currently no reliable vagal or noradrenergic biomarker of tVNS that produces replicable results across studies. It is likely that the reasons for this are multifactorial [see for a detailed discussion, (Burger et al., 2020a)]. Firstly, many studies included relatively small sample sizes, and the reported effects may have been underpowered. Secondly, baseline differences in tonic noradrenergic activation may also have an important influence on the efficacy (Murphy et al., 2011; Gee et al., 2014; van Kempen et al., 2019). Finally, stimulation settings, including stimulation sites (tragus vs. cymba concha; left vs. right ear) and parameters such as stimulation interval (30 s ON/OFF vs. continuous stimulation), intensity set-up (fixed or variable across participants), pulse widths, stimulation timing, among others, are not kept constant across experiments, impeding, to some extent, a full comparison of the results across labs. These changes might not be arbitrary, given that some of the settings may favor the efficacy of tVNS (e.g., stimulation of cymba conchae compared to the tragus (Yakunina et al., 2017); continuous vs. intermittent stimulation (Ventura-Bort et al., 2018); and long compared to short stimulation duration (Warren et al., 2019). We hope that the aforementioned standards may help overcome these challenges and improve the current knowledge about potential tVNS biomarkers.

Functional Neuroimaging

In comparison to HRV, pupil dilation and sAA, *functional magnetic resonance imaging fMRI* provides the possibility to confirm involvement of the central noradrenergic system by looking directly at LC and NTS activation as well as activation of possible target areas. Consequently, neuroimaging studies have tried to assess the modulatory role of VN activity on the LC-NE system activity in healthy adults (Kraus et al., 2007, 2013; Dietrich et al., 2008; Frangos et al., 2015; Yakunina et al., 2017; Badran et al., 2018b; Peng et al., 2018; Sclocco et al., 2019, 2020) and interictal migraine patients (Garcia et al., 2017; Zhang et al., 2019). The following results in functional activation are based on the comparison between real and sham stimulation at varying stimulation locations across the studies (see **Table 1**). Three studies that examined activation using 1.5T imaging (Dietrich et al., 2008) did not report any sham stimulation, therefore their results were based only on active stimulation compared to pre-stimulation baseline ($N = 4$). The authors found increased activation in the left LC as well as an increase in functional activation in the left thalamus (Dietrich et al., 2008). Conversely, a decrease in functional activation in limbic and temporal brain areas ($N = 6$) (Kraus et al., 2007) as well as in the LC and the NTS ($n = 8$) (Kraus et al., 2013) has also been shown. However, the reported functional activation of these three studies might have to be interpreted with caution due to the low sample sizes. Additionally, it is possible that the spatial precision afforded in data acquisition and data processing was not sufficient in these studies to reliably detect activations in LC and the NTS which are only a few millimeters wide. Furthermore, none of these studies reported information on the MRI head coil or smoothing kernel used which makes it difficult to assess Signal-to-Noise Ratio (SNR) and spatial precision of the results (Kraus et al., 2007, 2013; Dietrich et al., 2008). Studies with larger sample sizes using 3T scanners have shown *functional activation* in NTS (Frangos et al., 2015; Garcia et al., 2017; Yakunina et al., 2017; Sclocco et al., 2020), in the bilateral amygdala and left parahippocampal gyrus (Peng et al., 2018), which corresponds to the results of Frangos et al. (2015) that showed an increase in activation in the contralateral amygdala, nucleus accumbens and anterior thalamic nuclei. Moreover, a gradual increase and maximal activation in NTS during post-stimulation was observed (Frangos et al., 2015). In addition to

increased NTS activation, post stimulation effects, immediately after exhalatory-gated auricular vagal afferent nerve stimulation (eRAVANS), led to increased response to trigeminal sensory afference in nucleus raphe centralis and LC (Garcia et al., 2017). Yakunina et al. (2017) showed bilateral LC and NTS activation in unsmoothed data and indicated that this was also observed during real stimulation by placing electrodes at the inner surface of the tragus. Badran et al. (2018b) were not able to replicate these effects. However, this study used lower resolution fMRI (voxel size of 3 mm^3), which may explain the lack of activation observed in the NTS and LC. Some studies have also reported a *decrease in functional activation* in the bilateral hypothalamus and throughout the hippocampal formation in healthy adults (Frangos et al., 2015) as well as in the bilateral LC in interictal migraine patients (Zhang et al., 2019). This heterogeneous pattern of functional activations reported highlights once again the challenge of comparing and interpreting results from fMRI tVNS studies using different devices, electrodes, stimulation locations and session durations, as already previously reviewed (e.g., Yakunina et al., 2017; Peng et al., 2018). It is furthermore equally important to consider the varying stimulation and rest phases of study designs in different studies (e.g., 0.5 s pulse during each exhalation phase of respiration Garcia et al., 2017; 7 min on/2 min off stimulation Frangos et al., 2015; 30 s on/60 s off stimulation Yakunina et al., 2017; Peng et al., 2018), which might have affected the ability to detect functional activations in NTS and LC target areas. As there is considerable one possible focus in using tVNS fMRI to study evoked activation in NTS and other neurotransmitter source nuclei following stimulation, the fMRI studies of Yakunina et al. (2017) and Sclocco et al. (2019) used high resolution (e.g., 2.75 mm, and 1.2 mm isotropic resolution, respectively) and small Gaussian smoothing kernels (e.g., 2 mm), provide promising spatial precision in their methodological approach. Moreover, both studies compared results across various stimulation locations and observed most convincing LC activations using the left cymba conchae as an active stimulation location, which is in line with left cymba conchae being considered a good target for eliciting LC activation (Peuker and Filler, 2002). Both Yakunina et al. (2017) and Sclocco et al. (2019) provided details on co-registration methods and demonstrated sufficient spatial precision in data processing. The latter study used ultra-high-resolution fMRI at 7 Tesla with multi-band factor 2 to further increase SNR and demonstrated that exhalatory-gated tVNS enhanced NTS and LC/raphe targeting. Similar to Garcia et al. (2017), they observed increased activation in the LC as well as both dorsal and median raphe nuclei and in contrast to previous studies, they implemented short duration stimulation events (1s) extended over many minutes of time (Sclocco et al., 2019). Whilst many 3T fMRI studies may lack sufficient spatial precision to answer the question whether tVNS can target NTS and LC, recent studies suggest that larger sample sizes can also show NTS and LC response at this lower field strength (Sclocco et al., 2020), and previous 3T studies are more numerous and provide the strongest support that tVNS may indeed be a suitable tool for targeting the LC-NE system. Disorder specific brain circuits have been discussed as potential targets for tVNS in depression (Iseger et al., 2020) and tinnitus (Yakunina et al., 2018).

Taken together, when validating tVNS effects in various populations with the use of the most direct biomarker at hand for the LC-NE system, i.e., fMRI—a number of methodological considerations should be kept in mind over and above the usual need for appropriate stimulation parameters. Specifically, given that both NTS and LC span only a few millimeters, the extent of smoothing across studies should be considered. Frangos et al. (2015) pointed out the concerns of applying spatial smoothing to brainstem nuclei. Choosing a too high smoothing factor [e.g., 6 mm (Peng et al., 2018) or 8 mm (Yakunina et al., 2017)] could lead to an increased likelihood of false positives or to no observable activation in brainstem nuclei and thus, some chose to forgo smoothing brainstem data (Frangos et al., 2015; Yakunina et al., 2017). Similarly, ultra-high-resolution fMRI in the range of 1–2 mm voxel sizes also at higher field strengths seems warranted as well as customized high-precision spatial post-processing approaches optimized for the LC-NE system [see (Liu et al., 2017) for a review]. In addition to using comparable set-ups across studies, further research should also try to incorporate structural measures of the LC-NA system such as neuromelanin (NM)—sensitive magnetic resonance imaging (MRI) to anatomically identify the LC *in vivo* (Sasaki et al., 2006; Betts et al., 2017, 2019; Hämmerer et al., 2018; Priovoulos et al., 2018; Liu et al., 2019; Trujillo et al., 2019; Ye et al., 2020). Finally, the increased susceptibility of brainstem fMRI for low SNR and high physiological noise (Sclocco et al., 2018) could be counteracted by appropriate imaging paradigms as well as denoising or noise-control approaches (Brooks et al., 2013; Sclocco et al., 2018). If these recommendations are kept in mind, fMRI carries great potential as a more precise and direct tool for identifying activation in the LC-NE system using tVNS and in future may help to differentiate between tVNS responders vs. non-responders.

Toward Circuit-Based tVNS: Translational Approaches

Despite the growing interest in tVNS and in particular taVNS in clinical applications, many human studies remain in explorative frameworks and are typically confined to indirect readouts or neuronal activity of indirect fMRI responses (Yakunina et al., 2017; Burger et al., 2020a). Besides, imaging of small pontine nuclei such as the LC, NTS, or the raphe nucleus can be challenging in humans using MRI/fMRI, even at the purely anatomical level (Betts et al., 2019). Animal experimentation, on the other hand, can employ invasive techniques that allow researchers to gain detailed insights in molecular, anatomical, and neurophysiological mechanisms involved in VNS therapy. Thus, animal models enable a systematic investigation to be undertaken not only in terms of specificity of their readouts, such as cellular activity or level of neuromodulators, but also in terms of delineating parameter space for effective stimulation.

Effectiveness of VNS stimulation can be detected either directly with high temporal resolution i.e., *in vivo* electrophysiology as well as calcium imaging or more indirectly, after stimulation, using immunostaining or mRNA probes against immediate early genes products (C-Fos, Arc, Egr1) available in several animal models (Groves et al., 2005; Manta et al., 2009; Ay et al., 2016; Hulsey et al., 2017). Activation

of the NTS, LC, and raphe nucleus after VNS has also been monitored via extracellular electrophysiological recordings (Groves et al., 2005; Manta et al., 2009; Hulsey et al., 2017). Hulsey and colleagues mapped the stimulation space using LC neuron spiking activity as an output variable. Although this study was performed using invasive VNS, it was clearly shown that the application of low currents (0.1–1.2 mA) induced LC neuron firing, but higher currents (>1.2 mA) also activated neighboring Me5 neurons (Hulsey et al., 2017). This finding is of particular importance since different neuronal populations with distinct axonal projection could be potentially recruited depending on the set of stimulation parameters chosen. Also such findings can explain the broad scope of responses seen in human studies under sub-optimal parameters. Electrophysiological modulation in LC output regions has also been recorded upon VNS (Dorr and Debonnel, 2006; Manta et al., 2009; Alexander et al., 2017; Beaumont et al., 2017). These changes in neuronal activity in LC efferents have also been associated with long-lasting changes in the synaptic proteome in the amygdala and piriform cortex (Alexander et al., 2017). It is worth mentioning that similar studies are absent in the case of the promising non-invasive taVNS in animal models.

Despite Hulsey's rigorous approach toward parameter space exploration, a very rigid set of stimulation parameters is commonly used in animal models as well as in human studies. These involve current intensities varying between 0.25 and 1 mA, pulse frequency ranging between 20 and 30 Hz, a pulse width of 330–500 μs and a duty cycle of 30 s stimulation followed by a 5 min resting phase for 30–60 min (Manta et al., 2009; He et al., 2013b; Jiang et al., 2016; Vázquez-Oliver et al., 2020). Nevertheless, animal research offers the possibility to easily explore new sets of parameters such as variable waveforms or summation effects of multiple stimulation locations (Ay et al., 2016; Kaniusas et al., 2019a). In this respect, biphasic waveforms have been lately proposed since they can lead to larger recruitment of nerve fibers compared to monophasic waveforms (Kaniusas et al., 2019a). Monophasic, biphasic and triphasic stimulation patterns for different bursts lengths were recently compared (Kaniusas et al., 2020). This aspect of being able to manipulate the waveform, therefore, may allow us to tailor the strength of our stimulation depending on the specific disease condition. Furthermore, the majority of reports fail to provide a convincing rationale behind their parameter selection (Hosoi et al., 2000; Huston et al., 2007), stating them as "customized" and thus hindering the optimization of these stimulation parameters (Noller et al., 2019). Given that tVNS finds its application in a range of conditions, just as in the human studies noted above, it will be of prime importance to scrutinize factors such as the stimulation parameters, the anatomical location to deliver the electrical stimulation on the VN, and the design of the electrodes (Noller et al., 2019). Overall, optimization of stimulation parameters derived from animal research may provide an essential basis for optimal tVNS in human patients.

Nevertheless, electrophysiological read-outs might not always be the most suitable output signal to tune stimulation parameters. Even though specific stimulation parameters can evoke robust neuronal spiking, it can also lead to neurotransmitter depletion at the terminals (Yavich et al., 2005). Therefore, higher spiking rates do not necessarily translate into increasing levels of neuromodulators at the extracellular space. Thus, when spike rate is used as the only output optimization variable, the final results can be skewed. In this context, neurochemical approaches became a potent tool that is routinely implemented in animal models but is still far from being applicable in humans. Pioneering studies in the neurochemistry field using microdialysis identified glutamate release in the NTS of cats as a likely mode of vagal neurotransmission (Allchin et al., 1994). Since noradrenergic, cholinergic, or serotonergic activation downstream of the NTS likely mediates therapeutic effects of VNS, synaptic exhaustion can lead to a ceiling of neurotransmitter/neuromodulator levels at lower stimulation frequency as determined by microdialysis (Roosevelt et al., 2006; Follesa et al., 2007; Raedt et al., 2011; Manta et al., 2013). Nevertheless, if real-time feedback is intended for optimization of stimulation parameters using neuromodulator concentration as the output variable, the temporal resolution of microdialysis is too low. Here, electrochemical methods such as cyclic voltammetry and amperometry can be a suitable alternative given their subsecond time resolution (Kile et al., 2012). In particular, neuromodulators such as dopamine, adrenaline, noradrenaline, ATP, and serotonin can be electrochemically detected via fast-scan cyclic voltammetry or amperometry *in vivo* (Heien et al., 2004; John and Jones, 2007; Gourine et al., 2008; Njagi et al., 2010). Thus, fast electrochemical detection of neuromodulator concentration can help to optimize tVNS parameters for a personalized intervention in different pathologies (Mirza et al., 2019). Bringing together both, stimulation optimization and high-speed detection of neuromodulator release, will help to dissect the complex brain state dependence seen in human studies. It is worth noting that using neurotransmitter concentration as an output variable for stimulation parameter optimization can be easily implemented in animal models, with the advantage of multiple recordings in different regions simultaneously and high-density channel recordings (Zhang et al., 2018; Tomagra et al., 2019). Yet, due to its invasive nature, application in humans is precluded, which emphasizes the need for preclinical research on non-human primates.

A unique opportunity in animal research compared to humans will be the dissection of afferent and efferent pathways on a cellular and molecular level. Early retrograde tracing studies have helped us to understand how the auricular branch of the VN innervates brainstem nuclei (Jacquin et al., 1982; Takemura et al., 1987). The auriculotemporal nerve and auricular branch of the VN are thought to predominantly project to the NTS, dorsal vagal nucleus, motor nucleus of the VN, and paratrigeminal nucleus. A picture emerged where most innervation to these nuclei show a strong ipsilateral profile, although the area postrema is a notable exception, as it has bilateral innervation (Kalia and Sullivan, 1982). Despite these pioneering studies, new genetic and viral approaches in animals will continue to unlock the main cellular connectivity pathways involved in tVNS (Nassi et al., 2015). Such connectivity schemes

are likely to guide mechanistic approaches to optimally stimulate neuromodulatory systems and better anticipate off-targets effects. It is worthwhile to note that the auricular branch of the VN stimulation zone is innervated by sympathetic nerves as well. It has been therefore suggested that several sympathetic pathways could be stimulated while stimulating the auricular branch of the VN, which might lead to an activation of the NTS via the LC (Cakmak, 2019). This suggestion is novel since unidirectional NTS to LC activation is usually considered (Cakmak, 2019). On the other hand, there is accumulating evidence showing that the LC itself is not a functional neuroanatomical unit, but instead has multiple modules that differ in their projection targets and activity dynamics (Chandler et al., 2019). For example, circuits analysis using viral tracing, optogenetics, and chemogenetics have unraveled specific LC modules/circuits involved in analgesia, explorative behavior, or aversive learning modulation (Hirschberg et al., 2017; Borodovitsyna et al., 2018; Chandler et al., 2019). Moreover, it has been shown that NA is released in the hippocampus after 0.5 mA current stimulation but not in the cortex, while both structures are flooded by the neuromodulator when threshold current crosses more than 1.0 mA (Roosevelt et al., 2006). This exciting finding highlights: (1) the possibility of targeting different networks based on stimulation parameters and (2) the importance of understanding susceptibility of sub-circuits within the noradrenergic system regarding the stimulation parameters as well as different pathologies or brain states.

Animal research will reveal complex neuroanatomical connectivity implicated in taVNS with LC/NTS modularity. As a corollary, researchers will have to keep in mind that parameter optimization should be tuned specifically to the disease or brain state to be modulated, taking into account specific functional neuroanatomy. High throughput recording techniques such as calcium imaging and high-density *in vivo* electrophysiology coupled to molecular genetics and viral tracing will be needed together in this quest (Nassi et al., 2015; Schwarz et al., 2015; Totah et al., 2019). In addition, high-density channel electrochemical methods, behavioral studies and specific transgenic models of disease will also be required to provide a general view on tVNS/taVNS effects at the organismic level during normal conditions and in disease (Zhang et al., 2016, 2018; Tomagra et al., 2019; Vázquez-Oliver et al., 2020).

CONCLUSIONS

Given that the VN has been implicated in the pathophysiology of a number of disorders across many disciplines and phenomena on a behavioral and psychological level, VNS, and particularly non-invasive tVNS, has generated considerable interest. Whilst the mechanisms by which tVNS exerts psychological and physiological effects are increasingly, and more completely, understood, many early studies have been beset by inconsistencies around reporting. The development of internationally agreed consensus guidelines around reporting of tVNS studies should address these issues. Whilst tVNS represents a potential treatment option in many disorders and an interesting tool for experimental research, it needs to be studied in an objective and robust manner before its true place as a neuroimmunomodulatory intervention can be determined.

AUTHOR'S NOTE

If you are interested in being part of the tVNS consensus group and our efforts to strengthen tVNS research methodology, please contact the corresponding author. Finding consensus among so many authors involved is certainly a challenge. We therefore like these recommendations to be considered as work in progress.

AUTHOR CONTRIBUTIONS

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

FUNDING

The specific work presented here, did not receive any funding. DH is supported by the CBBS NeuroNetzwerk 17, SFB (Sonderforschungsbereich) 1315, Project B6, Research Training Group 2413 'SynAge' TP12, and ARUK SRF2018B-004. DQ acknowledges support from the Novo Nordisk Foundation (Excellence grant: NNF16OC0019856) and the Research Council of Norway (301767). VN acknowledges financial support via the US National Institutes for Health (NIH), Office of the Director (OT2-OD023867 to VN); National Center for Complementary and Integrative Health (NCCIH), NIH (P01-AT009965 to VN).

REFERENCES

Afanasiev, S. A., Pavliukova, E. N., Kuzmichkina, M. A., Rebrova, T. Y., Anfinogenova, Y., Likhomanov, K. S., et al. (2016). Nonpharmacological correction of hypersympatheticotonia in patients with chronic coronary insufficiency and severe left ventricular dysfunction. *Ann. Noninvasive Electrocardiol.* 21, 548–556. doi: 10.1111/anec.12349

Aihua, L., Lu, S., Liping, L., Xiuru, W., Hua, L., and Yuping, W. (2014). A controlled trial of transcutaneous vagus nerve stimulation for the treatment of pharmacoresistant epilepsy. *Epilepsy Behav.* 39, 105–110. doi: 10.1016/j.yebeh.2014.08.005

Alexander, G. M., Huang, Y. Z., Soderblom, E. J., He, X.-P., Moseley, M. A., and McNamara, J. O. (2017). Vagal nerve stimulation modifies neuronal activity and the proteome of excitatory synapses of amygdala/piriform cortex. *J. Neurochem.* 140, 629–644. doi: 10.1111/jnc.13931

Allchin, R. E., Batten, T. F., McWilliam, P. N., and Vaughan, P. F. (1994). Electrical stimulation of the vagus increases extracellular glutamate recovered from the nucleus tractus solitarii of the cat by *in vivo* microdialysis. *Exp. Physiol.* 79, 265–268. doi: 10.1113/expphysiol.1994.sp003761

Antonino, D., Teixeira, A. L., Maia-Lopes, P. M., Souza, M. C., Sabino-Carvalho, J. L., Murray, A. R., et al. (2017). Non-invasive vagus nerve stimulation acutely improves spontaneous cardiac baroreflex sensitivity in healthy young

men: a randomized placebo-controlled trial. *Brain Stimul.* 10, 875–881. doi: 10.1016/j.brs.2017.05.006

Aston-Jones, G., and Waterhouse, B. (2016). Locus coeruleus: from global projection system to adaptive regulation of behavior. *Brain Res.* 1645, 75–78. doi: 10.1016/j.brainres.2016.03.001

Ay, I., Nasser, R., Simon, B., and Ay, H. (2016). Transcutaneous cervical vagus nerve stimulation ameliorates acute ischemic injury in rats. *Brain Stimul.* 9, 166–173. doi: 10.1016/j.brs.2015.11.008

Ay, I., Sorensen, A. G., and Ay, H. (2011). Vagus nerve stimulation reduces infarct size in rat focal cerebral ischemia: an unlikely role for cerebral blood flow. *Brain Res.* 1392, 110–115. doi: 10.1016/j.brainres.2011.03.060

Badran, B. W., Brown, J. C., Dowdle, L. T., Mithoefer, O. J., LaBate, N. T., Coatsworth, J., et al. (2018a). Tragus or cymba conchae? Investigating the anatomical foundation of transcutaneous auricular vagus nerve stimulation (taVNS). *Brain Stimul.* 11, 947–948. doi: 10.1016/j.brs.2018.06.003

Badran, B. W., Dowdle, L. T., Mithoefer, O. J., LaBate, N. T., Coatsworth, J., Brown, J. C., et al. (2018b). Neurophysiologic effects of transcutaneous auricular vagus nerve stimulation (taVNS) via electrical stimulation of the tragus: a concurrent taVNS/fMRI study and review. *Brain Stimul.* 11, 492–500. doi: 10.1016/j.brs.2017.12.009

Badran, B. W., Jenkins, D. D., Cook, D., Thompson, S., Dancy, M., DeVries, W. H., et al. (2020). Transcutaneous auricular vagus nerve stimulation-paired rehabilitation for oromotor feeding problems in newborns: an open-label pilot study. *Front. Hum. Neurosci.* 14:77. doi: 10.3389/fnhum.2020.00077

Badran, B. W., Mithoefer, O. J., Summer, C. E., LaBate, N. T., Glusman, C. E., Badran, A. W., et al. (2018c). Short trains of transcutaneous auricular vagus nerve stimulation (taVNS) have parameter-specific effects on heart rate. *Brain Stimul.* 11, 699–708. doi: 10.1016/j.brs.2018.04.004

Badran, B. W., Yu, A. B., Adair, D., Mappin, G., DeVries, W. H., Jenkins, D. D., et al. (2019). Laboratory administration of transcutaneous auricular vagus nerve stimulation (taVNS): technique, targeting, and considerations. *J. Visual. Exp.* 143: 984. doi: 10.3791/58984

Banni, S., Carta, G., Murru, E., Cordeddu, L., Giordano, E., Marrosu, F., et al. (2012). Vagus nerve stimulation reduces body weight and fat mass in rats. *PLoS ONE.* 7:e44813. doi: 10.1371/journal.pone.0044813

Barbanti, P., Grazzi, L., Egeo, G., Padovan, A. M., Liebler, E., and Bussone, G. (2015). Non-invasive vagus nerve stimulation for acute treatment of high-frequency and chronic migraine: an open-label study. *J. Headache Pain* 16:61. doi: 10.1186/s10194-015-0542-4

Barbella, G., Cocco, I., Freri, E., Marotta, G., Visani, E., Franceschetti, S., et al. (2018). Transcutaneous vagal nerve stimulatio (t-VNS): an adjunctive treatment option for refractory epilepsy. *Seizure* 60, 115–119. doi: 10.1016/j.seizure.2018.06.016

Bauer, S., Baier, H., Baumgartner, C., Bohlmann, K., Fauser, S., Graf, W., et al. (2016). Transcutaneous vagus nerve stimulation (tVNS) for treatment of drug-resistant epilepsy: a randomized, double-blind clinical trial (cMPsE02). *Brain Stimul.* 9, 356–363. doi: 10.1016/j.brs.2015.11.003

Beaumont, E., Campbell, R. P., Andresen, M. C., Scofield, S., Singh, K., Libbus, I., et al. (2017). Cervical vagus nerve stimulation augments spontaneous discharge in second- and higher-order sensory neurons in the rat nucleus of the solitary tract. *Am. J. Physiol Heart Circ. Physiol.* 313, H354–H367. doi: 10.1152/ajpheart.00070.2017

Beste, C., Steenbergen, L., Sellaro, R., Grigoriadou, S., Zhang, R., Chmielewski, W., et al. (2016). Effects of concomitant stimulation of the GABAergic and norepinephrine system on inhibitory control—a study using transcutaneous vagus nerve stimulation. *Brain Stimul.* 9, 811–818. doi: 10.1016/j.brs.2016.07.004

Betts, M. J., Cardenas-Blanco, A., Kanowski, M., Jessen, F., and Düzel, E. (2017). *In vivo* MRI assessment of the human locus coeruleus along its rostrocaudal extent in young and older adults. *Neuroimage* 163, 150–159. doi: 10.1016/j.neuroimage.2017.09.042

Betts, M. J., Kirilina, E., Otaduy, M. C. G., Ivanov, D., Acosta-Cabronero, J., Callaghan, M. F., et al. (2019). Locus coeruleus imaging as a biomarker for noradrenergic dysfunction in neurodegenerative diseases. *Brain* 142, 2558–2571. doi: 10.1093/brain/awz193

Bianca, R., and Komisaruk, B. R. (2007). Pupil dilatation in response to vagal afferent electrical stimulation is mediated by inhibition of parasympathetic outflow in the rat. *Brain Res.* 1177, 29–36. doi: 10.1016/j.brainres.2007.06.104

Bikson, M., Esmaeilpour, Z., Adair, D., Kronberg, G., Tyler, W. J., Antal, A., et al. (2019). Transcranial electrical stimulation nomenclature. *Brain Stimul.* 12, 1349–1366. doi: 10.1016/j.brs.2019.07.010

Bonaz, B., Picq, C., Sinniger, V., Mayol, J. F., and Clarençon, D. (2013). Vagus nerve stimulation: from epilepsy to the cholinergic anti-inflammatory pathway. *Neurogastroenterol. Motil.* 25, 208–221. doi: 10.1111/nmo.12076

Boon, P., De Cock, E., Mertens, A., and Trinka, E. (2018). Neurostimulation for drug-resistant epilepsy: a systematic review of clinical evidence for efficacy, safety, contraindications and predictors for response. *Curr. Opin. Neurol.* 31, 198–210. doi: 10.1097/WCO.0000000000000534

Borges, U., Knops, L., Laborde, S., Klatt, S., and Raab, M. (2020). Transcutaneous vagus nerve stimulation may enhance only specific aspects of the core executive functions. A randomized crossover trial. *Front. Neurosci.* 14:523. doi: 10.3389/fnins.2020.00523

Borges, U., Laborde, S., and Raab, M. (2019). Influence of transcutaneous vagus nerve stimulation on cardiac vagal activity: not different from sham stimulation and no effect of stimulation intensity. *PLoS ONE* 14:e0223848. doi: 10.1371/journal.pone.0223848

Borodovitsyna, O., Flamini, M. D., and Chandler, D. J. (2018). Acute stress persistently alters locus coeruleus function and anxiety-like behavior in adolescent rats. *Neuroscience* 373, 7–19. doi: 10.1016/j.neuroscience.2018.01.020

Brack, K. E., Coote, J. H., and Ng, G. A. (2004). Interaction between direct sympathetic and vagus nerve stimulation on heart rate in the isolated rabbit heart. *Exp. Physiol.* 89, 128–139. doi: 10.1113/expphysiol.2003.002654

Brázdil, M., DoleŽalová, I., Koritáková, E., Chládek, J., Roman, R., Pail, M., et al. (2019). EEG Reactivity predicts individual efficacy of vagal nerve stimulation in intractable epileptics. *Front. Neurol.* 10:392. doi: 10.3389/fneur.2019.00392

Bretherton, B., Atkinson, L., Murray, A., Clancy, J., Deuchars, S., and Deuchars, J. (2019). Effects of transcutaneous vagus nerve stimulation in individuals aged 55 years or above: potential benefits of daily stimulation. *Aging* 11, 4836–4857. doi: 10.18632/aging.102074

Brock, C., Brock, B., Aziz, Q., Møller, H. J., Pfeiffer Jensen, M., Drewes, A. M., et al. (2017). Transcutaneous cervical vagal nerve stimulation modulates cardiac vagal tone and tumor necrosis factor-alpha. *Neurogastroenterol. Motil.* 29:e12999. doi: 10.1111/nmo.12999

Brooks, C. M., and Lange, G. (1977). Interaction of myogenic and neurogenic mechanisms that control heart rate. *Proc. Natl. Acad. Sci. U.S.A.* 74, 1761–1762. doi: 10.1073/pnas.74.4.1761

Brooks, J. C. W., Faull, O. K., Pattinson, K. T. S., and Jenkinson, M. (2013). Physiological noise in brainstem fMRI. *Front. Hum. Neurosci.* 7:623. doi: 10.3389/fnhum.2013.00623

Brown, G. L., and Eccles, J. C. (1934). The action of a single vagal volley on the rhythm of the heart beat. *J. Physiol.* 82, 211–241. doi: 10.1113/jphysiol.1934.sp003176

Burger, A. M., D'Agostini, M., Verkuil, B., and Diest, I. V. (2020a). Moving beyond belief: a narrative review of potential biomarkers for transcutaneous vagus nerve stimulation. *Psychophysiology* 57:e13571. doi: 10.1111/psyp.13571

Burger, A. M., Diest, I. V., Does, W. V., der, Hysaj, M., Thayer, J. F., Brosschot, J. F., et al. (2018). Transcutaneous vagus nerve stimulation and extinction of prepared fear: a conceptual non-replication. *Sci. Rep.* 8:11471. doi: 10.1038/s41598-018-29561-w

Burger, A. M., Van der Does, W., Brosschot, J. F., and Verkuil, B. (2020b). From ear to eye? No effect of transcutaneous vagus nerve stimulation on human pupil dilation: a report of three studies. *Biol. Psychol.* 152:107863. doi: 10.1016/j.biopsycho.2020.107863

Burger, A. M., Van der Does, W., Thayer, J. F., Brosschot, J. F., and Verkuil, B. (2019a). Transcutaneous vagus nerve stimulation reduces spontaneous but not induced negative thought intrusions in high worriers. *Biol. Psychol.* 142, 80–89. doi: 10.1016/j.biopsycho.2019.01.014

Burger, A. M., Van Diest, I., Van der Does, W., Korbee, J. N., Waziri, N., Brosschot, J. F., et al. (2019b). The effect of transcutaneous vagus nerve stimulation on fear generalization and subsequent fear extinction. *Neurobiol. Learn. Mem.* 161, 192–201. doi: 10.1016/j.nlm.2019.04.006

Burger, A. M., and Verkuil, B. (2018). Transcutaneous nerve stimulation via the tragus: are we really stimulating the vagus nerve? *Brain Stimul.* 11, 945–946. doi: 10.1016/j.brs.2018.03.018

Burger, A. M., Verkuil, B., Fenlon, H., Thijs, L., Cools, L., Miller, H. C., et al. (2017). Mixed evidence for the potential of non-invasive transcutaneous vagal nerve stimulation to improve the extinction and retention of fear. *Behav. Res. Ther.* 97, 64–74. doi: 10.1016/j.brat.2017.07.005

Burger, A. M., Verkuil, B., Van Diest, I., Van der Does, W., Thayer, J. F., and Brosschot, J. F. (2016). The effects of transcutaneous vagus nerve stimulation on conditioned fear extinction in humans. *Neurobiol. Learn. Mem.* 132, 49–56. doi: 10.1016/j.nlm.2016.05.007

Burneo, J. G., Faught, E., Knowlton, R., Morawetz, R., and Kuzniecky, R. (2002). Weight loss associated with vagus nerve stimulation. *Neurology* 59, 463–464. doi: 10.1212/WNL.59.3.463

Busch, V., Zeman, F., Heckel, A., Menne, F., Ellrich, J., and Eichhammer, P. (2013). The effect of transcutaneous vagus nerve stimulation on pain perception – an experimental study. *Brain Stimul.* 6, 202–209. doi: 10.1016/j.brs.2012.04.006

Butson, C. R., and McIntyre, C. C. (2005). Tissue and electrode capacitance reduce neural activation volumes during deep brain stimulation. *Clin. Neurophysiol.* 116, 2490–2500. doi: 10.1016/j.clinph.2005.06.023

Butt, M. F., Albusoda, A., Farmer, A. D., and Aziz, Q. (2020). The anatomical basis for transcutaneous auricular vagus nerve stimulation. *J. Anat.* 236, 588–611. doi: 10.1111/joa.13122

Cakmak, Y. O., Apaydin, H., Kiziltan, G., Gunduz, A., Ozsoy, B., Urey, H., et al. (2017). Rapid alleviation of parkinson's disease symptoms via electrostimulation of intrinsic auricular muscle zones. *Front. Hum. Neurosci.* 11:338. doi: 10.3389/fnhum.2017.00338

Cakmak, Y. O. (2019). Concerning auricular vagal nerve stimulation: occult neural networks. *Front. Hum. Neurosci.* 13:421. doi: 10.3389/fnhum.2019.00421

Capone, F., Assenza, G., Di Pino, G., Musumeci, G., Ranieri, F., Florio, L., et al. (2015). The effect of transcutaneous vagus nerve stimulation on cortical excitability. *J. Neural Transm.* 122, 679–685. doi: 10.1007/s00702-014-1299-7

Capone, F., Miccinilli, S., Pellegrino, G., Zollo, L., Simonetti, D., Bressi, F., et al. (2017). Transcutaneous vagus nerve stimulation combined with robotic rehabilitation improves upper limb function after stroke. *Neural Plasticity* 2017:7876507. doi: 10.1155/2017/7876507

Cha, W. W., Song, K., and Lee, H. Y. (2016). Persistent geotropic direction-changing positional nystagmus treated with transcutaneous vagus nerve stimulation. *Brain Stimul.* 9, 469–470. doi: 10.1016/j.brs.2016.03.011

Chakravarthy, K., Chaudhry, H., Williams, K., and Christo, P. J. (2015). Review of the uses of vagal nerve stimulation in chronic pain management. *Curr. Pain Headache Rep.* 19:54. doi: 10.1007/s11916-015-0528-6

Chandler, D. J., Jensen, P., McCall, J. G., Pickering, A. E., Schwarz, L. A., and Totah, N. K. (2019). Redefining noradrenergic neuromodulation of behavior: impacts of a modular locus coeruleus architecture. *J. Neurosci.* 39, 8239–8249. doi: 10.1523/JNEUROSCI.1164-19.2019

Chen, M., Yu, L., Ouyang, F., Liu, Q., Wang, Z., Wang, S., et al. (2015). The right side or left side of noninvasive transcutaneous vagus nerve stimulation: based on conventional wisdom or scientific evidence? *Int. J. Cardiol.* 187, 44–45. doi: 10.1016/j.ijcard.2015.03.351

Clancy, J. A., Mary, D. A., Witte, K. K., Greenwood, J. P., Deuchars, S. A., and Deuchars, J. (2014). Non-invasive vagus nerve stimulation in healthy humans reduces sympathetic nerve activity. *Brain Stimul.* 7, 871–877. doi: 10.1016/j.brs.2014.07.031

Colzato, L. S., Ritter, S. M., and Steenbergen, L. (2018a). Transcutaneous vagus nerve stimulation (tVNS) enhances divergent thinking. *Neuropsychologia* 111, 72–76. doi: 10.1016/j.neuropsychologia.2018.01.003

Colzato, L. S., Sellaro, R., and Beste, C. (2017). Darwin revisited: the vagus nerve is a causal element in controlling recognition of other's emotions. *Cortex* 92, 95–102. doi: 10.1016/j.cortex.2017.03.017

Colzato, L. S., Wolters, G., and Peifer, C. (2018b). Transcutaneous vagus nerve stimulation (tVNS) modulates flow experience. *Exp. Brain Res.* 236, 253–257. doi: 10.1007/s00221-017-5123-0

Cork, S. C. (2018). The role of the vagus nerve in appetite control: implications for the pathogenesis of obesity. *J. Neuroendocrinol.* 30:e12643. doi: 10.1111/jne.12643

Cristancho, P., Cristancho, M. A., Baltuch, G. H., Thase, M. E., and O'Reardon, J. P. (2011). Effectiveness and safety of vagus nerve stimulation for severe treatment-resistant major depression in clinical practice after FDA approval: outcomes at 1 year. *J. Clin. Psychiatry* 72, 1376–1382. doi: 10.4088/JCP.09m05888blu

De Couck, M., Cserjesi, R., Caers, R., Zijlstra, W. P., Widjaja, D., Wolf, N., et al. (2017). Effects of short and prolonged transcutaneous vagus nerve stimulation on heart rate variability in healthy subjects. *Auton. Neurosci.* 203, 88–96. doi: 10.1016/j.autneu.2016.11.003

De Ferrari, G. M., and Schwartz, P. J. (2011). Vagus nerve stimulation: from pre-clinical to clinical application: challenges and future directions. *Heart Fail. Rev.* 16, 195–203. doi: 10.1007/s10741-010-9216-0

De Icco, R., Martinelli, D., Bitetto, V., Fresia, M., Liebler, E., Sandrini, G., et al. (2018). Peripheral vagal nerve stimulation modulates the nociceptive withdrawal reflex in healthy subjects: a randomized, cross-over, sham-controlled study. *Cephalalgia* 38, 1658–1664. doi: 10.1177/0333102417742347

de Lartigue, G. (2016). Role of the vagus nerve in the development and treatment of diet-induced obesity. *J. Physiol.* 594, 5791–5815. doi: 10.1113/JP271538

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

De Taeye, L., Vonck, K., van Bochove, M., Boon, P., Van Roost, D., Mollet, L., et al. (2014). The P3 event-related potential is a biomarker for the efficacy of vagus nerve stimulation in patients with epilepsy. *Neurotherapeutics* 11, 612–622. doi: 10.1007/s13311-014-0272-3

Desbeaumes Jodoin, V., Richer, F., Miron, J.-P., Fournier-Gosselin, M.-P., and Lespérance, P. (2018). Long-term sustained cognitive benefits of vagus nerve stimulation in refractory depression. *J. ECT* 34, 283–290. doi: 10.1097/YCT.0000000000000502

Dietrich, S., Smith, J., Scherzinger, C., Hofmann-Preiß, K., Freitag, T., Eisenkolb, A., et al. (2008). A novel transcutaneous vagus nerve stimulation leads to brainstem and cerebral activations measured by functional MRI / funktionelle magnetresonanztomographie zeigt aktivierungen des hirnstamms und weiterer zerebraler strukturen unter transkutaner vagusnervstimulation. *Biomed. Tech/Biomed. Eng.* 53, 104–111. doi: 10.1515/BMT.2008.022

Dorr, A. E., and Debonnel, G. (2006). Effect of vagus nerve stimulation on serotonergic and noradrenergic transmission. *J. Pharmacol. Exp. Ther.* 318, 890–898. doi: 10.1124/jpet.106.104166

Du, X. J., Dart, A. M., and Riemersma, R. A. (1994). Sex differences in the parasympathetic nerve control of rat heart. *Clin. Exp. Pharmacol. Physiol.* 21, 485–493. doi: 10.1111/j.1440-1681.1994.tb02545.x

Ehlert, U., Erni, K., Hebisch, G., and Nater, U. (2006). Salivary alpha-amylase levels after yohimbine challenge in healthy men. *J. Clin. Endocrinol. Metab.* 91, 5130–5133. doi: 10.1210/jc.2006-0461

Ellrich, J. (2011). Transcutaneous vagus nerve stimulation. *Eur. Neurol. Rev.* 6, 254–256. doi: 10.17925/ENR.2011.06.04.254

Falkenberg, L. E., Westerhausen, R., Specht, K., and Hugdahl, K. (2012). Resting-state glutamate level in the anterior cingulate predicts blood-oxygen level-dependent response to cognitive control. *Proc. Natl Acad. Sci. U. S. A.* 109, 5069–5073. doi: 10.1073/pnas.1115628109

Fallgatter, A. J., Ehlis, A.-C., Ringel, T. M., and Herrmann, M. J. (2005). Age effect on far field potentials from the brain stem after transcutaneous vagus nerve stimulation. *Int. J. Psychophysiol.* 56, 37–43. doi: 10.1016/j.ijpsycho.2004.09.007

Fallgatter, A. J., Neuhauser, B., Herrmann, M. J., Ehlis, A.-C., Wagener, A., Scheuerpflug, P., et al. (2003). Far field potentials from the brain stem after transcutaneous vagus nerve stimulation. *J. Neural Transm.* 110, 1437–1443. doi: 10.1007/s00702-003-0087-6

Fang, J., Egorova, N., Rong, P., Liu, J., Hong, Y., Fan, Y., et al. (2017). Early cortical biomarkers of longitudinal transcutaneous vagus nerve stimulation treatment success in depression. *Neuroimage Clin.* 14, 105–111. doi: 10.1016/j.nicl.2016.12.016

Fang, J., Rong, P., Hong, Y., Fan, Y., Liu, J., Wang, H., et al. (2016). Transcutaneous vagus nerve stimulation modulates default mode network in major depressive disorder. *Biol. Psychiatry* 79, 266–273. doi: 10.1016/j.biopsych.2015.03.025

Ferrari, G. M. D., Crijns, H. J. G. M., Borggrefe, M., Milasinovic, G., Smid, J., Zabel, M., et al. (2011). Chronic vagus nerve stimulation: a new and promising therapeutic approach for chronic heart failure. *Eur. Heart J.* 32, 847–855. doi: 10.1093/eurheartj/ehq391

Finisguerra, A., Crescentini, C., and Urgesi, C. (2019). Transcutaneous vagus nerve stimulation affects implicit spiritual self-representations. *Neuroscience* 412, 144–159. doi: 10.1016/j.neuroscience.2019.05.059

Fischer, R., Ventura-Bort, C., Hamm, A., and Weymar, M. (2018). Transcutaneous vagus nerve stimulation (tVNS) enhances conflict-triggered adjustment

of cognitive control. *Cogn. Affect. Behav. Neurosci.* 18, 680–693. doi: 10.3758/s13415-018-0596-2

Follesa, P., Biggio, F., Gorini, G., Caria, S., Talani, G., Dazzi, L., et al. (2007). Vagus nerve stimulation increases norepinephrine concentration and the gene expression of BDNF and bFGF in the rat brain. *Brain Res.* 1179, 28–34. doi: 10.1016/j.brainres.2007.08.045

Frangos, E., Ellrich, J., and Komisaruk, B. R. (2015). Non-invasive access to the vagus nerve central projections via electrical stimulation of the external ear: FMRI evidence in humans. *Brain Stimul.* 8, 624–636. doi: 10.1016/j.brs.2014.11.018

Frangos, E., and Komisaruk, B. R. (2017). Access to vagal projections via cutaneous electrical stimulation of the neck: FMRI evidence in healthy humans. *Brain Stimul.* 10, 19–27. doi: 10.1016/j.brs.2016.10.008

Frøkjaer, J. B., Bergmann, S., Brock, C., Madzak, A., Farmer, A. D., Ellrich, J., et al. (2016). Modulation of vagal tone enhances gastroduodenal motility and reduces somatic pain sensitivity. *Neurogastroenterol. Motil.* 28, 592–598. doi: 10.1111/nmo.12760

Gancheva, S., Bierwagen, A., Markgraf, D. F., Bönhof, G. J., Murphy, K. G., Hatziagelaki, E., et al. (2018). Constant hepatic ATP concentrations during prolonged fasting and absence of effects of cerbomed nemos® on parasympathetic tone and hepatic energy metabolism. *Mol. Metab.* 7, 71–79. doi: 10.1016/j.molmet.2017.10.002

Garcia, R. G., Lin, R. L., Lee, J., Kim, J., Barbieri, R., Sclocco, R., et al. (2017). Modulation of brainstem activity and connectivity by respiratory-gated auricular vagal afferent nerve stimulation in migraine patients. *Pain* 158, 1461–1472. doi: 10.1097/j.pain.0000000000000930

Gaul, C., Diener, H.-C., Silver, N., Magis, D., Reuter, U., Andersson, A., et al. (2016). Non-invasive vagus nerve stimulation for PREVention and Acute treatment of chronic cluster headache (PREVA): a randomised controlled study. *Cephalalgia* 36, 534–546. doi: 10.1177/0333102415607070

Gee, J. W., de, Knapen, T., and Donner, T. H. (2014). Decision-related pupil dilation reflects upcoming choice and individual bias. *Proc. Natl. Acad. Sci. U.S.A.* 111, E618–E625. doi: 10.1073/pnas.1317557111

Genheimer, H., Andreatta, M., Asan, E., and Pauli, P. (2017). Reinstatement of contextual conditioned anxiety in virtual reality and the effects of transcutaneous vagus nerve stimulation in humans. *Sci. Rep.* 7:17886. doi: 10.1038/s41598-017-18183-3

Gidron, Y., Deschepper, R., De Couck, M., Thayer, J. F., and Velkeniers, B. (2018). The vagus nerve can predict and possibly modulate non-communicable chronic diseases: introducing a neuroimmunological paradigm to public health. *J. Clin. Med.* 7:371. doi: 10.3390/jcm7100371

Gil, K., Bugajski, A., and Thor, P. (2011). Electrical vagus nerve stimulation decreases food consumption and weight gain in rats fed a high-fat diet. *J. Physiol. Pharmacol.* 62, 637–646.

Giraudier, M., Ventura-Bort, C., and Weymar, M. (2020). Transcutaneous vagus nerve stimulation (tVNS) improves high confidence recognition memory but not emotional word processing. *Front. Psychol.* 11:1276. doi: 10.3389/fpsyg.2020.01276

Goadsby, P. J., Grosberg, B. M., Mauskop, A., Cady, R., and Simmons, K. A. (2014). Effect of noninvasive vagus nerve stimulation on acute migraine: an open-label pilot study. *Cephalalgia* 34, 986–993. doi: 10.1177/0333102414524494

Goldberger, J. J., Arora, R., Buckley, U., and Shivkumar, K. (2019). Autonomic nervous system dysfunction. *J. Am. College Cardiol.* 73:64. doi: 10.1016/j.jacc.2018.12.064

Gourine, A. V., Dale, N., Korsak, A., Llaudet, E., Tian, F., Huckstepp, R., et al. (2008). Release of ATP and glutamate in the nucleus tractus solitarii mediate pulmonary stretch receptor (Breuer–Hering) reflex pathway. *J. Physiol.* 586, 3963–3978. doi: 10.1113/jphysiol.2008.154567

Groves, D. A., Bowman, E. M., and Brown, V. J. (2005). Recordings from the rat locus coeruleus during acute vagal nerve stimulation in the anaesthetised rat. *Neurosci. Lett.* 379, 174–179. doi: 10.1016/j.neulet.2004.12.055

Guleyupoglu, B., Schestatsky, P., Edwards, D., Fregni, F., and Bikson, M. (2013). Classification of methods in transcranial electrical stimulation (tES) and evolving strategy from historical approaches to contemporary innovations. *J. Neurosci. Methods* 219, 297–311. doi: 10.1016/j.jneumeth.2013.07.016

Hämmerer, D., Callaghan, M. F., Hopkins, A., Kosciessa, J., Betts, M., Cardenas-Blanco, A., et al. (2018). Locus coeruleus integrity in old age is selectively related to memories linked with salient negative events. *Proc. Natl. Acad. Sci. U.S.A.* 115, 2228–2233. doi: 10.1073/pnas.1712268115

Hansen, N. (2019). Memory reinforcement and attenuation by activating the human locus coeruleus via transcutaneous vagus nerve stimulation. *Front. Neurosci.* 12:955. doi: 10.3389/fnins.2018.00955

Harden, C. L., Pulver, M. C., Ravdin, L. D., Nikolov, B., Halper, J. P., and Labar, D. R. (2000). A pilot study of mood in epilepsy patients treated with vagus nerve stimulation. *Epilepsy Behav.* 1, 93–99. doi: 10.1006/ebeh.2000.0046

Hasan, A., Wolff-Menzler, C., Pfeiffer, S., Falkai, P., Weidinger, E., Jobst, A., et al. (2015). Transcutaneous noninvasive vagus nerve stimulation (tVNS) in the treatment of schizophrenia: a bicentric randomized controlled pilot study. *Eur. Arch. Psychiatry Clin. Neurosci.* 265, 589–600. doi: 10.1007/s00406-015-0618-9

He, W., Jing, X., Wang, X., Rong, P., Li, L., Shi, H., et al. (2013a). Transcutaneous auricular vagus nerve stimulation as a complementary therapy for pediatric epilepsy: a pilot trial. *Epilepsy Behav.* 28, 343–346. doi: 10.1016/j.yebeh.2013.02.001

He, W., Jing, X.-H., Zhu, B., Zhu, X.-L., Li, L., Bai, W.-Z., et al. (2013b). The auriculo-vagal afferent pathway and its role in seizure suppression in rats. *BMC Neurosci.* 14:85. doi: 10.1186/1471-2202-14-85

Heien, M. L. A. V., Johnson, M. A., and Wightman, R. M. (2004). Resolving neurotransmitters detected by fast-scan cyclic voltammetry. *Anal. Chem.* 76, 5697–5704. doi: 10.1021/ac0491509

Hein, E., Nowak, M., Kiess, O., Biermann, T., Bayerlein, K., Kornhuber, J., et al. (2013). Auricular transcutaneous electrical nerve stimulation in depressed patients: a randomized controlled pilot study. *J. Neural Transm.* 120, 821–827. doi: 10.1007/s00702-012-0908-6

Hirschberg, S., Li, Y., Randall, A., Kremer, E. J., and Pickering, A. E. (2017). Functional dichotomy in spinal- vs prefrontal-projecting locus coeruleus modules splits descending noradrenergic analgesia from ascending aversion and anxiety in rats. *ELife* 6:e29808. doi: 10.7554/eLife.29808.027

Homma, S., Yamazaki, Y., and Karakida, T. (1993). Blood pressure and heart rate relationships during cervical sympathetic and vagus nerve stimulation in streptozotocin diabetic rats. *Brain Res.* 629, 342–344. doi: 10.1016/0006-8993(93)91343-Q

Hong, G.-S., Pintea, B., Lingohr, P., Coch, C., Randau, T., Schaefer, N., et al. (2019). Effect of transcutaneous vagus nerve stimulation on muscle activity in the gastrointestinal tract (transVaGa): a prospective clinical trial. *Int. J. Colorectal Dis.* 34, 417–422. doi: 10.1007/s00384-018-3204-6

Hosoi, T., Okuma, Y., and Nomura, Y. (2000). Electrical stimulation of afferent vagus nerve induces IL-1beta expression in the brain and activates HPA axis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 279, R141–R147. doi: 10.1152/ajpregu.2000.279.1.R141

Hou, P. W., Hsu, H. C., Lin, Y. W., Tang, N. Y., Cheng, C. Y., and Hsieh, C. L. (2015). The history mechanism, and clinical application of auricular therapy in traditional Chinese medicine. *Evid Based Complement. Alternat. Med.* 2015:495684. doi: 10.1155/2015/495684

Howland, R. H. (2014). Vagus nerve stimulation. *Curr. Behav. Neurosci. Rep.* 1, 64–73. doi: 10.1007/s40473-014-0010-5

Huang, F., Dong, J., Kong, J., Wang, H., Meng, H., Spaeth, R. B., et al. (2014). Effect of transcutaneous auricular vagus nerve stimulation on impaired glucose tolerance: a pilot randomized study. *BMC Complement. Alternat. Med.* 14:203. doi: 10.1186/1472-6882-14-203

Huang, H. (1974). *Ear Acupuncture*. Emmaus, PA: Rodale Press Emmaus.

Huang, J., Wang, Y., Jiang, D., Zhou, J., and Huang, X. (2010). The sympathetic-vagal balance against endotoxemia. *J. Neural Transm.* 117, 729–735. doi: 10.1007/s00702-010-0407-6

Huffman, W. J., Subramaniyan, S., Rodriguiz, R. M., Wetsel, W. C., Grill, W. M., and Terrando, N. (2019). Modulation of neuroinflammation and memory dysfunction using percutaneous vagus nerve stimulation in mice. *Brain Stimul.* 12, 19–29. doi: 10.1016/j.brs.2018.10.005

Hulsey, D. R., Riley, J. R., Loerwald, K. W., Rennaker, R. L., Kilgard, M. P., and Hays, S. A. (2017). Parametric characterization of neural activity in the locus coeruleus in response to vagus nerve stimulation. *Exp. Neurol.* 289, 21–30. doi: 10.1016/j.expneurol.2016.12.005

Huston, J. M., Gallowitsch-Puerta, M., Ochani, M., Ochani, K., Yuan, R., Rosas-Ballina, M., et al. (2007). Transcutaneous vagus nerve stimulation reduces serum high mobility group box 1 levels and improves survival in murine sepsis. *Crit.Care Med.* 35, 2762–2768. doi: 10.1097/01.CCM.0000288102.15975.BA

Hyvärinen, P., Yrttiaho, S., Lehtimäki, J., Ilmoniemi, R. J., Mäkitie, A., Ylikoski, J., et al. (2015). Transcutaneous vagus nerve stimulation modulates tinnitus-related beta- and gamma-band activity. *Ear Hear.* 36, e76–e85. doi: 10.1097/AUD.0000000000000123

Ikramuddin, S., Blackstone, R. P., Brancatisano, A., Toouli, J., Shah, S. N., Wolfe, B. M., et al. (2014). Effect of reversible intermittent intra-abdominal vagal nerve blockade on morbid obesity: the recharge randomized clinical trial. *JAMA* 312, 915–922. doi: 10.1001/jama.2014.10540

Iseger, T. A., van Bueren, N. E. R., Kenemans, J. L., Gevirtz, R., and Arns, M. (2020). A frontal-vagal network theory for major depressive disorder: implications for optimizing neuromodulation techniques. *Brain Stimul.* 13, 1–9. doi: 10.1016/j.brs.2019.10.006

Jacobs, H. I. L., Riphagen, J. M., Razat, C. M., Wiese, S., and Sack, A. T. (2015). Transcutaneous vagus nerve stimulation boosts associative memory in older individuals. *Neurobiol. Aging* 36, 1860–1867. doi: 10.1016/j.neurobiolaging.2015.02.023

Jacquin, M. F., Semba, K., Rhoades, R. W., and Egger, M. D. (1982). Trigeminal primary afferents project bilaterally to dorsal horn and ipsilaterally to cerebellum, reticular formation, and cuneate, solitary, supratrigeminal and vagal nuclei. *Brain Res.* 246, 285–291. doi: 10.1016/0006-8993(82)91177-5

Jalife, J., Slenter, V. A., Salata, J. J., and Michaels, D. C. (1983). Dynamic vagal control of pacemaker activity in the mammalian sinoatrial node. *Circ. Res.* 52, 642–656. doi: 10.1161/01.RES.52.6.642

Janner, H., Klausenitz, C., Gürtler, N., Hahnenkamp, K., and Usichenko, T. I. (2018). Effects of electrical transcutaneous vagus nerve stimulation on the perceived intensity of repetitive painful heat stimuli: a blinded placebo- and sham-controlled randomized crossover investigation. *Anesthesia Analgesia* 126, 2085–2092. doi: 10.1213/ANE.0000000000002820

Jiang, Y., Li, L., Ma, J., Zhang, L., Niu, F., Feng, T., et al. (2016). Auricular vagus nerve stimulation promotes functional recovery and enhances the post-ischemic angiogenic response in an ischemia/reperfusion rat model. *Neurochem. Int.* 97, 73–82. doi: 10.1016/j.neuint.2016.02.009

Jin, Y., and Kong, J. (2016). Transcutaneous vagus nerve stimulation: a promising method for treatment of autism spectrum disorders. *Front. Neurosci.* 10:609. doi: 10.3389/fnins.2016.00609

Jodoin, V. D., Lespérance, P., Nguyen, D. K., Fournier-Gosselin, M.-P., Richer, F., Centre Hospitalier de l'Université de Montréal, Canada. (2018). Effects of vagus nerve stimulation on pupillary function. *Int. J. Psychophysiol.* 98(3 Pt 1), 455–459. doi: 10.1016/j.ijpsycho.2015.10.001

John, C. E., and Jones, S. R. (2007). "Fast scan cyclic voltammetry of dopamine and serotonin in mouse brain slices," in *Electrochemical Methods for Neuroscience,* eds A. C. Michael and L. M. Borland (Francis:CRC Press/Taylor). Available onlie at: http://www.ncbi.nlm.nih.gov/books/NBK2579/

Johnson, R. L., and Wilson, C. G. (2018). A review of vagus nerve stimulation as a therapeutic intervention. *J. Inflamm. Res.* 11, 203–213. doi: 10.2147/JIR.S163248

Jongkees, B. J., Immink, M. A., Finisguerra, A., and Colzato, L. S. (2018). Transcutaneous vagus nerve stimulation (tVNS) enhances response selection during sequential action. *Front. Psychol.* 9:1159. doi: 10.3389/fpsyg.2018.01159

Joshi, S., Li, Y., Kalwani, R. M., and Gold, J. I. (2016). Relationships between pupil diameter and neuronal activity in the locus coeruleus, colliculi, and cingulate cortex. *Neuron* 89, 221–234. doi: 10.1016/j.neuron.2015.11.028

Juel, J., Brock, C., Olesen, S., Madzak, A., Farmer, A., Aziz, Q., et al. (2017). Acute physiological and electrical accentuation of vagal tone has no effect on pain or gastrointestinal motility in chronic pancreatitis. *J. Pain Res.* 10, 1347–1355. doi: 10.2147/JPR.S133438

Kaczmarczyk, R., Tejera, D., Simon, B. J., and Heneka, M. T. (2017). Microglia modulation through external vagus nerve stimulation in a murine model of Alzheimer's disease. *J. Neurochem.* 146, 76–85. doi: 10.1111/jnc.14284

Kalia, M., and Sullivan, J. M. (1982). Brainstem projections of sensory and motor components of the vagus nerve in the rat. *J. Comp. Neurol.* 211, 248–265. doi: 10.1002/cne.902110304

Kampusch, S., Kaniusas, E., and Széles, J. C. (2013). "New approaches in multi-punctual percutaneous stimulation of the auricular vagus nerve," in *2013 6th International IEEE/EMBS Conference on Neural Engineering (NER)* (San Diego, CA), 263–266. doi: 10.1109/NER.2013.6695922

Kaniusas, E. (2019). *Biomedical Signals and Sensors III: Linking Electric Biosignals and Biomedical Sensors*. Available online at: https://www.springer.com/de/book/9783319749167

Kaniusas, E., Kampusch, S., Tittgemeyer, M., Panetsos, F., Gines, R. F., Papa, M., et al. (2019a). Current directions in the auricular vagus nerve stimulation II – an engineering perspective. *Front. Neurosci.* 13:772. doi: 10.3389/fnins.2019.00772

Kaniusas, E., Kampusch, S., Tittgemeyer, M., Panetsos, F., Gines, R. F., Papa, M., et al. (2019b). Current directions in the auricular vagus nerve stimulation I – a physiological perspective. *Front. Neurosci.* 13:854. doi: 10.3389/fnins.2019.00854

Kaniusas, E., Samoudi, A. M., Kampusch, S., Bald, K., Tanghe, E., Martens, L., et al. (2020). Stimulation pattern efficiency in percutaneous auricular vagus nerve stimulation: experimental versus numerical data. *IEEE Trans. Biomed. Eng.* 67, 1921–1935. doi: 10.1109/TBME.2019.2950777

Kemp, J., Després, O., Pebayle, T., and Dufour, A. (2014). Age-related decrease in sensitivity to electrical stimulation is unrelated to skin conductance: an evoked potentials study. *Clin. Neurophysiol.* 125, 602–607. doi: 10.1016/j.clinph.2013.08.020

Keute, M., Boehrer, L., Ruhnau, P., Heinze, H.-J., and Zaehle, T. (2019a). Transcutaneous vagus nerve stimulation (tVNS) and the dynamics of visual bistable perception. *Front. Neurosci.* 13:227. doi: 10.3389/fnins.2019.00227

Keute, M., Demirezen, M., Graf, A., Mueller, N. G., and Zaehle, T. (2019b). No modulation of pupil size and event-related pupil response by transcutaneous auricular vagus nerve stimulation (taVNS). *Sci. Rep.* 9:11452. doi: 10.1038/s41598-019-47961-4

Keute, M., Ruhnau, P., Heinze, H.-J., and Zaehle, T. (2018). Behavioral and electrophysiological evidence for GABAergic modulation through transcutaneous vagus nerve stimulation. *Clin. Neurophysiol.* 129, 1789–1795. doi: 10.1016/j.clinph.2018.05.026

Khadka, N., Borges, H., Zannou, A. L., Jang, J., Kim, B., Lee, K., et al. (2018). Dry tDCS: Tolerability of a novel multilayer hydrogel composite non-adhesive electrode for transcranial direct current stimulation. *Brain Stimul.* 11, 1044–1053. doi: 10.1016/j.brs.2018.07.049

Kile, B. M., Walsh, P. L., McElligott, Z. A., Bucher, E. S., Guillot, T. S., Salahpour, A., et al. (2012). Optimizing the temporal resolution of fast-scan cyclic voltammetry. *ACS Chem. Neurosci.* 3, 285–292. doi: 10.1021/cn200119u

Koenig, J., Parzer, P., Haigis, N., Liebemann, J., Jung, T., Resch, F., et al. (2019). Effects of acute transcutaneous vagus nerve stimulation on emotion recognition in adolescent depression. *Psychol. Med.* 1–10. doi: 10.1017/S0033291719003490. [Epub ahead of print].

Koenig, J., Rash, J. A., Campbell, T. S., Thayer, J. F., and Kaess, M. (2017). A meta-analysis on sex differences in resting-state vagal activity in children and adolescents. *Front. Physiol.* 8:582. doi: 10.3389/fphys.2017.00582

Koenig, J., and Thayer, J. F. (2016). Sex differences in healthy human heart rate variability: a meta-analysis. *Neurosci. Biobehav. Rev.* 64, 288–310. doi: 10.1016/j.neubiorev.2016.03.007

Kong, J., Fang, J., Park, J., Li, S., and Rong, P. (2018). Treating depression with transcutaneous auricular vagus nerve stimulation: state of the art and future perspectives. *Front. Psychiatry* 9:20. doi: 10.3389/fpsyt.2018.00020

Koopman, F. A., Chavan, S. S., Miljko, S., Grazio, S., Sokolovic, S., Schuurman, P. R., et al. (2016). Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis. *Proc. Natl. Acad. Sci. U.S.A.* 113, 8284–8289. doi: 10.1073/pnas.1605635113

Krahl, S. E., and Clark, K. B. (2012). Vagus nerve stimulation for epilepsy: a review of central mechanisms. *Surg. Neurol. Int.* 3, S255–S259. doi: 10.4103/2152-7806.103015

Krahl, S. E., Senanayake, S. S., and Handforth, A. (2003). Right-sided vagus nerve stimulation reduces generalized seizure severity in rats as effectively as left-sided. *Epilepsy Res.* 56, 1–4. doi: 10.1016/s0920-1211(03)00122-0

Kraus, T., Hösl, K., Kiess, O., Schanze, A., Kornhuber, J., and Forster, C. (2007). BOLD fMRI deactivation of limbic and temporal brain structures and mood enhancing effect by transcutaneous vagus nerve stimulation. *J. Neural Transm.* 114, 1485–1493. doi: 10.1007/s00702-007-0755-z

Kraus, T., Kiess, O., Hösl, K., Terekhin, P., Kornhuber, J., and Forster, C. (2013). CNS BOLD fMRI effects of sham-controlled transcutaneous electrical nerve stimulation in the left outer auditory canal – a pilot study. *Brain Stimul.* 6, 798–804. doi: 10.1016/j.brs.2013.01.011

Krause, B., and Cohen Kadosh, R. (2014). Not all brains are created equal: the relevance of individual differences in responsiveness to transcranial electrical stimulation. *Front. Syst. Neurosci.* 8:25. doi: 10.3389/fnsys.2014.00025

Kreuzer, P. M., Landgrebe, M., Husser, O., Resch, M., Schecklmann, M., Geisreiter, F., et al. (2012). Transcutaneous vagus nerve stimulation: retrospective assessment of cardiac safety in a pilot study. *Front. Psychiatry* 3:70. doi: 10.3389/fpsyt.2012.00070

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

Kuo, T. B., Lin, T., Yang, C. C., Li, C. L., Chen, C. F., and Chou, P. (1999). Effect of aging on gender differences in neural control of heart rate. *Am. J. Physiol.* 277, H2233–H2239. doi: 10.1152/ajpheart.1999.277.6.H2233

Kuo, T. B. J., Lai, C. J., Huang, Y.-T., and Yang, C. C. H. (2005). Regression analysis between heart rate variability and baroreflex-related vagus nerve activity in rats. *J. Cardiovasc. Electrophysiol.* 16, 864–869. doi: 10.1111/j.1540-8167.2005.40656.x

Lamb, D. G., Porges, E. C., Lewis, G. F., and Williamson, J. B. (2017). Non-invasive vagal nerve stimulation effects on hyperarousal and autonomic state in patients with posttraumatic stress disorder and history of mild traumatic brain injury: preliminary evidence. *Front. Med.* 4:124. doi: 10.3389/fmed.2017.00124

Lange, G., Janal, M. N., Maniker, A., Fitzgibbons, J., Fobler, M., Cook, D., et al. (2011). Safety and efficacy of vagus nerve stimulation in fibromyalgia: a phase I/II proof of concept trial. *Pain Med.* 12, 1406–1413. doi: 10.1111/j.1526-4637.2011.01203.x

Lanska, D. J. (2002). Corning and vagal nerve stimulation for seizures in the 1880s. *Neurology* 58, 452–459. doi: 10.1212/WNL.58.3.452

Laqua, R., Leutzow, B., Wendt, M., and Usichenko, T. (2014). Transcutaneous vagal nerve stimulation may elicit anti- and pro-nociceptive effects under experimentally-induced pain—a crossover placebo-controlled investigation. *Auton. Neurosci. Basic Clin.* 185, 120–122. doi: 10.1016/j.autneu.2014.07.008

Lehtimäki, J., Hyvärinen, P., Ylikoski, M., Bergholm, M., Mäkel,ä, J. P., Aarnisalo, A., et al. (2013). Transcutaneous vagus nerve stimulation in tinnitus: a pilot study. *Acta Oto Laryngol.* 133, 378–382. doi: 10.3109/00016489.2012.750736

Lerman, I., Hauger, R., Sorkin, L., Proudfoot, J., Davis, B., Huang, A., et al. (2016). Noninvasive transcutaneous vagus nerve stimulation decreases whole blood culture-derived cytokines and chemokines: a randomized, blinded, healthy control pilot trial: noninvasive vagus nerve stimulation modulates peripheral inflammation. *Neuromodulation* 19, 283–290. doi: 10.1111/ner.12398

Leutzow, B., Lange, J., Gibb, A., Schroeder, H., Nowak, A., Wendt, M., et al. (2013). Vagal sensory evoked potentials disappear under the neuromuscular block – an experimental study. *Brain Stimul.* 6, 812–816. doi: 10.1016/j.brs.2013.03.005

Levine, Y. A., Koopman, F., Faltys, M., Zitnik, R., and Tak, P.-P. (2014). Neurostimulation of the cholinergic antiinflammatory pathway in rheumatoid arthritis and inflammatory bowel disease. *Bioelectron. Med.* 1, 34–43. doi: 10.15424/bioelectronmed.2014.00008

Levy, M. N., Martin, P. J., Lano, T., and Zieske, H. (1969). Paradoxical effect of vagus nerve stimulation on heart rate in dogs. *Circ. Res.* 25, 303–314. doi: 10.1161/01.RES.25.3.303

Lewine, J. D., Paulson, K., Bangera, N., and Simon, B. J. (2019). Exploration of the impact of brief noninvasive vagal nerve stimulation on EEG and event-related potentials: impact of nVNS on brain electrophysiology. *Neuromodulation* 22, 564–572. doi: 10.1111/ner.12864

Liporace, J., Hucko, D., Morrow, R., Barolat, G., Nei, M., Schnur, J., et al. (2001). Vagal nerve stimulation: adjustments to reduce painful side effects. *Neurology* 57, 885–886. doi: 10.1212/WNL.57.5.885

Liu, J., Fang, J., Wang, Z., Rong, P., Hong, Y., Fan, Y., et al. (2016). Transcutaneous vagus nerve stimulation modulates amygdala functional connectivity in patients with depression. *J. Affect. Disord.* 205, 319–326. doi: 10.1016/j.jad.2016.08.003

Liu, K. Y., Acosta-Cabronero, J., Cardenas-Blanco, A., Loane, C., Berry, A. J., Betts, M. J., et al. (2019). *In vivo* visualization of age-related differences in the locus coeruleus. *Neurobiol. Aging* 74, 101–111. doi: 10.1016/j.neurobiolaging.2018.10.014

Liu, K. Y., Marijatta, F., Hämmerer, D., Acosta-Cabronero, J., Düzel, E., and Howard, R. J. (2017). Magnetic resonance imaging of the human locus coeruleus: a systematic review. *Neurosci. Biobehav. Rev.* 83, 325–355. doi: 10.1016/j.neubiorev.2017.10.023

Liugan, M., Zhang, M., and Cakmak, Y. O. (2018). Neuroprosthetics for auricular muscles: neural networks and clinical aspects. *Front. Neurol.* 8:752. doi: 10.3389/fneur.2017.00752

Lv, H., Zhao, Y., Chen, J., Wang, D., and Chen, H. (2019). Vagus nerve stimulation for depression: a systematic review. *Front. Psychol.* 10:64. doi: 10.3389/fpsyg.2019.00064

Maffiuletti, N. A., Herrero, A. J., Jubeau, M., Impellizzeri, F. M., and Bizzini, M. (2008). Differences in electrical stimulation thresholds between men and women. *Ann. Neurol.* 63, 507–512. doi: 10.1002/ana.21346

Malik, M. (1996). Heart rate variability. Standards of measurement, physiological interpretation, and clinical use: task force of the European Society of Cardiology and the North American Society for Pacing and Electrophysiology. *Annals Noninv. Electrocardiol.* 1, 151–181. doi: 10.1111/j.1542-474X.1996.tb00275.x

Manta, S., Dong, J., Debonnel, G., and Blier, P. (2009). Enhancement of the function of rat serotonin and norepinephrine neurons by sustained vagus nerve stimulation. *J. Psychiatry Neurosci.* 34, 272–280.

Manta, S., El Mansari, M., Debonnel, G., and Blier, P. (2013). Electrophysiological and neurochemical effects of long-term vagus nerve stimulation on the rat monoaminergic systems. *Int. J. Neuropsychopharmacol.* 16, 459–470. doi: 10.1017/S1461145712000387

Marrosu, F., Serra, A., Maleci, A., Puligheddu, M., Biggio, G., and Piga, M. (2003). Correlation between GABAA receptor density and vagus nerve stimulation in individuals with drug-resistant partial epilepsy. *Epilepsy Res.* 55, 59–70. doi: 10.1016/S0920-1211(03)00107-4

McGough, J. J., Sturm, A., Cowen, J., Tung, K., Salgari, G. C., Leuchter, A. F., et al. (2019). Double-blind, sham-controlled, pilot study of trigeminal nerve stimulation for attention-deficit/hyperactivity disorder. *J. Am. Acad. Child Adolesc. Psychiatry* 58, 403–411.e3. doi: 10.1016/j.jaac.2018.11.013

Merrill, D. R., Bikson, M., and Jefferys, J. G. R. (2005). Electrical stimulation of excitable tissue: design of efficacious and safe protocols. *J. Neurosci. Methods* 141, 171–198. doi: 10.1016/j.jneumeth.2004.10.020

Mertens, A., Naert, L., Miatton, M., Poppa, T., Carrette, E., Gadeyne, S., et al. (2020). Transcutaneous vagus nerve stimulation does not affect verbal memory performance in healthy volunteers. *Front. Psychol.* 11:551. doi: 10.3389/fpsyg.2020.00551

Mertens, A., Raedt, R., Gadeyne, S., Carrette, E., Boon, P., and Vonck, K. (2018). Recent advances in devices for vagus nerve stimulation. *Expert Rev. Med. Devices* 15, 527–539. doi: 10.1080/17434440.2018.1507732

Minhas, P., Bansal, V., Patel, J., Ho, J. S., Diaz, J., Datta, A., et al. (2010). Electrodes for high-definition transcutaneous DC stimulation for applications in drug delivery and electrotherapy, including tDCS. *J. Neurosci. Methods* 190, 188–197. doi: 10.1016/j.jneumeth.2010.05.007

Mirza, K. B., Golden, C. T., Nikolic, K., and Toumazou, C. (2019). Closed-loop implantable therapeutic neuromodulation systems based on neurochemical monitoring. *Front. Neurosci.* 13:808. doi: 10.3389/fnins.2019.00808

Moher, D., Schulz, K. F., Altman, D., and CONSORT Group Consolidated Standards of Reporting Trials (2001). The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomized trials. *JAMA* 285, 1987–1991. doi: 10.1001/jama.285.15.1987

Moodithaya, S., and Avadhany, S. T. (2012). Gender differences in age-related changes in cardiac autonomic nervous function. *J. Aging Res.* 2012:679345. doi: 10.1155/2012/679345

Morris, G. L., Gloss, D., Buchhalter, J., Mack, K. J., Nickels, K., and Harden, C. (2013). Evidence-based guideline update: vagus nerve stimulation for the treatment of epilepsy. *Epilepsy Curr.* 13, 297–303. doi: 10.5698/1535-7597-13.6.297

Morris, J., Straube, A., Diener, H.-C., Ahmed, F., Silver, N., Walker, S., et al. (2016). Cost-effectiveness analysis of non-invasive vagus nerve stimulation for the treatment of chronic cluster headache. *J. Headache Pain* 17:43. doi: 10.1186/s10194-016-0633-x

Mourdoukoutas, A. P., Truong, D. Q., Adair, D. K., Simon, B. J., and Bikson, M. (2018). High-resolution multi-scale computational model for non-invasive cervical vagus nerve stimulation. *Neuromodulation* 21, 261–268. doi: 10.1111/ner.12706

Mridha, Z., de Gee, J. W., Shi, Y., Alkashgari, R., Williams, J., Suminski, A., et al. (2019). Graded recruitment of pupil-linked neuromodulation by parametric stimulation of the vagus nerve | *bioRxiv [Preprint]*. doi: 10.1101/2019.12.28.890111

Murphy, P. R., Robertson, I. H., Balsters, J. H., and O'connell, R. G. (2011). Pupillometry and P3 index the locus coeruleus-noradrenergic arousal function in humans. *Psychophysiology* 48, 1532–1543. doi: 10.1111/j.1469-8986.2011.01226.x

Murray, A. R., Atkinson, L., Mahadi, M. K., Deuchars, S. A., and Deuchars, J. (2016a). The strange case of the ear and the heart: the auricular vagus nerve and its influence on cardiac control. *Auton. Neurosci. Basic Clin.* 199, 48–53. doi: 10.1016/j.autneu.2016.06.004

Murray, A. R., Clancy, J. A., Deuchars, S. A., and Deuchars, J. (2016b). Transcutaneous vagus nerve stimulation (tVNS) decreases sympathetic nerve activity in older healthy human subjects. *FASEB J.* 30(1 Suppl), 754.3. doi: 10.1136/heartjnl-2016-309890.215

Napadow, V. (2019). When a white horse is a horse: embracing the overlap between acupuncture and neuromodulation. *J. Altern. Complement. Med.* 24, 621–623. doi: 10.1089/acm.2018.29047.vtn

Napadow, V., Edwards, R. R., Cahalan, C. M., Mensing, G., Greenbaum, S., Valovska, A., et al. (2012). Evoked pain analgesia in chronic pelvic pain patients using respiratory-gated auricular vagal afferent nerve stimulation. *Pain Med.* 13, 777–789. doi: 10.1111/j.1526-4637.2012.01385.x

Nassi, J. J., Cepko, C. L., Born, R. T., and Beier, K. T. (2015). Neuroanatomy goes viral! *Front. Neuroanat.* 9:80. doi: 10.3389/fnana.2015.00080

Nemeroff, C. B., Mayberg, H. S., Krahl, S. E., McNamara, J., Frazer, A., Henry, T. R., et al. (2006). VNS therapy in treatment-resistant depression: clinical evidence and putative neurobiological mechanisms. *Neuropsychopharmacology* 31, 1345–1355. doi: 10.1038/sj.npp.1301082

Nesbitt, A. D., Marin, J. C. A., Tompkins, E., Ruttledge, M. H., and Goadsby, P. J. (2015). Initial use of a novel noninvasive vagus nerve stimulator for cluster headache treatment. *Neurology* 84, 1249–1253. doi: 10.1212/WNL.0000000000001394

Neuhaus, A. H., Luborzewski, A., Rentzsch, J., Brakemeier, E. L., Opgen-Rhein, C., Gallinat, J., et al. (2007). P300 is enhanced in responders to vagus nerve stimulation for treatment of major depressive disorder. *J. Affect. Disord.* 100, 123–128. doi: 10.1016/j.jad.2006.10.005

Neuser, M. P., Teckentrup, V., Kühnel, A., Hallschmid, M., Walter, M., and Kroemer, N. B. (2019). Vagus nerve stimulation increases vigor to work for rewards. *BioRxiv [Preprint]*. 789982. doi: 10.1101/789982

Ng, G. A., Brack, K. E., and Coote, J. H. (2001). Effects of direct sympathetic and vagus nerve stimulation on the physiology of the whole heart—a novel model of isolated langendorff perfused rabbit heart with intact dual autonomic innervation. *Exp. Physiol.* 86, 319–329. doi: 10.1113/eph8602146

Nieuwenhuis, S., Aston-Jones, G., and Cohen, J. D. (2005). Decision making, the P3, and the locus coeruleus-norepinephrine system. *Psychol. Bull.* 131, 510–532. doi: 10.1037/0033-2909.131.4.510

Njagi, J., Chernov, M. M., Leiter, J. C., and Andreescu, S. (2010). Amperometric detection of dopamine *in vivo* with an enzyme based carbon fiber microbiosensor. *Anal. Chem.* 82, 989–996. doi: 10.1021/ac9022605

Nogier, P. M. F. (1957). Über die akupunktur der ohrmuschel. *Dt Ztschr Akup* 6, 25–35.

Noller, C. M., Levine, Y. A., Urakov, T. M., Aronson, J. P., and Nash, M. S. (2019). Vagus nerve stimulation in rodent models: an overview of technical considerations. *Front. Neurosci.* 13:911. doi: 10.3389/fnins.2019.00911

Paleczny, B., Seredyński, R., and Ponikowska, B. (2019). Inspiratory- and expiratory-gated transcutaneous vagus nerve stimulation have different effects on heart rate in healthy subjects: preliminary results. *Clin. Auton. Res.* doi: 10.1007/s10286-019-00604-0. [Epub ahead of print].

Panebianco, M., Zavanone, C., Dupont, S., Restivo, D. A., and Pavone, A. (2016). Vagus nerve stimulation therapy in partial epilepsy: a review. *Acta Neurol. Belgica* 116, 241–248. doi: 10.1007/s13760-016-0616-3

Pardo, J., Sheikh, S., Kuskowski, M., Surerus-Johnson, C., Hagen, M., Lee, J., et al. (2007). Weight loss during chronic, cervical vagus nerve stimulation in depressed patients with obesity. *Int. J. Obesity* 31, 1756–1759. doi: 10.1038/sj.ijo.0803666

Pavlov, V. A., and Tracey, K. J. (2012). The vagus nerve and the inflammatory reflex–linking immunity and metabolism. *Nat. Rev. Endocrinol.* 8, 743–754. doi: 10.1038/nrendo.2012.189

Peng, L., Mu, K., Liu, A., Zhou, L., Gao, Y., Shenoy, I. T., et al. (2018). Transauricular vagus nerve stimulation at auricular acupoints kindey (CO10), yidan (CO11), liver (CO12) and shenmen (TF4) can induce auditory and limbic cortices activation measured by fMRI. *Hear. Res.* 359, 1–12. doi: 10.1016/j.heares.2017.12.003

Penry, J. K., and Dean, J. C. (1990). Prevention of intractable partial seizures by intermittent vagal stimulation in humans: preliminary results. *Epilepsia* 31, S40–S43. doi: 10.1111/j.1528-1157.1990.tb05848.x

Perkins, D. O. (2002). Predictors of noncompliance in patients with schizophrenia. *J. Clin. Psychiatry* 63, 1121–1128. doi: 10.4088/JCP.v63n1206

Peterchev, A. V., Wagner, T. A., Miranda, P. C., Nitsche, M. A., Paulus, W., Lisanby, S. H., et al. (2012). Fundamentals of transcranial electric and magnetic stimulation dose: definition, selection, and reporting practices. *Brain Stimul.* 5, 435–453. doi: 10.1016/j.brs.2011.10.001

Peuker, E. T., and Filler, T. J. (2002). The nerve supply of the human auricle. *Clin. Anat.* 15, 35–37. doi: 10.1002/ca.1089

Polak, T., Markulin, F., Ehlis, A.-C., Langer, J. B. M., Ringel, T. M., and Fallgatter, A. J. (2009). Far field potentials from brain stem after transcutaneous vagus nerve stimulation: optimization of stimulation and recording parameters. *J. Neural Transm.* 116, 1237–1242. doi: 10.1007/s00702-009-0282-1

Premchand, R. K., Sharma, K., Mittal, S., Monteiro, R., Dixit, S., Libbus, I., et al. (2014). Autonomic regulation therapy via left or right cervical vagus nerve stimulation in patients with chronic heart failure: results of the ANTHEM-HF trial. *J. Cardiac Failure* 20, 808–816. doi: 10.1016/j.cardfail.2014.08.009

Priovoulos, N., Jacobs, H. I. L., Ivanov, D., Uludag, K., Verhey, F. R. J., and Poser, B. A. (2018). High-resolution *in vivo* imaging of human locus coeruleus by magnetization transfer MRI at 3T and 7T. *Neuroimage* 168, 427–436. doi: 10.1016/j.neuroimage.2017.07.045

Raedt, R., Clinckers, R., Mollet, L., Vonck, K., El Tahry, R., Wyckhuys, T., et al. (2011). Increased hippocampal noradrenaline is a biomarker for efficacy of vagus nerve stimulation in a limbic seizure model. *J. Neurochem.* 117, 461–469. doi: 10.1111/j.1471-4159.2011.07214.x

Rajkowski, J. (1993). Correlations between locus coeruleus (LC) neural activity, pupil diameter and behavior in monkey support a role of LC in attention. *Soc. Neurosc.* Available online at: https://ci.nii.ac.jp/naid/10021384962/en/

Rawat, J. K., Roy, S., Singh, M., Guatam, S., Yadav, R. K., Ansari, M. N., et al. (2019). Transcutaneous vagus nerve stimulation regulates the cholinergic anti-inflammatory pathway to counteract 1, 2-dimethylhydrazine induced colon carcinogenesis in albino wistar rats. *Front. Pharmacol.* 10:353. doi: 10.3389/fphar.2019.00353

Redgrave, J., Day, D., Leung, H., Laud, P. J., Ali, A., Lindert, R., et al. (2018). Safety and tolerability of transcutaneous vagus nerve stimulation in humans; a systematic review. *Brain Stimul.* 11, 1225–1238. doi: 10.1016/j.brs.2018.08.010

Rong, P., Liu, A., Zhang, J., Wang, Y., He, W., Yang, A., et al. (2014). Transcutaneous vagus nerve stimulation for refractory epilepsy: a randomized controlled trial. *Clin. Sci.* doi: 10.1042/CS20130518. [Epub ahead of print].

Rong, P., Liu, J., Wang, L., Liu, R., Fang, J., Zhao, J., et al. (2016). Effect of transcutaneous auricular vagus nerve stimulation on major depressive disorder: a nonrandomized controlled pilot study. *J. Affect. Disord.* 195, 172–179. doi: 10.1016/j.jad.2016.02.031

Roosevelt, R. W., Smith, D. C., Clough, R. W., Jensen, R. A., and Browning, R. A. (2006). Increased extracellular concentrations of norepinephrine in cortex and hippocampus following vagus nerve stimulation in the rat. *Brain Res.* 1119, 124–132. doi: 10.1016/j.brainres.2006.08.048

Roslin, M., and Kurian, M. (2001). The use of electrical stimulation of the vagus nerve to treat morbid obesity. *Epilepsy Behav.* 2, S11–S16. doi: 10.1006/ebeh.2001.0213

Rufener, K. S., Geyer, U., Janitzky, K., Heinze, H.-J., and Zaehle, T. (2018). Modulating auditory selective attention by non-invasive brain stimulation: differential effects of transcutaneous vagal nerve stimulation and transcranial random noise stimulation. *Eur. J. Neurosci.* 48, 2301–2309. doi: 10.1111/ejn.14128

Salman, I. M. (2015). Cardiovascular autonomic dysfunction in chronic kidney disease: a comprehensive review. *Curr. Hypertens. Rep.* 17:59. doi: 10.1007/s11906-015-0571-z

Sasaki, M., Shibata, E., Tohyama, K., Takahashi, J., Otsuka, K., Tsuchiya, K., et al. (2006). Neuromelanin magnetic resonance imaging of locus ceruleus and substantia nigra in Parkinson's disease. *Neuroreport* 17, 1215–1218. doi: 10.1097/01.wnr.0000227984.84927.a7

Sator-Katzenschlager, S. M., Scharbert, G., Kozek-Langenecker, S. A., Szeles, J. C., Finster, G., Schiesser, A. W., et al. (2004). The short- and long-term benefit in chronic low back pain through adjuvant electrical versus manual auricular acupuncture. *Anesthesia Analgesia* 98, 1359–1364. doi: 10.1213/01.ANE.0000107941.16173.F7

Schevernels, H., van Bochove, M. E., De Taeye, L., Bombeke, K., Vonck, K., Van Roost, D., et al. (2016). The effect of vagus nerve stimulation on response inhibition. *Epilepsy Behav.* 64, 171–179. doi: 10.1016/j.yebeh.2016.09.014

Schulz-Stübner, S., and Kehl, F. (2011). Treatment of persistent hiccups with transcutaneous phrenic and vagal nerve stimulation. *Intensive Care Med.* 37, 1048–1049. doi: 10.1007/s00134-011-2150-3

Schwarz, L. A., Miyamichi, K., Gao, X. J., Beier, K. T., Weissbourd, B., DeLoach, K. E., et al. (2015). Viral-genetic tracing of the input–output organization of a central noradrenaline circuit. *Nature* 524, 88–92. doi: 10.1038/nature 14600

Sclocco, R., Beissner, F., Bianciardi, M., Polimeni, J. R., and Napadow, V. (2018). Challenges and opportunities for brainstem neuroimaging with ultrahigh field MRI. *Neuroimage* 168, 412–426. doi: 10.1016/j.neuroimage.2017.02.052

Sclocco, R., Garcia, R. G., Gabriel, A., Kettner, N. W., Napadow, V., and Barbieri, R. (2017). "Respiratory-gated auricular vagal afferent nerve stimulation (RAVANS) effects on autonomic outflow in hypertension," in *2017 39th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC)* (Jeju), 3130–3133. doi: 10.1109/EMBC.2017.8037520

Sclocco, R., Garcia, R. G., Kettner, N. W., Fisher, H. P., Isenburg, K., Makarovsky, M., et al. (2020). Stimulus frequency modulates brainstem response to respiratory-gated transcutaneous auricular vagus nerve stimulation. *Brain Stimul.* 13, 970–978. doi: 10.1016/j.brs.2020.03.011

Sclocco, R., Garcia, R. G., Kettner, N. W., Isenburg, K., Fisher, H. P., Hubbard, C. S., et al. (2019). The influence of respiration on brainstem and cardiovagal response to auricular vagus nerve stimulation: a multimodal ultrahigh-field (7T) fMRI study. *Brain Stimul.* 12, 911–921. doi: 10.1016/j.brs.2019.02.003

Sellaro, R., de Gelder, B., Finisguerra, A., and Colzato, L. S. (2018). Transcutaneous vagus nerve stimulation (tVNS) enhances recognition of emotions in faces but not bodies. *Cortex* 99, 213–223. doi: 10.1016/j.cortex.2017.11.007

Sellaro, R., Steenbergen, L., Verkuil, B., van IJzendoorn, M. H., and Colzato, L. S. (2015a). Transcutaneous vagus nerve stimulation (tVNS) does not increase prosocial behavior in cyberball. *Front. Psychol.* 6:499. doi: 10.3389/fpsyg.2015.00499

Sellaro, R., van Leusden, J. W. R., Tona, K.-D., Verkuil, B., Nieuwenhuis, S., and Colzato, L. S. (2015b). Transcutaneous vagus nerve stimulation enhances post-error slowing. *J. Cogn. Neurosci.* 27, 2126–2132. doi: 10.1162/jocn_a_00851

Shikora, S., Toouli, J., Herrera, M. F., Kulseng, B., Zulewski, H., Brancatisano, R., et al. (2013). Vagal blocking improves glycemic control and elevated blood pressure in obese subjects with type 2 diabetes mellitus. *J. Obes.* 2013:245683. doi: 10.1155/2013/245683

Shim, H. J., Kwak, M. Y., An, Y.-H., Kim, D. H., Kim, Y. J., and Kim, H. J. (2015). Feasibility and safety of transcutaneous vagus nerve stimulation paired with notched music therapy for the treatment of chronic tinnitus. *J. Audiol. Otol.* 19, 159–167. doi: 10.7874/jao.2015.19.3.159

Silberstein, S. D., Calhoun, A. H., Lipton, R. B., Grosberg, B. M., Cady, R. K., Dorlas, S., et al. (2016a). Chronic migraine headache prevention with noninvasive vagus nerve stimulation: the EVENT study. *Neurology* 87, 529–538. doi: 10.1212/WNL.0000000000002918

Silberstein, S. D., Mechtler, L. L., Kudrow, D. B., Calhoun, A. H., McClure, C., Saper, J. R., et al. (2016b). Non-invasive vagus nerve stimulation for the acute treatment of cluster headache: findings from the randomized, double-blind, sham-controlled ACT1 study. *Headache* 56, 1317–1332. doi: 10.1111/head.12896

Silvanto, J., Muggleton, N., and Walsh, V. (2008). State-dependency in brain stimulation studies of perception and cognition. *Trends Cogn. Sci.* 12, 447–454. doi: 10.1016/j.tics.2008.09.004

Slenter, V. A., Salata, J. J., and Jalife, J. (1984). Vagal control of pacemaker periodicity and intranodal conduction in the rabbit sinoatrial node. *Circ. Res.* 54, 436–446. doi: 10.1161/01.RES.54.4.436

Sooksood, K., Stieglitz, T., and Ortmanns, M. (2009). "Recent advances in charge balancing for functional electrical stimulation," in *Conference Proceedings: Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual Conference* (Minneapolis), 2009, 5518–5521. doi: 10.1109/IEMBS.2009.5333181

Sooksood, K., Stieglitz, T., and Ortmanns, M. (2010). An active approach for charge balancing in functional electrical stimulation. *IEEE Trans. Biomed. Circ. Syst.* 4, 162–170. doi: 10.1109/TBCAS.2010.2040277

Stavrakis, S., Humphrey, M. B., Scherlag, B. J., Hu, Y., Jackman, W. M., Nakagawa, H., et al. (2015). Low-level transcutaneous electrical vagus nerve stimulation suppresses atrial fibrillation. *J. Am. Coll. Cardiol.* 65, 867–875. doi: 10.1016/j.jacc.2014.12.026

Steenbergen, L., Colzato, L. S., and Maraver, M. J. (2020). Vagal signaling and the somatic marker hypothesis: the effect of transcutaneous vagal nerve stimulation on delay discounting is modulated by positive mood. *Int. J. Psychophysiol.* 148, 84–92. doi: 10.1016/j.ijpsycho.2019.10.010

Steenbergen, L., Sellaro, R., Stock, A.-K., Verkuil, B., Beste, C., and Colzato, L. S. (2015). Transcutaneous vagus nerve stimulation (tVNS) enhances response selection during action cascading processes. *Eur. Neuropsychopharmacol.* 25, 773–778. doi: 10.1016/j.euroneuro.2015.03.015

Stefan, H., Kreiselmeyer, G., Kerling, F., Kurzbuch, K., Rauch, C., Heers, M., et al. (2012). Transcutaneous vagus nerve stimulation (t-VNS) in pharmacoresistant epilepsies: a proof of concept trial. *Epilepsia* 53, e115–e118. doi: 10.1111/j.1528-1167.2012.03492.x

Straube, A., Ellrich, J., Eren, O., Blum, B., and Ruscheweyh, R. (2015). Treatment of chronic migraine with transcutaneous stimulation of the auricular branch of the vagal nerve (auricular t-VNS): A randomized, monocentric clinical trial. *J. Headache Pain* 16:543. doi: 10.1186/s10194-015-0543-3

Sun, P., Zhou, K., Wang, S., Li, P., Chen, S., Lin, G., et al. (2013). Involvement of MAPK/NF-κB signaling in the activation of the cholinergic anti-inflammatory pathway in experimental colitis by chronic vagus nerve stimulation. *PLoS ONE* 8:e69424. doi: 10.1371/journal.pone.0069424

Szeska, C., Richter, J., Wendt, J., Weymar, M., and Hamm, A. O. (2020). Promoting long-term inhibition of human fear responses by non-invasive transcutaneous vagus nerve stimulation during extinction training. *Sci. Rep.* 10:1529. doi: 10.1038/s41598-020-58412-w

Sztajzel, J., Jung, M., and Bayes de Luna, A. (2008). Reproducibility and gender-related differences of heart rate variability during all-day activity in young men and women. *Ann. Noninvasive Electrocardiol.* 13, 270–277. doi: 10.1111/j.1542-474X.2008.00231.x

Takemura, M., Sugimoto, T., and Sakai, A. (1987). Topographic organization of central terminal region of different sensory branches of the rat mandibular nerve. *Exp. Neurol.* 96, 540–557. doi: 10.1016/0014-4886(87)90217-2

Teckentrup, V., Neubert, S., Santiago, J. C. P., Hallschmid, M., Walter, M., and Kroemer, N. B. (2020). Non-invasive stimulation of vagal afferents reduces gastric frequency. *Brain Stimul.* 13, 470–473. doi: 10.1016/j.brs.2019.12.018

Tekdemir, I., Aslan, A., and Elhan, A. (1998). A clinico-anatomic study of the auricular branch of the vagus nerve and Arnold's ear-cough reflex. *Surg. Radiol. Anat.* 20, 253–257. doi: 10.1007/s00276-998-0253-5

Thayer, J. F., and Lane, R. D. (2000). A model of neurovisceral integration in emotion regulation and dysregulation. *J. Affect. Disord.* 61, 201–216. doi: 10.1016/S0165-0327(00)00338-4

Thayer, J. F., and Lane, R. D. (2009). Claude bernard and the heart-brain connection: further elaboration of a model of neurovisceral integration. *Neurosci. Biobehav. Rev.* 33, 81–88. doi: 10.1016/j.neubiorev.2008.08.004

Tobaldini, E., Toschi-Dias, E., Appratto de Souza, L., Rabello Casali, K., Vicenzi, M., Sandrone, G., et al. (2019). Cardiac and peripheral autonomic responses to orthostatic stress during transcutaneous vagus nerve stimulation in healthy subjects. *J. Clin. Med.* 8:496. doi: 10.3390/jcm8040496

Tomagra, G., Picollo, F., Battiato, A., Picconi, B., De Marchis, S., Pasquarelli, A., et al. (2019). Quantal release of dopamine and action potential firing detected in midbrain neurons by multifunctional diamond-based microarrays. *Front. Neurosci.* 13:288. doi: 10.3389/fnins.2019.00288

Tona, K.-D., Revers, H., Verkuil, B., and Nieuwenhuis, S. (2020). Noradrenergic regulation of cognitive flexibility: no effects of stress, transcutaneous vagus nerve stimulation, and atomoxetine on task-switching in humans. *J. Cogn. Neurosci.* 32:1881–1895. doi: 10.1162/jocn_a_01603

Totah, N. K. B., Logothetis, N. K., and Eschenko, O. (2019). Noradrenergic ensemble-based modulation of cognition over multiple timescales. *Brain Res.* 1709, 50–66. doi: 10.1016/j.brainres.2018.12.031

Tran, N., Asad, Z., Elkholey, K., Scherlag, B. J., Po, S. S., and Stavrakis, S. (2019). Autonomic neuromodulation acutely ameliorates left ventricular strain in humans. *J. Cardiovasc. Transl. Res.* 12, 221–230. doi: 10.1007/s12265-018-9853-6

Trujillo, P., Petersen, K. J., Cronin, M. J., Lin, Y.-C., Kang, H., Donahue, M. J., et al. (2019). Quantitative magnetization transfer imaging of the human locus coeruleus. *Neuroimage* 200, 191–198. doi: 10.1016/j.neuroimage.2019.06.049

Tu, Y., Fang, J., Cao, J., Wang, Z., Park, J., Jorgenson, K., et al. (2018). A distinct biomarker of continuous transcutaneous vagus nerve stimulation treatment in major depressive disorder. *Brain Stimul.* 11, 501–508. doi: 10.1016/j.brs.2018.01.006

Usichenko, T., Hacker, H., and Lotze, M. (2017a). Transcutaneous auricular vagal nerve stimulation (taVNS) might be a mechanism behind the analgesic effects of auricular acupuncture. *Brain Stimul.* 10, 1042–1044. doi: 10.1016/j.brs.2017.07.013

Usichenko, T., Laqua, R., Leutzow, B., and Lotze, M. (2017b). Preliminary findings of cerebral responses on transcutaneous vagal nerve stimulation on experimental heat pain. *Brain Imaging Behav.* 11, 30–37. doi: 10.1007/s11682-015-9502-5

Uthman, B. M., Wilder, B. J., Penry, J. K., Dean, C., Ramsay, R. E., Reid, S. A., et al. (1993). Treatment of epilepsy by stimulation of the vagus nerve. *Neurology* 43, 1338–1345. doi: 10.1212/WNL.43.7.1338

Val-Laillet, D., Biraben, A., Randuineau, G., and Malbert, C. H. (2010). Chronic vagus nerve stimulation decreased weight gain, food consumption and sweet craving in adult obese minipigs. *Appetite* 55, 245–252. doi: 10.1016/j.appet.2010.06.008

Valsalva, A. M. (1704). *De Aura Humana Tractatus and Trajecti ad Rhenum Urecht*. Utrecht:Trajecti ad Rhenum.

van Kempen, J., Loughnane, G. M., Newman, D. P., Kelly, S. P., Thiele, A., O'Connell, R. G., et al. (2019). Behavioural and neural signatures of perceptual decision-making are modulated by pupil-linked arousal. *ELife* 8:e42541. doi: 10.7554/eLife.42541

Van Leusden, J. W. R., Sellaro, R., and Colzato, L. S. (2015). Transcutaneous vagal nerve stimulation (tVNS): a new neuromodulation tool in healthy humans? *Front. Psychol.* 6:102. doi: 10.3389/fpsyg.2015.00102

Vanneste, S., Martin, J., Rennaker, R. L., Kilgard, M. P. (2017). Pairing sound with vagus nerve stimulation modulates cortical synchrony and phase coherence in tinnitus: an exploratory retrospective study. *Sci. Rep.* 7:17345. doi: 10.1038/s41598-017-17750-y

Vargas Luna, J. L., Krenn, M., Cortés, J. A., and Mayr, W. (2013). Comparison of current and voltage control techniques for neuromuscular electrical stimulation in the anterior thigh. *Biomed. Tech.* 58:1–2. doi: 10.1515/bmt-2013-4021

Vázquez-Oliver, A., Brambilla-Pisoni, C., Domingo-Gainza, M., Maldonado, R., Ivorra, A., and Ozaita, A. (2020). Auricular transcutaneous vagus nerve stimulation improves memory persistence in naïve mice and in an intellectual disability mouse model. *Brain Stimul.* 13, 494–498. doi: 10.1016/j.brs.2019.12.024

Ventura-Bort, C., Wirkner, J., Genheimer, H., Wendt, J., Hamm, A. O., and Weymar, M. (2018). Effects of transcutaneous vagus nerve stimulation (tVNS) on the P300 and alpha-amylase level: a pilot study. *Front. Hum. Neurosci.* 12:202. doi: 10.3389/fnhum.2018.00202

Verkuil, B., and Burger, A. M. (2019). Transcutaneous vagus nerve stimulation does not affect attention to fearful faces in high worriers. *Behav. Res. Ther.* 113, 25–31. doi: 10.1016/j.brat.2018.12.009

Vieira, A., Reis, A. M., Matos, L. C., Machado, J., Moreira, A. (2018). Does auriculotherapy have therapeutic effectiveness? an overview of systematic reviews. *Complement. Ther. Clin. Pract.* 33, 61–70. doi: 10.1016/j.ctcp.2018.08.005

von Elm, E., Altman, D. G., Egger, M., Pocock, S. J., Gøtzsche, P. C., Vandenbroucke, J. P., et al. (2008). The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *J. Clin. Epidemiol.* 61, 344–349. doi: 10.1016/j.jclinepi.2007.11.008

Wang, D.-W., Yin, Y.-M., and Yao, Y.-M. (2016). Vagal modulation of the inflammatory response in sepsis. *Int. Rev. Immunol.* 35, 415–433. doi: 10.3109/08830185.2015.1127369

Wang, Z., Zhou, X., Sheng, X., Yu, L., and Jiang, H. (2015a). Unilateral low-level transcutaneous electrical vagus nerve stimulation: a novel noninvasive treatment for myocardial infarction. *Int. J. Cardiol.* 190, 9–10. doi: 10.1016/j.ijcard.2015.04.087

Wang, Z., Zhou, X., Sheng, X., Yu, L., Jiang, H. (2015b). Noninvasive vagal nerve stimulation for heart failure: was it practical or just a stunt? Int. *J. Cardiol.* 187, 637–638. doi: 10.1016/j.ijcard.2015.03.430

Wang, Z.engjian, Fang, J., Liu, J., Rong, P., Jorgenson, K., Park, J., Lang, C., et al. (2018). Frequency-dependent functional connectivity of the nucleus accumbens during continuous transcutaneous vagus nerve stimulation in major depressive disorder. *J. Psychiatric Res.* 102, 123–131. doi: 10.1016/j.jpsychires.2017.12.018

Warren, C. M., Tona, K. D., Ouwerkerk, L., van Paridon, J., Poletiek, F., van Steenbergen, H., et al. (2019). The neuromodulatory and hormonal effects of transcutaneous vagus nerve stimulation as evidenced by salivary alpha amylase, salivary cortisol, pupil diameter, and the P3 event-related potential. *Brain Stimul.* 12, 635–642. doi: 10.1016/j.brs.2018.12.224

Warren, C. M., van den Brink, R. L., Nieuwenhuis, S., and Bosch, J. A. (2017). Norepinephrine transporter blocker atomoxetine increases salivary alpha amylase. *Psychoneuroendocrinology* 78, 233–236. doi: 10.1016/j.psyneuen.2017.01.029

Weise, D., Adamidis, M., Pizzolato, F., Rumpf, J.-J., Fricke, C., and Classen, J. (2015). Assessment of brainstem function with auricular branch of vagus nerve stimulation in Parkinson's disease. *PLoS ONE* 10:e0120786. doi: 10.1371/journal.pone.0120786

Woodbury, D. M., and Woodbury, J. W. (1990), Effects of vagal stimulation on experimentally induced seizures in rats. *Epilepsia* 31, S7–S19. doi: 10.1111/j.1528-1157.1990.tb05852.x

Woods, A. J., Antal, A., Bikson, M., Boggio, P. S., Brunoni, A. R., Celnik, P., et al. (2016). A technical guide to tDCS, and related non-invasive brain stimulation tools. *Clin. Neurophysiol.* 127, 1031–1048. doi: 10.1016/j.clinph.2015.11.012

Wostyn, S., Staljanssens, W., De Taeye, L., Strobbe, G., Gadeyne, S., Van Roost, D., et al. (2017). EEG derived brain activity reflects treatment response from vagus nerve stimulation in patients with epilepsy. *Int. J. Neural Syst.* 27:1650048. doi: 10.1142/S0129065716500489

Xiong, J., Xue, F. S., Liu, J. H., Xu, Y. C., Liao, X., Zhang, Y. M., et al. (2009). Transcutaneous vagus nerve stimulation may attenuate postoperative cognitive dysfunction in elderly patients. *Med. Hypoth.* 73, 938–941. doi: 10.1016/j.mehy.2009.06.033

Yakunina, N., Kim, S. S., and Nam, E.-C. (2017). Optimization of transcutaneous vagus nerve stimulation using functional MRI. *Neuromodulation* 20, 290−300. doi: 10.1111/ner.12541

Yakunina, N., Kim, S. S., and Nam, E.-C. (2018). BOLD fMRI effects of transcutaneous vagus nerve stimulation in patients with chronic tinnitus. *PLoS ONE* 13:e0207281. doi: 10.1371/journal.pone.0207281

Yang, G., Xue, F., Sun, C., Liao, X., and Liu, J. (2017). Vagal nerve stimulation: a potentially useful adjuvant to treatment of sepsis. *J Anesth Perioper Med.* (2017). doi: 10.24015/JAPM.2017.0012

Yao, G., Kang, L., Li, J., Long, Y., Wei, H., Ferreira, C. A., et al. (2018). Effective weight control via an implanted self-powered vagus nerve stimulation device. *Nat. Commun.* 9:5349. doi: 10.1038/s41467-018-07764-z

Yavich, L., Jäkälä, P., and Tanila, H. (2005). Noradrenaline overflow in mouse dentate gyrus following locus coeruleus and natural stimulation: real-time monitoring by *in vivo* voltammetry. *J. Neurochem.* 95, 641–650. doi: 10.1111/j.1471-4159.2005.03390.x

Ye, R., Rua, C., O'Callaghan, C., Jones, P. S., Hezemans, F., Kaalund, S. S., et al. (2020). An *in vivo* probabilistic atlas of the human locus coeruleus at ultra-high field. *BioRxiv [Preprint]*. 932087. doi: 10.1101/2020.02.03.932087

Ylikoski, J., Lehtimäki, J., Pirvola, U., Mäkitie, A., Aarnisalo, A., Hyvärinen, P., et al. (2017). Non-invasive vagus nerve stimulation reduces sympathetic preponderance in patients with tinnitus. *Acta Oto Laryngol.* 137, 426–431. doi: 10.1080/00016489.2016.1269197

Yoo, P. B., Liu, H., Hincapie, J. G., Ruble, S. B., Hamann, J. J., and Grill, W. M. (2016). Modulation of heart rate by temporally patterned

vagus nerve stimulation in the anesthetized dog. *Physiol. Rep.* 4:e12689. doi: 10.14814/phy2.12689

Yu, L., Huang, B., Po, S. S., Tan, T., Wang, M., Zhou, L., et al. (2017). Low-level tragus stimulation for the treatment of ischemia and reperfusion injury in patients with ST-segment elevation myocardial infarction: a proof-of-concept study. *JACC Cardiovasc. Interv.* 10, 1511–1520. doi: 10.1016/j.jcin.2017.04.036

Yuan, H., and Silberstein, S. D. (2016a). Vagus nerve and vagus nerve stimulation, a comprehensive review: Part I. *Headache* 56, 71–78. doi: 10.1111/head.12647

Yuan, H., and Silberstein, S. D. (2016b). Vagus nerve and vagus nerve stimulation, a comprehensive review: Part II. *Headache* 56, 259–266. doi: 10.1111/head.12650

Zabara, J. (1985). Time course of seizure control to brief, repetitive stimuli. *Epilepsia* 28:604.

Zabara, J. (1992). Inhibition of experimental seizures in canines by repetitive vagal stimulation. *Epilepsia* 33, 1005–1012. doi: 10.1111/j.1528-1157.1992.tb01751.x

Zhang, S., Song, Y., Jia, J., Xiao, G., Yang, L., Sun, M., et al. (2016). "An implantable microelectrode array for dopamine and electrophysiological recordings in response to L-dopa therapy for Parkinson's disease," in *Conference Proceedings: . . . Annual International Conference of the IEEE Engineering in Medicine and Biology Society. IEEE Engineering in Medicine and Biology Society. Annual Conference* (Orlando), *2016*, 1922–1925. doi: 10.1109/EMBC.2016.7591098

Zhang, S., Song, Y., Wang, M., Xiao, G., Gao, F., Li, Z., et al. (2018). Real-time simultaneous recording of electrophysiological activities and dopamine overflow in the deep brain nuclei of a non-human primate with Parkinson's disease using nano-based microelectrode arrays. *Microsyst. Nanoeng.* 4, 1–9. doi: 10.1038/micronano.2017.70

Zhang, Y., Liu, J., Li, H., Yan, Z., Liu, X., Cao, J., et al. (2019). Transcutaneous auricular vagus nerve stimulation at 1 Hz modulates locus coeruleus activity and resting state functional connectivity in patients with migraine: an fMRI study. *Neuroimage Clin.* 24:101971. doi: 10.1016/j.nicl.2019.101971

Ziemann, U., Tam, A., Bütefisch, C., and Cohen, L. G. (2002). Dual modulating effects of amphetamine on neuronal excitability and stimulation-induced plasticity in human motor cortex. *Clin. Neurophysiol.* 113, 1308–1315. doi: 10.1016/S1388-2457(02)00171-2

Conflict of Interest: EK and SK are employed by company SzeleSTIM GmbH. JS received honoraria from SzeleSTIM GmbH and owns patents in the field of the auricular vagus nerve stimulation. EK, SK, and JS are shareholders of SzeleSTIM GmbH.

The remaining 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.

The reviewer TS declared a shared affiliation, with no collaboration, with one of the authors HJ, to the handling editor at the time of review.

Citation: Farmer AD, Strzelczyk A, Finisguerra A, Gourine AV, Gharabaghi A, Hasan A, Burger AM, Jaramillo AM, Mertens A, Majid A, Verkuil B, Badran BW, Ventura-Bort C, Gaul C, Beste C, Warren CM, Quintana DS, Hämmerer D, Freri E, Frangos E, Tobaldini E, Kaniusas E, Rosenow F, Capone F, Panetsos F, Ackland GL, Kaithwas G, O'Leary GH, Genheimer H, Jacobs HIL, Van Diest I, Schoenen J, Redgrave J, Fang J, Deuchars J, Széles JC, Thayer JF, More K, Vonck K, Steenbergen L, Vianna LC, McTeague LM, Ludwig M, Veldhuizen MG, De Couck M, Casazza M, Keute M, Bikson M, Andreatta M, D'Agostini M, Weymar M, Betts M, Prigge M, Kaess M, Roden M, Thai M, Schuster NM, Montano N, Hansen N, Kroemer NB, Rong P, Fischer R, Howland RH, Sclocco R, Sellaro R, Garcia RG, Bauer S, Gancheva S, Stavrakis S, Kampusch S, Deuchars SA, Wehner S, Laborde S, Usichenko T, Polak T, Zaehle T, Borges U, Teckentrup V, Jandackova VK, Napadow V and Koenig J (2021) International Consensus Based Review and Recommendations for Minimum Reporting Standards in Research on Transcutaneous Vagus Nerve Stimulation (Version 2020). Front. Hum. Neurosci. 14:568051. doi: 10.3389/fnhum.2020.568051

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: frontiersin.org/about/contact

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership