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Noise-induced hearing loss: From basic to clinical research

Edited by
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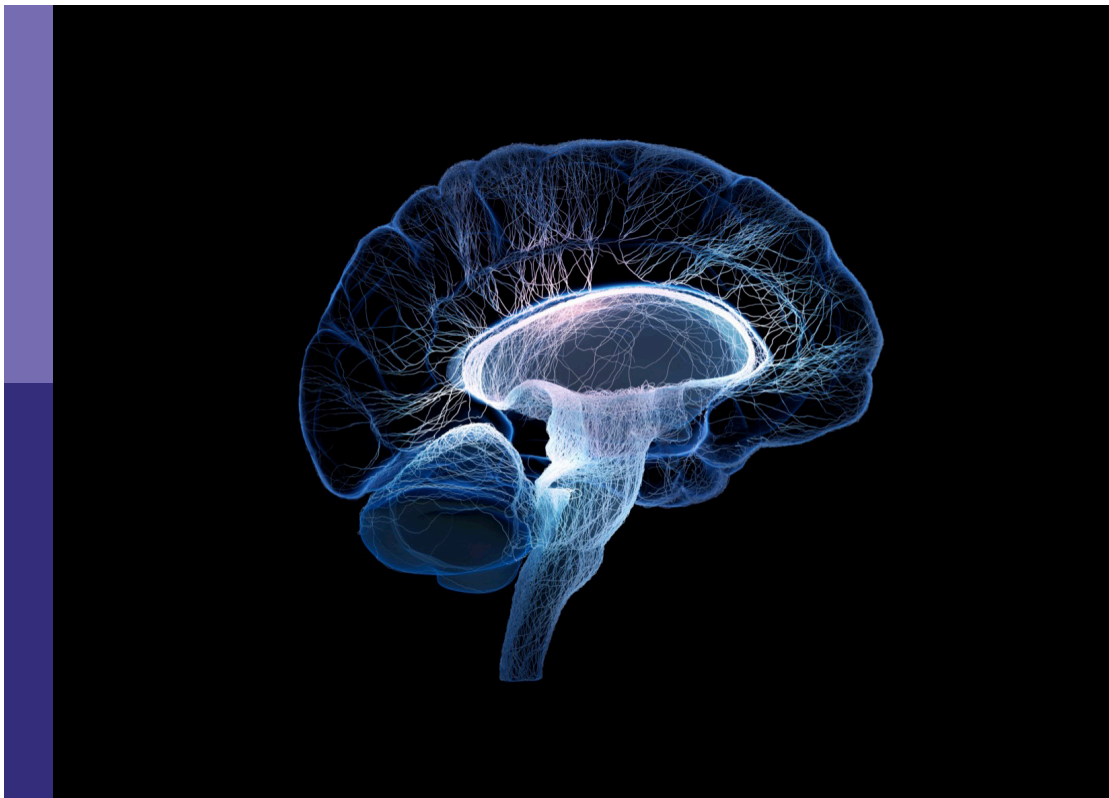

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Noise-induced hearing loss: From basic to clinical research

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Editorial: Noise-induced hearing loss: From basic to clinical research

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KEYWORDS

noise exposure, hearing loss, tinnitus, cochlear synaptopathy, coding-in-noise deficits

Editorial on the Research Topic
Noise-induced hearing loss: From basic to clinical research

Noise-induced hearing loss (NIHL) is one of the most common types of hearing loss among adults. The World Health Organization estimates that 10% of the world's population is exposed to sound levels that could potentially cause NIHL (Chadha et al., 2021). This Research Topic focused on NIHL was opened for submission from September 2021 to July 2022, with one opinion, four reviews, and eight original articles being included.

Exposure to industrial noise is one of the most common risks for NIHL. With the development of industrialization, non-Gaussian noise (also known as complex noise), which transients high-energy impulsive noise superimposed on the steady-state background noise, has been the primary noise type in the industry. Recent evidence showed that the temporal structure of complex noise could be expressed in the kurtosis metric (β), which is defined as the ratio of the fourth-order central moment to the squared second-order central moment of a distribution (Zhang et al., 2022a). Zhou et al. investigated the epidemiological characteristics of occupational NIHL among 1,050 manufacturing workers in China and found that kurtosis strengthens the association between noise exposure duration and noise intensity with high-frequency hearing loss. Shi et al. further validated the application of cumulative noise exposure (CNE) adjusted by kurtosis when evaluating occupational NIHL associated with non-Gaussian noise among 1,558 manufacturing workers from five industries in China. Their serial of studies demonstrated that the kurtosis-adjusted-CNE metric is more effective than CNE alone in assessing occupational NIHL among workers under non-Gaussian noise exposure. Recently, a draft guideline for measuring workplace noise exposure based on their work has been proposed in China (Zhang et al., 2022b).

NIHL is a complex condition with indiscernible mechanisms that result from exposure to loud sounds, and as research illustrates, is likely influenced by age, sex, genetics, underlying diseases, personal behaviors, and other physical and chemical hazards (Basner et al., 2014; Wang et al., 2021a). Chen et al. summarized primarily human studies as well as animal studies concerning the role of susceptible genes in NIHL, aims to provide insights into the

further exploration of NIHL prevention and specific treatment. Meanwhile, Kurabi and team theorized several possible molecular pathways might be involved in NIHL (Kurabi et al., 2017). Zhao et al. focused on the adenylate-activated kinase (AMPK) pathway, and found that early AMPK activation may protect hearing by increasing ATP storage and reducing the release of large quantities of p-AMPK, which could help to inhibit synaptic damage.

Despite numerous investigations into NIHL, treatment options are still limited and preventive measures are not well implemented. NIHL can be avoided if appropriate preventive measures are adopted (The, 2019). Bramati et al. provided insights into the Dangerous Decibels® program for the prevention of NIHL for noise-exposed workers. Their study showed greater effectiveness than the conventional educational-based intervention in a Brazilian population. In addition to occupational noise exposures, other noises may stem from everyday occurrences, and there are growing concerns about the increasing incidence of NIHL in children and adolescents who are potentially exposed to an array of loud sounds on a daily basis (Dillard et al., 2022). However, for non-occupational noise exposure, it is challenging to regulate as it would have to accommodate for the wide range of possible high-intensity sound sources, as there is high variability in activities that involve loud sounds for young people in their daily life. With the increasing application and contributions of neuroscience in recent NIHL studies, Pang and Gilliver proposed an opinion that neuroscience-informed approaches to reducing recreational NIHL for young people are required to meet the needs of the developing adolescent brain. Designing age-appropriate NIHL campaigns that consider these factors may increase the likelihood that interventions are efficacious and cost-effective.

Of late, several studies indicated that even moderate noise exposure could result in hearing difficulties in individuals with normal hearing thresholds, which has been referred to as "hidden hearing loss (HHL)" (Kohrman et al., 2020). Despite progress in pre-clinical models, evidence supporting the existence of HHL in humans remains inconclusive, and clinicians lack any non-invasive biomarkers that are sensitive to HHL (Bramhall et al., 2019; Wang et al., 2021b). Here, Valderrama et al. reviewed animal models of HHL as well as the ongoing research that aims to develop tools with which to diagnose and manage hearing difficulties associated with HHL. They discussed new research opportunities facilitated by recent methodological tools that may overcome a series of barriers that have hampered meaningful progress in diagnosing and treating of HHL.

Noise-induced synaptopathy (NIS) has been researched extensively as a potential cause of coding-in-noise deficits (CIND) and HHL. However, by using low-level, intermittent noise exposure mimicking the human experience in guinea pigs, Xia et al. found that degradations in signal processing were likely limited and not reflective of NIS and noise-induced HHL. Similarly, Pinsonnault-Skvarenina et al. also failed to find any significant association between noise exposure and auditory brainstem response outcomes, which might have detected cochlear synaptopathy in young factory workers with normal hearing. Ripley et al. further summarized the translational difficulties from animal data to human clinical, the technical challenges in quantifying NIS in humans, and the problems with the spontaneous rates theory on signal coding. The temporal fluctuation profile model was discussed as a potential alternative for signal coding at a high sound level against background noise, in association with the mechanisms of efferent control on the cochlea gain.

Cumulative damage from long-term noise exposure is also a major cause of age-related hearing loss, tinnitus, and even degraded learning and cognitive abilities (Manukyan, 2022). For noise-induced tinnitus, Hayes et al. developed the appetitive operant conditioning paradigm to assess acute and chronic sound-induced tinnitus in rats, which provides a platform for future investigations into the neural basis of tinnitus. For cognitive dysfunction related to noise exposure, Patel et al., exposed 6-month-old rats to an occupational-like noise and studied both hippocampal-dependent and striatal-dependent cognitive dysfunction. They highlighted that even mild noise exposure early in adulthood could have long-lasting implications for cognitive function later in life. Manohar et al. reviewed recent results that illustrate how NIHL deprives higher-order structures than the cochlea (such as the hippocampus) of the vital sensory information needed to carry out complex, higher-order functions.

We hope that this collection of articles on NIHL has provided readers with a comprehensive understanding of the current state of research in this area. Through the exploration of various influencing factors, mechanisms, prevention strategies, and non-auditory effects of NIHL, we have gained valuable insights into the complexities of this condition.

As we move forward, we encourage readers to use this information to guide their own research and clinical practices. Whether through the development of new prevention strategies or the advancement of early diagnosis and precise therapy, there is much work to be done in the NIHL area.

One key message that unites this entire collection is the importance of collaboration and interdisciplinary approaches to NIHL research. Only through the joint efforts of clinicians, scientists, engineers, and other stakeholders can we hope to make meaningful progress in our understanding and management of this population. We urge readers to join this effort and work toward a future where NIHL is a preventable and treatable condition.

Author contributions

All authors except QW were guest editors of the Research Topic. QW was the research assistant and secretary on the Research Topic. All authors wrote the paper and approved the submitted version.

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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.

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ORIGINAL RESEARCH
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Assessment of Occupational Hearing Loss Associated With Non-Gaussian Noise Using the Kurtosis-Adjusted Cumulative Noise Exposure Metric: A Cross-Sectional Survey

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Objective: There is little literature on the validity of kurtosis-adjusted noise energy metrics in human studies. Therefore, this study aimed to validate the application of cumulative noise exposure (CNE) adjusted by kurtosis in evaluating occupational hearing loss associated with non-Gaussian noise among manufacturing workers.

Methods: A cross-sectional survey was conducted on 1,558 manufacturing workers exposed to noise from five industries to collect noise exposure and hearing loss data. Both CNE and kurtosis-adjusted CNE (CNE′) were collapsed into 2-dB(A)•year bins, and the mean noise-induced permanent threshold shifts at 3, 4, and 6 kHz ($NIPTS_{346}$) in each bin were calculated. The contributions of CNE and CNE′ to noise-induced hearing loss (NIHL) were compared using the multiple linear regression. The degree of overlap of two linear regression equations (i.e., between CNE′ and $NIPTS_{346}$ for non-Gaussian noise and between CNE and $NIPTS_{346}$ for Gaussian noise) was used to evaluate the validity of the CNE′ using a stratified analysis based on age and sex.

Results: Multiple linear regression models showed that after kurtosis adjustment, the standardized regression coefficient of CNE increased from 0.230 to 0.255, and R^2 increased from 0.147 to 0.153. The linear relationship between $NIPTS_{346}$ and CNE′ or CNE showed that the regression line of non-Gaussian noise was closer to that of Gaussian noise when using CNE′ than using CNE. The mean difference in $NIPTS_{346}$ between the equations of non-Gaussian noise and Gaussian noise was significantly reduced from 4.32 to 1.63 dB HL after kurtosis adjustment ($t = 12.00$, $p < 0.001$). Through a stratified analysis, these significant decreases were observed in male and female workers, and workers aged ≥30 years old.

Conclusion: As a noise exposure metric combining noise energy and temporal characteristics, the kurtosis-adjusted-CNE metric was more effective than CNE alone in assessing occupational hearing loss among manufacturing workers in non-Gaussian noise environment. However, more studies are needed to verify the validity of the kurtosis-adjusted-CNE metric.

Keywords: kurtosis, non-Gaussian noise, hearing loss, cumulative noise exposure, manufacturing workers

INTRODUCTION

With the development of industrialization, non-Gaussian noise has been the main noise type in the industry. Non-Gaussian noise (also known as complex noise) comprises transient high-energy impulsive noise superimposed on the steady-state background noise (Suter, 2017). Unlike steady-state noise (Gaussian noise), which has a normal or Gaussian distribution of acoustic energy in time, non-Gaussian noise has a complex distribution of acoustic energy and changes over time. Some studies found that because of complex temporal characteristics, non-Gaussian noise caused more severe hearing loss than Gaussian noise (Lei et al., 1994; Hamernik et al., 2003, 2007). This phenomenon challenges the validity of the equal energy hypothesis (EEH), which assumes that the effects of noise exposure on the cochlea are proportional to noise energy, regardless of its distribution (Suter, 2017). Since existing noise standards (e.g., ISO 1999) have been established on the basis of EEH, which use A-weighted sound pressure level (L_{Aeq}) as the sole metric of noise exposure, their applicability to non-Gaussian noise is questionable. Zhang et al. (2020) found that ISO1999 underestimated noise-induced permanent threshold shift associated with non-Gaussian noise by 13.6 dB HL on average across the four audiometric test frequencies (2, 3, 4, and 6 kHz). The problem with existing noise standards is that they only rely on noise energy to quantify the noise exposure and ignore the effect of temporal characteristics on noise-induced hearing loss (NIHL). Therefore, it is necessary to develop new noise exposure metrics that can combine noise energy and temporal characteristics to effectively evaluate NIHL associated with different types of noise.

Temporal characteristics of noise waveform contain many elements such as the peak level, duration of an impulse, and inter-peak interval (Zhang et al., 2021b). Kurtosis (β) has been shown to incorporate these elements and can be used as a simple and feasible metric to indirectly reflect the temporal characteristics of noise (Erdreich, 1986; Hamernik and Qiu, 2001; Hamernik et al., 2003). Cumulative noise exposure (CNE) is a comprehensive metric combining noise intensity and noise exposure duration (ED), which can better represent noise energy than L_{Aeq} (Sulkowski and Lipowczan, 1982; Earshan, 1986). Studies showed that both kurtosis and CNE had a dose–response relationship with NIHL (Zhang et al., 2014; Xie et al., 2016). Thus, some scholars proposed that the CNE adjusted by kurtosis (kurtosis-adjusted CNE, CNE′) could be used as a new metric for effectively evaluating the risk of NIHL. To test this idea, Zhao et al. (2010) and Xie et al. (2016) conducted a human survey with sample sizes of 195 and 341 manufacturing workers, respectively. They took the dose–response curves between CNE (CNE′) and NIHL prevalence as an evaluation method and found that the non-Gaussian noise curve was closer to the Gaussian noise curve when using CNE′ than using CNE. However, there is still a research gap in that there are few studies on large sample sizes of workers in different industries to verify the validity of the CNE′ metric.

In this study, 1,558 manufacturing workers from five industries were included through a cross-sectional survey to test the application of CNE′ in assessing the occupational hearing loss associated with non-Gaussian noise. The contributions of CNE and CNE′ to noise-induced hearing loss (NIHL) were compared using the multiple linear regression and dose-effect curve.

MATERIALS AND METHODS

Subjects

A cross-sectional survey was conducted from 2019 to 2021. Industrial workers exposed to noise ($N = 2{,}065$) were recruited from 17 manufacturing enterprises in five industries in the Zhejiang province of China. Workers from the automotive (four factories), electronics (three factories), and metal products (four factories) industries were mainly exposed to non-Gaussian noise, while those from the textile (four factories) and paper-making (two factories) industries were primarily exposed to Gaussian noise. Each participant was informed of the purpose and design of this study and was asked to sign an informed consent form. The study protocol was approved by the ethics committee of the Zhejiang Center for Disease Control and Prevention, China (approval reference number: ZJCDC-T-043-R).

The criteria for inclusion were as follows: (1) consistently working in the same job category and work site for the entire employment period; (2) being employed at their current work for at least 1 year; (3) no history of military service or shooting activities; (4) no history of ear diseases, ear trauma, or hearing loss; (5) no family history of hearing loss; (6) no history of ototoxic drug use; (7) no co-exposure to noise and ototoxic chemicals or heavy metals confirmed by field investigation; and (8) no or minimal use of hearing protection devices (HPD). As a result, 1,558 workers were included from the original pool of 2,065 participants.

Field Investigation

Before the survey, a field investigation was conducted to understand the size and space of the workplaces, production processes, the distribution of noise resources, the noise type and noise level, the number of workers exposed to noise, and the use of HPD. The workplaces with stable work processes and machinery were selected for survey workplaces through the field investigation. Before recording, a hygienist confirmed with the manager of the workplace and each participant that this was the noise they were typically exposed to on an average working day.

Questionnaire Survey

A face-to-face questionnaire survey was administered by an occupational hygienist. The questionnaire collected the following information from each participant: general individual information (sex, age, history of military service or shooting activities, etc.), occupational history (factory, worksite, job type, length of employment, duration of daily noise exposure, HPD use, past work with noise exposure, etc.), and health condition (history of ear diseases, ear trauma, or hearing loss, ototoxic drug use, smoking or drinking, diabetes, etc.). All information was checked for errors and then stored in the database every day.

Noise Data Collection

A digital sound recorder (ASV5910-R, Hangzhou Aihua Instruments Co., Ltd., China) was used to record each participant's noise exposure over the course of a shift. The instrument is a specialized device for precise measurement and analysis of personal noise exposure. It is equipped with a 1/4-inch pre-polarized condenser microphone characterized by broad frequency response (20 Hz to 20 kHz), high sensitivity level (2.24 mV/Pa), and wide measurement range (40–141 dB[A]). Under a full charge, the recorder can work continuously for at most 23 h. The full-shift noise of each participant was recorded with a 32-bit resolution at 48 kHz sampling rate. The recording was saved on 32 GB micro SD card and then transferred to a computer for subsequent analysis.

Calculation of Noise Metrics

The MATLAB software was used to analyze the noise waveform for obtaining the kurtosis value and A-weighted sound pressure level normalized to a nominal 8-h working day ($L_{Aeq,8h}$). A kurtosis value was computed in each consecutive 40-s time window of the noise recording. The arithmetic mean of the calculated kurtosis values in a recording was calculated and used as the kurtosis metric (β). Kurtosis represents the impulsiveness of noise (Qiu et al., 2021). The greater the kurtosis, the higher the impulsiveness. Kurtosis value 10 was used to distinguish non-Gaussian noise from Gaussian noise (Davis et al., 2012). Noise with kurtosis greater than or equal to 10 was defined as non-Gaussian noise, while noise with kurtosis less than 10 was defined as Gaussian noise.

$L_{Aeq,8h}$ can be calculated by the formula in ISO 1999 2013:

$$L_{Aeq,8h} = L_{Aeq,T_e} + 10 * \lg\left(T_e / T_0\right) \quad (1)$$

where T_e is the effective duration of the working day in hours; T_0 is the reference duration (8 h); and $L_{Aeq,Te}$ is the L_{Aeq} for T_e. CNE, a comprehensive index combining noise intensity with exposure duration, is defined as:

$$CNE = 10 * \lg\left[\frac{1}{T_{ref}}\sum_{i=1}^{n}\left(T_i * 10^{L_{Aeq,8hi}/10}\right)\right] \quad (2)$$

where n is the number of stages working at different noise environments; T_i is the duration of noise exposure in years at the ith stage; $L_{Aeq,8hi}$ is the $L_{Aeq,8h}$ occurring over the time interval T_i; and $T_{ref} = 1$ year. Because all subjects in this study were restricted to work in the same noise environment for the entire employment period, n equaled to 1, and a simplified formula for Eq. (2) was given as follows:

$$CNE = L_{Aeq,8h} + 10 * lgT \quad (3)$$

where T is the duration of noise exposure. CNE′ could be used as a new metric for hearing loss risk assessment. It combines kurtosis (β), $L_{Aeq,8h}$, and exposure duration (T), and the calculation formula is shown as follows:

$$CNE' = L_{Aeq,8h} + \frac{\ln(\beta) + 1.9}{\lg 2} * \lg T \quad (4)$$

Pure-Tone Audiometry

Each participant was given a pure-tone audiometry and an otologic examination by a certificated audiologist. The audiometric test was performed in an audiometric room of a mobile physical examination vehicle using an audiometer (Interacoustics AD629, Denmark) with an air conduction headphone (HDA300). Before the test, the audiometer and the headphone were calibrated by the Zhejiang Institute of Metrology according to the Chinese standard (Verification Regulation of Audiological Equipment Pure-tone Audiometers, JJG 388–2012).

The test was performed at least 16 h after occupational noise exposure. Air conduction pure-tone hearing threshold levels at 0.5, 1, 2, 3, 4, 6, and 8 kHz were tested in both ears. Measured hearing thresholds at each frequency were adjusted by subtracting the age- and sex-specific hearing thresholds according to Table A.3 of ISO 1999 2013. The noise-induced permanent threshold shifts (NIPTS) at each frequency for each participant were calculated according to ISO 1999 2013. The mean NIPTS at 3, 4, and 6 kHz in both ears ($NIPTS_{346}$), representing the extent of hearing loss at high frequencies, was calculated for subsequent analysis.

Methods for Comparing the Contribution of CNE and CNE′ to $NIPTS_{346}$

The multiple linear regression analysis and dose-effect curve were used to compare the contribution of CNE to NIHL before and after kurtosis adjustment. In Model 1 of multiple linear regression, analysis, age, sex, and CNE were used as the independent variables, and $NIPTS_{346}$ was used as the dependent variable. In Model 2, age, sex, and CNE′ were used as the independent variables, and $NIPTS_{346}$ was used as the dependent variable. The standardized regression coefficient served as an indicator for comparing the contribution of CNE and CNE′ to $NIPTS_{346}$. In addition, the value of R^2, which represents the goodness-of-fit in the regression model, served as another evaluation indicator.

The dose-effect curves between CNE (CNE′) and $NIPTS_{346}$ for Gaussian and non-Gaussian noise were plotted. Both CNE and CNE′ were collapsed into 2-dB(A)•year bins, and the mean $NIPTS_{346}$ in each bin was calculated. In the dose-effect curves, the abscissa was the mid-value in each bin, while the ordinate was the mean $NIPTS_{346}$ in the corresponding bin. The differences in $NIPTS_{346}$ between the non-Gaussian noise curve and the Gaussian noise curve at each CNE bin (D_1) and the differences at each CNE′ bin (D_2) were calculated and compared. Considering the influence of age and sex in NIHL, a stratified analysis is needed. Study subjects were stratified by age and sex, respectively, and then, the dose-effect curves were plotted and analyzed.

Statistical Analysis

Continuous variables were expressed as mean with standard deviation or median with quartile. Continuous variables were compared between two groups using the *t*-test or non-parametric test. Categorical variables were expressed as proportions and were compared using the chi-square test. To compare the hearing loss caused by different noise types, an analysis of covariance was performed, in which $NIPTS_{346}$ served as the dependent variable, noise type (non-Gaussian or Gaussian noise) served as the fixed factor, while age (≥30 years or < 30 years), sex (male or female), and CNE served as the covariates for controlling the differences in age, sex, and noise energy between two groups. The independent *t*-test was used to compare the differences between D_1 and D_2. $p < 0.05$ was considered significant.

RESULTS

General Information of Noise Exposure

Table 1 shows the general noise exposure information for 1,558 workers in five industries. Of them, 64.4% were male. The mean age of subjects was 34.2 ± 9.3 years. The mean $L_{Aeq,8h}$ was 89.6 ± 7.1 dB(A), and the average exposure duration was 7.3 ± 6.5 years. Among all participants, 928 workers, mainly from automotive, electronics, and metal products manufacturing industries, were exposed to non-Gaussian noise, while 630 workers, mainly from textile and paper-making industries, were exposed to Gaussian noise.

Comparison of $NIPTS_{346}$ Between Non-Gaussian Noise Group and Gaussian Noise Group

The analysis of the covariance model in **Table 2** shows that the least-squares means of $NIPTS_{346}$ between non-Gaussian noise group and Gaussian noise group were 23.53 ± 0.34 dB HL (95% *CI* 22.85–24.21) and 21.53 ± 0.43 dB HL (95% *CI* 20.69–22.37), respectively. The least-squares mean difference (2.00 dB HL) of $NIPTS_{346}$ between the two groups was significant ($p = 0.001$).

Multiple Linear Regression Analyses Between $NIPTS_{346}$ and Key Factors

Table 3 shows the results of the multiple linear regression analyses. The two models and each factor (e.g., age, sex, CNE, and CNE′) had statistical significance ($p < 0.001$). From Model 1 to Model 2, the standardized regression coefficient of CNE increased from 0.230 to 0.255 (increased by 10.9%), while the standardized regression coefficient of age decreased from 0.231 to 0.200 (reduced by 13.4%). In Model 1, the order of the standard regression coefficient was age > CNE > sex; in Model 2, the order of the standard regression coefficient was CNE′ > age > sex. R^2 increased from 0.147 for Model 1 to 0.153 for Model 2, an increase of 4.1%. R^2_{CNE} and $R^2_{CNE'}$ in the non-Gaussian group were 0.732 and 0.770, respectively, an increase of 5.2%.

The Dose-Effect Relationships Between $NIPTS_{346}$ and CNE or CNE′

The simple linear regression model was used to fit the dose-effect curve between $NIPTS_{346}$ and CNE or CNE′. **Figure 1A** demonstrates the linear regression equation between $NIPTS_{346}$ and CNE for both the non-Gaussian noise group and the Gaussian noise group. The simple linear regression equation of the Gaussian noise group was $NIPTS_{346} = 0.540CNE—29.707$, $R^2 = 0.871$. The equation of non-Gaussian noise group was $NIPTS_{346} = 0.613CNE'—32.415$, $R^2 = 0.723$. The regression line of non-Gaussian noise (continuous line) was above the line of Gaussian noise (dotted line) with a significant distance between them. **Figure 1B** shows the linear relationship between $NIPTS_{346}$ and CNE′. The equation of the Gaussian noise group remained unchanged, while that of the non-Gaussian noise group was changed to $NIPTS_{346} = 0.526CNE'—26.697$, $R^2 = 0.770$. After CNE was adjusted by kurtosis, the regression line of non-Gaussian noise was closer to that of Gaussian noise, and R^2 of non-Gaussian noise had an increase of 6.5% (from 0.723 to 0.770). **Table 4** shows the mean difference in $NIPTS_{346}$ between the non-Gaussian noise equation and the Gaussian noise equation at each bin before and after the kurtosis adjustment. The two independent samples *t*-test showed that the mean D_2 of $NIPTS_{346}$ was 1.63 dB HL, which was significantly lower than D_1 (4.32 dB HL; $t = 12.00$, $p < 0.001$).

Figures 2A,B show the linear regression equations for male workers when using both CNE and CNE′, and **Figures 2C,D** for female workers. When using CNE, the regression line of non-Gaussian noise for both males and females was above that of Gaussian noise with a significant distance between them (male: mean $D_1 = 3.47$ dB HL; female: mean $D_1 = 5.26$ dB HL). When CNE′ was used, the regression line of non-Gaussian

TABLE 1 | The general information of noise exposure for participants from five industries.

	N	Male (%)	Age (year)	ED (year)	$L_{Aeq,8h}$ [dB(A)]	CNE [dB(A)·year]	Kurtosis*
Automotive	589	81.3	32.6 ± 8.2	5.4 ± 4.9	87.7 ± 4.2	93.5 ± 5.6	15.0 (9.1, 25.1)
Electronics	262	47.3	31.6 ± 8.0	5.8 ± 5.2	84.6 ± 6.0	90.4 ± 7.9	24.9 (15.4, 44.0)
Metal products	194	68.0	38.3 ± 9.4	9.7 ± 8.1	91.1 ± 6.9	99.2 ± 9.2	16.0 (7.2, 48.5)
Textile	422	49.8	33.2 ± 8.5	8.6 ± 6.7	94.9 ± 7.9	102.6 ± 8.8	5.1 (3.3, 11.2)
Paper making	91	61.5	46.9 ± 9.8	11.9 ± 8.6	88.9 ± 4.5	98.2 ± 6.0	7.8 (4.8, 12.6)
Total	1,558	64.4	34.2 ± 9.3	7.3 ± 6.5	89.6 ± 7.1	96.4 ± 8.8	12.9 (6.6, 25.0)

*ED: exposure duration; CNE: cumulative noise exposure. * kurtosis value was expressed as the median with quartile.*

TABLE 2 | Comparison of least-squares mean of $NIPTS_{346}$ between non-Gaussian noise and Gaussian noise.

Noise type	Least-squares mean	Standard error	95% CI	*p*
Non-Gaussian noise	23.53	0.34	22.85–24.21	0.001
Gaussian noise	21.53	0.43	20.69–22.37	

TABLE 3 | The multiple linear regression analyses between $NIPTS_{346}$ and key factors.

	Unstandardized coefficient	Standardized coefficient	*t*	*p*
Model 1: $NIPTS_{346} = b_0 + b_1Age + b_2Sex + b_3CNE$			$R^2_{model\ 1}$ = 0.147	R^2_{CNE} = 0.732*
Intercept (b_0)	−20.462		−4.965	$p < 0.001$
Age (b_1)	0.271	0.231	6.852	$p < 0.001$
Sex (b_2)	1.849	0.081	2.588	$p < 0.001$
CNE (b_3)	0.326	0.230	6.965	$p < 0.001$
Model 2: $NIPTS_{346} = b_0 + b_1Age + b_2Sex + b_3CNE'$			$R^2_{model\ 2}$ = 0.153	$R^2_{CNE'}$ = 0.770+
Intercept (b_0)	−16.968		−4.818	$p < 0.001$
Age (b_1)	0.234	0.200	5.687	$p < 0.001$
Sex (b_2)	2.008	0.088	2.835	$p < 0.001$
CNE′ (b_3)	0.286	0.255	7.405	$p < 0.001$

**R^2_{CNE} was the R^2 for CNE in the linear regression model between mean $NIPTS_{346}$ and CNE (collapsed into 2-dB(A)·year bins) in the non-Gaussian noise group.*
+$R^2_{CNE'}$ was the R^2 for CNE′ in the linear regression model between mean $NIPTS_{346}$ and CNE′ (collapsed into 2-dB(A)·year bins) in the non-Gaussian noise group.

TABLE 4 | A decrease in $NIPTS_{346}$ difference between the two equations of non-Gaussian and Gaussian noise after the kurtosis adjustment.

Factor	Mean D_1 (dB HL)	Mean D_2 (dB HL)	*t*	*p*
Total	4.32	1.63	12.00	<0.001
Male	3.47	0.96	20.11	<0.001
Female	5.26	2.04	14.25	<0.001
Age ≥ 30	4.10	1.13	15.80	<0.001
Age < 30	2.70	2.53	0.38	0.707

D_1, the difference between the two linear regression equations of non-Gaussian and Gaussian noise at each unadjusted CNE bin. D_2, the difference between the two linear regression equations of non-Gaussian and Gaussian noise at each CNE′ bin.

noise for males nearly overlapped with that of Gaussian noise (mean $D_2 = 0.96$ dB HL). For females, the regression line of non-Gaussian noise was also very close to that of Gaussian noise (mean $D_2 = 2.04$ dB HL). The mean difference when using CNE (D_1) was significantly higher than that when using CNE′ (D_2) for both males ($t = 20.11$, $p < 0.001$) and females ($t = 14.25$, $p < 0.001$).

Figures 3A,B show the regression lines for workers aged 30 years or older, and **Figures 3C,D** for workers less than 30 years old. For workers aged ≥30, the line of non-Gaussian noise was above that of Gaussian noise when using CNE and became close to the line of Gaussian noise when using CNE′. The mean difference of $NIPTS_{346}$ between two lines significantly

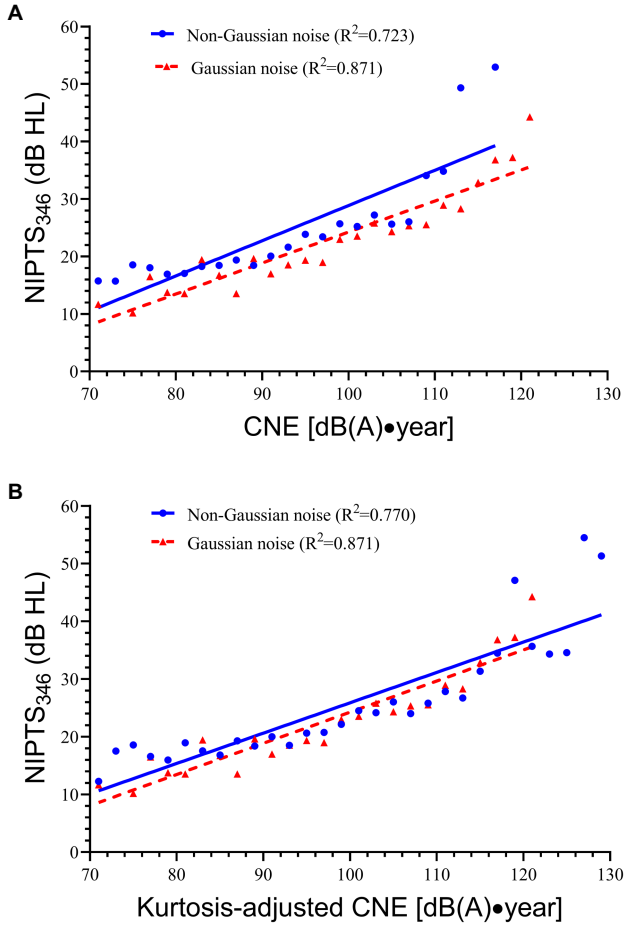

FIGURE 1 | The linear relationship between $NIPTS_{346}$ and CNE or CNE′ for all subjects. **(A)** The linear relationship between $NIPTS_{346}$ and CNE. The regression equation for Gaussian noise is $NIPTS_{346} = 0.540CNE - 29.707$, $R^2 = 0.871$. The regression equation for non-Gaussian noise is $NIPTS_{346} = 0.613CNE' - 32.415$, $R^2 = 0.723$. **(B)** The linear relationship between $NIPTS_{346}$ and CNE′. The regression equation for non-Gaussian noise is $NIPTS_{346} = 0.526CNE' - 26.697$, $R^2 = 0.770$.

decreased after CNE was adjusted by kurtosis (mean $D_1 = 4.10$ dB HL, mean $D_2 = 1.13$ dB HL, $t = 15.80$, $p < 0.001$). For workers aged <30, the mean difference of $NIPTS_{346}$ when using CNE (mean $D_1 = 2.70$ dB HL) was a little higher than CNE′ (mean $D_2 = 2.53$ dB HL), although the difference was not statistically significant ($t = 0.38$, $p = 0.707$).

DISCUSSION

An analysis of covariance showed that the least-squares mean of $NIPTS_{346}$ in the non-Gaussian group was significantly higher than that in the Gaussian noise group ($p = 0.001$), indicating that non-Gaussian noise resulted in more hearing loss than Gaussian noise under the same noise energy exposure. Other studies reported similar results. Li et al. (2021) compared the difference of hearing loss between general machinery

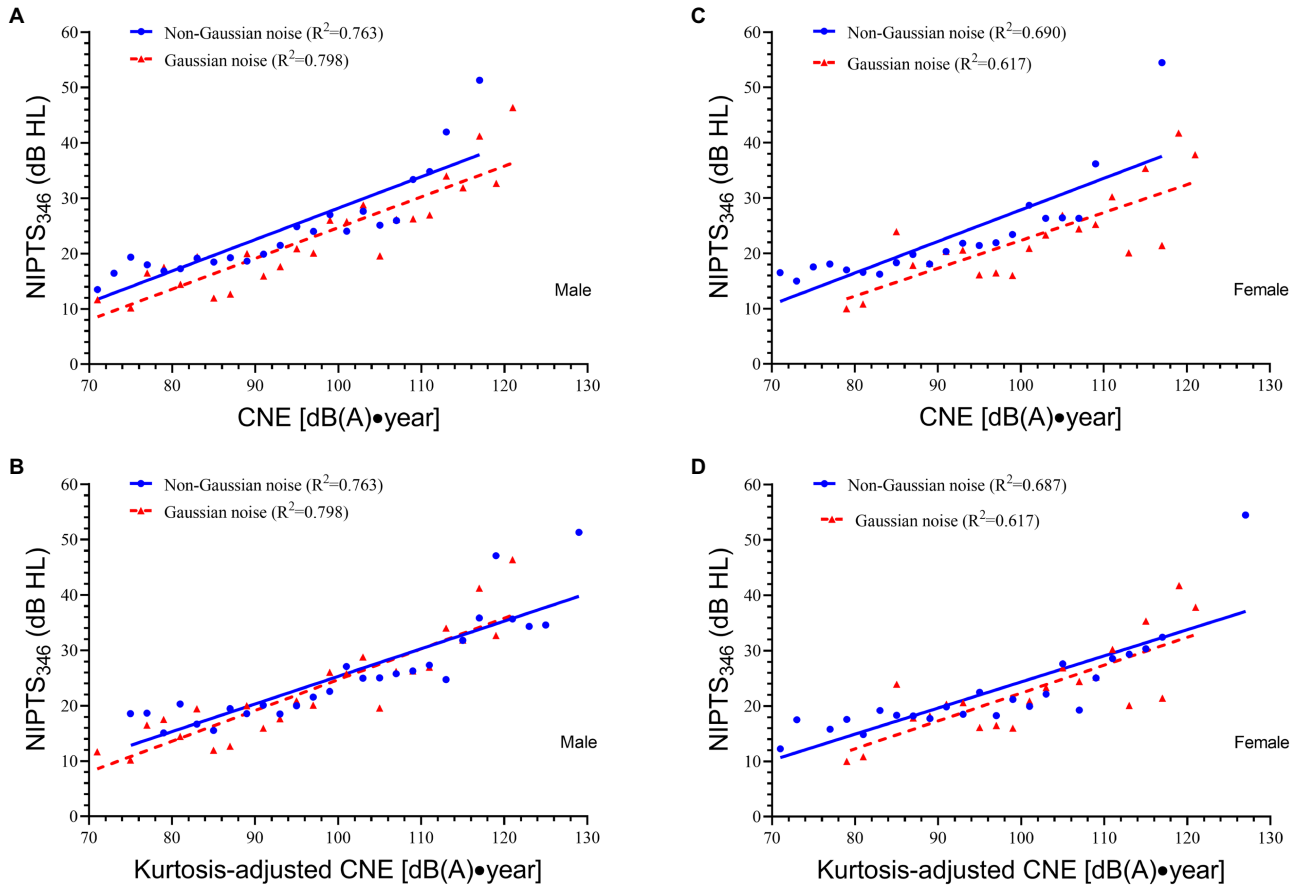

FIGURE 2 | The linear relationship between $NIPTS_{346}$ and CNE or CNE′ for male and female workers. **(A)** The linear relationship between $NIPTS_{346}$ and CNE for male workers. The regression equation for Gaussian noise is $NIPTS_{346} = 0.556CNE - 30.910$, $R^2 = 0.798$. The regression equation for non-Gaussian noise is $NIPTS_{346} = 0.568CNE' - 28.599$, $R^2 = 0.763$. **(B)** The linear relationship between $NIPTS_{346}$ and CNE′ for male workers. The regression equation for non-Gaussian noise is $NIPTS_{346} = 0.499CNE' - 24.598$, $R^2 = 0.763$. **(C)** The linear relationship between $NIPTS_{346}$ and CNE for female workers. The regression equation for Gaussian noise is $NIPTS_{346} = 0.504CNE - 28.037$, $R^2 = 0.617$. The regression equation for non-Gaussian noise is $NIPTS_{346} = 0.571CNE' - 29.231$, $R^2 = 0.690$. **(D)** The linear relationship between $NIPTS_{346}$ and CNE′ for female workers. The regression equation for non-Gaussian noise is $NIPTS_{346} = 0.472CNE' - 22.825$, $R^2 = 0.687$.

manufacturing workers exposed to non-Gaussian noise and workers exposed to Gaussian noise (such as spinning and weaving) and found that the former had a higher threshold level of hearing. Xie et al. (2021) reported that workers in industries with high kurtosis (such as furniture, hardware, automotive, machinery, steel, and electrical equipment manufacturing industries) suffered from more severe hearing loss than workers in industries with low kurtosis values. Shi et al. (2021) conducted a meta-analysis on 30 studies covering a wide range of industries and found that workers exposed to non-Gaussian noise had 2.2 times higher risk of high-frequency NIHL than those exposed to Gaussian noise.

The increased risk of hearing loss may be associated with the complex temporal structure of non-Gaussian noise. The degree to which noise intensity deviates from Gaussian distribution (i.e., the impulsiveness of noise) is responsible for excessive hearing loss. Kurtosis is a statistics metric of the extent to which the tails of distribution differ from the tails of the Gaussian distribution. The more impulsive the noise, the greater the kurtosis. Zhang et al. (2021b) reported that kurtosis was significantly associated with the difference of peak SPL (L_{peak}) minus its $L_{Aeq,8h}$ across different types of work. The temporal structure of a non-Gaussian noise can be indirectly characterized by estimating the kurtosis. Qiu and his colleagues exposed chinchillas to noise with different kurtosis but equal energy and found that noise with higher kurtosis caused more severe hair cell loss (Qiu et al., 2006, 2007, 2013). Zhang et al. (2021a) found besides $L_{Aeq,8h}$ and exposure duration, kurtosis was a risk factor for occupational NIHL and had a dose-effect relationship with $NIPTS_{346}$. These findings suggest that noise energy is a necessary metric while kurtosis is also an important metric in assessing the hearing loss associated with non-Gaussian noise, and solely noise energy metrics may underestimate the hearing loss caused by non-Gaussian noise (Suvorov et al., 2001; Seixas et al., 2012; Zhang et al., 2020). Qiu et al. (2013) found that different temporal structure of noises might produce the same

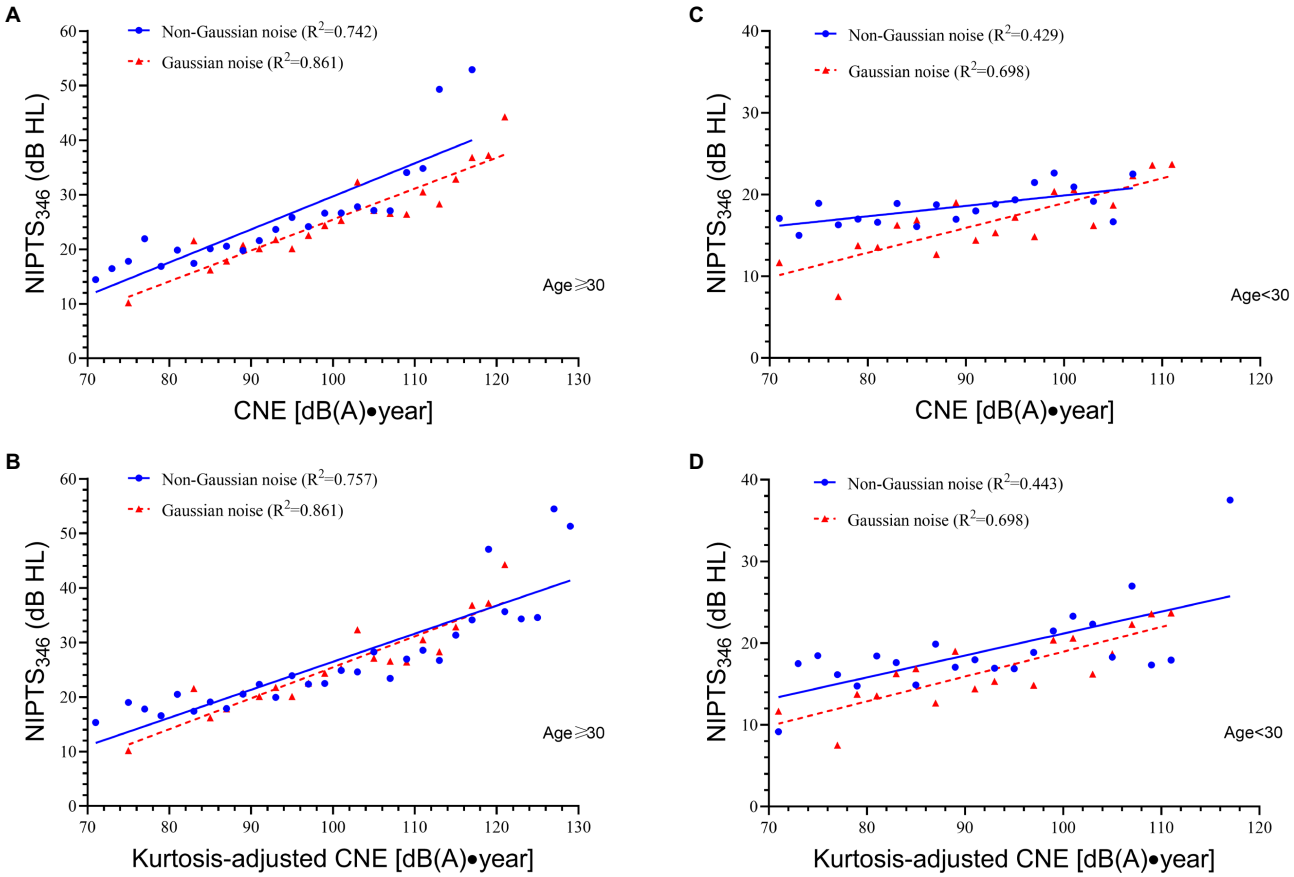

FIGURE 3 | The linear relationship between $NIPTS_{346}$ and CNE or CNE′ for workers aged ≥30 and aged <30. **(A)** The linear relationship between $NIPTS_{346}$ and CNE for workers aged ≥30. The regression equation for Gaussian noise is $NIPTS_{346}$=0.567CNE−31.269, R^2=0.861. The regression equation for non-Gaussian noise is $NIPTS_{346}$=0.606CNE′−30.916, R^2=0.742. **(B)** The linear relationship between $NIPTS_{346}$ and CNE′ for workers aged ≥30. The regression equation for non-Gaussian noise is $NIPTS_{346}$=0.514CNE′−24.925, R^2=0.757. **(C)** The linear relationship between $NIPTS_{346}$ and CNE for workers aged <30. The regression equation for Gaussian noise is $NIPTS_{346}$=0.304CNE−11.397, R^2=0.698. The regression equation for non-Gaussian noise is $NIPTS_{346}$=0.128CNE′−7.132, R^2=0.429. **(D)** The linear relationship between $NIPTS_{346}$ and CNE′ for workers aged <30. The regression equation for non-Gaussian noise is $NIPTS_{346}$=0.268CNE′−5.640, R^2=0.443.

kurtosis value; however, for the same kurtosis, the detailed temporal structure of noise exposure did not have a strong influence on hearing trauma, while different kurtosis levels had significant influence on hearing trauma. Therefore, kurtosis and energy are sufficient and necessary metrics to evaluate NIHL. A combination of noise energy and kurtosis (e.g., kurtosis-adjusted CNE) has the potential to be used to evaluate the hearing loss associated with non-Gaussian noise.

This study aimed to validate the applicability of kurtosis-adjusted CNE (CNE′) in assessing NIHL. Multiple linear regression models in **Table 3** showed the most significant standard regression coefficient in Model 1 was age, while the largest one in Model 2 was CNE′. From Model 1 to Model 2, the impact of age on NIHL decreased while the impact of CNE and kurtosis increased, indicating that kurtosis adjustment made the contribution of CNE′ to NIHL greater than that of CNE. An increase of R^2 after kurtosis adjustment implied an improvement in regression goodness-of-fit, suggesting that CNE′ was a better measure for assessing NIHL associated with non-Gaussian noise than CNE. This result was supported by a study by Xie et al. (2016) that reported an increase of R^2 of CNE after kurtosis adjustment using the multiple regression analysis. The larger sample size in this study (928 non-Gaussian-exposed workers) might be more convincing in terms of the validity of CNE′ than that (178 non-Gaussian-exposed workers) in Xie et al.'s study.

Figure 1 illustrates the linear relationship between $NIPTS_{346}$ and CNE or CNE′ for all subjects. Before the kurtosis adjustment, the regression equation of non-Gaussian noise had higher levels of $NIPTS_{346}$ than that of Gaussian noise (mean D_1 = 4.32 dB HL), which was consistent with the above finding that non-Gaussian noise caused more severe hearing loss than Gaussian noise. Thus, as shown in **Figure 1A**, the regression line of non-Gaussian noise was above that of Gaussian noise. However, after CNE was adjusted by kurtosis, the difference of $NIPTS_{346}$ between the two lines was significantly reduced, and the regression line of non-Gaussian noise nearly overlapped that of Gaussian noise when using CNE′ (1.63 dB HL left), which indicated that there was an equivalent noise-induced effect for the two groups. This result suggested CNE′ could

be used to evaluate the hearing loss caused by different types of noise (e.g., Gaussian and non-Gaussian noise). Zhao et al. (2010) and Xie et al. (2016) came to similar conclusions. They plotted the dose–response curve between CNE (CNE′) and NIHL prevalence and found that the curve of non-Gaussian noise almost overlapped that of Gaussian noise when using CNE′. Zhang et al. (2021b) also plotted the dose–response curves and further calculated the differences in NIHL prevalence between the non-Gaussian noise group and Gaussian noise group; the authors found that after kurtosis adjustment, the average difference of NIHL prevalence significantly decreased from 7.63% to 1.12%. These findings suggested that CNE′ was able to consistently estimate the prevalence of hearing loss across varied noise environments using a single metric.

In this study, the multiple regression analysis demonstrated age and sex were risk factors affecting NIHL. This result was supported by previous studies (Gates et al., 1990; Pearson et al., 1995; Sriopas et al., 2017; Nyarubeli et al., 2019). Thus, this study used a stratified analysis based on age and sex to observe the role of CNE′ alone in NIHL. **Figure 2** illustrated that in male or female workers, the use of CNE′ could significantly reduce the difference of hearing loss between non-Gaussian noise and Gaussian noise ($p < 0.001$). Especially for male workers, the regression line of non-Gaussian noise nearly overlapped that of Gaussian noise (mean $D_2 = 0.96$ dB HL). Xie et al. (2016) also conducted a stratified analysis and obtained the same conclusion among male workers. **Figures 3A,B** demonstrated in workers aged ≥30, the regression line of non-Gaussian noise nearly overlapped that of Gaussian noise (mean $D_2 = 1.13$ dB HL), and the distance between two lines was significantly reduced ($t = 15.80$, $p < 0.001$) after kurtosis adjustment.

In this study, the effectiveness of CNE′ among workers aged <30 was not significant, which was a limitation for this study. The reason was related to the insufficient sample size of these young workers in specific bins of CNE (CNE′), especially in 70–78 CNE (CNE′) bins, which increased the variability of data and resulted in low R^2 values (e.g., 0.429–0.698) of regression lines. For example, for Gaussian-exposed workers, the sample size of the 70–72 CNE bin or the 76–78 CNE bin was only one and that of the 72–74 and 74–76 CNE bin was zero. Therefore, greater sample sizes of young workers exposed to low noise level are needed in further studies. In addition, methodologies to verify the effectiveness of CNE need to be further improved.

CONCLUSION

As a noise exposure metric combining noise energy and temporal characteristics, the kurtosis-adjusted-CNE metric was more effective than CNE alone in assessing NIHL among manufacturing workers in the non-Gaussian noise environment. More epidemiological studies are needed to verify the validity of the kurtosis-adjusted-CNE metric.

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 the Zhejiang Center for Disease Control and Prevention, China (approval reference number: ZJCDC-T-043-R). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ZS: investigation, formal analysis, and writing—original draft. XW and HX: methodology and investigation. XG: investigation and data curation. LZ: formal analysis and visualization. MZ: conceptualization, funding acquisition, writing—review and editing, and supervision. All authors contributed to the article and approved the submitted version.

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REVIEW
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Unexpected Consequences of Noise-Induced Hearing Loss: Impaired Hippocampal Neurogenesis, Memory, and Stress

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Noise-induced hearing loss (NIHL), caused by direct damage to the cochlea, reduces the flow of auditory information to the central nervous system, depriving higher order structures, such as the hippocampus with vital sensory information needed to carry out complex, higher order functions. Although the hippocampus lies outside the classical auditory pathway, it nevertheless receives acoustic information that influence its activity. Here we review recent results that illustrate how NIHL and other types of cochlear hearing loss disrupt hippocampal function. The hippocampus, which continues to generate new neurons (neurogenesis) in adulthood, plays an important role in spatial navigation, memory, and emotion. The hippocampus, which contains place cells that respond when a subject enters a specific location in the environment, integrates information from multiple sensory systems, including the auditory system, to develop cognitive spatial maps to aid in navigation. Acute exposure to intense noise disrupts the place-specific firing patterns of hippocampal neurons, "spatially disorienting" the cells for days. More traumatic sound exposures that result in permanent NIHL chronically suppresses cell proliferation and neurogenesis in the hippocampus; these structural changes are associated with long-term spatial memory deficits. Hippocampal neurons, which contain numerous glucocorticoid hormone receptors, are part of a complex feedback network connected to the hypothalamic-pituitary (HPA) axis. Chronic exposure to intense intermittent noise results in prolonged stress which can cause a persistent increase in corticosterone, a rodent stress hormone known to suppress neurogenesis. In contrast, a single intense noise exposure sufficient to cause permanent hearing loss produces only a transient increase in corticosterone hormone. Although basal corticosterone levels return to normal after the noise exposure, glucocorticoid receptors (GRs) in the hippocampus remain chronically elevated. Thus, NIHL disrupts negative feedback from the hippocampus to the HPA axis which regulates the release of corticosterone. Preclinical studies suggest that the noise-induced changes in hippocampal place

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cells, neurogenesis, spatial memory, and glucocorticoid receptors may be ameliorated by therapeutic interventions that reduce oxidative stress and inflammation. These experimental results may provide new insights on why hearing loss is a risk factor for cognitive decline and suggest methods for preventing this decline.

Keywords: hippocampus, neurogenesis, noise-induced hearing loss, memory, spatial navigation, stress, glucocorticoid receptor (GCR)

INTRODUCTION

Intense noise primarily damages the sensory hair cells and spiral ganglion neurons; their destruction reduces the flow of acoustic information to numerous structures within the central auditory pathway as well as other parts of the brain that utilize auditory information to carry out complex processes such as formulating an emotional response to a baby's cry, reacting viscerally when called to supper or exiting a train when your station is announced over the intercom. In order to respond effectively in these situations, the neural activity relayed through the ascending auditory pathway must be quickly and continuously integrated with information being processed in other parts of the brain such as those involved with motor control, cognition, emotion, and memory.

The hippocampus is generally considered as a structure involved in the formation of new memories, cognitive maps, and spatial navigation. Although the hippocampus lies outside the classical auditory pathway, it nevertheless responds to sound (Bickford and Wear, 1995; Moxon et al., 1999; Moita et al., 2003) and other sensory stimuli (Tamura et al., 1992; Cooper et al., 1998; Levy et al., 2004; Martin et al., 2007; Zheng et al., 2010; Gener et al., 2013). Consequently, severe noise-induced hearing loss (NIHL) would be expected to deprive the hippocampus of auditory information, for example, remembering a sequence of telephone numbers or series of instructions on how to exit a building. In the past decade, there has been growing interest in understanding how hearing loss affects the hippocampus, much of this motivated by clinical studies showing that blast wave-induced hearing loss is associated with memory and cognitive impairments as well as epidemiological studies suggesting that hearing loss significantly increases the risk of developing dementia (Lin et al., 2011a). How hearing loss disrupts memory and cognitive function is a major unanswered question with enormous clinical implications (Slade et al., 2020; Johnson et al., 2021). To provide insights on how NIHL could disrupts hippocampal function, the following section will briefly review some of the structural and functional characteristics of the hippocampus associated with auditory processing.

HIPPOCAMPUS OVERVIEW

The hippocampus is located in the medial portion of the temporal lobe adjacent to the inferior horn of the lateral ventricle (Lavenex, 2012; Wible, 2013; Fogwe et al., 2021). The two major components of the hippocampus are the dentate gyrus and the hippocampus proper, or Cornu Ammonis (CA) with three subdivisions in rodents, CA1, CA2, and CA3 (**Figure 1A**). The CA, shaped like a ram's horn, wraps around dentate gyrus. The main afferent and efferent fibers in the hippocampus travel together in two major bundles, the fornix and subiculum. The fornix relays information between the hippocampus and multiple brain regions, principally the septal nuclei, preoptic nuclei, striatum, orbital cortex, cingulate cortex, thalamus, and mammillary body. Fibers in the subiculum relay information between the hippocampus and the entorhinal cortex and amygdala, which in turn connect to other areas such as the cingulate, olfactory bulb, and orbital cortex. Many of these structures form reciprocal connections with the hippocampus. Information from the entorhinal cortex is relayed through the performant pathway to the dentate gyrus. The dentate gyrus, in turn, transmits this information through mossy fibers to CA3 where it is relayed by Schaffer collaterals to neurons in CA1. Information from CA3 and CA1 is subsequently relayed through the fornix, fimbria, and subiculum to other regions of the brain. The output of the CA1 region of the hippocampus can also be directly relayed to the entorhinal cortex.

Pyramidal cells in the hippocampus receive glutamatergic excitatory inputs on numerous spines located on the apical dendritic shaft. The apical dendrites are oriented perpendicular to the pyramidal cells, whose somas are aligned in a thin layer along the CA axis. This stereotypic orientation causes the extracellular currents from individual neurons to summate and generate large field potentials. The primary axon of most pyramidal cells connects to neurons in the cerebral cortex, but collateral side branches emerging from the pyramidal cells form excitatory synapses on basket cell interneurons, which in turn synapse back onto the pyramidal cells. When activated, basket cells release the inhibitory neurotransmitter, gamma aminobutyric acid (GABA), generating recurrent negative feedback inhibition that dampens the activity of the pyramidal cells. Neurological conditions that reduce recurrent inhibition from the basket cells can lead to hyperactivity in pyramidal cells resulting in epileptic seizures and hippocampal sclerosis (Arellano et al., 2004; Cossart et al., 2005).

The hippocampus, considered part of the limbic system, forms connections with regions of the brain involved with emotion such as the amygdala, hypothalamus, and mammillary body (Miller and O'Callaghan, 2005; Cui et al., 2013; Kim et al., 2015). The hippocampus also contributes to the formation of new memories, cognitive maps, and spatial navigation (Moscovitch et al., 2005; Hartley et al., 2014; Ekstrom et al., 2017). The hippocampus contains place cells that respond vigorously when an animal moves into or through a specific place in the environment often in a specific direction. Place-specific neural firing has been observed in both pyramidal

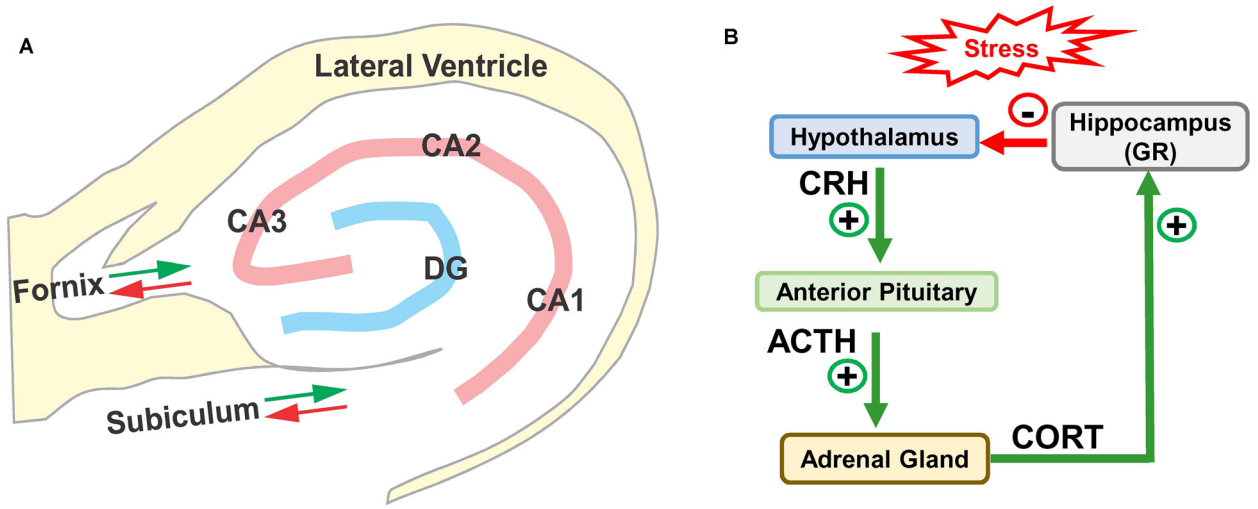

FIGURE 1 | (A) Schematic of hippocampus and surrounding lateral ventricle. Three subdivisions of the rodent Cornu Ammonis (CA1, CA2, and CA3) surrounding the dentate gyrus (DG). Only the major afferent and efferent pathways (red/green arrows) through the subiculum and fornix are shown. **(B)** Schematic of hypothalamic-pituitary adrenal (HPA) axis with hippocampus. Stress stimulates the release (+) of corticotropic releasing hormone (CRH) from the hypothalamus, which binds to receptors in anterior pituitary causing the release (+) of adrenocorticotropic hormone (ACTH) which stimulates the release of corticosterone (CORT). CORT binds to glucocorticoid receptors (GRs) in the hippocampus, which provides negative feedback (–) to the hypothalamus suppressing the release of CRH, ACTH, and CORT.

cells in CA neurons and granule cells in the dentate gyrus (Harvey et al., 2009; Bartsch et al., 2010; Mizuseki et al., 2011; Schmidt et al., 2012). These findings led to the hypothesis that hippocampal place cells are used to construct cognitive maps of the environment (O'Keefe, 1991; Krupic et al., 2018). In line with this view, patients with hippocampal lesions often suffer from amnesia and have difficulty on spatial navigation tasks and remembering where they have been (Banta Lavenex et al., 2014; Schoberl et al., 2019).

Sensory Inputs to Hippocampus

The ability to spatially navigate through the environment to find food in a remote location requires an ongoing stream of multisensory information that can be compared against a cognitive map of the surroundings. Spatial navigation is believed to rely on three sources of information (Ravassard et al., 2013). These include visual cues from distant objects (O'Keefe, 1991), self-motion perceptual information (Gothard et al., 1996; Pastalkova et al., 2008; e.g., vestibular, proprioceptive), and information gleaned from other sensory systems (e.g., auditory, somatosensory, olfactory; Gener et al., 2013; Geva-Sagiv et al., 2015; Schinazi et al., 2016). The relative importance of these navigational cues varies with the nature of the task and the subject's innate capabilities. In a brightly illuminated room, a rodent traveling through an eight arm radial maze to locate food in the northeast arm of the maze could use visual cues, together with odor, somatosensory, and auditory cues to navigate to the correct location. Although the visual acuity of rodents is poorer than that of primates (Prusky et al., 2000; Cruz-Martin and Huberman, 2012), they nevertheless use visual cues together with other forms of sensory information to remember where food can be found on subsequent searches of the maze.

Rats, however, are nocturnal animals and on a dark night, visual cues would be greatly reduced. Consequently, olfactory, somatosensory, and auditory cues would take on greater significance for navigating to the correct location. The sensory cues employed in spatial navigation also depend on the innate abilities of the species. Echo locating bats flying on a dark night and searching for its home in a dark cave would rely heavily on echolocation using auditory processing skills to return home.

Neurogenesis, Memory, and Spatial Navigation

Neurogenesis refers to a process in which new neurons are generated from stem cells in the adult brain. Neurogenesis has been well established in the subventricular zone and hippocampus of adult rodents and non-human primates (Cinini et al., 2014), but in humans the evidence remains controversial (Boldrini et al., 2018; Sorrells et al., 2018; Kumar et al., 2019). The hippocampus in the rodent brain contains a stem cell niche. Approximately 9,000 new cells are born in the hippocampus of a young rat each day; most of these differentiate into neurons, migrate, and establish functional connections with other cells within a neural network (Cameron et al., 1993; Hastings and Gould, 1999; van Praag et al., 2002; Kuhn et al., 2018). There is a growing body of evidence linking hippocampal neurogenesis to the formation and retention of new memories related to spatial navigation, recognition, and declarative memory (Snyder et al., 2005; Aimone et al., 2006; Opitz, 2014; Bird, 2017). Aging, chronic stress, excess alcohol consumption, and cranial irradiation suppress neurogenesis, induce apoptosis and disrupts the formation of hippocampal dependent memories (Shors et al., 2002; Lucassen et al., 2006; Nixon, 2006;

Warner-Schmidt and Duman, 2006; Winocur et al., 2006; Kubera et al., 2011). Conversely, antidepressant drugs, glucocorticoid antagonists, and exercise tend to enhance neurogenesis and improve memory (Encinas et al., 2006; Mayer et al., 2006; Oomen et al., 2007; Blackmore et al., 2009; ElBeltagy et al., 2010).

Hippocampus and Stress

The hippocampus is especially vulnerable to several forms of trauma including chronic stress (Sapolsky, 1986; McEwen, 1994; Royo et al., 2006). High levels of stress activate the hypothalamic-pituitary-adrenal (HPA) axis (**Figure 1B**); this stimulates the release of corticotrophin-releasing hormone (CRH) from the hypothalamus, which in turn promotes the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland. ACTH binds to receptors on cells in the adrenal gland, which leads to the release of corticosterone (CORT), a stress hormone in rodents (cortisol in humans). The released CORT crosses the blood-brain barrier into bloodstream where it can bind to the high affinity mineralocorticoid receptors (MRs) and low affinity glucocorticoid receptors (GRs). Under normal conditions, low-levels of CORT mainly binds to and activates MRs (see Ogita et al., 2012; Mifsud and Reul, 2018), but during periods of stress, CORT increases to high enough levels that it activates GRs. GRs are expressed on cells throughout the brain, but are heavily expressed on cells in the hippocampus (de Kloet et al., 1993; de Kloet and Meijer, 2019). The hippocampus is believed to be especially vulnerable to stress because it contains one of the highest densities of GRs in the brain (Joels et al., 2018). Indeed, high levels of CORT suppress hippocampal neurogenesis by hyperphosphorylating huntingtin, reducing brain derived neurotrophic factor (BDNF; see Agasse et al., 2020) and inducing dendritic atrophy on hippocampal pyramidal neurons. These negative effects are prevented by GR antagonists (Cameron and Gould, 1994; Magarinos and McEwen, 1995; Mayer et al., 2006; Warner-Schmidt and Duman, 2006; Morales-Medina et al., 2009).

Auditory Inputs and Hippocampal Function

Auditory stimuli, such as a fire alarm, could affect the hippocampus indirectly by stimulating the release of CORT, or alternatively by stimulating the release of neurotransmitters from sound-sensitive neurons, such as those in the amygdala or septum, that project to neurons in the hippocampus (McDonald, 1998; Janak and Tye, 2015; Xiao et al., 2018) or from the lemniscal portion of the auditory pathway (Bickford et al., 1993; Moxon et al., 1999). Auditory evoked responses have been recorded from the dentate gyrus and CA of the hippocampus; the latency of the first peak of the evoked response in rats is approximately 30 ms, about 15 ms longer than the response from the inferior colliculus (Hall and Borbely, 1970; Jirsa et al., 1992). The threshold for eliciting a neural response from the hippocampus is roughly 25 to 35 dB higher than in the inferior colliculus. Of potential interest is the fact that the response amplitude from hippocampus can be enhanced by high-dose salicylate, an ototoxic drug that depresses the neural output of the cochlea (Chen et al., 2014). The early portion of the sound-evoked hippocampal response is largely abolished by destruction of the entorhinal cortex, which relays information to the hippocampus through the subiculum and perforant pathway (Deadwyler et al., 1981). Hippocampal neurons exhibit a range of specialized responses to sounds; some neurons exhibit directional sensitive responses while others respond to changes in frequency, intensity, tempo, and duration (Brown and Buchwald, 1973; Sakurai, 2002; Ruusuvirta et al., 2010; Geva-Sagiv et al., 2016). These acoustic features could be used to construct declarative memories as well as spatial and non-spatial cognitive maps.

Neural activity in the hippocampus is modified by auditory experience, especially sounds with biological significance (Deadwyler et al., 1981; Moita et al., 2003). After rats are trained on an operant auditory discrimination task, granule cells in the dentate gyrus acquire the "ability to distinguish" between two different auditory tokens by responding more robustly to positively reinforced sounds vs. unreinforced/negative stimuli. Hippocampal neurons acquire this preferential response to auditory stimulation through positive reinforcement (Deadwyler et al., 1981; Foster et al., 1988). Destruction of the perforant pathway, which relays auditory information from the entorhinal cortex to the hippocampus, largely abolishes neural responses to both positive and negative sounds (i.e., a non-selective effect). In contrast, lesions of the septal pathway impair auditory discrimination by reducing the difference in neural response magnitude to positive vs. negative auditory tokens (i.e., a selective effect). Thus, the septal pathway appears to relay information about the positive and negative attributes of the conditioned auditory stimulus (Foster et al., 1988).

Hippocampal neurons show evidence of both auditory working memory and reference memory. This is illustrated by a study in which rats were trained to discriminate between pairs of tones based on their temporal order vs. the similarity or difference in the pitch of the tones. To assess working memory, rats were trained to make a Go response if the current tone was different from the preceding tone and not respond (No-Go) if the current tone was the same as the previous tone (Sakurai, 1994). Alternatively, reference memory was assessed by training rats to make a Go response if the two sequential stimuli were both high-tones and to make a No-Go response if the two sequential tones were both low-tones (Sakurai, 1994). After training, some hippocampal neurons preferentially responded (i.e., produced more spike discharges or probability of firing to one task than the other) on the working memory task; others preferentially responded on the reference memory task and some responded on both the working memory and reference memory tasks. After operant reinforcement training, hippocampal neurons "formed memories" on how to differentially respond on an auditory working memory task vs. an auditory reference memory task (Sakurai, 1993, 1998).

Hippocampal place cells preferentially fire action potentials when an animal enters a specific location in its environment. The specificity and reliability of place cell firing is affected by information gleaned from external and internal sensory cues acquired by navigating through the environment on multiple occasions. Place cell performance is often evaluated in rodents using an eight-arm radial maze with food bait placed in one or more arms of the maze. While traversing through the maze,

olfactory and somatosensory systems provide useful proximal cues while vision provides distal information (Quirk et al., 1990; Markus et al., 1994; Gener et al., 2013; Geva-Sagiv et al., 2016). However, in echolocating bats flying about in the dark, hippocampal place cells create an auditory map-like representation of a physical space using cues gained from echolocation (Geva-Sagiv et al., 2015).

Besides preferential response to physical place, hippocampal neurons are also able to create non-physical maps along a continuous auditory dimension such as sound frequency (Aronov et al., 2017). After rats were trained to physically change sound frequency by manipulating a joystick, the firing of hippocampal neurons increased as the rat shifted stimulus frequency in the direction of the target frequency; this occurred independent of other factors. Hippocampal neural firing occurred around discrete frequency fields only when the rat performed the task, whereas the same neurons did not respond when the same frequency was presented alone outside the situational environment. These non-spatial auditory frequency-fields often overlapped spatial navigation place-fields suggesting a common hippocampal mechanism not only for coding spatial navigation but also other non-spatial cognitive tasks such as remembering the correct sequence of sounds as in a melody.

Because the hippocampus is considered important for memory storage, it may come as no surprise that human functional imaging studies have implicated the hippocampus in storage of complex auditory information such as auditory hallucinations (Silbersweig et al., 1995; Takebayashi et al., 2002; Suzuki et al., 2003; Lefebvre et al., 2016) and simpler sounds such as the buzzing or ringing of the phantom sound of tinnitus (Lockwood et al., 1998; Chen et al., 2015). The phantom sound of tinnitus and musical hallucination often emerge following NIHL (Humes et al., 2006; Yankaskas, 2013) and other forms of acquired hearing loss (Rosanski and Rosen, 1952; Hammeke et al., 1983; Aizenberg et al., 1991; Tanriverdi et al., 2001).

NOISE-INDUCED HEARING LOSS

Permanent NIHL, Decreased Neurogenesis, and Memory Deficits

Recent epidemiological studies indicate that hearing loss is a risk factor for dementia and cognitive decline (Lin et al., 2011b; Deal et al., 2017; Su et al., 2017), suggesting the possible involvement of the hippocampus. Moreover, combat personnel exposed to intense blasts not only develop hearing loss (Cave et al., 2007), but also memory and/or other cognitive impairments (Belanger et al., 2009). It is unclear if these cognitive impairments result from the hearing loss *per se* or other factors such as the direct traumatic effect of the blasts on the brain as suggested by animal studies showing blast-induced neuropathology and tau protein expression in the hippocampus (Säljö et al., 2009; Sajja et al., 2015).

The hippocampus is a major site of neurogenesis in the adult brain (Kaplan and Bell, 1984; Eriksson et al., 1998; Snyder et al., 2009) and recent animal studies have shown that NIHL can chronically suppress hippocampal neurogenesis (Kraus et al., 2010; Liu et al., 2016; Manohar et al., 2020). A persistent decline in neurogenesis was first reported in 2010 after adult rats had been unilaterally exposed to intense continuous noise (2 h, 126 dB SPL, narrowband noise, 12 kHz) and evaluated several months post-exposure. The unilateral noise exposure destroyed virtually all outer hair cells (OHCs) and inner hair cells (IHCs) over the basal two-thirds of the cochlea (**Figure 2A**), but it did not damage the hair cells in the contralateral cochlea that had been protected with an ear plug (Kraus et al., 2010). Neurogenesis was evaluated several months after the exposure by labeling hippocampal brain sections from noise-exposed and control rats with doublecortin (DCX), a protein expressed in developing neural precursor cells (Brown et al., 2003). In normal controls, DCX-labeled soma were arranged in a band running along the subgranular zone of the dentate gyrus (**Figure 2B**) and an elaborate network of processes extended from the soma of these neurons. In noise-exposed rats, by contrast, the number of DCX-labeled somas was greatly reduced and few neural processes emanated from the somas of these neural precursors (**Figure 2C**). Although only one ear was noise-damaged, the number of DCX-positive cells was reduced in both the ipsilateral and contralateral dentate gyrus by ∼30% (**Figure 2D**). Ki67 immunolabeling was used to assess the rate of hippocampal cell division at the time of sacrifice several months post-exposure (Scholzen and Gerdes, 2000). Ki67 immunolabeling was reduced by more than 50% in the subgranular zone of the ipsilateral and contralateral hippocampus (**Figure 2E**). These results suggested that cochlear hearing loss might result in long-term cognitive or spatial navigation deficits.

Subsequent experiments conducted in adult mice bilaterally exposed to broadband noise (123 dB SPL, 2 h) revealed significant cognitive impairments on the Morris Water Maze test several months post-exposure (Liu et al., 2016). Noise-exposed mice with significant permanent hearing loss and massive OHC loss in the basal half of the cochlea had significantly more difficulty learning the location of the hidden platform (i.e., working memory deficits). Several weeks later, the noise-exposed mice also had more difficulty remembering where the hidden platform had been previously located (i.e., reference memory deficits). The chronic working memory deficits and reference memory deficits were associated with a bilateral decline in hippocampal neurogenesis (∼27%) and cell proliferation (∼26%). Moreover, the learning and remembering deficits were positively correlated with the degree of hearing loss. At the time when the memory tests were performed (∼3-months post-exposure), there was no evidence of long-term oxidative stress in the hippocampus. In addition, CORT hormone levels were normal ruling out stress as a causal factor. Similarly, postnatal mice exposed to intense noise near the onset of hearing not only suffered from severe hearing loss in adulthood, but also suffered from chronic spatial learning and memory deficits and decreased neurogenesis several months after the noise exposure (Tao et al., 2015). These results indicate that NIHL in early life is a risk factor for learning and memory deficits in later life.

A persistent reduction in hippocampal neurogenesis was also observed in adult rats exposed to three blast waves with a peak pressure of 188 dB SPL (Newman et al., 2015). The bilateral blast

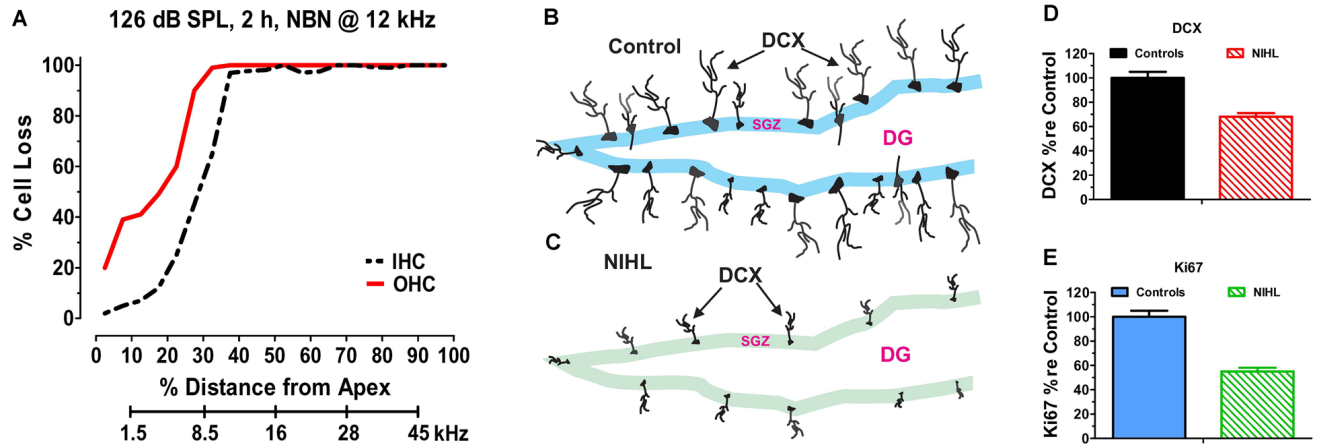

FIGURE 2 | Noise-induced hearing loss suppresses hippocampal cell proliferation and neurogenesis. **(A)** Cochleogram showing massive loss of outer hair cells (OHC) and inner hair cells (IHC) in the noise-exposed cochlear several months after a 2-h unilateral exposure to narrowband noise (NBN) centered at 12 kHz and presented at 126 dB SPL. Percent cell loss plotted as function of percent distance from the apex of the cochlear. Cochlear place related to frequency using rat tonotopic map on lower abscissa. **(B)** Schematic of dentate gyrus (DG) of hippocampus from normal control showing immunolabeled doublecortin (DCX) soma in the subgranular zone (SGZ); note extensive immunolabeled processes emanating from soma. **(C)** Schematic of DG of hippocampus several months after a noised induced hearing loss (NIHL) showing immunolabeled DCX) soma in the subgranular zone (SGZ). Note reduced number of DCX soma and paucity of labeled processes in the NIHL hippocampus compared to normal control (panel **B**). **(D)** Schematic showing relative number (% re Control: percentage relative to control) of DCX labeled neurons in hippocampus of normal control rats (100%) and rats with noise-induced hearing loss (NIHL). **(E)** Schematic showing relative number (% re Control: percentage relative to control) of Ki67 labeled neurons in hippocampus of normal control rats (100%) and rats with noise-induced hearing loss (NIHL).

exposure produced hair cell lesions in both ears. Approximately 25% of the OHCs and IHCs were missing over much of the cochlea, but in the extreme base of the cochlea, hair cell losses exceeded 85%. Hippocampal neurogenesis, assessed by DCX-labeling, was reduced by ∼40% in the dentate gyrus several months after the exposure (Newman et al., 2015).

In subsequent experiments, working memory and reference memory were assessed approximately 3-months after rats were exposed to six blasts with a peak intensity of 185 dB peak SPL (Manohar et al., 2020). This bilateral exposure caused a severe hearing loss and greatly reduced the neural output of the cochlea as reflected in the compound action potential. Neurogenesis assessed by DCX-labeling was reduced by ∼46%; this reduction was largely due to decreased cell proliferation rather than a decline in the proportion of new cells that differentiated into neurons, consistent with earlier results (Kraus et al., 2010). The blast-exposed rats performed as well as control rats learning the location of the hidden platform on the Morris Water Maze test (i.e., normal working memory). However, when retested several weeks later, the blast-exposed rats had difficulty remembering where the hidden platform had previously been located; results indicative of impaired memory consolidation (i.e., reference memory deficit). Thus, blast-wave induced hearing loss only caused a deficit in reference memory unlike previous work in mice in which working memory was also impaired (Liu et al., 2016). It has been suggested that active learning promotes the survival of new hippocampal neurons (Anderson et al., 2011; Shors et al., 2012; Curlik et al., 2013). However, in noise-exposed mice, active training on a Morris Water Maze task had minimal effect in promoting neuron survival (Liu et al., 2016).

Acute Noise Exposure and Hippocampal Place Cells

The firing pattern of hippocampal place cells remain relatively stable for months as long as testing occurs in the same environment (Save et al., 2000; Agnihotri et al., 2004). Although visual, olfactory and somatosensory cues are considered the primary signals regulating place cell firing, auditory stimuli also appear to be important (Moita et al., 2003, 2004). The subtle contribution of auditory cues is illustrated when place cell fields are mapped out in an eight arm radial maze before and after an intense noise exposure. During baseline testing before the noise exposure, hippocampal place cells consistently fired at specific locations within the maze (**Figure 3A**). To determine the impact of intense noise exposure on place cell firing, rats were exposed for 30 min to a 104 dB SPL, 4 kHz tone (Goble et al., 2009). Place cell firing patterns were greatly disrupted after the noise exposure (**Figure 3B**). The original place field was shrunken and distorted and new place fields emerged. Instead of only firing at a specific location within the maze, cells began to respond at multiple locations within the maze. The disruptions of place field firing patterns began immediately after the noise exposure and persisted for at least 24 h. These results indicate that noise-induced changes in cochlear function results in unexpected changes in place-cell firing. Because cognitive function was not assessed, it is unclear if this noise exposure disrupted spatial navigation. It is unclear if the functional changes in place cell firing are

temporary or permanent, but given that this noise exposure was not too severe, it seems likely that the place cell firing patterns might be restored as hearing loss recovers following the noise exposure.

Acute Noise Exposure and Hippocampal Long-Term Potentiation

Neural circuits in the hippocampus exhibit different forms of synaptic plasticity. The most well studied form of synaptic plasticity is long-term potentiation (LTP), a prolonged increase in synaptic strength that occurs following repeated stimulation of a synapse such as the Schaffer-CA1 or perforant-dentate synapses. LTP has been considered a form of synaptic learning and memory. Repetitive auditory stimulation can influence hippocampal function (Angelucci et al., 2007; Deschaux et al., 2011; Kraus and Canlon, 2012; Nguyen et al., 2018) raising the possibility that intense noise exposure might disrupt hippocampal LTP and spatial navigation. Indeed, a 1-min exposure to high intensity sound stimulation (110 dB SPL), but not low intensity (80 dB SPL) stimulation disrupted hippocampal LTP for more than 24 h. However, neither the low or high intensity sounds failed to disrupt learning and memory performance assessed with the Morris water maze (de Deus et al., 2017). While short, intense noise exposures can easily disrupt hippocampal LTP (Cunha et al., 2018), noise-induced disruption of LTP does not appear to be a predictor of impaired spatial memory.

Chronic Intermittent Noise Exposure, Neurogenesis, and Memory

From a mechanistic perspective, it may be important to distinguish between the chronic vs. acute effects of NIHL on the hippocampus. Stress hormones began to rise once noise levels reach 85 dB SPL and they continue to increase up to 110 dB, the highest intensity evaluated (Burow et al., 2005). However, this increase is normally temporary because CORT binds to GRs; this triggers negative feedback onto the hypothalamus depressing the release of CORT even if the stressful noise is continued (Dallman et al., 1992; Romero, 2004). Consequently, after a single, intense (114 dB SPL), short duration (10 min) noise exposure, CORT levels rise to a peak roughly 15 min after the start of the noise, but then return to baseline approximately 50 min after the noise is turned off (Windle et al., 2013; **Figure 4A**). In cases of very short duration, moderately intense noise exposure such as this, the hearing loss and cochlear damage are likely negligible.

On the other hand, a single, very high intensity noise exposure lasting several hours (126 dB SPL, 2-h, narrowband noise at 12 kHz) is likely to cause significant hearing loss and hair cell damage (Hayes et al., 2019). Immediately after such a traumatic noise exposure CORT levels are transiently elevated, but after several days CORT levels recover to baseline and remain stable for weeks afterwards (**Figure 4B**). Although, basal CORT levels are normal, GRs are significantly upregulated in rats with NIHL compared to controls (**Figures 5A,B**); GR expression had increased roughly two-fold above normal (**Figure 5C**). In contrast, mineralocorticoid receptor (MR) expression levels in the NIHL rats were similar to controls (**Figure 5D**). The chronic upregulation of hippocampal GRs would likely disrupt negative feedback to the HPA axis, potentially contributing to a blunted response to stress (see **Figure 1B**).

However, if an intense noise (100 dB SPL) is repeatedly turned on (4-h) and off (20 h) for 30 days, CORT levels are chronically elevated. CORT levels are the highest 30 min after the noise is turned off on day1 (**Figure 4C**). CORT measurements obtained 24 h after the noise was turned off on day-15 and day-30 are only slightly lower than those obtained shortly after the noise was turned off on day-1 (Samson et al., 2007). Thus, the persistent elevation of CORT during chronic intermittent noise exposure could create a condition of unremitting stress leading to disruption of the HPA axis (Hebert and Lupien, 2007). The chronic stress to the HPA axis could only be alleviated if the subject habituates or adapts to the noise-induced stress response (Day et al., 2009; Masini et al., 2012). However, if chronic stress is unpredictable, it can chronically disrupt the HPA axis resulting in a blunted stress response, decreased neurogenesis, and increased inflammation (Algamal et al., 2018; Blossom et al., 2020; Parul et al., 2021).

The consequences of persistent exposure to intermittent noise are illustrated by a study in which rats were exposed for 15 days to 100 dB SPL noise for 2 h/day. The rats were then evaluated 15-days post-exposure when they exhibited a relatively mild NIHL (Shukla et al., 2019). CORT levels were significantly elevated several weeks after the intermittent noise exposure. Moreover, cell proliferation and neurogenesis were greatly reduced and spatial memory was impaired, consistent with the persistent increase in CORT after the exposure. However, if the rats were pretreated with an adenosine A2a receptor agonist, which exerts cytoprotective effects by increasing adenosine availability, the noise-induced hearing loss and disruptions of the hippocampus were greatly reduced (Fredholm, 2007; Wong et al., 2010). The protection of the hippocampus induced by this adenosine agonist is consistent with previous reports seen with other antioxidants and neuroprotective compounds (Herrera et al., 2003; Hinduja et al., 2015; Daulatzai, 2016).

NIHL Accelerates Cognitive Decline in Alzheimer Models

With the worldwide increase in longevity, the prevalence of dementia and Alzheimer's disease (AD) is expected to surge creating tremendous social and economic burdens (Alzheimer's Association Report, Alzheimer's Association Report; Bennett et al., 2021; Farina et al., 2022). To understand the biological basis of the diseases, many rodent models of AD have been developed (Gotz et al., 2018) providing researcher with the opportunity to investigate the contribution of environmental factors such as NIHL in disease progression (Cui et al., 2015; Gai et al., 2017; Jafari et al., 2020; Paciello et al., 2021). In one study, triple transgenic AD mice were repeatedly noise exposed as young adults and cognitive and hippocampal function evaluated months afterwards, but before the expected time of cognitive decline. Prior noise exposure accelerated the onset of short-term and long-term memory decline in the AD mice. These early memory deficits were associated with

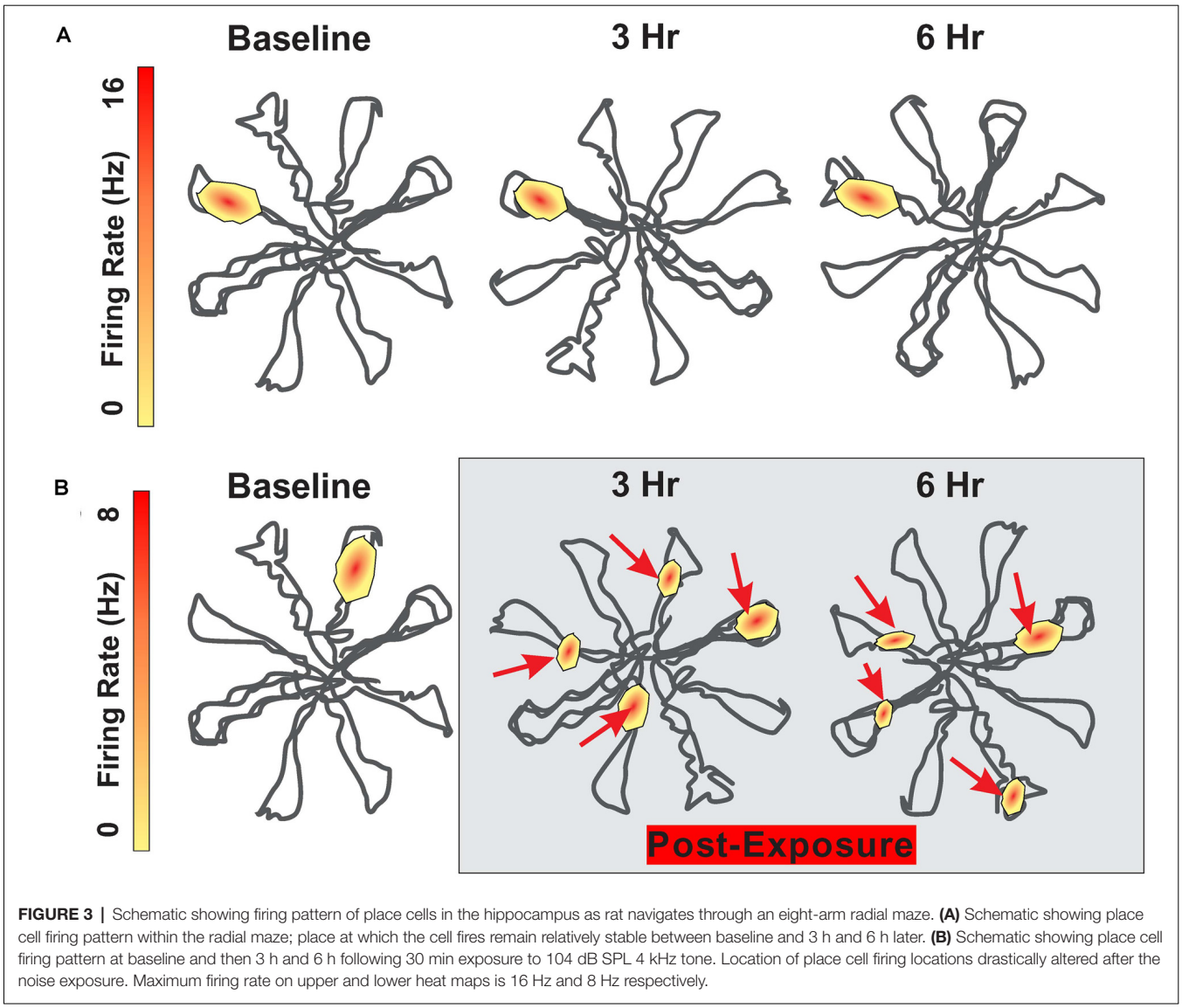

FIGURE 3 | Schematic showing firing pattern of place cells in the hippocampus as rat navigates through an eight-arm radial maze. **(A)** Schematic showing place cell firing pattern within the radial maze; place at which the cell fires remain relatively stable between baseline and 3 h and 6 h later. **(B)** Schematic showing place cell firing pattern at baseline and then 3 h and 6 h following 30 min exposure to 104 dB SPL 4 kHz tone. Location of place cell firing locations drastically altered after the noise exposure. Maximum firing rate on upper and lower heat maps is 16 Hz and 8 Hz respectively.

abnormal synaptic function, increased neuroinflammation and enhanced tau protein expression in the hippocampus and were accompanied by noise-induced functional and morphological changes in the auditory cortex (Paciello et al., 2021).

Others have observed temporary (<7 days) increases in Aβ and amyloid precursor protein in the rat hippocampus after chronic noise stress (100 dB SPL, 4 h/day, 28-days; Cui et al., 2015). The short-lasting increases in the AD proteins are likely related to repeated daily stress induced by the 28-day intermittent noise exposure. If these daily stressful noise exposures were to continue over many months or years they could eventually lead to the chronic buildup of toxic AD proteins and long-term memory deficits. It has been known for many years that long-term exposure to moderate intensity intermittent noise can result in permanent NIHL (Johnson et al., 1976; Melnick, 1991); however, the preceding results suggest that prolonged exposure to unpredictable intermittent noise could also contribute to cognitive decline and dementia as suggested by epidemiological studies.

OTHER TYPES OF PERIPHERAL HEARING LOSS

Reduced Neurogenesis With Conductive Hearing Loss and Ototoxicity

While this review has focused on NIHL as a disruptor of neurogenesis and memory, other types of peripheral hearing losses that deprive the hippocampus of auditory information might be expected to have similar effects. Indeed, clinical studies indicate that prolonged conductive hearing impairment in early life contributes to chronic auditory processing deficits, poorer social skills, language, reading, and cognitive deficits (Zinkus and Gottlieb, 1980; Reichman and Healey, 1983; Bidadi et al., 2008;

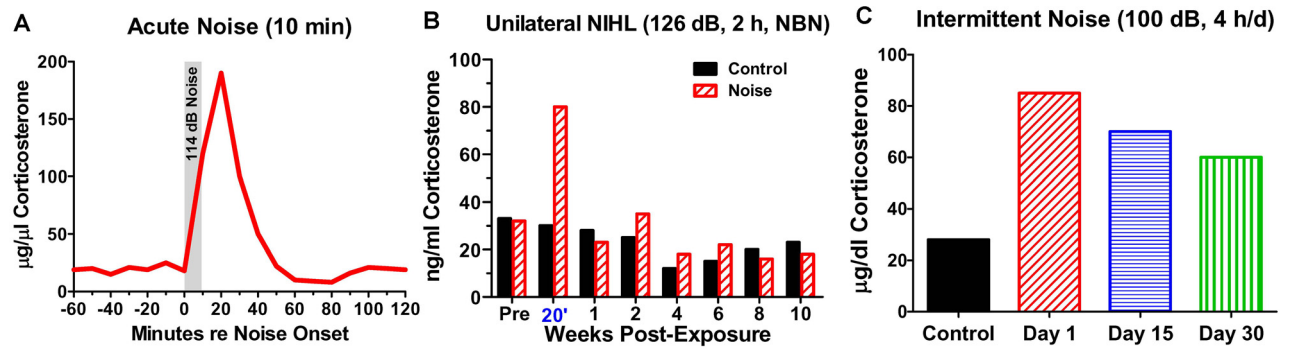

FIGURE 4 | Noise-induced changes in corticosterone. **(A)** Schematic showing rapid rise and fall of serum corticosterone following a 10-min exposure to white noise presented at 114 dB SPL. **(B)** Schematic illustrating corticosterone levels measured over 12 weeks in sham control rats and rats exposed unilaterally for 2-h to 126 dB SPL narrowband noise (NBN) centered at 12 kHz. Corticosterone greatly elevated in noise group 20' post-exposure, but levels decline to normal 1-week post-exposure. No significant difference in long-term basal corticosterone levels between control and noise-exposed group. **(C)** Schematic illustrating the rise in serum corticosterone following chronic intermittent noise presented at 100 dB SPL for 4-h/day over a period of 30 days. On day 1, corticosterone measured 30-min post-exposure while on day 15 and day 30, corticosterone was measured 24-h after the exposure.

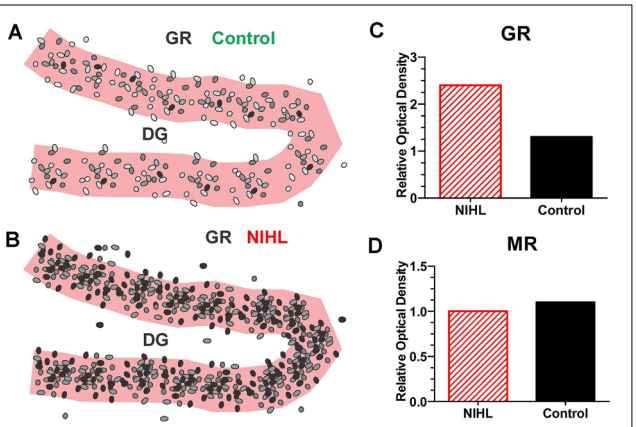

FIGURE 5 | Severe NIHL alters glucocorticoid receptor (GR) expression in hippocampus. **(A)** Schematic of dentate gyrus (DG) in hippocampus showing immunolabeling of GR receptors (black, gray round, oval symbols schematically illustrate the relative intensity of immunolabeling.) in normal control. **(B)** Schematic of DG in hippocampus showing GR immunolabeling several months after induction of severe unilateral noise-induced hearing loss (NIHL; 126 dB SPL, 2 h, NBN centered at 12 kHz). **(C)** Schematic of relative optical density of GR immunolabeling in DG in rats with severe chronic NIHL compared to controls. **(D)** Schematic of relative optical density of mineralocorticoid receptor (MR) immunolabeling of rats with chronic NIHL compared to controls.

Williams and Jacobs, 2009; Purcell et al., 2016). These findings suggest that chronic conductive hearing loss could negatively impact the hippocampus by reducing the flow of auditory information to the brain without damaging the sensorineural elements in the cochlea. Indeed, when auditory inputs to young mice were suppressed by surgically occluding one or both ear canals for 5 weeks, hippocampal cell proliferation and neurogenesis were suppressed and these effects were more severe when both ears were blocked (Kurioka et al., 2021). Unilateral conductive hearing loss suppressed neurogenesis bilaterally in the hippocampus, similar to the effects seen with unilateral NIHL (Kraus et al., 2010). Stress hormone levels were elevated 1 week after surgically occluding both ear canals raising the possibility that chronic stress was responsible for decreased neurogenesis. However, one argument against this view is that stress hormones are unlikely to remain elevated during the entire 5 week of ear canal occlusion because GRs in the hippocampus provide negative feedback to the hypothalamus that prevents the chronic release of stress hormones (Hayes et al., 2019). The validity of this hypothesis could be tested by regularly monitoring stress hormone levels over the period during which chronic ear canal blockade occurred.

Clinical reports suggest that temporary conductive hearing loss in early life, when the nervous system is rapidly developing, could contribute to permanent cognitive and memory deficits (Reichman and Healey, 1983; Williams and Jacobs, 2009). Support for this hypothesis comes from studies in which postnatal rats were subjected to a temporary bilateral conductive hearing loss. Hearing largely recovered when rats reached adulthood. Nevertheless, the rats with early-age temporary conductive hearing losses manifested significant deficits on working memory and reference memory tasks when they reached adulthood. These cognitive deficits were associated with reduced hippocampal cell proliferation, a decrease in hippocampal LTP and fewer hippocampal dendritic spines and post-synaptic densities (Zhao et al., 2018).

Cisplatin Ototoxicity

Many drugs used clinical to treat cancer such as platinum-based antitumor drugs or life threatening bacterial diseases such as aminoglycoside antibiotics are ototoxic (Rybak, 1986; Arslan et al., 1999). These drug have long been known to cause permanent cochlear hearing loss by damaging the sensory hair cells, support cells, and spiral ganglion neurons in the cochlea. However, platinum based antitumor drugs such as cisplatin and carboplatin which block cell division could have potentially devastating effects on the hippocampus by suppressing cell proliferation and neurogenesis in the hippocampus. Cisplatin,

one of the most widely used antineoplastic agent, has a number of well-known side effects including ototoxicity (Helson et al., 1978; Ravi et al., 1995), nephrotoxicity (Fillastre and Raguenez-Viotte, 1989), and neurotoxicity (Cavaletti et al., 1996). Other less well recognized complications include memory and attention impairments often referred to a "chemobrain" (Troy et al., 2000; Hede, 2008; Chiu et al., 2017). Cisplatin, which blocks cell division, crosses the blood-brain barrier (Nakagawa et al., 1996) and when administered *in vivo* to rodents robustly suppressed cell division and neural progenitors in the dentate gyrus. Cisplatin also damaged synapses, increased pro-apoptotic gene expression and enhanced cell death for at least 6 weeks following treatment (Dietrich et al., 2006; Andres et al., 2014; Manohar et al., 2014; Hinduja et al., 2015). Rats treated with high-dose cisplatin exhibited both learning and memory deficits on the Morris water maze test of spatial memory; these deficits were unlikely due to nonspecific health effects because swim speed and distance traveled in the cisplatin group did not differ from controls (Oz et al., 2015). These deficits were attributed to cisplatin's neurotoxic effects on the hippocampus; however, it is possible that cisplatin-induced hearing loss is also a factor.

Cyclodextrin Ototoxicity

Other ototoxic drugs such as the aminoglycoside antibiotics induce serious side effects such as nephrotoxicity, neurotoxicity, anemia, and thrombocytopenia (Snavely and Hodges, 1984; Prayle et al., 2010) making it difficult to disentangle the effect of cochlear hearing loss from more generalized effects on the central nervous system and hippocampus in particular. Unlike cisplatin and aminoglycoside antibiotics that are accompanied by numerous side effects, it may be possible to rapidly induce a hearing loss with minimal side effects with a single high dose of cyclodextrins (Crumling et al., 2017). 2-Hydroxypropyl-beta-cyclodextrin (HPβCD), which chelates cholesterol, is used to treat Niemann-Pick C1, a fatal neurological disorder caused by the intracellular buildup of lipids. High doses of HPβCD initially destroy the OHCs causing a 40 dB hearing loss (Liu et al., 2020). Approximately 6 weeks later the IHCs, organ of Corti, and spiral ganglion neurons degenerate resulting in a significant hearing loss and nearly total loss of OHCs and IHCs over most of the cochlea. Such lesions would deprive the central auditory pathway and hippocampus of nearly all auditory information. Approximately 4 months after treatment with 4,000 mg/kg of HPβCG, our preliminary studies revealed a massive reduction DCX immunolabeling in the dentate gyrus of the hippocampus. Because HPβCD has few side effect, these results suggest that massive cochlear damage may be sufficient to suppress neurogenesis. However, further research is needed to determine HPβCD-induced hearing loss that disrupts spatial learning and memory.

FUTURE DIRECTIONS

Preventing Cognitive Decline

If NIHL and other forms of peripheral hearing loss impair memory and increases the risk of dementia, then hearing restoration could conceivably slow or reverse these losses. Among elderly patients with profound postlingual deafness, only 25% had normal cognitive scores prior to cochlear implantation (Mosnier et al., 2015). However, 1 year after cochlear implantation, the percentage of subjects with normal cognitive function increased to 40%. Prior to cochlear implantation, 20% had abnormal cognitive scores on three of six cognitive tests, but this declined to 5% post-implantation. Implantation also resulted in improved speech perception, enhanced quality of life, and decreased depression. There was a strong relationship between scores on long-term memory and speech in noise possibly due to the fact that working memory is important for understanding speech in noise (Javanbakht et al., 2021).

Hearing aids assist individuals with moderate hearing loss to understand speech in quiet and noise by reducing the cognitive load (Glick and Sharma, 2020) and improving communication in social interactions. However, it is unclear whether hearing aids prevent cognitive decline. Some have found that hearing aids provide no benefits (Dawes et al., 2015) while others have reported positive results. In an experimental trial of adults with mid-moderate hearing loss, 6 months use of hearing aids improved global cognitive function, executive function, visual working memory, and increased cognitive processing speed (Glick and Sharma, 2020). Evoked potential measurements indicated that these improvements were correlated with restoration of more normal cortical. Over a 25-year longitudinal study, use of a hearing aid among individuals with self-reported hearing loss slowed cognitive decline (Amieva et al., 2015). Other reports indicate that hearing aids improve executive function and working memory with greater benefit for females than males (Sarant et al., 2020). The improved speech intelligibility in noise that hearing aids provide would be expected to enhance short-term working memory (Rudner et al., 2012; Neher et al., 2018), but it is unclear if hearing aids enhance long-term memory given that hearing loss is more detrimental to long-term than short-term memory (Rönnberg et al., 2011; Ng et al., 2014). Only 20% of individuals that would benefit from a hearing aid actually own one (Chien and Lin, 2012). Thus, increasing the acceptance of hearing aids among potential beneficiaries represents a significant opportunity for improving both hearing as well as better brain health.

Physical Activity, Neurogenesis, Memory, and Cognition

Although there is considerable interest in identifying pharmacological interventions to prevent dementia and AD, life style changes in the form of increased physical activity may offer significant benefit. Exercise greatly enhance neurogenesis, learning and memory in animal models (van Praag et al., 1999a,b), effects associated with increased expression of brain derived neurotrophic factor (BDNF) in the hippocampus (Adlard et al., 2004; Okamoto et al., 2021) and decreased amyloid protein levels in transgenic AD mice (Adlard et al., 2005). Epidemiological studies suggest that physical activity and fitness significantly reduces cognitive decline and AD (Laurin et al., 2001; Lytle et al., 2004; Podewils et al., 2005; Ross et al., 2016). These benefits may be mediated by various molecules

released during physical exercise (Tari et al., 2019) such as BDNF which enhances learning, and prevents cognitive decline (Cotman and Berchtold, 2002; Cotman and Engesser-Cesar, 2002). Physical exercise also upregulates insulin-like growth factor-1 (IGF-1), a neuroprotective molecule. Low levels of IGF-1 are associated with AD whereas high levels are linked to increased hippocampal volume (Westwood et al., 2014) and enhanced learning and memory (Cetinkaya et al., 2013). IGF-1 also promotes hippocampal cell proliferation that had been depressed by prior cisplatin treatment (Janelsins et al., 2010). These observations are consistent with several epidemiological studies indicating that physical activity protects against cognitive decline and AD (Laurin et al., 2001; Sofi et al., 2011). Exercise also slows the progression of hearing loss in animal models of presbycusis (Han et al., 2016) consistent with epidemiological studies (Gispen et al., 2014; Haas et al., 2016; Kawakami et al., 2022). In an increasingly sedentary world, a consistent moderate, daily dose of physical exercise may promote better hearing and brain health.

LIMITATIONS

The animal studies discussed in this review indicate that chronic or acute noise exposure can suppress hippocampal neurogenesis and impair spatial learning and memory, but further work is needed to address a number of important questions. In cases of significant permanent NIHL, the literature indicates that these deficits persist up to 3–4 months post-exposure, but longer duration studies are needed to determine if these deficits continue or improve with longer recovery times. If the deficits get worse over time, then it would be important to evaluate potential mechanisms that contribute to this decline and to identify therapeutic interventions to prevent this.

The noise-induced disturbances in place cell function (**Figure 3**) represents an acute effect of an acute noise exposure that would likely only cause a temporary hearing loss. Future studies are needed to determine if more severe noise exposures cause permanent disruption of hippocampal place cell function. While the acute noise-induced changes in place cell function suggest that they could contribute to permanent disturbances in spatial navigation and memory impairment, we are unaware of any studies in which the noise-induced functional changes in place cell function have been correlated with a long-term, persistent decline in neurogenesis or long-term deficits on spatial memory acquisition or memory retention. Future studies aimed at investigating the relationships between hippocampal place cell dysfunction and neurogenesis and the relationship between place cell dysfunction and spatial memory deficits represent promising areas of future research.

Because many urban environments are characterized by moderate intensity intermittent and unpredictable noise exposures that cause little threshold shift, it would be important to determine if prolonged exposure to such noise permanently disrupts neurogenesis, learning and memory. Indeed, there is growing interest in noise-exposure that cause little threshold shift due to synaptopathy that reduce the flow of auditory information to the central nervous system (Kujawa and Liberman, 2015; Shi et al., 2016). Well-controlled animal studies could evaluate this. Intense exposures that induce permanent NIHL increase GR expression in the hippocampus. The chronic upregulation of hippocampal GR expression would presumably disrupt negative feedback in the HPA axis. Because GRs are ubiquitously expressed throughout the central nervous system, it could be useful to determine if GRs are up or downregulated elsewhere in the brain. This review tended to focus on noise-induced stress as a major factor in suppressing hippocampal neurogenesis, but because conductive hearing loss can suppress neurogenesis (Zhao et al., 2018; Kurioka et al., 2021), auditory deprivation, independent of stress may be sufficient to suppress hippocampal neurogenesis. To test the role of auditory deprivation as a major factor in suppressing hippocampal neurogenesis independent of stress, it would be important to determine if stress hormones and stress hormone receptors change or remain stable following auditory deprivation.

An important clinical question is whether the noise-induced disruptions to neurogenesis, learning and memory can be reversed by increased physical activity, an enriched environment or pharmacologic interventions and if so, what is the optimal time to do so. These and other related questions provide a framework that could be addressed in future preclinical studies.

AUTHOR CONTRIBUTIONS

SM, DD, and G-DC: conceptualization, visualization, and writing. LL, JW, Y-CC, and LC: conceptualization and writing. RS: conceptualization, visualization, writing, and funding acquisition. All authors contributed to the article and approved the submitted version.

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REVIEW
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Animal-to-Human Translation Difficulties and Problems With Proposed Coding-in-Noise Deficits in Noise-Induced Synaptopathy and Hidden Hearing Loss

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Noise induced synaptopathy (NIS) and hidden hearing loss (NIHHL) have been hot topic in hearing research since a massive synaptic loss was identified in CBA mice after a brief noise exposure that did not cause permanent threshold shift (PTS) in 2009. Based upon the amount of synaptic loss and the bias of it to synapses with a group of auditory nerve fibers (ANFs) with low spontaneous rate (LSR), coding-in-noise deficit (CIND) has been speculated as the major difficult of hearing in subjects with NIS and NIHHL. This speculation is based upon the idea that the coding of sound at high level against background noise relies mainly on the LSR ANFs. However, the translation from animal data to humans for NIS remains to be justified due to the difference in noise exposure between laboratory animals and human subjects in real life, the lack of morphological data and reliable functional methods to quantify or estimate the loss of the afferent synapses by noise. Moreover, there is no clear, robust data revealing the CIND even in animals with the synaptic loss but no PTS. In humans, both positive and negative reports are available. The difficulty in verifying CINDs has led a re-examination of the hypothesis that CIND is the major deficit associated with NIS and NIHHL, and the theoretical basis of this idea on the role of LSR ANFs. This review summarized the current status of research in NIS and NIHHL, with focus on the translational difficulty from animal data to human clinicals, the technical difficulties in quantifying NIS in humans, and the problems with the SR theory on signal coding. Temporal fluctuation profile model was discussed as a potential alternative for signal coding at high sound level against background noise, in association with the mechanisms of efferent control on the cochlea gain.

Keywords: noise induced synaptopathy (NIS), ribbon synapses, temporal processing, coding-in-noise deficit, cochlear efferent, fluctuation profile, auditory nerve

INTRODUCTION

Noise induced hearing loss (NIHL) is typically defined and quantified by the permanent threshold shift (PTS) caused by noise exposure (Berger et al., 1978). In recent years, however, this concept has been expanded by the finding in animal studies that noise can cause a significant amount of damage to the ribbon synapses between inner hair cells (IHC) and spiral ganglion neurons (SGN) in the cochlea without PTS (Kujawa and Liberman, 2009; Moser et al., 2013; Starr and Rance, 2015; Moser and Starr, 2016; Song et al., 2016; Kaur et al., 2019; Kim et al., 2019; Liu et al., 2019). After a brief, 2-h exposure of noise at 100–106 dB SPL, these studies have reported an initial loss of up to 50% of ribbon synapses. Auditory nerve malfunctions are expected in association with such massive damage and synapse loss, but these could not be detected by routine audiology assessment focused on thresholds because of the absent PTS. Damage and loss of ribbon synapses, as well as associated functional deficits, can be collectively described as noise-induced synaptopathy (NIS) (Chen et al., 2019a). However, before the functional deficits were detailed and the nature of the deficits was uncovered, the concept of noise induced hidden hearing loss (NIHHL) was proposed to umbrella any potential problems resulting from this pathology (Plack et al., 2014; Le Prell and Clavier, 2017; Liberman, 2017; Liberman and Kujawa, 2017; Chen et al., 2019a; Huet et al., 2019; Kohrman et al., 2020). One of the primary potential problems of interest is coding-in-noise deficit (CIND), which describes an impaired ability to perceive sound in background noise. CIND has been speculated as the major problem in subjects with NIHHL or NIS without PTS due to the selective damage and loss of the ribbon synapses innervating auditory nerve fibers with low spontaneous rates (LSR ANFs) by noise exposure and the unique role of LSR ANFs in signal coding against high level background noise.

Noise induced hidden hearing loss has been of special interest in the field of audiology since the first report on the noise induced synaptic loss without PTS in CBA mice (Kujawa and Liberman, 2009), and it continues to gain attraction as noise-induced synaptic damage may also occur in humans.

Abbreviations: ABR, auditory brainstem response; AMD, amplitude modulation detection; ANFs, auditory nerve fibers; ANCOVAs, analyses of covariance; CAP, compound action potential; CF, characteristics frequencies; CIND, coding-in-noise deficit; CN, cochlear nucleus; AVCN, anterior ventral CN; PCVN, posterior ventral CN; DCN, dorsal CN; CRM, co-ordinate response measure; DPOAE, distortion-product otoacoustic emission; DTT, digit triplet test; EAR, efferent acoustic reflex; ECochG, electrocochleography; EFR, envelope following response; HC, hair cells; IHC, inner HC; OHC, outer HC; HIN, hearing-in-noise; IPD, interaural phase difference; ITD, interaural time differences; MEMR, middle ear muscle reflex; NESI, Noise Exposure Structured Interview; NIS, noise induced synaptopathy; NIHHL, noise induced hidden hearing loss; OC, olivocochlear; MOC, medial OC; LOC, lateral OC; PSTH, peristimulus time histograms; PTA, pure tone average; PTS, permanent threshold shift; SR, spontaneous rate; L/M/HSR, low/medial/high SR; SGN, spiral ganglion neuron; SPiN, speech perception in noise; (T)MTF, (temporal) modulation transfer function; TTS, temporary threshold shift.

Clarification and Differentiation Between Concepts of Noise Induced Synaptopathy and Noise Induced Hidden Hearing Loss

Some concepts have been used in this field widely, but their definitions may not always be clear and are sometimes misused. For example, the terms NIS and NIHHL are sometimes used interchangeably. It is beneficial to make a clear differentiation between the two. In this review, NIS covers not only the noise induced loss of but also damage to cochlear ribbon synapses, as well as the associated consequences to cochlear function. Moreover, NIS can occur with or without NIHL, which is typically defined by PTS. However, NIS usually refers to cases without PTS in this review, unless otherwise stated. In any case, NIS mainly refers to cochlear pathology. In contrast, NIHHL refers to any hearing problems caused by noise other than hearing loss defined as PTS. It is notable that while NIHHL caused directly by NIS is likely to result from cochlear dysfunction, it could also reflect changes in central mechanisms.

To date, although there have been a significant number of studies on the topics of NIS and NIHHL, many knowledge gaps remain. The pathology associated with noise-induced synaptic damage and loss is largely understood based on studies using laboratory animals. Since morphological evaluation of cochlear synapses is almost impossible in humans due to ethical limitations, animal data have been used to interpret or predict synaptopathy in humans—a practice that is necessary but not ideal. In doing so, large differences in noise exposures used with animals in laboratory settings and those experienced by human beings have been generally ignored (see details in section "Noise Induced Synaptopathy Studies in Animal Models and Difficulty in Translation" below).

Perceptual difficulty in background noise, which can be referred to as a coding-in-noise deficit (CIND, the term that will be used in this review), has been thought to be the major problem in NIHHL. The theoretical base underlying this idea is the functional categorization of auditory nerve fibers (ANFs) related to spontaneous rate (SR) and the bias of noise damage to the synapses innervating ANFs with low SR (LSR). However, a selective loss of LSR ANFs has only been reported in two animal studies (Furman et al., 2013; Song et al., 2016) and cannot be confirmed in humans due to technical difficulties in recording single unit ANF function. So far, there are no reliable objective measurements that can precisely verify and quantify NIS in humans (see Section "Measurements Based on Middle Ear Muscle Reflex in NIS Detection" below). In behavioral studies attempting to verify CIND in humans with a history of noise exposure but no PTS, contradictory results have been reported (see Section "Is Coding-in-Noise Deficit Really the Major Problem of Noise Induced Synaptopathy and Noise Induced Hidden Hearing Loss?" below). This may be related to technical errors in some cases, but it suggests a larger problem with the idea that a selective loss of LSR ANFs is the primary pathophysiological mechanism underlying NIS and NIHHL.

In this review, previous studies will be summarized to verify the gaps in knowledge associated with the translation from animal models to humans, to clarify relevant concepts and to address existing confusions. The review will re-examine the theory of SR-based functional categories, and the role of ANFs in different SR groups in the coding of high-level sounds against background noise. It will also address challenges with the traditional view and will discuss a new model and its difficulties. Limitations and controversies in studies of NIS and NIHHL will be discussed in detail to facilitate the planning of future research.

NOISE INDUCED SYNAPTOPATHY STUDIES IN ANIMAL MODELS AND DIFFICULTY IN TRANSLATION

Across species, ribbon synapses between IHCs and SGNs show similarity in their functions and structures (Nouvian et al., 2006; Moser and Starr, 2016; Wagner and Shin, 2019). Moreover, this synapse appears to be universally sensitive to noise damage in the animal models investigated so far. The sensitivity of the ribbon synapses between IHCs and SGNs to noise damage was first investigated by Pujol et al. (1990, 1993, 1996), Puel et al. (1994, 1998), and Pujol and Puel (1999). Due to methodological limitations at the time, synaptic damage from noise or glutamate agonists were considered temporary and therefore did not elicit much attention in the field of hearing research. However, noise damage to this synapse became a hot topic about a decade later following a report in CBA mice showing a significant synaptic loss after a brief noise exposure that did not cause PTS (Kujawa and Liberman, 2009). Unlike the earlier publications, the new research used immunohistology staining against the pre- and post-synaptic structures, which allowed for the counting of synaptic puncta over the whole IHCs so as to quantify the number of synapses (see review Chen et al., 2019a). In the study using CBA mice (Kujawa and Liberman, 2009), the initial loss of the ribbon synapses was more than 60% in the frequency region above 8 kHz after a 2-h expose to a band of noise at 100 dB SPL. The synaptic loss in this mouse strain was largely irreversible with a recovery of less than 10%, leading to a 50% permanent loss in synapses. Interestingly, it was a dominant opinion for many years that noise-induced synaptic loss was irreversible. Connected to this idea, NIS was conceptually narrowed as noise-induced synaptic loss. Lately, however, evidence has accumulated in favor of the idea that noise-induced synaptic loss is largely or partially reversible. A recovery of synaptic counts has been found in guinea pigs (Liu et al., 2012; Shi et al., 2013; Song et al., 2021), rats (Ruttiger et al., 2013; Singer et al., 2013; Bing et al., 2015), and other strains of mice (Shi et al., 2015; Kaur et al., 2019; Kim et al., 2019). Moreover, functional deficits in ANF units have been found to develop with recovery of the synaptic count (Song et al., 2016). This finding is consistent with the idea of synaptic repair and suggests that the repaired synapses are not healthy. In addition, intrinsic mechanisms involving neurotrophins (Sly et al., 2016; Suzuki et al., 2016) and cochlear efferent regulation (Maison et al., 2013; Boero et al., 2018; Ohata et al., 2021) involved in the maintenance and repair of ribbon synapses have been identified (see review Chen et al., 2019a). It is now more accepted that part of the interrupted ribbon synapses can be repaired or re-established, at least partially. It is also possible that damage and repair may occur across surviving synapses. ***Since the repaired/re-established synapses may not be normal but rather have some functional deficits, the concept of NIS should cover not only the loss of synapses, but also the pathology of survived and repaired synapses.***

One of the challenges in human studies of NIS and NIHHL is the difficulty obtaining morphological evidence for cochlear ribbon synapses. Ideally, animal data on cochlear pathology can be used to predict the effects of noise on human cochleae. However, this approach is hampered by a noticeable limitation of the studies using laboratory animals: the type of noise exposure. In order to create a significant amount of damage/loss of synapses, the noise has usually been presented at the highest level possible that does not cause PTS (around 100 dB SPL in mice, and 105 dB SPL in guinea pigs and rats). Noise exposure at such a level can cause a significant amount of synaptic loss within a short period (e.g., 2 h). Moreover, stationary, continuous noise exposure has usually been used. While the animal data suggests the possibility of NIS in humans, direct translation is not valid because the noise used in the animal studies is unlike what humans experience outside of laboratory settings. The noise frequently experienced by humans that has raised the most concern comes from traffic (Munzel and Sorensen, 2017; Nieuwenhuijsen et al., 2017; Zare Sakhvidi et al., 2018; Munzel et al., 2020), recreational events (Ivory et al., 2014; Fulbright et al., 2017), working in industrial settings (Stucken and Hong, 2014; Lie et al., 2016), and military activity (Pfannenstiel, 2014; Nakashima and Farinaccio, 2015). For the purpose of this review, noise related to military activity will not be considered because of its limited relevance to the general population. Several general features differentiate the noise experienced by humans from that used in previous NIS studies with animals. First, the noise produced by traffic, industrial settings, and recreational events is generally of a much lower sound level than what has been used to cause NIS in animal studies, especially when the use of hearing preservation methods/devices is taken into consideration under current safety standards. Currently, safety regulations ensure that the noise levels rarely exceed 90 dB SPL. Furthermore, the long-term equivalent (Leq) sound level of noise generated by traffic is generally lower than 80 dBA, indicating that even though noise levels of traffic may frequently peak at very high levels, those instances will only last for very short periods of time (Jagniatinskisa et al., 2017; Oiamo et al., 2017). Secondly, the noise experienced by humans in real life is temporally fluctuated in level (Barlow and Castilla-Sanchez, 2012; Masullo et al., 2016), not stationary as what is used in the laboratory studies. Thirdly, the noise experienced by humans is generally intermittent or repeated interruptedly, with damaging doses accumulating across long periods of time.

The resting time between the segments of noise exposure obviously allows for the recovery or repair of potential damage and likely changes the consequence of consecutive noise exposure on the synapse. Therefore, the pathology caused by such noise may be different from what is caused by a brief exposure at high

level. Related to the level difference in noise exposure, is the need to validate the "equal energy" hypothesis, which is generally accepted for NIHL (Ward et al., 1981; Gomez Estancona et al., 1983; Lindgren and Axelsson, 1983; Roberto et al., 1985; Fredelius et al., 1987; Borg and Engstrom, 1989). However, this hypothesis may not hold in the development of NIS. In one study, noise of 84 dB SPL was presented continuously to CBA mice for 168 h, resulting a much higher total dose than the brief noise of 2 h at 100 dB SPL (Maison et al., 2013). This noise exposure did not cause a significant loss of ribbon synapses compared to the large amount of synaptic loss in the same strain of mice after the brief noise exposure (Kujawa and Liberman, 2009). All these discrepancies make it invalid to predict NIS in humans using the animal data that is currently available.

HOW CAN NOISE INDUCED SYNAPTOPATHY BE QUANTIFIED IN HUMANS WITH POTENTIAL NOISE INDUCED HIDDEN HEARING LOSS?

Great efforts have been expended to quantify potential NIS in human subjects. However, efforts in this regard are largely hindered by the fact that it is almost impossible to observe the synaptic status of cochleae directly in humans due to ethical restrictions. Limited post-mortem cochlear analysis has shown synaptic damage in subjects with noise-exposure history but normal hearing thresholds and OHCs (Zeng and Shannon, 1995; Viana et al., 2015). However, the synaptic loss in such samples cannot be fully attributed to noise due to the involvement of aging.

Can the loss of ANFs by synaptic damage be verified functionally? Theoretically, there are many measurements that can quantify the loss of ANF function. However, to do such measurements in a clinically applicable, non-invasive manner appears to be very challenging. Presently, several objective methods have been proposed for detecting NIS in human subjects. Many of them aim to measure the change of transient cochlear responses, while other studies aim to evaluate ANF responses phase-locked to amplitude modulation.

Measurements Based Upon Transient Responses

Auditory nerve fibers will not function at all when synapses with IHCs are lost and ANFs connected by damaged synapses will have a reduced firing rate in response to sound (Song et al., 2016). Therefore, NIS in NIHHL will reduce cochlear neural output to the auditory brain. For example, a reduction of wave I amplitude of the auditory brainstem response (ABR) has been seen in animal studies of NIS (e.g., Kujawa and Liberman, 2009). In humans, such a reduction has been reported in subjects with tinnitus (Schaette and McAlpine, 2011). However, several issues suggest caution in interpreting this result as a validation of ABR for NIS quantification. Firstly, it is not clear if and how the tinnitus in this study was related to noise exposure and therefore NIS, although noise exposure is one of the major causes of tinnitus. Secondly, wave I may not be ideal for estimating NIS clinically due to its small amplitude ($<0.2\ \mu V$) and large amplitude variation. These features suggest that the ABR wave I may not be a reliable measurement for identifying NIS.

Several alternative ABR measures have been proposed for NIS detection. Instead of measuring wave I directly, one study reported using the amplitude ratio between the waves V and I (Mehraei et al., 2016). The idea underlying this measurement is that, while wave I is reduced by NIS, wave V is likely not reduced or even increased as the result of increased central gain in subjects with hidden hearing loss (HHL) (Plack et al., 2014). Other alternatives are to measure shifts in wave V latency with masking (Mehraei et al., 2016; Gottschalk and Domschke, 2017) and to measure changes in the ratio between the summating potential (SP) and the compound action potential (CAP) in electrocochleography (ECochG) (Phillipson, 2017; Kara et al., 2020).

Auditory brainstem response- and CAP-based amplitude measurements tend to have poor reliability in humans due to poor signal-to-noise ratios in far field recordings. Currently, clinical ECochG measurement is usually conducted with electrode placed in the external ear canal. While the CAP amplitude obtained in such ECochG is larger than the ABR wave I recorded from scalp electrodes, it is still not adequate for a reliable quantification of NIS in NIHHL. Larger ECochG can be obtained by using an electrode on the tympanic membrane or needle electrodes placed on the cochlear promontory. However, such electrodes are less likely to be accepted by subjects.

It is worth noting that all of these measures focus on transient responses of ANFs to acoustic onsets. This conflicts with the idea that noise exposure primarily damages synapses to LSR ANFs because LSR ANFs do not contribute to the on-responses of ANFs (Bourien et al., 2014). If noise-induced synaptic loss is really limited or biased to LSR ANFs, transient responses should be relatively insensitive.

Even with these limitations, positive results have been reported using ABR to identify reduced cochlear output. For example, reduced wave I amplitude and increased V/I ratio has been found in subjects with a high risk of NIS (Suresh and Krishnan, 2020), replicating similar results in subjects with tinnitus (Schaette and McAlpine, 2011). Additionally, lower ABR wave I amplitudes have been reported in veterans with significant history of noise exposure (Bramhall et al., 2021). In another study, however, the CAP amplitude was not found to be correlated with hearing in noise function (Parker, 2020), although this study did not address NIS explicitly.

Measurements of Phase Locking for Noise Induced Synaptopathy Evaluation

The second approach to evaluating changes in cochlear function with NIS is to measure phase-locked responses to amplitude modulation, also named envelope following responses (EFR) (Bharadwaj et al., 2015; Shaheen et al., 2015; Galvez-Contreras et al., 2017; Kalia et al., 2017; Kobel et al., 2017; Le Prell and Clavier, 2017). This approach is likely to be superior to measurements based upon transient responses for several

reasons: (1) EFR reflects ongoing responses and is not related to the onset of stimulation. (2) Depending on the carrier frequency, different regions of the cochlea can be targeted to ensure testing of ANF function from regions of interest. (3) Unlike transient responses that are generated from all categories of ANFs when tested at high sound levels, EFR can selectively target LSR ANFs by using high-level carriers to saturate HSR ANFs. In this approach, shallow modulation depths are favored (Bharadwaj et al., 2014, 2015; Chen et al., 2019b; Fan et al., 2020), because when AM is presented at high levels, temporal amplitude fluctuations with shallow modulation depth are in the range where HSR ANFs are saturated. (4) The EFR test can be easily combined with masking methods to identify CIND.

Since NIS is thought to disproportionately occur in synapses with LSR ANFs, AM responses tested at high sound levels should be significantly attenuated. LSR fibers are also thought to be more important for signal encoding in high-level background noise (Joris and Yin, 1992; Moser and Starr, 2016; Plack et al., 2016; Kobel et al., 2017; Liberman and Kujawa, 2017), because they are robust with respect to masking (Costalupes, 1985; Young and Barta, 1986). Therefore, AM responses should be better suited to detect coding deficits in noise than transient responses such as ABR and CAP, which are dominated by onset responses from high-SR fibers (Bourien et al., 2014). This inference is supported by a study that found a more robust decrease in EFR phase-locking than in ABR wave I amplitude in CBA mice with cochlear synaptopathy [established by an octave-band noise (8–16 kHz) exposure at 98–99 dB SPL for 2 h; Shaheen et al., 2015].

In this study, Shaheen reported changes in the temporal modulation transfer function (TMTF) in mice with NIS (Shaheen et al., 2015). An AM signal with a high carrier frequency was used to target the cochlear region most likely to have NIS. The EFR was recorded in the far field using scalp electrodes. TMTFs from the control mice showed a bandpass pattern with the best modulation frequency located close to 1 kHz. The ANF origin of this peak was identified by the loss of this peak in the mice exposed to a noise that caused a significant amount of synaptic loss in the high frequency region. Similar changes in TMTF were reported in mice treated with cochlear application of ouabain (Parthasarathy and Kujawa, 2018). In this report, ouabain was applied with a dose that selectively killed LSR ANFs. In one of our previous studies in guinea pigs, we measured EFR in both the near field (recording from a round window electrode) and far field (from a scalp electrode) (Chen et al., 2019b). When a high frequency carrier (16 kHz) was used, a significant reduction in near-field EFR amplitude was seen across a wide range of modulation frequencies (from ∼100 to ∼1000 Hz), suggesting that the damage to ribbon synapses reduced phase locked responses of ANFs in a way that was not selective to modulation frequency. However, such a reduction was not seen in the far-field EFR recorded from the scalp. This result indicates that the sensitivity of the far-field EFR is low.

In human studies, positive reports are available showing a reduction of EFR amplitude in subjects with potential NIHHL (Bramhall et al., 2021). A recent study also proposed recording EFR with multi-band complex tones to measure the impact of NIS on cochlear responses (Wang et al., 2019). This approach likely reduces EFR testing time and allows for the evaluation of NIS across a larger frequency range more efficiently. However, the application of EFR for the purpose of identifying NIS needs to be optimized. A promising new approach involves the measurement of EFR to stimuli with rectangular envelopes, since these should be more sensitive to neural damage and less sensitive to changes in the cochlear amplifier. Several studies suggest that these may be more sensitive to NIS (Verhulst et al., 2018; Vasilkov et al., 2021) and more predictive of CIND (Mepani et al., 2021).

Measurements Based on Middle Ear Muscle Reflex in Noise Induced Synaptopathy Detection

The middle ear muscle reflex (MEMR) plays a role in protecting the cochlea from damage by loud sounds. This reflex is defined by an increased stiffness of the middle ear ossicular chain due to the contraction of middle ear muscles. In humans, the stapedius muscle is the major player in the MEMR. When it is evoked, excitation of IHCs and SGNs is reduced (Simmons, 1960; Borg, 1968). Since the activation of this reflex depends on the strength of the input from auditory nerves, the loss of ANFs due to synaptopathy may reduce the MEMR. Utilizing the MEMR to detect NIS has recently been explored, and it is a compelling idea considering the measurement of this acoustic reflex can easily and non-invasively be integrated into clinical audiology assessments (Valero et al., 2018).

As outlined by Bharadwaj et al. (2019), the hypothesis that the MEMR can be used as an objective measure of NIS detection stems from the likelihood that LSR ANFs play an important role in the MEMR circuit (Liberman and Kiang, 1984; Rouiller et al., 1986; Kobler et al., 1992; Bharadwaj et al., 2019). Since noise is thought to selectively damage synapses with LSR ANFs (Furman et al., 2013; Song et al., 2016), the MEMR should be weakened in subjects with NIS.

The connection between the loss of ANFs and MEMR function has been examined in animal studies. In one study, reflex growth function was measured in mice with varying degrees of NIS (Valero et al., 2018). To avoid attenuation by anesthesia, the mice were tranquilized briefly with isoflurane to allow for the fixation of plastic couplers in their ear canals with cyanoacrylate (Valero et al., 2016). Their surgically affixed head-plates were then secured atop a freely spinning platform on which they could walk at will; the MEMR was tested 15 min after the isoflurane was removed (Valero et al., 2018). The results indicated that both MEMR threshold elevation and magnitude reduction were scaled linearly with percentage of synapse loss, which ranged from 4 to 50% in the 22–45 kHz region. When the reflex elicitor was filtered to stimulate the region with the most synaptopathy, there was a stronger correlation between MEMR change and synaptic loss. Conversely, the correlation was the weakest in the non-synaptopathic region. Since the MEMR was not eliminated but obtained at higher thresholds even in subjects with 50% loss of ANFs, it is possible that ANFs in all three SR categories drive the MEMR (Valero et al., 2018) and NIS induces MEMR deficits due to the reduced number of ANFs. Therefore, MEMR is likely to be a useful metric of NIS.

Positive results have been found in human studies as well (Wojtczak et al., 2017; Mepani et al., 2020; Shehorn et al., 2020). For example, Shehorn et al. (2020) examined relationships between level-dependent speech intelligibility (rollover) and the wideband MEMR in adult participants aged 21–54 with normal hearing thresholds. The subjects were grouped based upon whether they had sought hearing help. Lifetime noise exposure was determined by self-report *via* the Noise Exposure Structured Interview (NESI), which resulted in marginally higher scores in the help-seeking group. The study found that the MEMR magnitude of help-seeking individuals was weaker. To determine the role of various factors (as covariates), including participant group, gender, age, pure tone average (PTA), tinnitus, ABR wave I amplitude, NESI, the side of the MEMR elicitor and elicitor level on MEMR magnitude (the dependent variable), analyses of covariance (ANCOVAs) using general mixed-effects models were performed. The results showed that the significant predictors were, in order of inclusion, elicitor level, elicitor side, and NESI. There was a significant interaction between NESI and elicitor level: for low-elicitor levels particularly, MEMR magnitude decreased with increasing lifetime noise exposure (Shehorn et al., 2020). This study thus agrees with the abovementioned animal research (Valero et al., 2016, 2018).

On the other hand, other studies have found negative results for the use of the MEMR in NIS. For example, one study showed no evidence in human participants for changes in MEMR threshold or growth related to NESI score when using a contralateral BBN elicitor (Causon et al., 2020). Another study examined the relationship between MEMR thresholds and tinnitus, difficulties with speech perception in noise (SPiN) and noise exposure (Guest et al., 2019a). The results of this work also revealed no relation between MEMR and noise exposure. However, the authors of this study refer to a prior study by Wojtczak et al. (2017), which revealed a large reduction in MEMR amplitude (by a factor of roughly four) in participants with tinnitus compared to a control group. In that study, all participants with tinnitus reported excessive and repeated noise exposure. This highlights the possible impact of methodological differences on the likelihood of detecting a relationship.

Validation and Comparison Across the Objective Measurements

There are noticeable discrepancies in many of the studies of objective measurements of cochlear synaptopathy. Several studies have been conducted with the intention of examining these discrepancies and offering a comparison of the objective measurements detailed above (Guest et al., 2019a; Kaur et al., 2019; Prendergast et al., 2019).

The work by Guest et al. (2019b) assessed the reliability of seven specific measures that fall within the three types of measurement discussed in sections "Measurements Based Upon Transient Responses," "Measurements of Phase Locking for Noise Induced Synaptopathy Evaluation," and "Measurements Based on Middle Ear Muscle Reflex in Noise Induced Synaptopathy Detection." The measures examined in the study were ABR wave I amplitude, ABR wave I growth, ABR wave V latency shift in noise masking, EFR amplitude, EFR growth with stimulus modulation depth, MEMR threshold and an MEMR across-frequency difference measure. The participants of the study consisted of 30 women aged 18–30 and were of a single sex due to known sex differences in electrophysiological response amplitudes. Each participant attended two test sessions, during which all seven measures were assessed. Pure-tone audiometry and distortion-product otoacoustic emissions (DPOAE) were also assessed during the test sessions, to ensure normal cochlear mechanical function.

In addition to examining the reliability of each measure individually, the study also made 18 comparisons across the proxy measures of synaptopathy. The results of the study indicate that measures of EFR amplitude and MEMR threshold are highly reliable measures in humans. The results also indicate that ABR wave I amplitude can be a highly reliable measure if proper care is taken regarding consistency in electrode placement, participant state, and other factors influenced by the researcher or clinician. It should be noted that clicks were used to elicit the ABR in this study, as well as research-grade recording equipment. If adopting ABR amplitude measures, the authors advised that the investigator assess the reliability of their own ABR measurements due to the lower ABR reliability found in their own work (Guest et al., 2019a). Similar results were found in a study that examined the test-retest reliability of raw measures, which found good reliability in MEMR threshold and moderate reliability in ABR wave I amplitude (Kamerer et al., 2019). However, despite the strong reliability of these raw amplitude and threshold measures, no correlations were observed between any of the proxy measures of cochlear synaptopathy. This broadly suggests that the participants did not possess synaptopathy or that the proxy measures were not sensitive to synaptopathy (Guest et al., 2019b).

In a separate study, proxy measures including ABR and EFR were evaluated by examining the effects of age and noise exposure (Prendergast et al., 2019). This study consisted of 156 participants, all with hearing thresholds within normal limits. Lifetime noise exposure was quantified using a structured interview aimed at determining the amount of time spent in environments with noise exceeding 85 dBA. In addition to ABR and EFR, psychophysical tasks such as interaural phase difference (IPD) and amplitude modulation detection (AMD) thresholds were examined, as well as the co-ordinate response measure (CRM) and digit triplet test (DTT) speech tasks. In short, the results of this study showed no evidence of age- or noise-induced cochlear synaptopathy *via* the proxy measures that were examined. Focusing on EFR and ABR for the purpose of this review, this work found no evidence for a relationship between age or noise exposure and EFR or ABR amplitudes. Therefore, by using these proxy measures, the results suggest that there is minimal effect of recreational noise exposure on auditory function for individuals with normal audiograms, which is inconsistent with the predicted effects of synaptopathy (Prendergast et al., 2019).

At this moment, it is too early to make a clear conclusion regarding which (if any) of the objective measures can be used to reliably verify NIS in humans.

IS CODING-IN-NOISE DEFICIT REALLY THE MAJOR PROBLEM OF NOISE INDUCED SYNAPTOPATHY AND NOISE INDUCED HIDDEN HEARING LOSS?

Coding-in-noise deficit refers to a coding deficit in background noise, specifically when examined with signals presented at relatively high sound levels or speech presented at normal levels with high-level background noise. This deficit has been hypothesized to be the major hearing problem associated with NIS and NIHHL (Furman et al., 2013; Bharadwaj et al., 2014; Plack et al., 2014; Kobel et al., 2017; Le Prell and Clavier, 2017; Liberman, 2017; Liberman and Kujawa, 2017; Hesse and Kastellis, 2019; Huet et al., 2019; De Siati et al., 2020; Hertzano et al., 2020; Henry, 2021), based on the functional categorization of ANFs by SR and the disproportionate impact of noise damage on the synapses innervating LSR ANFs. Compared to ANFs with high SR (HSR), LSR ANFs have higher thresholds and larger dynamic ranges (Liberman, 1978, 1982, 1988; Liberman and Beil, 1979; Taberner and Liberman, 2005), and are therefore more important for signal coding at high sound levels (Schalk and Sachs, 1980; Winter et al., 1990). Moreover, LSR ANFs appear to function better for coding signals masked by high-level background noise (Costalupes et al., 1984). Unfortunately, ribbon synapses innervating this group of ANFs are more sensitive to noise damage (Fucci et al., 1997a,b; Furman et al., 2013; Song et al., 2016).

While the hypothesis sounds reasonable, the supporting evidence is weak. At present, there are no solid data from animal studies showing CIND in subjects with NIS. Our group examined the coding of amplitude modulation in background noise using the envelope-following response recorded from the round window and did not find any differences between the control and the noise-exposed group with significant synaptic loss (Chen et al., 2019b; Fan et al., 2020). A positive result has only been reported in a single study that used a paradigm of pre-inhibition of a startle response to airpuffs. A noise burst presented in a background noise was used as the pre-inhibitor (Lobarinas et al., 2017). The report showed reduced pre-inhibition in the noise-exposed group, suggesting a deficit in hearing the noise burst in the background noise. However, noise-induced damage to ribbon synapses was not documented in the study. It is notable that the coding-in-noise deficit was seen only in the rats that were exposed to the noise at 109 dB SPL, but not at 106 dB SPL, which should have been adequate to produce significant NIS. Two limitations make the data interpretation difficult: (1) the pre-pulse inhibition of the startle responses involves the central auditory system, which may compensate for changes in cochlear function related to synaptopathy; and (2) the signal-to-noise ratio (SNR) between the pre-pulse inhibitor and the masker must be at least 20 dB in order to show clear inhibition in this paradigm. This is much higher than the SNR used in most signal-in-noise tasks, such as speech-in-noise measures conducted at ratios between −10 and +10 dB (Billings et al., 2017; Maamor and Billings, 2017; Best et al., 2018; Billings and Madsen, 2018; Yeend et al., 2018).

In human subjects with histories of noise exposure but normal hearing thresholds, there is a lack of consensus concerning the existence of CIND or hearing-in-noise (HIN) problems as well as a lack of morphological evidence and functional data indicating loss or damage of ribbon synapses. There exist many negative publications (Fulbright et al., 2017; Grinn et al., 2017; Grose et al., 2017; Le Prell and Clavier, 2017; Prendergast et al., 2017a, 2019; Yeend et al., 2017; Guest et al., 2018, 2019a; Valderrama et al., 2018), while positive reports are also available (Alvord, 1983; Kujala et al., 2004; Stone et al., 2008; Kumar et al., 2012; Stamper and Johnson, 2015; Liberman et al., 2016; Tepe et al., 2017; Meehan et al., 2019). For example, the study by Grinn et al. (2017) looked at the effects of long-term self-reported noise exposure as well as a loud recreational event on several audiologic measurements including ECochG. One of the main findings of the study was that there was no evidence of noise-induced decreases in human CAP amplitude in either the retrospective or prospective analyses. Contrarily, the study by Liberman et al. (2016), which assessed college students categorized into low-risk and high-risk groups based on self-report of noise exposure, found an increased SP/AP ratio in the high-risk group. As shown by the examples above, comparisons across studies are difficult due to differences in methodology and subject characteristics. Inconsistent and unreliable methods for quantifying noise exposure (e.g., self-report measures), coupled with a lack of morphological information renders it impossible to confirm the existence of NIS. Moreover, the methods used to identify CIND vary across different studies, some of which need to be validated (see section "The Role of Temporal Processing Deficits in Coding-in-Noise Deficit" for details). Therefore, it remains a mystery as to whether CIND is the major functional deficit in NIS and NIHHL.

QUESTIONING THE ROLE OF LOW SPONTANEOUS-RATE AUDITORY NERVE FIBERS IN CODING-IN-NOISE DEFICIT

Equivocal results and a lack of consensus from studies investigating CIND in both animals and human studies with (potential) NIS makes it necessary to re-examine the hypothesis that CIND is the major deficit associated with NIS and NIHHL, as well as the theoretical basis of this idea (i.e., that noise damage to ribbon synapses innervating LSR ANFs is the major pathology of NIS without PTS and that LSR ANFs are critical for coding signals at high levels and in background noise).

It is important to note that synaptic damage by noise is biased, but not limited, to synapses innervating low-SR ANFs. Since this group of ANFs constitutes only a small proportion of the total population of ANFs (Liberman, 1978), medium-SR (MSR) and even HSR ANFs are not spared when 50% of synapses are lost following a damaging noise exposure. Secondly, interrupted synapses can be repaired, including those innervating LSR ANFs. In Guinea pigs, a significant reduction of LSR ANFs was observed shortly after a noise exposure that initially destroyed ∼50% of

synapses in the high frequency region. However, the percentage distribution of ANFs across SR groups recovered 1 month later in spite of an ∼18% loss in the total number of remaining synapses (Song et al., 2016). This suggests that synapses with low-SR ANFs are also partially re-established.

The idea that the coding of high-level signals relies purely on L/MSR ANFs (because HSR ANFs firing rates are saturated at high levels) has also recently been challenged (Carney, 2018). This idea is based on the assumption that ANFs code sound level *via* average firing rate. This assumption was challenged by many aspects of Carney's review. For example, for such a coding scheme to work, the increase in the average firing rate with sound level must be larger than the variability change in firing rate with sound level. Since variability also increases with sound level, this would require rate-level functions that accelerate with level to compensate for the variability increase; such rate-level functions are not seen for any type of ANF (Siebert, 1965; Viemeister, 1988; Winter and Palmer, 1991; Delgutte, 1996; Heinz et al., 2001; Colburn et al., 2003; see Carney, 2018 for more details).

Models that combine ANFs of different thresholds and dynamic ranges, and over a range of characteristics frequencies (CFs) have been proposed to explain psychophysical level discrimination, which is roughly constant for wideband sound (Delgutte, 1987; Viemeister and Bacon, 1988; Winter and Palmer, 1991; see review Delgutte, 1996). These models are forced to make problematic assumptions. For example, the wide dynamic range of LSR ANFs does not exist for CFs below 1500 Hz (Winter and Palmer, 1991). The limited dynamic range of low-CF LSR ANFs is consistent with the importance of cochlear compression for creating the wide dynamic range of ANFs at higher CFs (Yates et al., 1992) and with physiological evidence based on ANF responses, suggesting that cochlear gain is relatively low for low CFs (Sewell, 1984; Cooper and Yates, 1994).

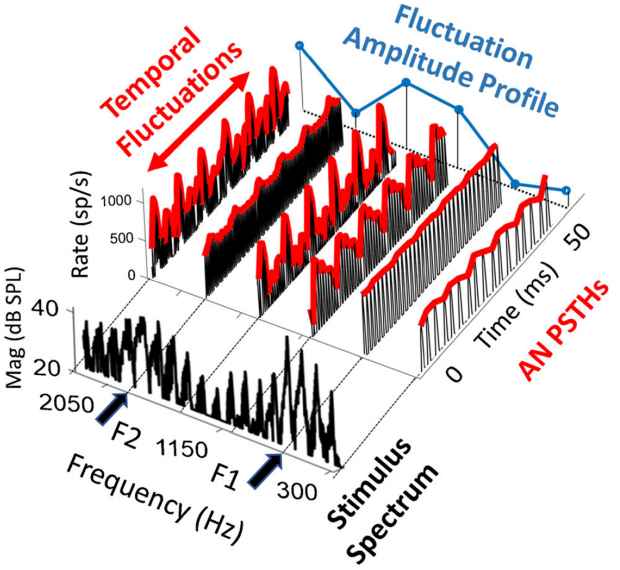

FIGURE 1 | Fluctuation profile of ANF response to vowel /ae/ spectrum, which is in the foreground. The model peristimulus time histograms (PSTHs) of HSR ANFs are presented at formant peaks (F1 = 700 and F2 = 1800 Hz) and troughs. Temporal fluctuation is large at trough frequencies and small nor none at the formants, forming the dips in the fluctuation amplitude that mirrors the formants. Adapted from Carney (2018).

FLUCTUATION PROFILE MODEL FOR COCHLEAR CODING OF HIGH-LEVEL SOUND

After challenging the idea that the coding of high-level sound relies on LSR ANFs, Carney proposed a model for the coding of high-level spectra *via* HSR ANFs, called the temporal fluctuation profile model (Carney, 2018). Temporal fluctuations exist in complex signals such as speech (**Figure 1**). For example, in voiced speech sounds, all harmonics are integer multiples of the fundamental frequency (f_0) such that neighboring harmonics are separated by f_0. The amplitude envelope arising from the combination of harmonics is thus modulated at this frequency, giving rise to ANF firing patterns that fluctuate at f_0. For average speech levels (65–70 dB SPL), temporal fluctuation of HSR ANF responses is minimal near spectral peaks (e.g., formants) because they are saturated. However, HSR ANFs in spectral troughs are not saturated and show strong temporal fluctuation by the responses phase-locked to f_0 (e.g., in the single unit study in cats; Schilling et al., 1998). The contrast in fluctuation strength between ANFs tuned to peaks versus trough frequencies gives rise to a temporal fluctuation-profile, which mirrors the spectrum of the voiced speech sound. To make this model work, the signal presentation level must be able to generate differences in temporal fluctuation between spectral peaks and troughs. At very high levels, it is possible that HSR ANF responses will be saturated (and thus non-fluctuating) at both spectral peaks and troughs, such that the model would not work. Therefore, the usefulness of the model appears to be limited to a narrow range of levels (where peaks but not troughs give rise to saturated responses in HSR ANFs).

This model can be used to interpret potential problems in coding speech and other high-level sounds in subjects with NIS. While this model reasonably illustrates the potential contribution of HSR ANFs to the coding of these high-level sounds, the contribution of L/MSR ANFs that are NOT saturated is ignored. When L/MSR ANFs are included, the fluctuation contrast across frequency should be reduced in comparison with a model including only HSR ANFs, because L/MSR ANFs are not saturated and may show little difference in temporal fluctuation between spectral peaks and troughs, at least in healthy cochleae. Interestingly, if NIS is associated with a selective loss or damage to synapses serving L/MSR ANFs, NIS should lead to stronger fluctuation contrast across frequency, thereby predicting better coding for speech. This conflicts with the idea that the damage and loss of L/MSR ANFs in NIS should negatively impact the coding of speech and other high-level sounds; rather, speech coding should be improved by the enhanced fluctuation profile resulting from the lost contribution of L/MSR ANFs.

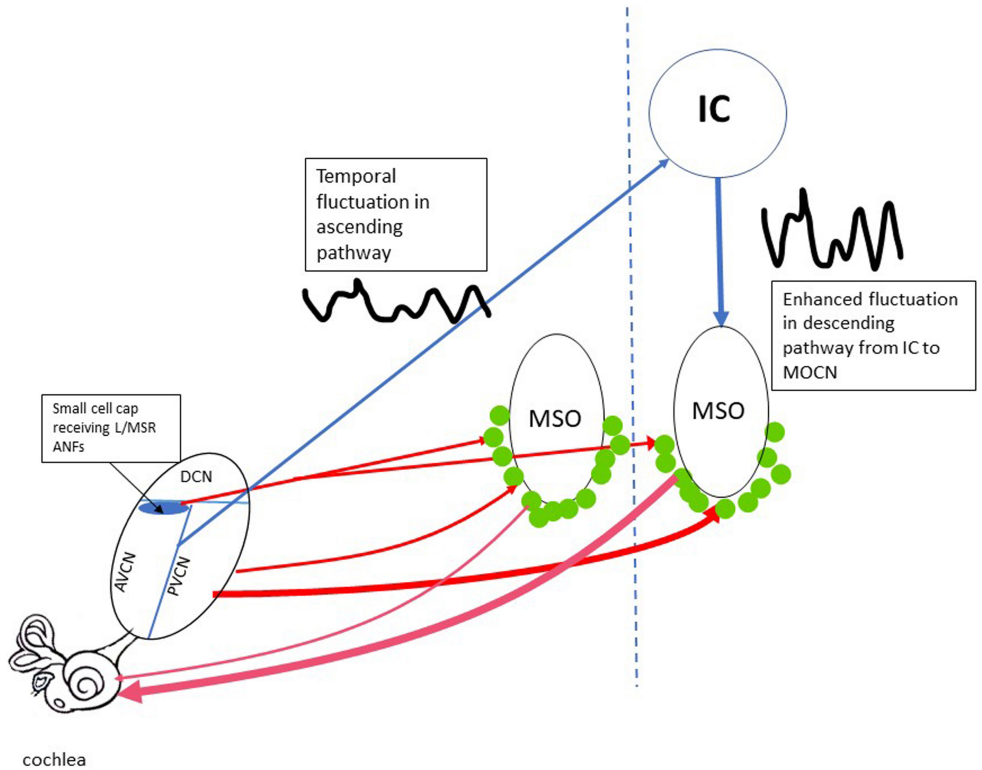

FIGURE 2 | The efferent feedback loops controlling the OHC gain. The short loops going through lower brainstem are marked by redlines. The thickness of the line represents the relative strength in the typical loop from PVCN to MOCNs (green dots). The loop from the small cell cap in AVCN to MOCN is thought to be selective receiving input from L/MSR ANFs. The relative strength of this loop is unknown. The long feedback loop (blue lines) includes the projection from both AVCN and PVCN cores to IC, which is sensitive to the low-frequency temporal fluctuation. The fluctuation is inherited and enhanced in the descending projection from IC to MOCNs. AVCN/PVCN, anterior/posterior ventral cochlear nucleus; DCN, dorsal cochlear nucleus; MOCN, medium olive cochlea neurons; MSO, medium superior olive; IC, inferior colliculus.

THE ROLE OF COCHLEAR EFFERENT IN NOISE INDUCED SYNAPTOPATHY AND FLUCTUATION PROFILE MODEL

The Potential Role of Medial Olivocochlear Control in Fluctuation Profile Model

The fluctuation profile appears to be inherited in the midbrain (inferior colliculus, IC), where neurons have response rates that vary systematically with the frequency and amplitude of low-frequency fluctuations on their inputs from cochlear nuclei (CN) (Joris et al., 2004). The low-frequency fluctuations of ANF responses are accentuated by CN neurons which, either directly or *via* other brainstem nuclei, may relay fluctuation profiles to IC neurons, in which the profile is somehow enhanced (Joris et al., 2004). Furthermore, the fluctuation profiles in IC may provide a feedback control mechanism *via* efferent control to the cochlear gain in a way that can possibly enhance the contrast of the fluctuation profile (Carney, 2018).

Olivocochlear neurons (OCNs) in the lower brainstem are a direct source of cochlear efferent control. They are divided into two groups: medial (MOC) and lateral (LOC) neurons. The function of MOC neurons is better understood; these are known to control OHC gain. Carney's model proposes two feedback loops for this gain control mechanism as summarized in **Figure 2**. In the long loop (blue lines in **Figure 2**), the fluctuation profile established in the ANFs is mapped in CN, which projects to IC and then down to MOC neurons (ANF-CN-IC-MOC-cochlea). In the short loop through the lower brain stem (ANF-CN-MOC-cochlea, red lines in **Figure 2**), there is a branch receiving projections of L/MSR ANFs in the small cell cap in the marginal shell of the anteroventral cochlear nucleus (AVCN), which then project to MOC neurons (Ye et al., 2000). A large majority of neurons in the small cell cap in cat AVCN have LSRs and very wide dynamic ranges (Ghoshal and Kim, 1996), consistent with the fact that their inputs arise from L/MSR ANFs (Leake and Snyder, 1989; Liberman, 1991; Ryugo, 2008). However, the feedback control in this branch is sensitive to firing rate and not temporal fluctuation. On the other hand, it is widely accepted that the ascending pathway from CN to MOC neurons is mainly through the posterior ventral cochlear nucleus (PVCN) (**Figure 2**; see review by Guinan, 2006), which is not specific to input from L/MSR ANFs (Thompson and Thompson, 1991; de Venecia et al., 2005; see review Guinan, 2006). In Guinea pigs, lesions in PVCN, but not the other subdivisions, produce long-term

decreases in the strength of the MOC-mediated efferent acoustic reflex (EAR). The degree of cell loss within the dorsal part of the PVCN determines the effect of the lesion on the strength of the EAR, as measured in the adaptation of distortion product otoacoustic emissions (DPOAEs) (de Venecia et al., 2005). The authors suggest that multipolar cells within the PVCN have the distribution and response characteristics appropriate to be the MOC reflex interneurons. It is an open question as to whether the PVCN-MOC branch of the lower brainstem loop relies upon input from the LSR ANFs and whether the output of this branch is regulated by efferent projections from fluctuation-sensitive neurons in the IC downstream. There is evidence suggesting that PVCN neurons responsible for the MOC EAR are likely those with chopper histograms and sharp tuning (Brown et al., 2003). However, the SRs and dynamic ranges of these neurons have not been specified.

At this moment, the relative importance between the short and the long loop is unclear. In a study by Gummer et al. (1988) a few medial efferent neurons showed a short latency (5 ms), which is consistent with a direct input from CN neurons to MOC neurons (1988). However, the group delays were longer for most neurons (8.2 ± 1 ms), indicating the involvement of a another relay, likely in the IC (Gummer et al., 1988). Alternatively, the long group delays could be accounted for by a direct CN connection plus a long delay in medial efferent dendrites.

Inferior colliculus neurons are sensitive to low-frequency fluctuations from the ascending pathway (Joris et al., 2004). They have bandpass modulation transfer functions (MTFs) with best modulation frequencies near the fundamental frequency (f_0) of male speech (Krishna and Semple, 2000; Carney et al., 2016); MOC neurons also have bandpass MTFs (Gummer et al., 1988). This indicates that they are likely excited by descending inputs from IC neurons, although it is not clear why the frequencies of human speech would have any relevance for the animals used in those studies. Nevertheless, Carney suggests that the fluctuations in the descending pathway from IC to MOC neurons can enhance the fluctuation profile in ANFs: those ANFs in channels near formant peaks produce less fluctuation, which would result in a weaker MOC excitation through the IC-MOC regulation and then less or no cochlear gain reduction, while those ANFs in channels near troughs produce stronger fluctuations that would excite MOC neurons more strongly, resulting in a greater decrease of cochlear gain. Therefore, the ANFs in the trough channels would be farther away from the level of saturation because of this regulation, while the ANFs in the peak channels would remain saturated.

To make this hypothesis work, one must assume that, when the cochlea is stimulated with temporarily fluctuated signals at a low level, a larger MOC efferent inhibition of cochlear gain would be seen, at least within a certain level range. However, this level effect is opposite to what has been observed in previous studies observing the efferent suppression of otoacoustic emissions and CAP. In such studies, the suppressor is presented contralaterally (contralateral suppression, or CS) for an easy differentiation of the afferent response from the suppressing stimulus. Available data unanimously show larger CS in both OAE (Moulin et al., 1993; Zhang et al., 2007) and CAP (Puria et al., 1996) with a higher suppressor level. However, in those studies, all CS signals are generally stationary. If fluctuation plays a dominant role, as suggested above, the level effect would be opposed by the activity of the long loop when a fluctuated suppressor (such as an AM tone or noise) is used: there would be greater CS for a low-level suppressor (at least within a certain range). However, this may not be seen because efferent control in the short loop through the small cell cap is not determined by temporal fluctuation but rather by overall firing rates of ANF inputs. The efferent control in this pathway should have a larger CS effect at a higher CS level. In one study, efferent suppression of OAEs was observed using an AM signal to evoke contralateral suppression. While the result showed that the suppression was increased with modulation depth, the suppression was observed at only one suppressor level (Maison et al., 1997). Level effects for efferent suppression of OAE and CAP with fluctuated suppressors have never been observed.

Furthermore, speculation about cochlear gain control regulating IHC/ANF saturation conflicts with the fact that the gain reduction is NOT observed for high sound levels but for low levels close to response threshold. This is seen in CAP (Wiederhold and Kiang, 1970) and single ANF responses (Guinan and Stankovic, 1996) evaluated with medial olivocochlear body (MOCB) stimulation, as well as in studies of contralateral suppression of DPOAEs (Chery-Croze et al., 1993; Kujawa et al., 1993; Zhang et al., 2007; Atcherson et al., 2008; Sun, 2008; Chambers et al., 2012; Danesh and Kaf, 2012) and CAP (Kawase and Liberman, 1993; May and McQuone, 1995; Puria et al., 1996; Popelar et al., 2001; Chabert et al., 2002; Najem et al., 2016). Therefore, such gain control is not likely to enhance fluctuation profiles in ANF responses to high-level sound.

Protective Effect of Efferent on Ribbon Synapses

Evidence is available for the protective role of medial efferent function against noise damage to the synapse. For example, exposure to a noise of 84 dB SPL for 168 h caused a 40% loss of afferent synapses in mice in which surgical de-efferentation to OHCs (not de-efferentation of LOC) was created in the olivocochlear body (OCB) pathway (Maison et al., 2013), while the synaptic loss by this noise was minimal in the control mice. The evidence for MOC protection of OHCs also comes from genetic studies. For example, a point knock-in in a subunit of nicotinic receptor alpha 9 enhanced efferent inhibition and reduced noise induced hearing loss in mice (Taranda et al., 2009; Boero et al., 2018).

While the medial efferent feedback provides EAR *via* the regulation of OHC gain, the functional role of the lateral efferents targeting afferent terminals underneath IHCs is much less understood. However, a few studies have provided positive data indicating a protective role of the lateral efferents. Noise exposure has been found to reduce the strength of OC function (Sliwinska-Kowalska and Kotylo, 2002; Peng et al., 2010). This reduction is likely related to noise-induced damage to the afferent system (such as NIS, which weakens the EAR circuit). Other results have suggested a protective effect against noise damage by LOC fibers. Evidence shows that dopaminergic LOC fibers

may exert tonic inhibition to prevent excitotoxicity (Ruel et al., 2001). Moreover, selective removal of LOC neurons has shown to increase cochlear nerve excitotoxicity (Darrow et al., 2007; Lendvai et al., 2011).

THE ROLE OF TEMPORAL PROCESSING DEFICITS IN CODING-IN-NOISE DEFICIT

Temporal Processing Disorders in Noise Induced Synaptopathy Without Permanent Threshold Shift

While CIND is questionable as the major problem resulting from NIS, temporal processing deficits have been demonstrated as being associated with NIS in both animal and human subjects. Auditory system signal processing is highly distinguishable from that of other sensory systems, such as vision, due to its high temporal resolution (Hirsh, 1959; Ronken, 1970; Leshowitz, 1971). Temporal processing disorders have been reported in subjects with presbycusis (Schneider and Hamstra, 1999; Pichora-Fuller and Souza, 2003; Gordon-Salant, 2005; Martin and Jerger, 2005; Walton, 2010; Humes et al., 2012), also known as age-related hearing loss, and in subjects with auditory neuropathy (Kumar and Jayaram, 2005; Vlastarakos et al., 2008; Narne, 2013; Lobarinas et al., 2020). Since NIS is a type of auditory neuropathy, temporal processing difficulties are likely to occur in subjects with NIS.

The pathological locus of NIS is the ribbon synapses between the IHCs and the SGNs, which happens to be the first speed-limiting site of auditory processing along the ascending auditory pathway. It is well recognized that the primary function of the presynaptic ribbons is to facilitate neurotransmission through the synapses (Moser et al., 2006; Safieddine et al., 2012; Moser and Starr, 2016). Therefore, damage to ribbons would be expected to give rise to limitations of auditory processing speed (Buran et al., 2010; Jing et al., 2013), likely resulting in temporal processing disorders.

In a single unit study, a development of temporal processing deficits was clearly associated with ribbon synapse repair after a damaging noise exposure (Song et al., 2016). In this study, a noise exposure of 105 dB SPL for 2 h was given to albino Guinea pigs. This noise led to an initial synaptic loss of approximately 50% in the high frequency region. Within the month following the noise exposure, temporal coding deficits developed along with partial recovery of the number of ribbon synapses. The temporal coding deficits manifested as a delayed onset peak of ANF firing as well as a reduced peak rate. Since the deficits were not seen shortly after the noise exposure, but only a month later after the synaptic count had largely recovered, it was concluded that the repaired synapses had presented problems with encoding signal onset (Song et al., 2016). A second study executed by the same researchers reported similar temporal processing deficits as measured in ABR and CAP in guinea pigs exposed to the same noise (Shi et al., 2013).

Temporal processing disorders resulting from noise exposure have also been investigated in human participants, both objectively and behaviorally. For instance, past objective studies have used ABR wave V in order to identify temporal coding deficits in humans following noise exposure (Mehraei et al., 2016; Prendergast et al., 2017a). In the study by Mehraei et al. (2016), it was found that the masking-induced wave-V latency shifts were correlated with changes in ABR wave-I amplitude, which may reflect the number of functional ANFs. In the mice observed in the study, it was demonstrated that NIS reduced wave-I amplitude growth with sound level. Notably, the amount of wave-V latency shift in noise was also reduced. Among the human participants in this study, those with small masking-induced wave-V latency shifts (which likely would be associated with smaller ABR wave-I amplitude and a larger loss of synapses) performed poorer on a sound localization task requiring discrimination of interaural time differences (ITD) in sound envelopes (Mehraei et al., 2016). This result suggests that NIS may result in temporal processing deficits. In another objective study, a correlation was found between poor envelope following responses (EFR) and poor ITD threshold, which was representative of poor temporal resolution (Bharadwaj et al., 2015). Poor EFR (i.e., reduced amplitude and/or phase-locking value) has also been reported as evidence of temporal processing disability in subjects with NIS (Bharadwaj et al., 2014; Shaheen et al., 2015; Parthasarathy and Kujawa, 2018; Prendergast et al., 2019).

A connection between CIND and temporal processing disorders has also been found in humans from behavioral studies. In one study, Snell et al. found that individuals with poorer gap detection thresholds showed significantly poorer word scores as the level of background babble increased (Snell et al., 2002), suggesting that temporal processing could play an important role in understanding speech in noise. More evidence is available for temporal processing deficits with NIS. In one study, participants who had been exposed to noise had trouble discriminating a temporally fluctuating noise from a more stationary noise than those without noise exposure (Stone et al., 2008). In another study, noise-exposed train drivers were found to perform poorer than controls in various tests of temporal processing ability, including gap detection, modulation detection and duration pattern detection. The poorer temporal resolution was also correlated with poor speech recognition in noise (Kumar et al., 2012). In light of evidence for the functional role of ribbon synapses in temporal processing and the sensitivity of the synapse to noise, as well as the apparent connection between temporal processing deficits and difficulty of hearing in noise, it is reasonable to assume that noise damage may cause CIND by degrading temporal processing.

However, reports refuting the connection between temporal processing deficits and CIND from NIS also exist. For instance, one study examined the auditory processing abilities of middle-aged participants with normal hearing thresholds by measuring AM detection thresholds. In this study, no clear relationship between noise exposure and auditory perception was found (Yeend et al., 2017). In another study, a significant but weak correlation was found between speech-in-noise deficits and temporal processing deficits in noise-exposed groups with normal hearing thresholds (Prendergast et al., 2017b). In both of those reports with negative results, the subjects in the noise group were selected based upon self-report and might not have had NIS.

Is Temporal Processing Disorder a Concern for Evaluating Coding-in-Noise Deficit?

The review above suggests that temporal processing deficits are likely to occur in individuals with NIS and may give rise to speech-in-noise deficits (or CIND). Logically, the evaluation of CIND should take temporal processing deficits into account, since temporal processing is involved in the detection of signals in background noise. As outlined in section "Noise Induced Synaptopathy Studies in Animal Models and Difficulty in Translation," the real-world noise experienced by humans tends to be temporally modulated. In such noise, it is possible to detect signals in the dips of the masker. However, such listening in the dips likely depends on robust temporal processing. To mimic real life hearing in noise challenges, maskers used in experiments investigating coding ability in background noise should also be temporally modulated. However, this issue has received scant attention in research designs—particularly in studies of CIND with NIS in animal models.

In behavioral studies with human participants, both stationary and modulated maskers (such as multi-talker babble) have been used in speech-in-noise tests in order to examine potential deficits in subjects with NIS, but the temporal characteristics of the masker have received insufficient focus, and there are no comprehensive comparisons of the masking effect from maskers with varying temporal features. For example, in one study reviewed above, stationary noise was used as the masker and no differences were found between the noise-exposed group and the control group in the speech-in-noise task (Prendergast et al., 2017b). In another study examining the effect of noise-induced tinnitus on speech-in-noise understanding in young adults, participants with noise-induced tinnitus showed worse speech-in-noise performance than non-tinnitus controls regardless of whether the masker was stationary or modulated (Gilles et al., 2016). However, there was no control group without noise exposure used in this study. Only the study by Kumar et al. (2012) appeared to confirm worse speech-in-noise performance in noise-exposed participants by using multi-talker babble as the masker (Kumar et al., 2012). However, the masking effect of the multi-talker babble was not compared to a stationary masker. It is therefore evident that a valid comparison cannot be made across the available studies to differentiate the effects of masker types (stationary versus modulated). To date, there are no comprehensive evaluations of whether a temporally modulated masker is superior to a stationary masker in a speech-in-noise test used to identify CIND in subjects with NIHHL.

IS THE SYNAPTIC DAMAGE AND REPAIR RESPONSIBLE FOR THE TEMPORARY THRESHOLD SHIFT AND RECOVERY?

Cochlear threshold recovery after a non-PTS-inducing noise exposure co-occurs with synapse count recovery and/or repair of damaged synapses. This co-occurrence has been considered by some researchers as evidence supporting synaptic repair as the *mechanism* of temporary threshold shift (TTS) recovery (Robertson, 1983; Puel et al., 1997; Shi et al., 2015; Wang et al., 2015). However, this idea conflicts with our understanding of the physiological mechanisms that determine cochlear threshold. It is well recognized that noise-induced reductions in auditory sensitivity are mainly due to damage to outer hair cells (OHC), which provide active gain for soft sounds (Hudspeth, 1997; Szalai et al., 2011). Threshold recovery following TTS is associated with a full recovery of OHC function, demonstrated by a recovery of otoacoustic emissions (OAE) (Subramaniam et al., 1994; Chang and Norton, 1996; Kujawa and Liberman, 2009) and cochlear microphonics (CM) (Wang et al., 1992, 2011; Chen et al., 1995; Chen and Liu, 2005; Chen and Zhao, 2007). In addition, repair of stereocilia and the tectorial membrane have been considered as potential mechanisms underlying the resolution of TTS in several studies (Sohmer, 1997; Nordmann et al., 2000; Wang et al., 2002, 2011; Tsuprun et al., 2003). To the extent that noise-induced damage to OHCs and surrounding structures is reversible, this reversibility provides a reasonable account for the recovery of cochlear thresholds following noise exposure.

Noise-induced IHC and synapse damage and repair are less likely to be involved in threshold recovery. Each IHC is innervated by more than 10 SGNs, and noise damage tends to be selective to synapses innervating high-threshold fibers that have low spontaneous spike rates (SR) (Furman et al., 2013; Song et al., 2016). Damage/repair or disruption of these synapses should not result in any change in thresholds, similar to results obtained *via* ouabain-induced cochlear damage at low doses (Bourien et al., 2014). This is further supported by differences in the time courses for the recovery of ABR threshold and CAP amplitude, which are related to the total number of ANFs that are functional. In a series of experiments using Guinea pigs, we found that ABR threshold shifts induced by brief noise exposures at 106 dB SPL were completely recovered a week later, with continuing recovery of CAP amplitudes and synapse counts occurring well after that time point. The hypothesis that moderate damage to IHCs and their synapses with SGNs may not impact thresholds is also supported by the finding that up to a 60% loss of SGNs, due to the selective IHC death induced by carboplatin in chinchillas, does not affect cochlear thresholds (Salvi et al., 2016).

Extant data cannot fully rule out changes in synaptic sensitivity that may occur in parallel with damage and repair of OHCs and surrounding structures. Since OHCs provide positive feedback in sound conduction, such changes in synaptic sensitivity would need to be observed by stimulation bypassing these OHC-based effects (to rule out the slim possibility that a temporary reduction in synaptic sensitivity is responsible for the noise-induced TTS).

TEMPORARY CONCLUSION AND FUTURE TASKS

Figure 3 presents a summary of this review in the attempt to show what we current know as well as gaps in our knowledge concerning NIS and NIHHL.

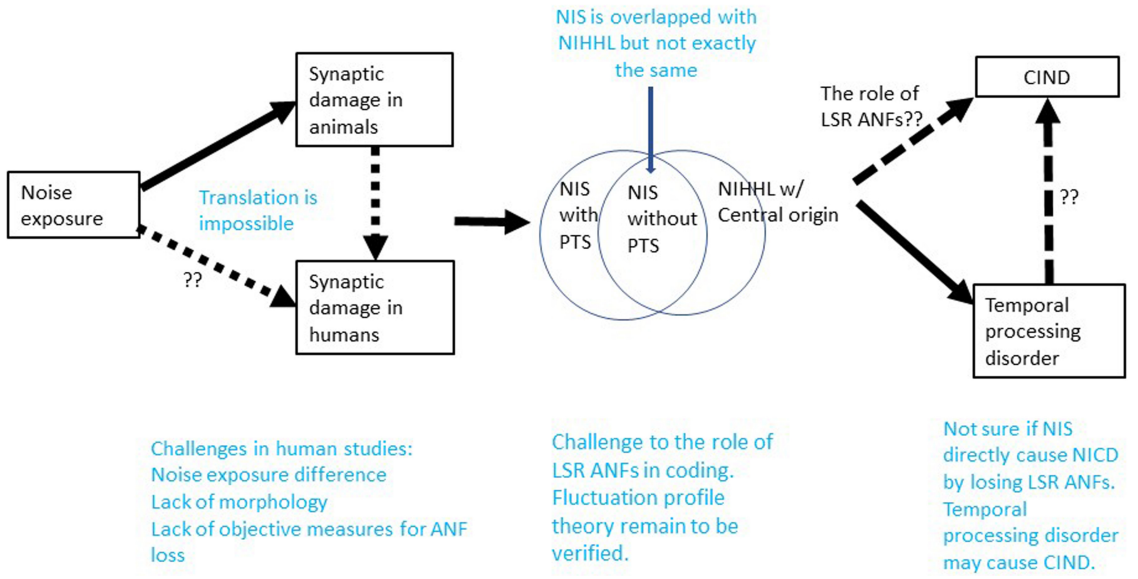

FIGURE 3 | Summary on what we know and what we do not know in the research of NIS and NIHHL. Note that NIS without PTS is overlapped with NIHHL of peripheral origin. The dashed lines without question markers indicate where the connection remain to be speculation and need to be verified.

Conceptually, NIS is largely but incompletely overlapped with NIHHL, since NIS can occur with or without PTS. While NIS refers to the peripheral effect of noise, NIHHL should also cover problems with central origin.

Studies of NIS and NIHHL have received wide interest for more than 10 years since the discovery of substantial ribbon synapse loss following a brief noise exposure that did not cause PTS in CBA mice (Kujawa and Liberman, 2009). Unlike what was seen in this earliest report, many studies have found that the initial synapse loss can be partially recovered. Based upon two available single unit studies in Guinea pigs, the noise damage appears to be biased to synapses innervating LSR ANFs (Furman et al., 2013; Song et al., 2016). However, in one of the two reports, this issue was observed dynamically, and the result showed that a normal distribution of ANFs across SR category was re-established with the partial recovery of total synaptic count.

Translation of animal data on NIS to humans is challenging due to the large differences in noise exposure used in animal research studies and the noise experienced in real human life. Hypothesized NIS in humans is also difficult to confirm due to a lack of morphological data and reliable objective tests that can quantify a loss of ANF function.

Functionally, CIND has been considered to be the major functional problem associated with NIS and NIHHL based upon the theory of SR-based ANF categorization and the finding of a disproportionate loss of LSR ANF after noise exposure. However, CIND has yet to be confirmed as a consequence of NIS in either animals or humans, suggesting a possible problem with the hypothesis. The hypothesis that speech encoding (and speech-in-noise encoding) is compromised in NIS because it depends disproportionately on LSR ANFs, which are selectively damaged by noise exposure, is further challenged by the fluctuation profile model. This model contends that speech is more robustly encoded by fluctuation profiles conveyed *via* HSR ANFs, and that LSR ANFs play a more important role in efferent control *via* the LOC and MOC. However, there are several unresolved issues for this model that remain to be validated, including the role of MOC function in this model.

Temporal processing disorders have been shown to be the most likely functional deficit associated with NIS, and these may be connected to hearing difficulties in noise, particularly with temporally modulated maskers. However, further research is required in humans, with particular attention paid to: (1) better quantifications of noise exposure and consistent use of control groups, (2) better quantifications or evaluations of NIS, (3) careful comparisons of maskers with different temporal characteristics to allow for the evaluation of the impact of temporal processing deficits on hearing in noise.

AUTHOR CONTRIBUTIONS

SR, ZZ, and SA: conceptualization, visualization, and writing. LX and JW: conceptualization, visualization, writing, and funding acquisition. All authors: contributed to the article and approved the submitted version.

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Synaptopathy in Guinea Pigs Induced by Noise Mimicking Human Experience and Associated Changes in Auditory Signal Processing

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Noise induced synaptopathy (NIS) has been researched extensively since a large amount of synaptic loss without permanent threshold shift (PTS) was found in CBA mice after a brief noise exposure. However, efforts to translate these results to humans have met with little success—and might not be possible since noise exposure used in laboratory animals is generally different from what is experienced by human subjects in real life. An additional problem is a lack of morphological data and reliable functional methods to quantify loss of afferent synapses in humans. Based on evidence for disproportionate synaptic loss for auditory nerve fibers (ANFs) with low spontaneous rates (LSR), coding-in-noise deficits (CIND) have been speculated to be the major difficulty associated with NIS without PTS. However, no robust evidence for this is available in humans or animals. This has led to a re-examination of the role of LSR ANFs in signal coding in high-level noise. The fluctuation profile model has been proposed to support a role for high-SR ANFs in the coding of high-level noise in combination with efferent control of cochlear gain. This study aimed to induce NIS by a low-level, intermittent noise exposure mimicking what is experienced in human life and examined the impact of the NIS on temporal processing under masking. It also evaluated the role of temporal fluctuation in evoking efferent feedback and the effects of NIS on this feedback.

Keywords: temporal processing, coding-in-noise deficit, cochlear efferent, fluctuation profile, Guinea pigs, noise-induced synaptopathy

Abbreviations: ABR, auditory brainstem response; AM, amplitude modulation; ANF, Auditory nerve fibers; CtBP2, C-terminal binding protein 2; CAP, compound action potential; CIND, coding-in-noise deficit; CS, contralateral suppression; EFR, envelop following responses; L/M/HSR ANF, low/medial/high SR ANF; MD, modulation depth; MF, modulation frequency; MOCN, Medial olive-cochlea neurons; nfEFR, near-field EFR; NIS, noise induced synaptopathy; NIHHL, noise induced hidden hearing loss; SR, spontaneous rate; PSD, post-synaptic density; TMTF, temporal modulation transfer function.

INTRODUCTION

The concept of noise induced hearing loss (NIHL) has been greatly enriched by the discovery of massive synaptic loss in cochleae without permanent threshold shifts (PTS) in animal studies (Kujawa and Liberman, 2009; Moser et al., 2013; Starr and Rance, 2015; Moser and Starr, 2016; Song et al., 2016; Kaur et al., 2019; Kim et al., 2019; Liu et al., 2019). Noise-induced synaptopathy (NIS) without PTS and noise-induced hidden hearing loss have become hot topics in hearing research since then. Due to the difficulty obtaining morphological data for cochlear synaptic loss cause by noise in humans, animal data has been used to interpret or predict NIS in human subjects. However, this translation has not been validated since the noise exposures used in the animal studies are mostly brief (e.g., 2 h) exposures at the maximum level that does not cause PTS (100–106 dB SPL). Such noise is not likely to be experienced by human subjects, for which traffic noise (Munzel and Sorensen, 2017; Nieuwenhuijsen et al., 2017; Zare Sakhvidi et al., 2018; Munzel et al., 2020), recreational noise (Ivory et al., 2014; Fulbright et al., 2017), noise in industrial settings (Stucken and Hong, 2014; Lie et al., 2016) and in military activity (Pfannenstiel, 2014; Nakashima and Farinaccio, 2015) are the major concerns. Except for military noise, these other common noise types do not have ongoing levels over 100 dB SPL. In industrial settings, which used to be major sources of NIHL, noise levels received by human ears rarely exceed 90 dB SPL under current safety regulations. The noise from traffic and recreational events may frequently peak at very high levels, but only lasts for very short periods of time (Jagniatinskisa et al., 2017; Oiamo et al., 2017). On the other hand, the noise in all of the above situations is amplitude modulated (Barlow and Castilla-Sanchez, 2012; Masullo et al., 2016), not stationary like the noise used in the laboratory studies. Moreover, noise-induced damage of human hearing accumulates over many years in which noise exposure is intermittent. Therefore, synaptic damage by the real-life noise is likely different from the damage created by the noise used in laboratory studies.

Functionally, NIS without PTS is associated with the concept of noise induced hidden hearing loss (NIHHL). Based on a selective loss of afferent synapses innervating auditory nerve fibers (ANFs) with low spontaneous rates (LSR) in two animal studies (Furman et al., 2013; Song et al., 2016) and the theory that LSR ANFs are necessary for signal coding in high level background noise, coding-in-noise deficits (CIND) or deficits of hearing in noise (DHIN) have been considered to be the major hearing problem associated with NIHHL (Plack et al., 2014; Le Prell and Clavier, 2017; Liberman, 2017; Liberman and Kujawa, 2017; Chen et al., 2019a; Huet et al., 2019; Kohrman et al., 2020). However, there is no reliable evidence supporting the existence of CIND in either animals or human subjects. The equivocal results have challenged the proposed unique role of LSR ANFs in coding high-level sounds and led to a reconsideration of high SR (HSR) ANFs in high-level signal coding. For example, the recently proposed fluctuation profile model suggests that high-level sounds are mainly coded by HSR ANFs (Carney, 2018). Interestingly, this model posits that efferent control of cochlear gain is part of mechanism and is sensitive to temporal fluctuation of auditory input although no evidence for this is reported.

In the present study, we aimed to (1) examine NIS without PTS by using a temporarily modulated noise with a long-term equivalent (Leq) sound level of 90 dB SPL, presented intermittently over a month to mimic noise exposures in human subjects, (2) to determine whether the resulting NIS impacts the ability of subjects to use temporal cues for coding masked signals, and (3) to evaluate the role of temporal fluctuations in contralateral suppression of the compound action potential (CAP) and determine whether this is affected by NIS.

MATERIALS AND METHODS

Outline of Subjects and Main Procedures

A total of 20 adult albino guinea pigs (Hartley) were obtained from Charles River, Canada for this study; 10 in the control and the noise groups, respectively. After the animals were recruited (at the age of 1.5–2.5 months), their external ears were checked for abnormalities. The animals were then tested with frequency-specific auditory brainstem responses (ABR) to ensure normal hearing sensitivity. In this baseline test, the envelope following response (EFR) was also measured in the far-field. Following the baseline test, the animals in the noise group were subjected to a noise exposure over a one-month period. One-month after the noise exposure or two months after the baseline test, ABR and EFR were repeated on the animals in each group, followed by a set of near-field recordings (from the round window), including the transient CAP and in response to amplitude modulation (AM, or near-field EFR—nfEFR). Following the terminal evaluation, the animals were sacrificed, and their cochleae were harvested for a morphological evaluation of ribbon synapse count. All of the procedures were approved by the University Committee of Laboratory Animals (protocol# 20-024).

Noise Exposure

Multi-talker babble was modified to be more suitable for Guinea pig hearing by shifting it to 2-16 kHz using a noise vocoder approach implemented in Matlab (Dorman et al., 1997) (see detail in **Supplementary Material**). The frequency-shifted multi-talker noise was presented in a sound booth *via* a four-speaker array (Pyramid TW-67 Super Tweeters; Brooklyn, NY, United States), which was suspended 40 cm above the sound booth floor. Throughout the noise exposure, the animals were awake and unrestrained in a metal wire cage inside the sound booth with free access to water and food (Chen et al., 2019b; Fan et al., 2020; Zhang et al., 2020). The animals were exposed to the noise presented an Leq of 90 dB SPL for 8–12 h per day. This was done on every other day to allow for a day of rest following each episode of noise exposure. The total duration of the noise exposure was 122 h, making the total energy of the noise roughly equal to the 2-h exposure at 106 dB SPL that has been used in

previous studies in Guinea pigs (Chen et al., 2019b; Fan et al., 2020; Zhang et al., 2020).

Auditory Brainstem Response and Envelop Following Responses

All electrophysiological evaluations were performed in an electromagnetically shielded sound booth. Guinea pigs were anesthetized with a mixture of ketamine and xylazine by intraperitoneal injection for the ABR and EFR baseline tests. The initial dose was 40 and 10 mg/kg for ketamine and xylazine, respectively, and 1/3 of the initial dose was added as needed to maintain the anesthesia as needed (judged by the toe-pinching reflex) when the test was exceptionally longer than 1 h. Throughout the experiment, the body temperature of the animal was kept at 38°C with a thermostatic heating pad. In the terminal evaluation, all of the tests were completed with the animals under urethane (i.p., 1.5 g/kg).

An auditory signal processing station (RZ6) from Tucker-Davis Technologies (TDT System III; Alachua, FL, United States) was used to generate the signals for auditory stimulation and to record the biological responses. The acoustic signals for all the auditory responses were delivered in open field *via* a broadband speaker (FT28D, Fostex). Maskers for EFR recording were also delivered in open field *via* an additional FT28D speaker.

Both ABR and EFR were recorded with three subdermal electrodes, with the recording electrode inserted at the vertex and the reference and grounding electrodes positioned posterior to the external auditory canals. The biological signals picked up by the electrodes were sent to an RA16PA preamplifier, which amplified the signal 20 times.

Auditory brainstem response (ABR) was evoked by 10-ms tone bursts (tone bursts) with a rise/fall time of 0.5 ms. The tone bursts were presented 21.1/s for the ABR and ABR thresholds were measured from 1 to 32 kHz in octave steps. For each trial, the response was averaged 1000 times; fewer averages were collected if a clear response was visible. At each frequency, tone bursts were presented in a descending sequence from 90 dB SPL toward threshold, which was defined as the lowest sound level at which a repeatable Wave-III was visible.

Envelop following responses (EFR) was evaluated in response to 16 kHz AM tones that were presented at a moderately high level of 75 dB SPL. The AM tones were presented in a sweeping pattern, and they had a duration of 500 ms and a rise/fall time of 5 ms. The modulation frequency (MF) was initially set from 113 to 1513 Hz in 100 Hz steps to get a TMTF, which was evaluated at two modulation depths (MD): 30 and 60%. The EFR was sampled at 24.414 kHz over a 500-ms time window to cover the length of the stimuli. The response of the first 50 ms was set to zero to avoid the impact of the onset response. In each trial, EFR was averaged 50 times before it was converted into the frequency domain by a Fast Fourier Transformation. The spectral peak at each MF was measured in dB as the phase-locked response to the MFs.

Following the testing in quiet, the effect of the masker on EFR was evaluated at the best MF (best modulation frequency), i.e., the MF at which the greatest response occurred by each of the two maskers: one was a high pass filtered white noise with a cutoff at 4 kHz (the stationary masker) and the other was the multi-talker noise used for the noise exposure (the masker with fluctuation). Each masker was played at 75 dB SPL [yielding a 0 dB signal-to-noise ratio (signal-to-noise ratio)].

To mitigate the impact of random changes in EFR with time, each masked EFR was sandwiched by two control recordings (without masking). This strategy was also used for the recording of the nfEFR. The two EFRs without masking were averaged and the effect of masking was calculated as the difference of magnitude in dB between the EFRs with and without masking.

Compound Action Potential and Near-Field Envelop Following Responses Recording From Round Window

Under anesthesia *via* urethane (i.p., 1.5 g/kg), a silver ball electrode was placed on the round window membrane after the mastoid was surgically opened. To secure the electrode in place, the silver wire was fixed to the mastoid with dental cement. The other end of the silver wire as well as the reference and grounding electrodes were connected to the preamplifier and then to the TDT system, exactly the same way as for the ABR and EFR recordings. A plastic tube was embedded in the dental cement to provide ventilation of the middle ear, preventing the buildup of negative pressure. During the surgery and recording, the animal was placed on a thermostatic heating pad to maintain a body temperature of 38°C. The nfEFR was measured and analyzed the same way as the scalp EFR, except that the number of averages in each trial was 25 instead of 50.

The transient CAP was evoked by a 16 kHz tone burst with 2-ms duration (0.5 ms rise/fall) from 90–10 dB SPL to obtain I/O functions. The stimuli were delivered in open field *via* a FT28D speaker. The effect of contralateral suppression (CS) was observed in CAP evoked by 16 kHz tone bursts. The CS signal was delivered in closed field *via* a MF-1 speaker with tubing. Three types of signals were used as CS stimuli: (1) 16 kHz tone without modulation, (2) 16 kHz tone sinusoidally modulated by 93 Hz at 30% MD, and (3) at 60% MD. With each type of CS signal, the CS effect was observed at three CS levels: 75-, 63-, and 51-dB SPL. Therefore, the CS effect was observed under 9 conditions (3 types at 3 levels).

Similar to the masking effect test, each CAP with CS was sandwiched by two records without CS to mitigate the impact of random variation of the CAP over time. The two controls were averaged for the calculation of the CS effect, which was the difference in the CAP with and without CS.

Synapse Count Observation

The morphological evaluation was carried out in accordance with previously published procedures (Liu et al., 2012; Shi et al., 2013; Song et al., 2016; Chen et al., 2019b; Fan et al., 2020; Zhang et al., 2020). To begin, the cochlear tissues were dissected after being fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS). They were then permeabilized with 1% Triton X-100 in PBS for 1 h, incubated in 5% goat serum in PBS for an additional 1 h, and then incubated overnight at 4°C with primary antibodies against both C-terminal binding protein 2

(CtBP2) and post-synaptic density-95 (PSD95) (mouse IgG1 to CtBP2; BD Biosciences, Franklin Lakes, NJ, United States: cat. # 612044, 1:200; mouse IgG2a to PSD95; Millipore, Billerica, MA, USE: cat. # MAB1596, 1:600). After the reaction, the tissues were washed and treated with the corresponding secondary antibodies (A21124 and A21131, respectively; Invitrogen, Carlsbad, CA, United States) at room temperature for 2 h, and then mounted on microscope slides.

A confocal laser-scanning microscope (LSM 710 META; Zeiss, Shanghai, China) with a 63 × water-immersion objective was used to obtain confocal images at specified frequency positions based on frequency-distance mapping (Viberg and Canlon, 2004). Next, image stacks were exported to ImageJ image-processing software (National Institutes of Health, Bethesda, MD, United States). In order to obtain the puncta densities, over 10 successive inner hair cells at each frequency position of the cochlea were selected to count the puncta of CtBP2 and PSD95.

Data Analysis

The ABR and EFR were repeated at two time points (baseline and end test) in each of the control and noise group, generating 4 data sets which were labeled as ctrl-young, ctrl-old in the control group, and pre-noise and post-noise in the noise group. Useful data was not obtained from every subject due to unexpected recording problems. The exact sample size was specified for each test result, either by the number in the brackets in the figure legends or as stated in the figure legend.

All data in this report are presented as means ± standard error of mean (SEM). To analyze the data, the data were first evaluated for normality and equal variances. Parametric tests were performed for data passing the normality and equal variance tests, otherwise, non-parametric tests were applied. All statistics were done using SigmaPlot 14. For data with multiple factors, ANOVAs were followed by *post hoc* pairwise evaluations. $P < 0.05$ was used as the criterion for significance.

RESULTS

Auditory Brainstem Response

The hearing status of the animals was examined with ABR in the noise group before and one month after the noise exposure to confirm that the noise exposure did not cause PTS. ABR was also tested in the control group across the times of the experiment to rule out any age-related change in auditory sensitivity. **Figure 1** shows ABR thresholds tested from the two groups at two time points. The ABR-frequency curves measured at the two time points in the control group were largely overlapping, indicating that there was no age effect on the ABR threshold. This was supported by the insignificant difference (F1 = 0.712, $p = 0.422$) between the repeated tests in the two-way repeated measure (RM) ANOVA against the test and frequency. A two-way ANOVA was conducted to compare the Ctrl-old and Post-noise groups (with frequency as a co-variant) to determine whether noise exposure had any impact on thresholds. No significant effect of group was seen between the two groups (F1 = 0.156, $p = 0.694$).

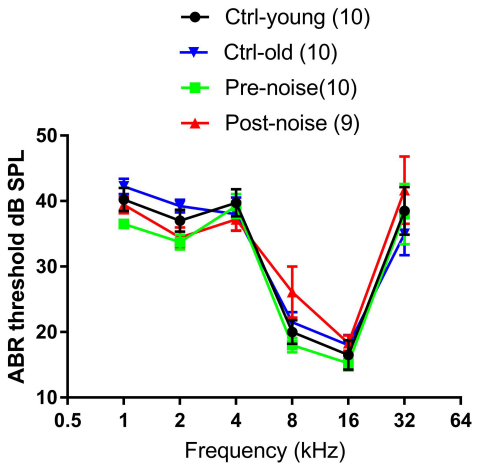

FIGURE 1 | The effect of age and noise on ABR thresholds. Ctrl-young and Pre-noise were the baseline thresholds taken at 1.5–2 months of age from both the control and noise groups before the noise exposure. Ctrl-old was measured at 4–5 months of age (from the control group), which matched the age of the noise group for the ABR tested one month after the noise exposure.

Synapse Count Observation

The ribbon synapses were identified by immunohistochemistry. The densities of the synapses were compared between the groups and with the previous data to verify the amount of synaptic loss from the noise exposure used in this study. **Figure 2** shows representative images of immunostaining against CtBP2 (red dots) and PSD (green dots) from both a control animal and a subject exposed to the noise (one month after). The images were taken from the high frequency region of the cochlea. The images show that the synaptic puncta are distributed mostly along the bottom of inner hair cells in the control cochlea (indicated by the curve along the bottom of an inner hair cell in **Figure 2A**), while the distribution is less organized, or widely distributed in the noise-exposed cochlea (as shown in the circulated area in **Figure 2B**).

Figure 3 compares the ribbon densities (stained against CtBP2) across groups. The data from a previous study were taken for the synaptic counts after a brief-noise exposure at a higher level (106 dB SPL, 2 h; noise 1) (Song et al., 2016) to compare with the low-level noise (∼90 dB SPL) given periodically over one month with a roughly equal dose in the present study (122 h, noise 2). Since the ribbon puncta are mostly paired with PSDs (**Figure 2**), the ribbon counts were used to indicate the number of synapses. This practice is supported by previous studies, which have shown that the numbers of CtBP2 puncta and the postsynaptic puncta are similarly changed after noise damage (Maison et al., 2013; Shi et al., 2013, 2016; Wang et al., 2015). **Figure 3A** shows the ribbon density-frequency map (or density cochleogram). **Figure 3B** compares the density averaged over the frequency region above 4 kHz. This average was 18.15 ± 0.387 in the control group and 15.18 ± 0.185 in the group exposed to the brief noise (noise 1, 16% lower than control). Average density was 16.99 ± 0.12 after the long-term noise exposure (noise 2,

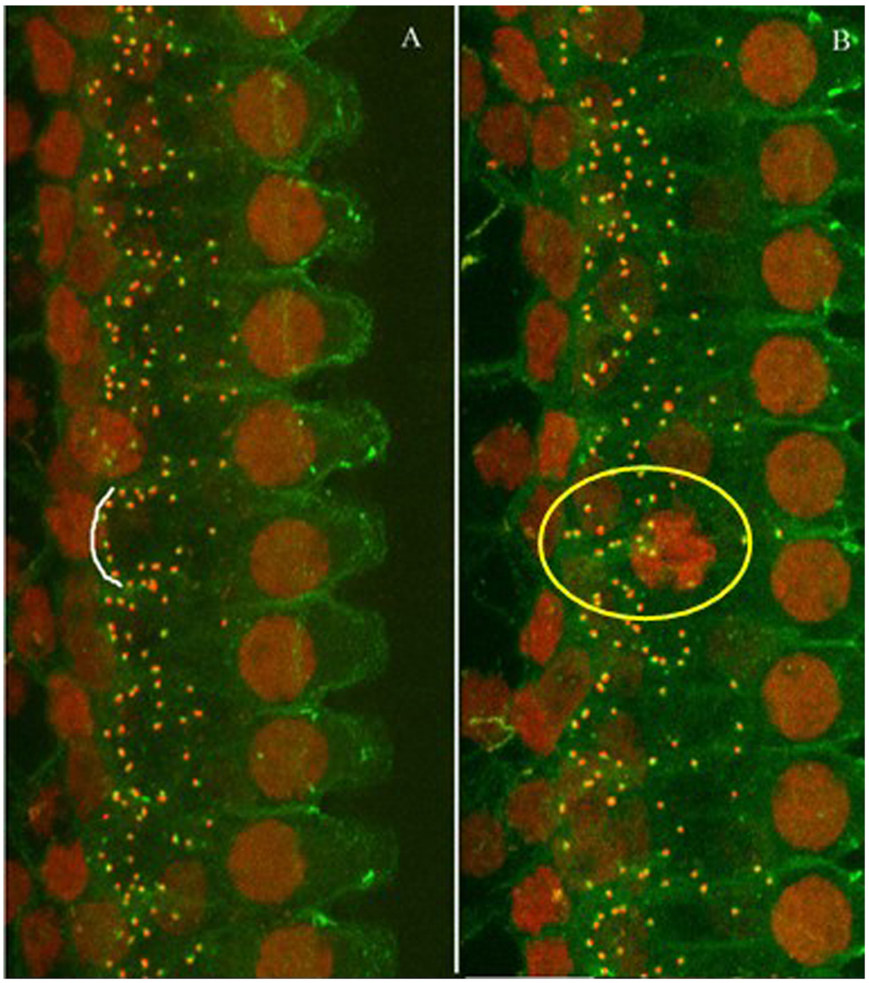

FIGURE 2 | Representative images of immunostaining against CtBP2 (red dots) and PSD (green dots). **(A)** Control animal. **(B)** Noise-exposed animal (one-month post-noise exposure). The images were taken from the high frequency region of the cochlea. The distribution of the puncta was mostly alone the bottom of the IHCs as (the white arc), but less organized in the noise-exposed cochlea (see the puncta in the yellow circle).

6.2% lower than control). A one-way ANOVA on rank (Kruskal-Wallis) showed a significant overall difference between groups ($H_2 = 19.79$, $p < 0.001$). *Post hoc* pairwise tests (Dunn's method) showed significant differences between the control and noise 1 groups ($Q = 4.445$, $p < 0.001$) and between the noise 1 and noise 2 groups ($Q = 3.029$, $p < 0.007$), but not between control and noise 2 groups ($Q = 1.983$, $p = 0.142$).

Envelop Following Responses and Near-Field Envelop Following Responses

Temporal Modulation Transfer Functions

Both EFR and nfEFR were observed to show the impact of the noise exposure on temporal processing, and to determine whether the damage to cochlear function would be reflected in the response recorded from scalp. **Figure 4** shows the impact of noise and age on the TMTF as assessed *via* EFR. TMTFs at 30% MD and 60% MD from the two groups at the two time points are given in **Figures 3A,B**, respectively. The TMTFs measured with 60% MD for the Ctrl-old and post-noise groups were largely overlapping at high MFs, while the TMTFs measured with 30% MD for the post-noise group diverged from the Ctrl-old TMTFs at high MFs, with the largest difference at 1213 Hz. At this MF, the difference between the groups was statistically significant (Mann-Whitney Rank Sum Test, $T = 59$, $p = 0.013$).

However, the significant change in the EFR TMTF was not seen in the nfEFR. **Figure 5** shows the nfEFR TMTFs between the groups. Since the nfEFR can only be recorded in the terminal test, they are shown only for the old age group without noise exposure (Ctrl-old) and the old age group post-noise exposure (Post-noise). Unlike the TMTFs in the far-field recording, those obtained in the near field are largely overlapping for both groups.

Effect of Stationary and Temporally Modulated Maskers

The impact of masker types on masking effect was observed in both EFR (**Figures 6A–C**) and nfEFR (**Figures 6D–F**) between the stationary masker and modulated multi-talker babble. The

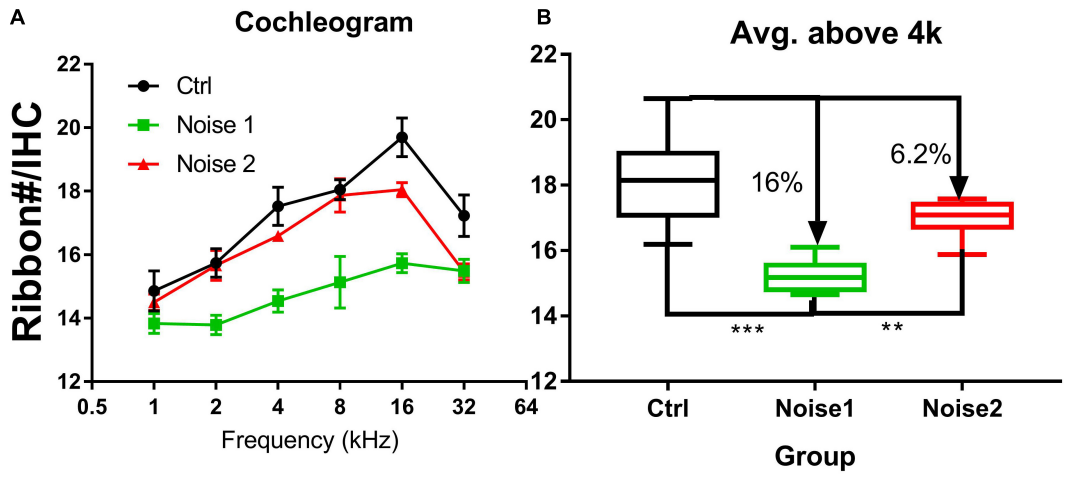

FIGURE 3 | Synapse density comparison across groups (n = 8 in every group). **(A)** The cochleogram of synaptic density. **(B)** The averaged synaptic density in the frequency region above 4 kHz. The synapse density is calculated from ribbons (Ctbp2). Noise 1 refers to a brief noise at 106 dB SPL for 2 h [data taken from a previous study Song et al. (2016)]. Noise 2 refers to the noise exposure examined in the present study (multi-talker noise, repeated over a period of 1 month for 122 h with an Leq of roughly 90 dB SPL). The density was compared between groups. ***$p < 0.001$, **$p < 0.01$.

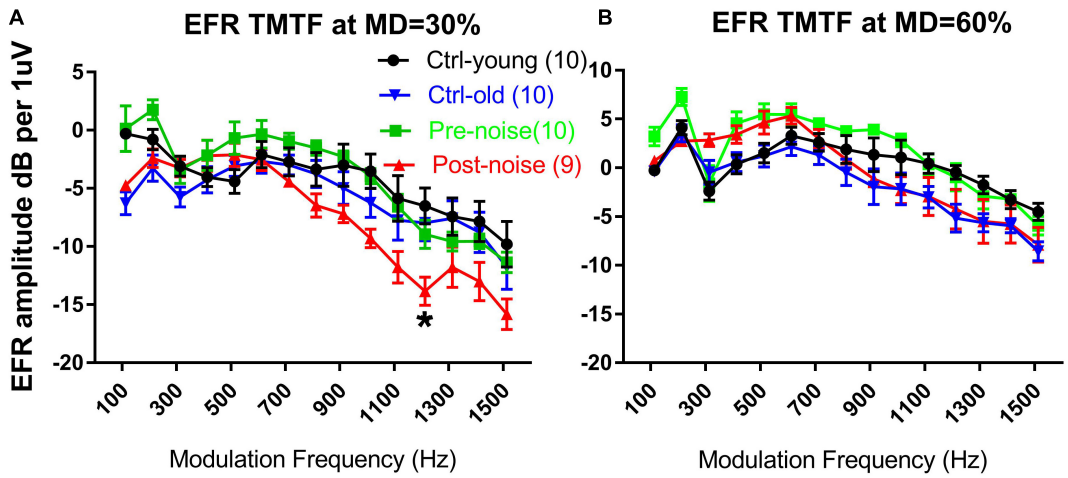

FIGURE 4 | The impact of age and noise on EFR TMTFs measured in response to AM at 30% **(A)** and 60% **(B)** MD. The post-noise TMFT curve obtained with 30% MD diverged from the pre-noise and ctrl-old TMTFs. A significant difference in EFR amplitude was seen between the ctrl-old and the post-noise TMTF at 1213 Hz MF. *$p < 0.05$.

effect of each masker at the best modulation frequency of each subject was calculated as the difference of the response amplitude with and without masking, or the attenuation of the response by masking in dB. Universally, the masking effect was much larger when the stationary masker was used than when the modulated masker was used. For example, under 30% MD in the post-noise testing (**Figure 6A**), the effect of masking on EFR amplitude using multitalker noise was 0.753 ± 0.328 dB, while the effect of masking using the HP masker was 5.318 ± 0.66 dB. A paired t-test indicated that this difference was significant ($t = 6.625$, $p < 0.001$). However, the difference between the two maskers (**Figure 6C**) did not show much variation between the groups and between the two tests within each group. For example, the difference between the two maskers with respect to their effects on the EFR at 30% MD in the post-noise test was not significantly smaller than in the pre-noise control (3.509 ± 0.569 versus 4.564 ± 1.842, paired t-test: $t = -1.185$, $p = 0.27$). This negative result is inconsistent with the idea that noise-induced synaptic damage impairs signal coding in modulated maskers.

The masking to nfEFR by the two maskers were shown also at two MDs (**Figures 6D,E**, respectively, for 30 and 60% MD). Similar to the result in EFR, the masking effect by the high-pass noise appeared to be larger than that of multi-talker masker and the masking effect by the two maskers appeared to be larger in the Post-noise group. A two-way ANOVA was performed at each MD for the factors of group and masker type. The analysis revealed a

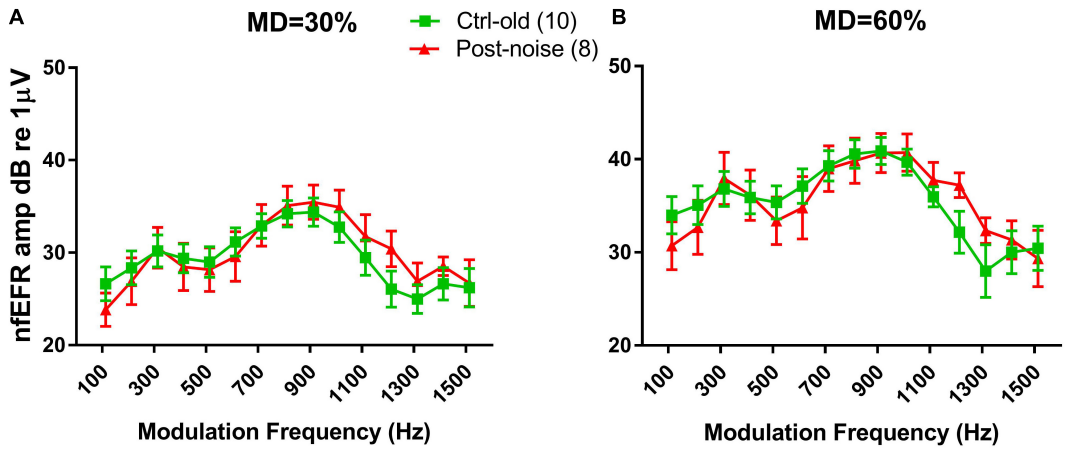

FIGURE 5 | TMTFs of nfEFRs at 30% MD **(A)** and 60% MD **(B)**. The results for the two groups are largely overlapping.

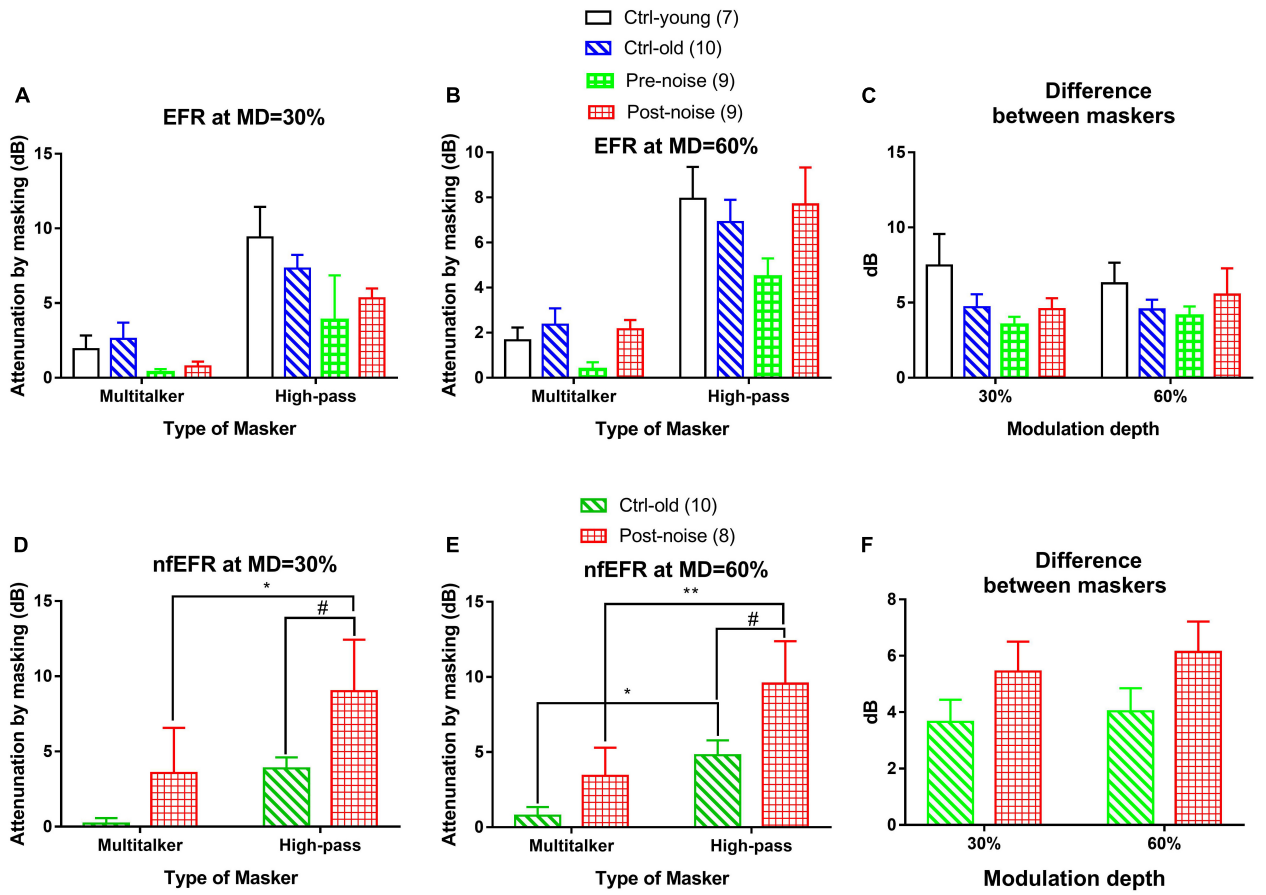

FIGURE 6 | The effect of both the modulated (multi-talker) and stationary (high-pass) maskers at the two MDs [30% **(A,D)** and 60% **(B,E)**] for both the EFR **(A,B)** and nfEFR **(D,E)**, as well as the difference in the masking effect between the two maskers [**(C)** for EFR and **(F)** for nfEFR]. Overall, the high-pass noise produced more masking than the multitalker noise. No significant difference was seen between the two maskers with respect to EFR amplitude **(C)**. For the nfEFR, the HP noise resulting in a greater masking effect for the post-noise group than for the control [# in **(D,F)**]. The difference between the two maskers in nfEFR **(F)** was much larger in the noise group as seen in the two-way ANOVA. However, *post hoc* tests found no significant differences within each MD. The number of "*" symbols show the significance level of the *post hoc* comparisons within each group, while the number of "#" symbols show the significance level of the *post hoc* comparisons within each masker: one for $p < 0.05$, two for $p < 0.01$, and three for $p < 0.001$.

significant effect of both masker type (F_1 = 7.401 and p = 0.010 for MD = 30%, F_1 = 15.716 and $p < 0.001$ for MD = 60%), and group (F_1 = 6.458 and p = 0.016 for MD = 30%, F_1 = 8.339 and p = 0.007 for MD = 60%).

Post hoc comparisons (Holm-Sidak method) revealed a significant effect of group within the stationary masker for both 30% MD (t = 2.175, p = 0.037) and 60% MD (t = 2.622, p = 0.013) (marked by "#" in **Figures 6D,E**). Further, significant effect of masker type marked by "*" was seen within the Post-noise group (t = 2.183, p = 0.036) at 30% MD, and within the Ctrl-old group (t = 2.358, p = 0.025) as well as the Post-noise group (t = 3.210, p = 0.003) at 60% MD.

Further, the masking effect difference between the two masker to the nfEFR (**Figure 6F**) was also examined by a two-way ANOVA. A significant group effect was found for group (F_1 = 4.192, p = 0.049), but not for MD. However, the *post hoc* comparisons (Holm-Sidak method) revealed no significant difference between groups within either of the MDs (30% MD; t = 1.330, p = 0.193, 60% MD; t = 1.566, p = 0.127).

Transient Compound Action Potential and Contralateral Suppression

The impact of modulated auditory input on medial olive cochlea (MOC) efferent control was observed *via* contralateral suppression on transient CAP, which was measured in response to 16 kHz tone bursts. **Figure 7A** shows CAP waveforms from one subject at levels from 90 to 20 dB SPL. The peak-to-peak value was read from the first negative peak to the next positive peak. Since the CAP was contaminated by the summating potential at sound levels above 70 dB SPL, the input/output (I/O) function was measured up to this level. **Figure 7B** shows the typical CS effect on an exemplary CAP I/O function. The suppression effected by the three CS signals was quite similar and was larger at lower levels of CAP-evoking tone bursts.

The CS effect was calculated in dB using the formula 20log[(CAP without CS)/(CAP with CS)]. Since the CS effect was larger at lower levels, the low-level average was calculated across the 30, 25, and 20 dB SPL tone bursts levels. **Figures 8A–C** show the CS effect caused by each of the three CS signal types (16 kHz stationary tone, and the same tone amplitude modulated at 30% and 60% MD). For each stimulus, CS effects were measured at three CS signal levels (75, 63 and 51 dB SPL). Overall, three trends can be seen for the CS effect across level and type of CS signal: (1) a larger CS effect is seen with a higher CS level, with no exception for the modulated CS signal (AM tone) as we hypothesized by the stronger fluctuation in HSR ANFS in response to a low sound level, (2) there is no obvious difference in the CS effect across the CS signal types, (3) CS effects were not reduced but rather increased in the noise group; suggesting that NIS did not impair MOC regulation on cochlear gain. Since the CAP suppression by the two lower CS signals was very small, further analysis focused only on the CS effect produced by the CS signal at 75 dB SPL to show the potential impact of CS type and group (**Figure 8C**). A two-way ANOVA performed for this purpose revealed a significant effect of group (F = 18.823, $p < 0.001$) but no significant effect of CS type (F = 1.747, p = 0.199). *Post hoc* comparisons were then performed (Holm-Sidak method) and revealed a significant difference between the Ctrl-old and the Post-noise groups within tone bursts signal type (t = 2.227, p = 0.031) and within the 30% AM signal type (t = 3.316, p = 0.002).

DISCUSSION

In the present study, the noise exposure was similar to that occurring in human experience in terms of level and temporal features and was applied at a lower level (around 90 dB SPL). The permanent reduction of synaptic density in the high frequency region was only 6% (**Figure 3**)— much lower than our previous reports after noise exposure at a high sound level in Guinea pigs (106 dB SPL, 2 h). We applied the noise exposure for 122 h to make the total energy of this exposure equivalent to that of the brief noise exposure at higher levels (100–106 dB SPL) used in previous studies that found a massive loss of afferent synapses in rodent cochleae (Kujawa and Liberman, 2009; Liu et al., 2012; Furman et al., 2013; Shi et al., 2013; Song et al., 2016; Chen et al., 2019b; Fan et al., 2020; Zhang et al., 2020). The equal-energy hypothesis is a rule of thumb, which states that noise exposure with equal energy should produce an equal amount of damage or NIHL even if presented at a different intensity level, at least after adjusting for kurtosis. This rule has been supported by many researchers in the field (Ward et al., 1981; Gomez Estancona et al., 1983; Lindgren and Axelsson, 1983; Roberto et al., 1985; Fredelius et al., 1987; Borg and Engstrom, 1989; Qiu et al., 2007). However, the results of the present study suggest that noise-induced synaptic loss is an exception to this rule. This is consistent with a previous report in which much less synaptic loss was found in CBA mice after a continuous noise exposure for 168 h at 84 dB SPL (Maison et al., 2013) as compared with a previous report using a more common brief noise exposure (100 dB SPL, 2 h) on the same strain of mice. The brief noise exposure yielded less total energy than the 168-h exposure at 84 dB SPL but produced 50% more synaptic loss in the high frequency region (Kujawa and Liberman, 2009).

Coding-in-noise deficits have been thought to be the most likely functional hearing difficulty associated with NIS without PTS (Plack et al., 2014; Le Prell and Clavier, 2017; Liberman, 2017; Liberman and Kujawa, 2017; Chen et al., 2019a; Huet et al., 2019; Kohrman et al., 2020). While great efforts have been made to verify the existence of CIND after NIS, results have been equivocal. To the best of our knowledge, only one animal study has found positive evidence for CIND after noise exposure, which was done in rats (Lobarinas et al., 2017). However, this study did not measure synaptic loss and several technical limitations make it difficult to interpret the result [see our review (Chen et al., 2019a) for details]. In human studies, reports with negative results (Fulbright et al., 2017; Grinn et al., 2017; Grose et al., 2017; Le Prell and Clavier, 2017; Prendergast et al., 2017a, 2019; Yeend et al., 2017; Guest et al., 2018, 2019; Valderrama et al., 2018) have been more plentiful than those with positive results (Alvord, 1983; Kujala et al., 2004; Stone et al., 2008; Kumar et al., 2012; Stamper and Johnson, 2015; Liberman et al., 2016;

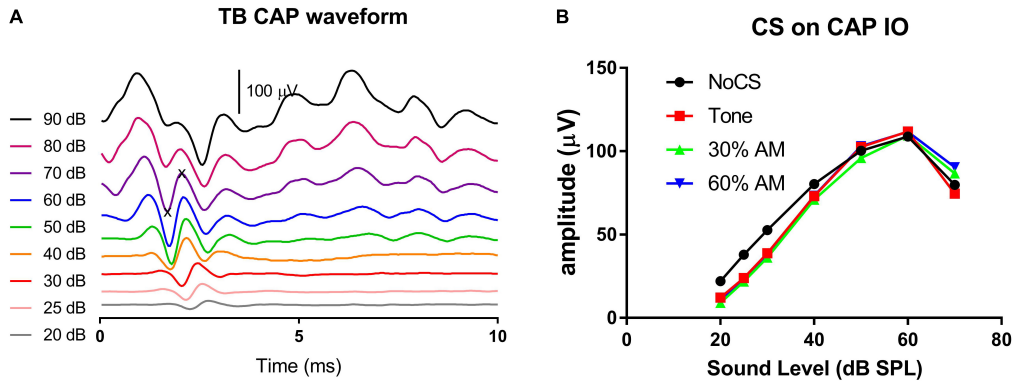

FIGURE 7 | CAP waveforms across sound levels **(A)** and the exemplary CS effect on CAP I/O functions **(B)**. Three CS signals (tones, AM with 30% and 60% modulation depths, respectively) were all presented at 75 dB SPL. They show a similar CS effect, which were larger at lower levels of tone bursts evoking CAP. The CAP amplitude was measured between "x" symbols **(A)**.

Tepe et al., 2017; Meehan et al., 2019). The variability in results could be partially rooted in methodological error or measurement inconsistency, including imprecise quantification of noise exposure based on different types of self-report, a lack of objective measurement of synaptic loss or its functional consequences, and different approaches to measuring CIND [see our recent review for detail (Ripley et al., 2022)]. However, the lack of robust evidence for the CIND expected to occur with NIS and NIHHL should cause us to question the theoretical foundation on which this expectation is based, which assumes a unique rule for LSR ANFs for coding signals in high-level background noise. In a recent review, this assumption has been challenged systematically (Carney, 2018).

While CIND remains to be proved to be the major functional difficulty associated with NIHHL, temporal processing disorders have been verified in subjects with (potential) auditory neuropathy including NIS (Bharadwaj et al., 2014, 2015; Shaheen et al., 2015; Mehraei et al., 2016; Prendergast et al., 2017b, 2019; Parthasarathy and Kujawa, 2018). It is reasonable to expect a deterioration in temporal processing ability after synaptic damage in the cochlea considering the function and importance of ribbon synapses in temporal processing. We have demonstrated this deficit in a single unit study in Guinea pigs with NIS (Song et al., 2016). Such temporal processing deficits may explain signal processing difficulties in noise, since poorer word scores tested with background babble have been found in conjunction with poorer temporal resolution as evaluated *via* gap-detection (Snell et al., 2002). In studies of NIHL, poorer temporal processing has also been correlated with noise-exposure history (Stone et al., 2008) and poorer speech perception in noise (Kumar et al., 2012).

In the present study, temporal processing was assessed *via* EFR TMTFs measured in both far field (EFR) and near field (nfEFR). A deterioration in temporal processing would be evident if the response amplitude was reduced, specifically shown as a sharper drop with increasing modulation frequency. Temporal processing deficits were found in a reduction of far field EFR at high modulation frequencies in the Post-noise group as compared to the Ctrl-old group. This was seen at the 30% MD only (**Figure 4A**). However, the TMTFs were largely overlapping between groups in nfEFR, suggesting a central origin of the changes in far-field EFR. A noise-induced change in far-field EFR TMTF has been reported previously in mice (Shaheen et al., 2015). In this study, band-pass TMTFs were reported with a peak close to a 1000 Hz MF. The ANF origin of this peak response was supported by its disappearance (or reduction) in the test after NIS was established. In the present Guinea pig study, however, the TMTFs showed a low-pass characteristic. The impact of noise was evidenced by a reduction of TMTFs at higher MFs but only when measured with AM at a 30% MD. A shallow MD has been recommended since it should be more sensitive to NIS which might be limited or biased to synapses connecting inner hair cells with low and medium SR ANFs (Bharadwaj et al., 2014). This is supported by the differences in TMTFs measured at two MDs in the present study (**Figures 4A,B**). In addition to the species difference, the EFRs in Shaheen's study were evaluated with an AM signal at 100% MD, and the NIS was more severe than in the present report (Shaheen et al., 2015).

One of the most common ways to evaluate hearing in noise is to test signal perception or coding with masking. To evaluate if the signal coding in noise depends on temporal processing, the masker should be temporarily modulated to allow for signal detection in the temporal dips of the masker. However, this technical matter has received little attention (Souchal et al., 2018; Chen et al., 2019b; Ralli et al., 2019; Zhang et al., 2020). In the present study, we compared the effects of a stationary masker with those of a modulated masker. We hypothesized that if NIS reduces the ability to detect a signal in the dips of a masker, the masking effect should be greater with a modulated masker, such that the differences between the two maskers should be decreased. However, this hypothesis was not supported by our results. The masking effect on the EFR by both maskers was not larger in the Post-noise group than the Control group (**Figure 6A**), and there was no difference between groups in EFR as a function of masker type (**Figure 6C**). Interestingly, there was a significant difference between the two maskers in nfEFR, but the difference between the

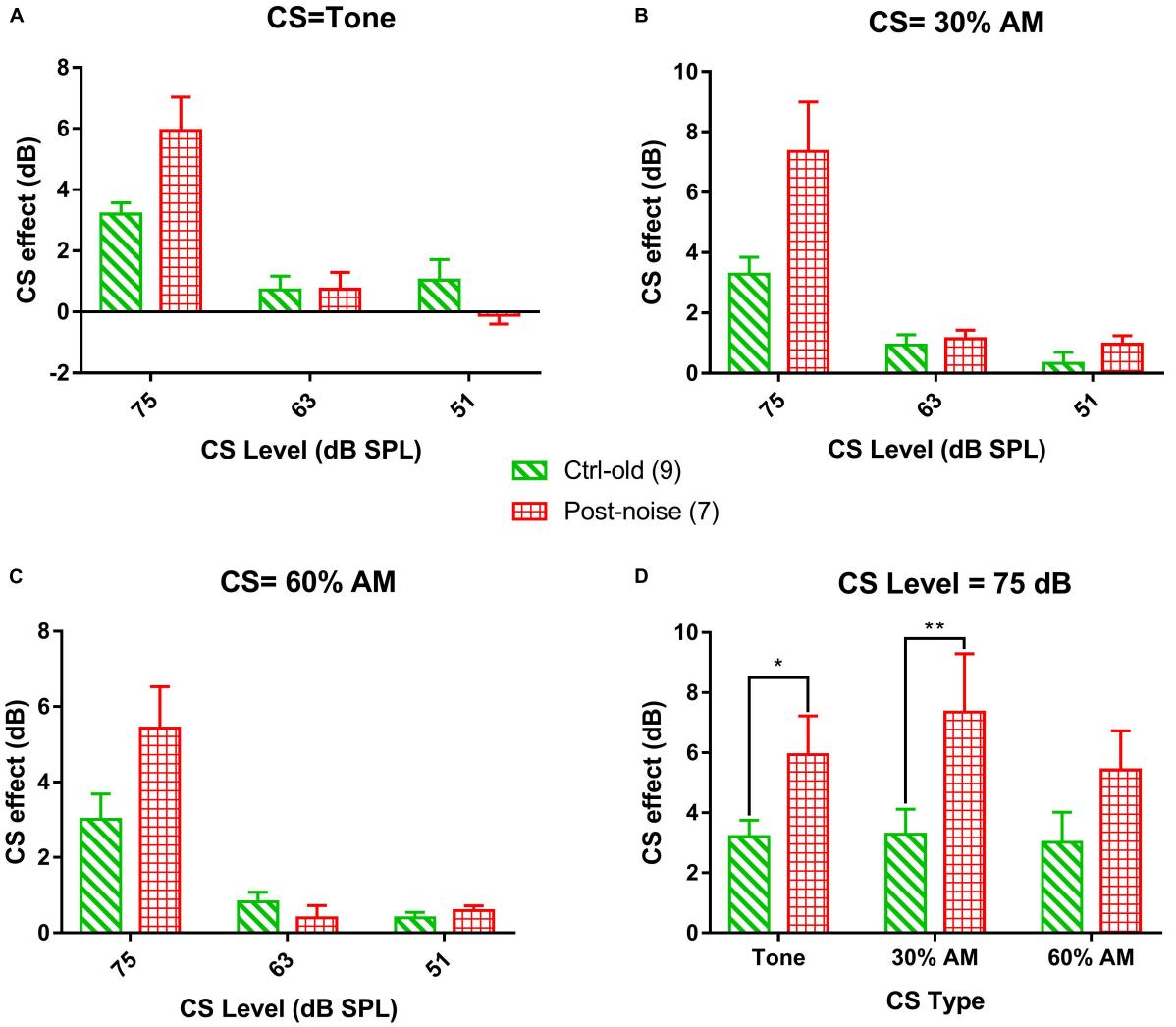

FIGURE 8 | The CS effect on transient CAP in response to 16 kHz. tone bursts at the low-level average (the average of the stimulation levels of 20, 25, and 30 dB SPL). The CS signals are 16 kHz tone bursts, and AM with 30% and 60% modulation, respectively, across three levels (75-, 63- and 51-dB SPL). **(A–C)** The effect of CS level on the CAP across groups, showing a decreased CS effect with decreasing CS level, consistent across the three types of CS signals. **(D)** Comparison of CS effect across CS type and group with 75 dB SPL CS signals.

maskers was larger in the post-noise group, in opposition to our hypothesis. Therefore, the present study does not provide clear evidence for NIS-related deterioration in signal detection in noise *via* using temporal cues. It is important to mention that in the present study, the amplitudes of nfEFR were reduced in the Post-noise group (shown in **Figures 6D,E** as the larger attenuation in the Post-noise group). This is consistent with our previous studies using high level noise exposures (Chen et al., 2019b; Zhang et al., 2020), although the synaptic loss in the present study was much less.

An alternative model of signal coding at high sound levels was proposed by Carney after challenging a unique role for LSR ANFs in this process (Carney, 2018). The so-called fluctuation profile model is specific for the coding of complex signals like speech at high levels *via* HSR ANFs. In voiced speech, the amplitude of the signal is modulated at the fundamental frequency, and these temporal stimulus fluctuations modulate the firing rates of ANFs. At average speech levels (65–70 dB SPL), fluctuations in ANF firing rate are expected to be minimal or absent at formant peaks because these ANFs are saturated due to the high stimulus sound level. However, HSR ANFs in spectral troughs are not saturated and thus have strongly modulated firing patterns. Therefore, the distribution of temporal fluctuations of HSR ANF firing rates across frequency provides a profile that mirrors the spectrum of the speech. Extending from this theory, Carney proposed that temporal fluctuations in neural firing in the ascending auditory pathway may play an important role in controlling efferent feedback *via* medial olivo-cochlear neurons (MOCN). Temporal fluctuations in ANF responses are inherited by the cochlear nucleus and inferior colliculus (Gummer et al., 1988;

Krishna and Semple, 2000; Joris et al., 2004; Carney et al., 2016). Carney suggested that sensitivity to such temporal fluctuations in the inferior colliculus may have an important role in regulating cochlear gain *via* the descending pathway from inferior colliculus to MOCN, providing a mechanism for enhancing fluctuation profile contrast. This is because ANFs near formant peaks show minimal to no fluctuation in firing rate (due to saturation) and therefore produce less excitation at the inferior colliculus and MOCN, resulting in less gain reduction than at frequencies near formant troughs. If this is correct, a modulated stimulus (such as an AM signal) should produce a stronger gain reduction *via* MOC feedback.

The efferent control to the cochlea is divided into two parts: (1) lateral efferent control from efferent neurons surrounding the lateral superior olive to the terminals of the type I afferent neurons under inner hair cells; (2) medial efferent control from MOCNs to the bodies of outer hair cells. The function of MOC control is much better understood as to regulate the active gain of outer hair cells, which in turn changes the response of ANFs to sound. While the pathway and function of efferent control *via* the lower brainstem have been comprehensively explored, corticofugal control from higher level auditory centers (such as the inferior colliculus) are less understood [see reviews (Terreros and Delano, 2015; Guinan, 2018; Fuchs and Lauer, 2019)].

MOC feedback is usually examined using a CS paradigm *via* otoacoustic emissions (OAE) or CAP. However, in most studies, CS signals are not temporarily modulated. In studies evaluating the effect of CS signal level, suppression has been found to be larger at higher CS signal levels [e.g., (Moulin et al., 1993; Zhang et al., 2007) for OAE and (Puria et al., 1996) for CAP]. In the present study, we used CAP to compare the amount of CS achieved with a stationary versus modulated suppressor signal. We predicted that, if temporal fluctuation is critical in MOC feedback, the modulated CS would produce a larger CS effect, which would be more so if presented at a relatively low level because HSR ANFs are not saturated at this level. However, our results did not show significant differences between the stationary and modulated suppressor signals with respect to CS. Moreover, the CS effect was always greater at a higher CS level, regardless of CS types. This negative result may not be adequate to fully reject a dominant role of temporal fluctuation of HSR ANFs in modulating MOCN-mediated efferent control. It is likely that a feedback loop relying upon average rate, rather than fluctuation, also exists (Carney, 2018), and these would have opposing effects. A role for stimulus fluctuation in MOC efferent control may therefore be difficult to detect.

The role of MOC efferent control in the development of NIS has been verified in mice: the degree of NIHHL was found to be positively correlated with the activity level of cholinergic receptors that were regulated by genetic manipulation (Boero et al., 2018, 2020). However, it is not clear if noise exposure itself can change MOC control of outer hair cells. Overall, our results did not show a reduction of CS to CAP in the noise group. Instead, there was an enhancement of the CS effect. Further studies are needed to confirm this enhancement in subjects with a larger amount of synaptic damage and loss.

In conclusion, the present study demonstrated that modulated, intermittent noise exposure common in real life is less effective in causing NIS. The risk of NIS without PTS and NIHHL may thus be lower than previously thought. With the smaller amount of NIS established by the noise exposure in this study, degradations in signal processing were likely limited and not reflective of those occurring with more severe NIS and NIHHL. Interestingly, while temporal processing dysfunction was seen in the far-field EFR TMTF, corresponding changes were not shown *via* nfEFR, suggesting a central origin for the changes in temporal processing. In contrast, a greater effect of masking on the EFR with NIS was only found in near-field measures, suggesting a peripheral origin for this effect along with central compensation. This result devalues the usefulness of EFR in evaluating the coding deficits associated with NIS. Furthermore, the temporal processing dysfunction did not appear to be related to the masking effect, given the different origins and the lack of any significant difference between the masking effect found with a stationary versus modulated masker. Finally, the results were not supportive of a role of temporal fluctuation in the MOC efferent control on cochlear gain.

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 animal study was reviewed and approved by Dalhousie University Committee of Laboratory Animals.

AUTHOR CONTRIBUTIONS

SR, XY, and SA: conceptualization, visualization, and writing. LX and JW: conceptualization, visualization, writing, and funding acquisition. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.935371/full#supplementary-material

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REVIEW
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The Role of Genetic Variants in the Susceptibility of Noise-Induced Hearing Loss

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Noised-induced hearing loss (NIHL) is an acquired, progressive neurological damage caused by exposure to intense noise in various environments including industrial, military and entertaining settings. The prevalence of NIHL is much higher than other occupational injuries in industrialized countries. Recent studies have revealed that genetic factors, together with environmental conditions, also contribute to NIHL. A group of genes which are linked to the susceptibility of NIHL had been uncovered, involving the progression of oxidative stress, potassium ion cycling, cilia structure, heat shock protein 70 (HSP70), DNA damage repair, apoptosis, and some other genes. In this review, we briefly summarized the studies primary in population and some animal researches concerning the susceptible genes of NIHL, intending to give insights into the further exploration of NIHL prevention and individual treatment.

Keywords: genes, noised-induced hearing loss, noise prevention, susceptibility, genetic variants

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INTRODUCTION

The auditory system helps people to hear sound, understand language, and even distinguish people or objects by recognizing different sounds. Any organic or functional impairment of the auditory pathway can lead to hearing impairment. According to a WHO report (Castaneda et al., 2019), more than 100 million people in East Asia are at risk of disabling hearing loss, leading to lifelong disability, and deafness has become one of the major problems affecting their life quality. Sensorineural hearing loss may be caused by pathological changes in the Corti's organ of the inner ear, the auditory nerve, or the auditory cortex. It is characterized by the impairment of sound perceptive and analytic ability, and classified as drug-induced hearing loss, presbycusis, hereditary hearing loss, noise-induced hearing loss (NIHL) and others. Although cochlear implant technology has been increasingly advanced in the treatment of hearing loss, its therapeutic effect varies with different lesion sites, therefore, sensorineural hearing loss remains one of the most challenging medical problems.

Noise pollution is one of the seven public hazards in modern society. NIHL, one of the hot spots of social concern, is the second cause of hearing loss in adults, and more than 6% of the global population is affected by NIHL according to WHO data (Sliwinska-Kowalska and Zaborowski, 2017). NIHL is an acquired hearing loss caused by long-term exposure of the auditory system to noise generated by construction, entertainment, industrial production, military equipment or others, and its incidence is only behind presbycusis among all the types of sensorineural hearing loss

(Miao et al., 2019). The A-frequency weighting network (dBA) is normally utilized to measure the levels of noise in decibels (dB) of sound pressure, indicating the risk of NIHL (Varela-Nieto et al., 2020). Moreover, the principal requirements for the diagnosis of NIHL are high-frequency hearing impairment, jeopardous amount of noise exposure and recognizable high-frequency audiometric notch or bulge (Coles et al., 2000). Addition to the auditory symptoms such as hearing descending, hearing allergy, tinnitus, the noise damage may also present as mental disorder, digestive disorder or some other organic dysfunction (Skogstad et al., 2016; Hahad et al., 2019).

Long-term noise exposure can lead to damage of peripheral auditory system, including the structure of cochlea hair cells, cilia, supporting cells, and tectorial membrane (Wang et al., 2002), hitting the external layer of hair cells the most, and the Corti's organ and spiral ganglion may also undergo degenerative changes (Henderson et al., 2006). The main manifestations of which are increased hearing threshold, decreased auditory sensitivity and speech resolution, tinnitus, and auditory hypersensitivity. A "V"-shaped depression appears at 4k Hz on the audiogram, which is called "V"-shaped notch hearing loss (Carroll et al., 2017). Low intensity or short time noise exposure can cause temporary changes of the auditory nerve synaptic transmitter, resulting in temporary hearing loss which could return to normal after the noise ceased, in terms of temporary threshold shift (TTS) (Kurabi et al., 2017). High intensity or long-time noise exposure causes damages on both hair cells and auditory nerve, resulting in hearing loss that could not be restored, which is called permanent threshold shift (PTS), and eventually leads to sensorineural hearing loss (Liberman, 2016).

Long-term noise exposure may also cause damage to the central auditory system, which mainly occurred in the cochlear nucleus, olivary nucleus, medial geniculate body, inferior colliculus, hippocampus and auditory cortex (Kujawa and Liberman, 2009; Eggermont, 2017). Most previous studies believed that the auditory cortex was the most vulnerable part under noise exposure, but Cheng et al. (2016) showed that the hippocampus may be more sensitive than the auditory cortex, mainly manifested as headache, dizziness, irritability, insomnia, memory loss and even serious mental problems (Eraslan et al., 2015). Long-term noise exposure can increase the expression of corticotropin releasing-hormone (CRH) in the hippocampus and decrease the inhibition of the hypothalamic-pituitary-adrenal (HPA) axis, which may worsen depression and anxiety (Valentino et al., 2010).

In this study, we searched papers published in English and Chinese *via* PubMed, Embase, Scopus, and Web of Science database, intending to provide an overview of current knowledge relevant to the pathogenesis and susceptibility genes to NIHL.

PATHOGENESIS OF NOISED-INDUCED HEARING LOSS

Environmental and genetic factors can both contribute to NIHL. Environmental factors include noise intensity, noise spectrum characteristics, noise exposure time, etc. Genetic factors mainly refer to NIHL susceptibility genes. Presently, there are four main theories about the pathogenesis of NIHL, including mechanical theory, vascular theory, metabolic theory, and immunoinflammatory theory.

Mechanical Theory

According to the mechanical theory, the internal tissue structure damage of cochlea caused by noise with over 130 dB intensity is mainly attributed to the mechanical damage (Patterson and Hamernik, 1997). High intensity noise impacts the Corti's organ and forms a strong liquid eddy current in the cochlear duct, which can cause the rupture of the vestibular membrane and lead to the fusion of endolymph and perilymph. The cytotoxic K^+ in endolymph could reach the tympanic scala through the orifice of the cupula cochleae and then reach the lymphatic space of the Corti's organ, where the contact of K^+ with hair cells leads to the destruction of cochlear sensory epithelial cells, atrophy of stria vascularis and degeneration of auditory nerve fibers. The other ways of mechanical injury were the rupture of the reticular laminae of the basilar membrane or the separation of the stereocilium of the outer hair cells from the cuticular plate, which can cause the K^+-rich endolymph to come into contact with the hair cells. In more serious cases, noise-induced mechanical force can also cause the Corti's organ to peel off from the basilar membrane (Spoendlin and Brun, 1973; Rajguru, 2013).

Vascular Theory

Vascular theory believes that long-term strong noise exposure may lead to vasoconstriction around the cochlear sensory epithelium, swelling vascular endothelial cell, narrowing vascular lumen, slowed blood flow velocity, decrease in local blood perfusion, increase in blood viscosity, accumulation of platelets and red blood cells in capillaries, and obvious thickening of capillary walls. All of the aforementioned factors may ultimately lead to cochlear ischemia and hypoxia, resulting in decreased activity of otoprotective enzymes, accumulation of cellular metabolites in cells, and damage to cochlear hair cells and the Corti's organ (Kim et al., 2018). Significant inner ear injury occurs when the perilymph oxygen partial pressure decreases by about 20% (Wu et al., 2014).

Metabolic Theory

According to the metabolic theory, noise exposure could lead to extensive metabolic changes in the auditory system. Overexpression of free radicals including reactive oxygen species (ROS) and reactive nitrogen species (RNS) in cochlea leads to the formation of lipid peroxides and accelerates hair cells apoptosis (Zhang et al., 2017). The isoconstrictive vascular substances such as isoprostaglandin and 8-iso-prostaglandin F2α could also be released from cochlear vascular system and the Corti's organ (Honkura et al., 2016). Strong noise exposure results in abnormal influx of K^+ ions, leading to depolarization of membrane potential and abnormal influx of Ca^{2+}, which is termed as calcium overload (Vicente-Torres and Schacht, 2006; Wang et al., 2007a). Excitotoxicity caused by large amount of glutamate release leads to edema and vacuolation of inner hair cells, neurotrophic factor deficiency, and mitochondrial

dysfunction, inducing acute hair cell damage (Fridberger et al., 1998). Besides, cytokines and chemokines such as tumor necrosis factor (TNF-α), IL-6 and IL-1β are upregulated, which make contributions to the cascading amplification of exogenous and endogenous apoptotic signaling pathways, promoting the release of pro-apoptotic proteins, leading to the activation of Caspase-3, chromatin concentration, and DNA damage. Le Prell et al. (2007) believed that metabolic injury played a key role in the pathogenesis of NIHL.

Immunoinflammatory Theory

Macrophages are the main natural immune cells in the cochlea and are important drivers of inflammation and tissue repair after noise exposure. In normal condition, cochlear macrophages inhabit spiral ligaments, spiral ganglion, basilar membrane and stria vascularis (Shi, 2010). The distribution, phenotype, number, morphology and functional state of cochlear macrophages were significantly changed after noise exposure (He W. et al., 2020). Due to the existence of tight junctions, the infiltration of macrophages and monocytes into the scala media is mainly confined to the scala tympani cavity beneath the basilar membrane, avoiding the damage and apoptosis of hair cells (Frye et al., 2018). After noise exposure, signaling pathway such as Toll-like receptor 4 (TLR-4)/nuclear factor kappa-B (NF-κB) and mitogen-activated protein kinase (MAPK)/c-Jun N-terminal kinase (JNK) were activated in cochleae, leading to upregulation of downstream inflammatory factors and chemokines including TNF-α, IL-6, IL-12, IL-1β, intercellular cell adhesion molecule-1 (ICAM-1), and monocyte chemoattractant protein-1 (MCP-1) (Wang et al., 2007b; Zhang G. et al., 2019). The release of theses cytokines and chemokines set off chain inflammatory reactions (Frye et al., 2019).

DIFFERENCES IN INDIVIDUAL SUSCEPTIBILITY TO NOISED-INDUCED HEARING LOSS

Noised-induced hearing loss (NIHL) is ranked as the highest incidence of industrial injury in the United States (Ralli et al., 2017), where 16% adult hearing loss are caused by exposure to industrial noise. While NIHL is a typical type of hearing loss, the causes of it are attributable to both environmental and genetic factors. Long-term exposure to noise is a prominent environmental factor for NIHL, but some studies found that not every worker who exposed to the same level of noise would develop NIHL, and the severity of NIHL also varies greatly (Irion, 1981; Hood, 1987). Taylor et al. (1965) detected the hearing threshold of workers in a textile factory and found that the workers with similar length of service had different hearing threshold which ranged from 10 to 70 dB.

In recent years, with studies of large-scale samples, it is known that even for the subjects exposed to the noise environment with similar density and duration, their hearing threshold shifts has significant individual differences (Lu et al., 2005). It reveals that there is a great difference in the susceptibility to NIHL among the population.

RESEARCH METHODS OF NOISED-INDUCED HEARING LOSS SUSCEPTIBLE GENE

After a comprehensive analysis of some experimental studies, we summarized the methods of population research for NIHL susceptible genes as follows: sufficient number of subjects with history of noise exposure were selected as the research object, strict inclusion criteria were established, and the population whose hearing threshold locates higher than 25dB was recruited into case group, whose hearing thresholds was less than or equal to 25dB was selected into control group. The candidate genes of the two groups were detected by implementing case-control study.

There are mainly three methods for the selection of candidate NIHL genes: (1) selection of genes that have been preliminarily confirmed in animal models (2) selection of susceptibility genes that have been reported in other types of deafness; and (3) according to the pathogenic mechanism of NIHL, detect relevant genes in the corresponding pathways.

At present, the techniques for detecting susceptible genes include microarray chip, polymerase chain reaction (PCR) - restriction enzyme digestion, quantitative reverse transcription PCR, amplification refractory mutation system (ARMS)-PCR, high-throughput sequencing, and whole-exome sequencing (WES) etc. Genetic screening was carried out and compared between the two population to determine the susceptible genes which might have important influence on the pathogenesis and development of NIHL.

SUSCEPTIBILITY GENES OF NOISE-INDUCED HEARING LOSS

According to the pathogenesis of NIHL, recent studies have revealed a large group of genes that are linked to NIHL involving oxidative stress, potassium ion cycling, cilia structure, heat shock protein genes 70, DNA damage repair, apoptosis, monogenic NIHL genes and others. The distribution of major susceptibility genes and the functions they are involved in is shown in **Figure 1**. The summary of NIHL susceptible genes and their locus is concluded in **Table 1**.

Antioxidant Genes

According to the metabolic theory, oxidative stress plays a major role in the pathomechanisms of NIHL (Spoendlin and Brun, 1973; Rajguru, 2013; Chen et al., 2020). Mutations of oxidative stress related genes would disturb the balance of the oxidative and antioxidative system in the cochlea, thus fail to eliminate the oxidative damage of ROS, leading to the structural and functional disorders of the cochlea which ultimately result in hearing loss.

ATPase Plasma Membrane Ca^{2+} Transporting 2 (ATP2B2, PMCA2)

ATP2B2, encoding plasma membrane calcium-transporting ATPase isoform2 (PMCA2), is located on human chromosome region 3p25, and played an important role on intracellular

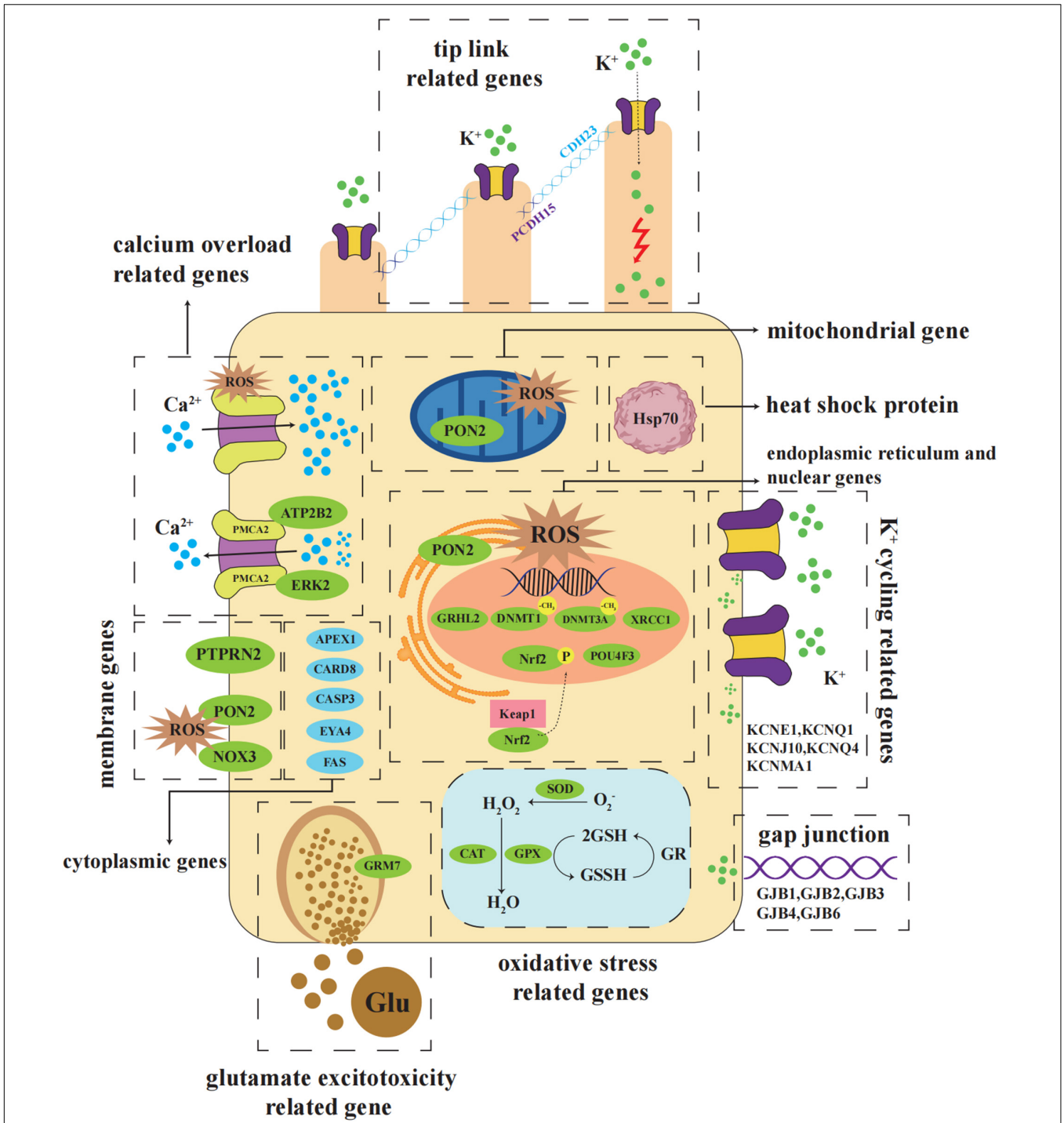

FIGURE 1 | Schematic diagram of major NIHL susceptible genes distributed in the hair cells. NIHL susceptible genes are involved in the progression of oxidative stress, potassium ion cycling, calcium overload, glutamate excitotoxicity, DNA damage repair, apoptosis, and other biochemical processes. They are distributed in various locations in cells, including membrane, cytoplasm, nucleus, mitochondria, and endoplasmic reticulum. Abbreviations: Glu, Glutamate; GR, Glutathione reductase; GSH, Glutathione; GSSH, Glutathione oxidized; Keap1, Kelch-like ECH associated protein 1; PMCA2, Plasma membrane calcium-transporting ATPase 2; ROS, Reactive oxygen species.

calcium homeostasis (Yang et al., 2013). In an animal experiment, Kozel et al. (2002) hypothesized that *Atp2b2*$^{+/-}$ mice may be more susceptible to NIHL. Recently, Li X. et al. (2016) designed a study to investigate whether genetic variability in *ATP2B2* was associated with high susceptibility to NIHL in Chinese Han nationality population. However, no significant main effect

was observed for *ATP2B2* gene single-nucleotide polymorphisms (SNPs) (rs1719571, rs3209637 and rs4327369) in their study because of the small sample size. In another case-control study of 760 Chinese textile workers, the results indicated that the rs3209637 C genotype of *ATP2B2* may lead to a greatly increased incidence of NIHL. Meanwhile, the analysis also demonstrates that *ATP2B2* SNPs (rs1719571, rs14154, and rs3209637) have a great effect on NIHL (Zhang S. et al., 2019).

Catalase

Catalase (CAT) is a ubiquitous enzyme in all organisms, functioning as a key antioxidant enzyme in the defense against oxidative stress. Catalase encoded by *CAT* gene can decompose hydrogen peroxide (H_2O_2), maintain the balance of redox in the body, and reduce the oxidative damage of cochlea caused by oxidative stress. Yang et al. (2015) screened 719 unrelated Chinese Han adults, including 225 healthy volunteers and 494 noise-exposed workers, and found that rs208679 and rs769217 SNPs were significantly associated with the susceptibility to NIHL. For rs208679 recessive effect, GG genotype showed significantly augmented risk when exposing to noise less than 85dB, while for rs769217 dominant effect, TT/TC combined genotypes significantly increased the risk of NIHL when noise intensity was between 85dB-92dB.

Glutathione Peroxidase 1

The GPX protein belongs to the glutathione peroxidase (GPx) family, which reduces H_2O_2 and organic hydroperoxides originated from Fenton and Haber Weiss reactions coupling with other glutathione (GSH) and GSH reductase redox systems (Evans and Halliwell, 1999). GPx oxidizes GSH into glutathione oxidized (GSSH), while glutathione reductase (GR) reduces GSSH into GSH. Moreover, H_2O_2 is catalyzed and broke down into H_2O by GPx and CAT to achieve antioxidant effects (**Figure 1**). Ohlemiller et al. (2000) performed research to investigate the association between cellular *Gpx1* gene and the susceptibility to NIHL in mice. The significant results revealed that *Gpx*-deficient mice showed increased susceptibility to NIHL. Wen et al. (2014) scrutinized the relationship between SNPs of GPX1 gene rs3448, rs1050450, rs1800668, and rs1987628, and the risk of developing NIHL among Chinese Han population. They clarified that GPX1 SNP rs1987628 may be a risk factor of NIHL. Another study of a limited sample set using genotyping kit to analyze the SNPs discovered that the individuals carrying rs1987628 GA genotype of GPX1 had a higher NIHL risk than those carrying the GG genotype (Li J. Y. et al., 2020).

Glutathione S-Transferase

Glutathione S-transferase (GST) can catalyze the binding of a variety of endogenous or exogenous compounds to reduced glutathione, which serves as an important protective antioxidant factor in the cochlea. Shen et al. (2012) analyzed the polymorphism of *GST* gene in 444 workers with NIHL and 445 workers with normal hearing to find out the relationship between the polymorphism and the susceptibility to NIHL. The results showed that null genotype of *GSTM1* rs10712361 had a higher risk of NIHL comparing with wild-type genotype. Lin et al. (2009) found that individuals carrying all genotypes with *GSTM1* null, *GSTT1* null, and *GSTP1 lle* (Guo et al., 2017)/*lle* (Guo et al., 2017) were more susceptible to NIHL.

Nuclear Factor Erythroid 2-Related Factor 2

NRF2, existing widely in tissues, is a key transcription factor in the regulation of oxidative stress. When affected by oxidative stress, NRF2 dissociates from Kelch like epichlorohydrin associated protein 1 (Keap1), a negative regulator of NRF2, and is transferred to the nucleus to recognize and bind antioxidant response elements (ARE) (Sivandzade et al., 2019). Thus, the transcription of downstream antioxidant enzyme genes is initiated, including heme oxygenase 1 (*HO-1*), superoxide dismutase (*SOD*), triphosphopyridine nucleotide (*NADPH*), *GST*, *GR* and *GPx* (He F. et al., 2020). Honkura et al. (2016) explored the contribution of *Nrf2* to cochlear protection *via* $Nrf2^{-/-}$ mice models. They found that *Nrf2* deficiency could exacerbate NIHL as auditory brainstem response (ABR) threshold shifts of the $Nrf2^{-/-}$ mice was significantly larger than the wild-type mice at 7 days post-exposure. Although noise exposure does not obviously change the expression of *Nrf2* target genes, the potent NRF2-activating drug, CDDO-Im used before the noise exposure could preserve the integrity of hair cells and improve post-exposure hearing level. Wang et al. (2019) found that persons with a G allele (*NRF2* tagSNP rs6726395) in addition to rs77684420 and the rs6726395, rs1962142, rs6721961, and rs77684420 haplotype had associations that may be more susceptible to NIHL.

Triphosphopyridine Nucleotide Oxidase-3

The NOX family of ROS-generating NADPH oxidases consists of 7 members: NOX1 to NOX5, DUOX1 and DUOX2. In particular, NOX3 is almost exclusively expressed in the inner ear, and it has been demonstrated to generate superoxide constitutively which is converted to H_2O_2 by SOD, which can in turn participate in cell signaling events (Krause, 2004; Forman et al., 2010). In a previous study, a significant reduction in the intensity of *NOX3* immunolabeling was observed in the inner sulcus region of the cochlea after noise exposure, and down-regulation of *NOX3* may represent an endogenous protective mechanism to reduce oxidative stress in the noise-exposed cochlea (Vlajkovic et al., 2013). Xin et al. (2021) conducted a case-control study in five factories in China, and illustrated the association between rs12195525 and NIHL susceptibility. For further exploration, Lavinsky et al. (2015) verified that *Nox3* is involved in NIHL susceptibility in $Nox3^{het}/Nox3^{het}$ and $Nox3^{het}/+$ mutant mice, which was frequency specific at 8 kHz. Besides, the significant and highly potential association of rs33652818 with ABR at 8 and 4 kHz was observed.

Paraoxonase-2

PON2 gene, localized in endoplasmic reticulum (ER), mitochondria and nuclear envelope, is involved in the process of defending ROS, ER stress, mitochondrial superoxide formation, and apoptosis (Altenhofer et al., 2010; Witte et al., 2011). Li X. et al. (2016) studied the polymorphisms of rs12026, rs7785846, and rs12704796 in *PON2* in 221 patients with NIHL and 233

TABLE 1 | Summary of NIHL susceptible genes and their locus.

Groups of genes	Gene	Full name	Genetic locus	References
Antioxidant genes	*APEX1*	Apurinic/Apyrimidinic endodeoxyribonuclease 1	rs1130409, rs1760944	Shen et al., 2016; Ding et al., 2019
	ATP2B2 (PMCA2)	ATPase plasma membrane Ca^{2+} transporting 2	rs1719571, rs3209637, rs14154	Kozel et al., 2002; Li X. et al., 2016; Yan et al., 2013; Zhang S. et al., 2019
	CAT	Catalase	rs769217, rs208679, rs7943316, rs769214, rs475043, rs12273124, rs494024, rs564250	Konings et al., 2007; Xia et al., 2011; Yang et al., 2015; Li T. et al., 2020
	GPX1	Glutathione peroxidase 1	rs1987628	Wen et al., 2014; Li J. Y. et al., 2020
	GST	Glutathione S-transferase	rs1695, rs1049055, rs10712361	Lin et al., 2009; Shen et al., 2012; Loukzadeh et al., 2019; Zong et al., 2019; Li Y. H. et al., 2020
	NFE2L2 (NRF2)	Nuclear factor erythroid 2-related factor 2	rs77684420, rs6726395, rs1962142, rs6721961	Honkura et al., 2016; Wang et al., 2019
	NOX3	NADPH Oxidase 3	rs12195525, rs33652818	Lavinsky et al., 2015; Xin et al., 2021
	PON2	Paraoxonase 2	rs12026, rs7785846, rs12704796, rs987539, rs7493, rs7786401	Cao et al., 2013; Li X. et al., 2016; Bhatt et al., 2020; Wu et al., 2020; Zhou H. et al., 2020
	SOD1	Superoxide dismutase 1	rs2070424, rs10432782	Liu et al., 2010b
	SOD2	Superoxide dismutase 2	rs4880, rs2855116	Ohlemiller et al., 1999; Fortunato et al., 2004; Liu et al., 2010a; Wang et al., 2014; Wang J. et al., 2017
Potassium ion cycling related genes	*KCNQ1*	Potassium voltage-gated channel subfamily Q member 1	rs800336, rs2056892, rs2011750, rs2283158, rs2283179, rs2283205, rs231899, rs760419, rs163171, rs8234, rs7945327, rs11022922, rs718579, rs463924	Van Laer et al., 2006; Pawelczyk et al., 2009; Ding et al., 2020
	KCNQ4	Potassium voltage-gated channel subfamily Q member 4	rs34287852, rs2769256, rs727146, rs4660468, rs12143503, rs4660470	Van Laer et al., 2006; Pawelczyk et al., 2009; Guo et al., 2018b; Zhou W. H. et al., 2020
	KCNE1	Potassium voltage-gated channel subfamily E regulatory subunit 1	rs915539, rs2070358, rs1805127, rs1805128	Van Laer et al., 2006; Ding et al., 2020
	KCNJ10	Potassium voltage-gated channel subfamily J member 10	rs1130183, rs1186675	Van Laer et al., 2006; Pawelczyk et al., 2009; Bhatt et al., 2020
	KCNMA1	Potassium calcium-activated channel subfamily M alpha 1	rs696211, rs1436089	Konings et al., 2009b; Zhang X. et al., 2019
	GJB1 (Cx32)	Gap Junction Protein Beta 1	rs747181, rs1997625	Van Laer et al., 2006; Pawelczyk et al., 2009
	GJB2 (Cx26)	Gap Junction Protein Beta 2	rs3751385, rs5030700, rs137852540	Van Laer et al., 2006; Pawelczyk et al., 2009; Zhou et al., 2016
	GJB3 (Cx31)	Gap Junction Protein Beta 3	rs476220	Van Laer et al., 2006
	GJB4 (Cx30.3)	Gap Junction Protein Beta 4	rs1998177, rs755931	Van Laer et al., 2006; Pawelczyk et al., 2009
	GJB6 (Cx30)	Gap Junction Protein Beta 6	rs945370, rs2065796, rs2065797	Van Laer et al., 2006
	SLC12A2	Solute carrier family 12 member 2	rs1962291, rs1560637, rs790153, rs790156, rs10089	Van Laer et al., 2006
Cilia structure related genes	*CDH23*	Cadherin related 23	rs1227049, rs1227051, rs3802711, rs3752752, rs41281334	Yang et al., 2006; Kowalski et al., 2014; Yu et al., 2016; Bhatt et al., 2020; Jiao et al., 2020; Jiang et al., 2021
	PCDH15	Protocadherin related 15	rs11004085, rs7095441, rs1100085, rs10825122, rs1930146, rs2384437, rs4540756, rs2384375	Konings et al., 2009b; Xu et al., 2017a,b
	MYH14	Myosin heavy chain 14	rs667907, rs588035	Konings et al., 2009b; Fu et al., 2016

(Continued)

TABLE 1 | (Continued)

Groups of genes	Gene	Full name	Genetic locus	References
Heat shock protein genes 70	*HSPA1A*	Heat shock protein family A member 1A	rs1043618, rs1061581	Li et al., 2017
	HSPA1B	Heat shock protein family A member 1B	rs2763979	Konings et al., 2009a; Chang et al., 2011
	HSPA1L	Heat shock protein family A member 1L	rs2075800, rs2227956	Chang et al., 2011; Li Y. H. et al., 2016; Li et al., 2017
DNA damage repair related genes	*DNMT1*	DNA methyltransferase 1	rs2228611	Guo et al., 2018a
	DNMT3A	DNA methyltransferase 3 alpha	rs749131, rs1550117	Guo et al., 2018a
	EYA4	EYA transcriptional coactivator and phosphatase 4	rs3777781, rs212769, rs3813346, rs9321402, rs9493627	Zhang et al., 2015; Yang Q. et al., 2016; Yang et al., 2017
	OGG1	8-Oxoguanine DNA glycosylase	rs1052133	Shen et al., 2014
Apoptosis related genes	*CASP3*	Caspase 3	rs1049216, rs6948	Wu et al., 2017
	ERK2 (MAPK1)	Extracellular signal-regulated kinase 2	Null (animal experiment)	Kurioka et al., 2015
	JNK1 (MAPK8)	C-Jun N-terminal kinases 1	rs11598320, rs8424	Sun et al., 2021
Other NIHL susceptible genes	*AUTS2*	Activator of transcription and developmental regulator	rs35075890	Niu et al., 2021
	CARD8	Caspase recruitment domain family member 8	rs2043211	Miao et al., 2021
	DFNA5 (GSDME)	Gasdermin E	rs2521758	Zhang et al., 2015
	FAS	Fas cell surface death receptor	rs1468063, rs2862833	Xu et al., 2021
	FOXO3	Forkhead box O3	rs2802292, rs10457180, rs12206094	Guo et al., 2017, Guo et al., 2018c; Jiao et al., 2017
	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	rs6489721	Wan et al., 2020
	GRHL2	Grainyhead like transcription factor 2	rs3735715, rs1981361, rs666026, rs611419	Li X. et al., 2013; Zhang et al., 2015; Xu et al., 2016; Yang Q. Y. et al., 2016; Li X. et al., 2020
	GRM7	Glutamate metabotropic receptor 7	rs1485175, rs1920109, rs9826579	Yu et al., 2018a,b
	HDAC2	Histone deacetylase 2	rs10499080, rs6568819	Wang et al., 2021
	HOTAIR	HOX transcript antisense RNA	rs4759314	Wang B. et al., 2017
	IL-6	Interleukin 6	rs1800795	Braga et al., 2014
	ITGA8	Integrin subunit alpha 8	rs10508489	Xia et al., 2011
	NCL	Nucleolin	rs7598759	Grondin et al., 2015
	NOTCH1	Notch receptor 1	rs3124594, rs3124603	Ding et al., 2018
	NRN1	Neuritin 1	rs3805789	Liu et al., 2021
	PER1	Period circadian regulator 1	rs2585405	Chen et al., 2021
	POU4F3	POU class 4 homeobox 3	rs1368402, rs891969	Xu et al., 2016
	PTPRN2	Protein tyrosine phosphatase receptor type N2	rs10081191	Niu et al., 2021
	SIK3	Salt-inducible kinase 3	rs493134, rs6589574, rs7121898	Yin et al., 2020
	STAT3	Signal transducer and activator of transcription 3	rs1053005	Gao et al., 2021
	TSP	Thrombospondin	Null (animal experiment)	Smeriglio et al., 2019
	UBAC2	UBA domain containing 2	rs3825427	Wan et al., 2022
	WHRN	Whirlin	rs12339210	Jiang et al., 2021
	XPO5	Exportin 5	rs11077	Wang et al., 2020
	XRCC1	X-Ray repair cross complementing 1	rs1799782	Ding et al., 2019

subjects with normal hearing by logistic regression analysis. It was found that rs12026 CG and CG + GG genotypes and rs7785846 CT and CT + TT genotypes were highly susceptible to NIHL. Wu et al. (2020) confirmed these results that *PON2* gene affects the NIHL susceptibility of cochlea.

Superoxide Dismutase 1 and 2

Superoxide Dismutase (SOD) is an important antioxidant enzyme in organisms and the primary substance for scavenging ROS in the body, which is involved in the reaction of superoxide anion (O_2^-) and H^+ to produce H_2O_2. It plays an important role on blocking cell damages caused by ROS and repairing the damaged cells in time. Liu et al. (2010a,b) analyzed the audiometric data of 2400 Chinese Han people exposed to occupational noise, and selected the 10% most susceptible and the 10% most resistant individuals as subjects to collect DNA samples. It has been found that the *SOD1* AA genotype at the rs2070424 was protective against NIHL, while the *SOD1* GG genotype of rs10432782 and the CT genotype of rs4880 (*SOD2* V16A SNP) was associated with higher occurrence of NIHL. However, the above results were not in agreement with a former research based on a Swedish population, which suggests that *SOD* genetic polymorphism may confer a race-specific contribution (Carlsson et al., 2005).

Potassium Ion (K^+) Cycling Related Genes

As an important charge carrier in the process of sound sensory conduction, K^+ can be secreted to the endolymph, and then utilized by the sensory hair cells of the inner ear through the mechanically sensitive K^+ channel, and this ion circulation ensures the generation of hearing. The related genes which has been proved susceptible to NIHL are illustrated in **Figure 2**.

Potassium Voltage-Gated Channel Subfamily E Regulatory Subunit 1 and Potassium Voltage-Gated Channel Subfamily Q Member 1

KCNE1 encodes a regulatory subunit of the KCNQ1 potassium channel-complex. Both KCNE1 and KCNQ1 are necessary for normal hearing. Pawelczyk et al. (2009) performed a study to clarify the hypothesis that genetic variability in genes of the potassium recycling pathway may be a risk factor for the development of NIHL. The significant results revealed that the AA genotype in rs2070358 appeared more frequently in resistant individuals than in susceptible ones, while genotype GG was more often among susceptible subjects. Recently, another study (Ding et al., 2020) was designed to investigate the association between genetic mutations in the *KCNE1* gene and susceptibility to NIHL in the Chinese population. Their results showed that the rs3453 C allele and the rs1805127 G allele were associated with increased susceptibility to NIHL.

Potassium Voltage-Gated Channel Subfamily Q Member 4

Potassium Voltage-Gated Channel Subfamily Q Member 4 (KCNQ4) is a voltage-gated potassium channel that plays essential roles on maintaining ion homeostasis and regulating hair cell membrane potential. Guo et al. (2018b) conducted a genetic association study to scrutinize the association between *KCNQ4* polymorphism and susceptibility to NIHL. They detected that rs4660468 CT/TT genotype and T allele may increase the susceptibility. In another study among Chinese population, the SNPs of rs4660468, rs4660470, rs34287852 in *KCNQ4* were genotyped by Zhou W. H. et al. (2020). They identified that the risk of developing NIHL in subjects carrying TA genotype of rs4660470 was 2.197 times than the one carrying TT genotypes, suggesting that the mutant allele A of rs4660470 in *KCNQ4* may be a risk factor for developing NIHL.

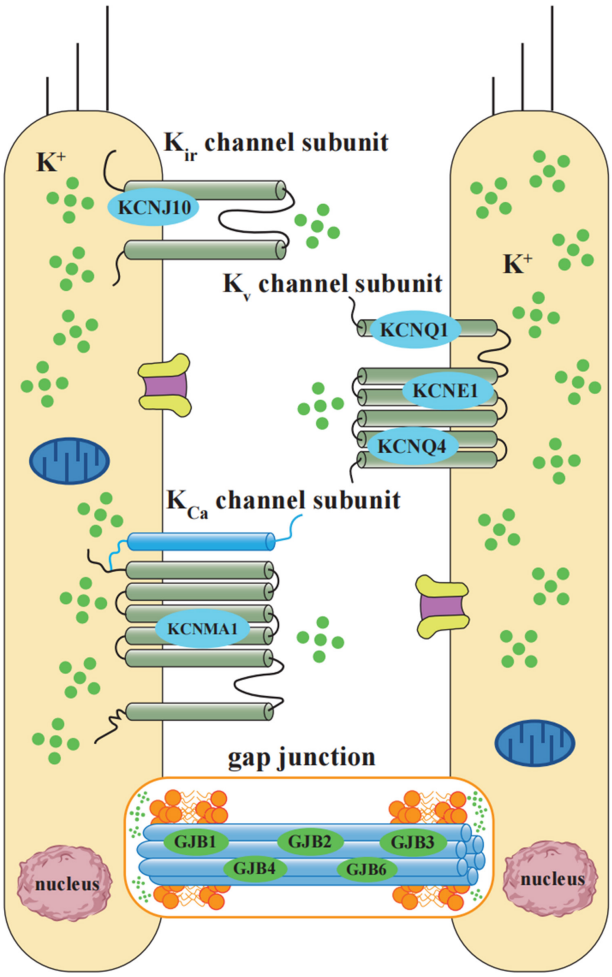

FIGURE 2 | Schematic diagram of potassium ion cycling related NIHL susceptibility genes. K^+ cycling related NIHL susceptibility genes include K^+ channel proteins and gap junction proteins. According to the operational mechanism and structures, NIHL susceptibility related K^+ channels can be classified into 3 groups: inward rectifier (K_{ir}, including *KCNJ10*), voltage-gated (K_V, including *KCNQ1*, *KCNE1*, and *KCNQ4*), and Ca^{2+}activated (K_{Ca}, including *KCNMA1*). Gap junctions between hair cells and non-sensory cells are primarily formed by a family of connexin proteins, which is encoded by gene *GJB1*, *GJB2, GJB3, GJB4,* and *GJB6.* Gap junction-mediated intercellular communication plays an essential role in K^+ exchange.

Potassium Inwardly Rectifying Channel Subfamily J Member 10

KCNJ10 encodes the inward-rectifying potassium channel that is expressed in the brain, the inner ear, and kidney. Pawelczyk et al. (2009) conducted a study to explore the putative hypothesis that genetic variations in ten genes associated with the potassium recycling pathway in the inner ear may influence susceptibility to the development of NIHL. Their results discovered that the polymorphism of rs1130183 in *KCNJ10* may be a risk factor for the development of NIHL. In addition, Bhatt et al. (2020) performed research to investigate the relationship between candidate genetic variants and NIHL in young musicians, they also identified that *KCNJ10* rs1130183 showed significant association with the distortion product otoacoustic emission (DPOAE) signal-to-noise ratio (SNR) in the right ear.

Gap Junction Protein Beta 2 (Connexin 26, Cx26)

GJB2, encoding a gap junction protein expressed in the inner ear, has been considered to be involved in the potassium recycling pathway in the cochlea. Van Eyken et al. (2007) performed a study to investigate the association between the *GJB2* 35delG mutation and the development of NIHL. Frustratingly, the results suggested that 35delG carriers had no increased susceptibility to the development of NIHL. However, in an animal study, Zhou et al. (2016) established a *Connexin26* knockdown mouse model to investigate the relationship between *Connexin26* gene and NIHL. Their results indicated that decreased *Connexin26* expression may contribute to the increased susceptibility to NIHL and promote the cell degeneration in the Corti's organ.

Cilia Structure Related Genes

Tip links of the hair cells play a crucial role in the process of mechano-electrical transduction (MET), transforming the mechanical sound stimuli into electrical signals (Sakaguchi et al., 2009). The main constituent of tip links are cadherin related 23 (CDH23) and procadherin related 15 (PCDH15), atypical members of the cadherin superfamily. Cadherin is a calcium-dependent cellular adhesion glycoprotein, which plays an important role in cell recognition, migration, tissue differentiation, the composition of adult tissues and embryonic development. The polymorphism of those genes is closely related to the susceptibility to NIHL. Besides, the damage of MYH14, located at the tip links between hair cells and hair cells, hair cells and supporting cells, also leads to susceptibility to NIHL.

Cadherin Related 23

Cadherin Related 23 (CDH23) is an important protein which is mainly expressed in the cilia of inner hair cells and vestibular membrane (Wilson et al., 2001). Anchored to ciliated microfilaments by actin, it forms a protein network with myosin VIIA for functional activity (Boeda et al., 2002). Its primary function is to maintain the structure and function of hair cell cilia and the ion composition of endolymph, which ensure the mechanical-electrical conversion of sound waves can be carried out normally during the transduction of sound waves in the inner ear (Siemens et al., 2004). It was evidenced in adult mice that *Cdh23* mutant mice were susceptible to NIHL. The results showed that the threshold of compound action potential (CAP) was increased by about 50dB at 12 kHz and 30 kHz frequency, which was more than twice that of wild type mice (Holme and Steel, 2004). Kowalski et al. (2014) selected 314 workers with the worst hearing as the experimental group and 313 workers with the best hearing as the control group from 3860 workers database exposed to the same noise environment. Statistical analysis showed that the genotype of the SNP rs3752752 located in exon 21 was closely related to NIHL susceptibility, in which CC genotype was more common in susceptible population, while CT genotype appeared more frequently in the group with better noise tolerance. Another study (Yang et al., 2006) revealed that individuals with rs3802721TT genotype, rs1227049CC genotype and GG genotype at the end of exon 7 were more susceptible to NIHL.

Procadherin Related 15

PCDH15 encodes a membrane protein that mediates calcium-dependent cell adhesion. It is considered that tip-link is composed of proteins encoded by *PCDH15* and *CDH23* genes (Rowlands et al., 2000). The protein encoded by the *PCDH15* forms the lower part of the tip-link, and the CDH23 forms the upper part. *In vitro*, the extracellular components of PCDH15 and CDH23 form parallel homodimers, and the homodimers are arranged in a Ca^{2+} dependent antiparallel manner (Ahmed et al., 2006). In recent years, it has been found that there is a correlation between *PCDH15* gene polymorphism and NIHL susceptibility. Zhang et al. (2014) selected 476 workers with NIHL and 475 workers with normal hearing from a factory in China for a case-control study. There is no difference in sex ratio, noise exposure years and exposure intensity between the two groups. It was found that the allele frequency and genotypes of rs1104085 were significantly correlated with NIHL susceptibility, that is, the susceptibility of variant allele CT or CC genotype was significantly lower than that of wild type TT homozygotes. Besides, SNPs of rs1100085, rs10825122, rs1930146, rs2384437, rs4540756, and rs2384375 were also found to have correlations with NIHL.

Myosin Heavy Chain 14

The *MYH14* is located on chromosome 19 and encodes myosin-binding protein C. It is an ATP-dependent molecular motor involved in cytoskeletal rearrangement and ion gate control. MYH14 was first identified as the causative gene for neurogenic deafness in 2004 (Donaudy et al., 2004). Konings et al. (2009a) conducted an association study of NIHL based on a candidate gene approach. They found two SNPs in *MYH14* (rs667907 and rs588035) that resulted in significant associations in the Polish sample set and significant interactions with noise exposure level in the Swedish sample set. Fu et al. (2016) established *Myh14* knockout mice using CRISPR/Cas9 technology and clarified the role of *MYH14* in the cochlea and NIHL. They found that $Myh14^{-/-}$ mice were more susceptible to high-intensity noise compared to control mice. After acoustic trauma, more pronounced loss of outer hair cells was observed in $Myh14^{-/-}$ mice than in wild-type controls, suggesting that *Myh14* may

play a beneficial role in protecting the cochlea after acoustic overstimulation in CBA/CaJ mice.

Heat Shock Protein Genes 70

Heat shock protein genes (HSPs) can be overexpressed in the inner ear by stimulation such as physiological stress, ototoxic drugs, high temperature and noise. Among them, HSP70 is a dominant type of heat stress protein which has great protective effect. Gratton et al. (2011) observed the difference of cochlear membrane labyrinth gene expression between noise-susceptible experimental group and noise-tolerant control group. It was found that the protein contents of HSP70 and HSP40 in the control group were significantly higher than those in the experimental group, indicating that the expression of *HSP70* gene may play an important role on protecting animals from NIHL. Lei et al. (2017) used Meta analysis to comprehensively analyze the relationship between *HSP70* polymorphism and NIHL susceptibility, and concluded that the polymorphism of rs1061581 and rs2227956 may be closely related to the susceptibility to NIHL. Li et al. (2017) screened 286 NIHL patients by measuring the hearing threshold of iron and steel workers, and selected another 286 normal hearing workers in the same noise environment as the control group. It was found that the proportion of TT genotype of rs2763979 in Chinese Han population was higher than that of CC/TC genotype in the NIHL group.

DNA Damage Repair Related Genes

Eyes Absent Homolog 4

Eyes Absent Homolog 4 (EYA4) is a member of the eye absent family of proteins that encode transcriptional activator-related proteins and plays an important role on regulating tissue-specific differentiation during embryonic development (Borsani et al., 1999). It also participates in a variety of biological activities including maintaining the development and maturation of the Corti's organ (Wayne et al., 2001). Zhang et al. (2015) investigated the relationship between the polymorphisms of *EYA4* and the risk of developing NIHL. The results of this study showed that rs3777781 and rs212769 in the *EYA4* gene were significantly associated with the risk of NIHL. In rs3777781, carriers of the AT and AA genotypes had a reduced risk of NIHL compared to subjects carrying the TT genotype. In rs212769, carriers of the AG and AA genotypes had an increased risk of NIHL compared to subjects with the GG genotype. In another case-control study (Yang et al., 2017), subjects carrying the rs3813346 TT genotype had a higher risk of NIHL than subjects carrying the GG genotype in the noise intensity > 85 dB group. In contrast, in the cumulative noise exposure (CNE) > 98 dB-year group, haplotype CGT showed a protective role in the development of NIHL compared to haplotype TGC, suggesting that genetic polymorphisms in the *EYA4* gene may be a genetic susceptibility factor for NIHL.

8-Oxoguanine DNA Glycosylase

Human 8-hydroxyguanine glycosylase (hOGG1) is a DNA repair enzyme in the base excision repair pathway, whose main function is to recognize and excise 8-oxo G in the DNA double strand and repair damaged DNA. Shen et al. (2014) designed research to investigate the relationship between the gene polymorphism (*hOGG1* Ser326Cys) of rs1052133 and susceptibility to high frequency hearing loss. The *hOGG1* Cys/Cys genotype was found to be a possible risk factor for high-frequency hearing loss, and stratified analysis revealed it was also associated with risk factors such as years of work in noisy jobs, noise exposure level and smoking. Thus, they concluded that the *hOGG1* Cys/Cys genotype may be a risk factor for high frequency hearing loss in the Chinese Han population.

Apoptosis Related Genes

Extracellular Signal-Regulated Kinase 2

Extracellular signal-regulated kinase (ERK) is a member of the MAPK cascades which is a key signaling pathway that control a multitude of cellular processes such as cell survival, protein synthesis, cell proliferation, growth, migration, and apoptosis (Cargnello and Roux, 2011). Recently, accumulative evidences indicate that ERK is involved in response to cellular stress such as noise exposure. When activated by stimulation, ERK2 transfers from the cytoplasm to the nucleus, result in the activation of downstream transcription factors who would further execute kinds of cellular functions (Seger et al., 1991). Kurioka et al. (2015) revealed that conditional *Erk2* knockout mice were more susceptible to noise damage and had slower recovery from NIHL compared to control mice. Furthermore, they detected a significant lower survival rate of inner hair cells in *Erk2* knockout mice. Their results suggest that *Erk2* is essential to the survival of hair cells in NIHL. However, to the best of our knowledge, the research concerning *ERK2* polymorphisms in NIHL population is nearly a piece of blank.

C-Jun N-Terminal Kinases 1

C-Jun N-terminal kinase (JNK), also known as stress-activated protein kinase (SAPK), is a member of the MAPK family (Hollville et al., 2019). The JNK stress pathways are involved in many different intracellular signaling pathways that control diverse cellular processes such as cell growth, differentiation, transformation, and most importantly, apoptosis (Zeke et al., 2016). Sun et al. (2021) conducted a study to explore the effect of *JNK1* polymorphisms on the sensitivity of NIHL, and the results indicated that the rs11598320 TT genotype and the rs8428 TT genotype may be associated with a higher risk of NIHL. Interestingly, a previous study has also reported that prednisone, a well-known steroid clinically used in the treatment of hearing loss, could inhibit the IL-1β-induced activation of *JNK1* (Hong and Jang, 2014).

Other Noised-Induced Hearing Loss Susceptible Genes

Caspase Recruitment Domain Family Member 8

Inflammation is a complex process that is thought to contribute to the development of NIHL. CARD8 is an important component of the inflammasome and has been implicated in inflammation. Miao et al. (2021) conducted a study to investigate the relationship between *CARD8* gene polymorphisms and NIHL

risk and to infer the underlying mechanisms. They verified three SNPs (rs2043211, rs1062808 and rs12459322) in a Chinese population including 610 NIHL cases and 612 normal hearing controls. The haplotype AGG (rs2043211-rs1062808-rs12459322), the AA genotype and A allele of rs2043211 were found associated with a reduced risk of NIHL.

Fas Cell Surface Death Receptor

Reactive oxygen species (ROS) production in the cochlea and blood caused by noise exposure leads to the processes of oxidative stress, lipid peroxidation, and DNA damage, during which *FAS* is activated. Xu et al. (2021) conducted case-control research to investigate the relationship between genetic polymorphisms in the *FAS* gene and NIHL risk. 692 NIHL workers and 650 controls were genotyped for four SNPs, among which two polymorphisms, rs1468063 and rs2862833, were associated with NIHL. Individuals harboring rs1468063-TT or rs2862833-AA genotypes had a decreased risk of NIHL.

Forkhead Box O3

FOXO3 is a gene with a variety of biological functions and is closely related to mammalian longevity. It regulates specific activation of transcription factors to exert effects on cell differentiation, apoptosis, cell cycle, DNA damage repair and oxidative stress (Stefanetti et al., 2018). Through the study of the animal model of NIHL, Gilels et al. (2017) found that the outer hair cells of *Foxo3* knockout mice were more seriously damaged than those of normal mice after the same intensity of noise exposure, and the severity of hearing loss increased significantly, indicating that *Foxo3* is an important protective gene for mice to maintain hearing after noise exposure. Guo et al. (2017) conducted research to explore the effects of *FOXO3* polymorphisms on individual NIHL susceptibility. The results proved that individuals with the G allele of rs2802292, G allele of rs10457180, T allele of rs12206094 and the haplotype GAC and others (TGT/GGT/GGC/GAT) (rs2802292-rs10457180-rs12206094) are associated with an increased risk of NIHL in a Chinese population. In addition, they revealed that GT-GG genotype in *FOXO3* may be a risk factor for occupational NIHL (Guo et al., 2018c). They concluded that the genetic polymorphisms rs2802292, rs10457180, rs12206094 and rs12212067 within *FOXO3* have the potential to be biomarkers for noise exposed impairment for workers.

Grainyhead-Like 2

Grainyhead-Like 2 (GRHL2) is a transcription factor that expressed in epithelial tissues, it not only plays a central role in embryonic development, but also contributes to epithelial cell maintenance (Peters et al., 2002). Li X. et al. (2013) conducted a study to evaluate the contribution of the *GRHL2* polymorphisms to NIHL susceptibility in a Chinese population and found that the subjects carrying rs611419 AT/TT were more resistant to NIHL compared with those carrying the AA genotype. In addition, another study revealed that the CC genotype of rs1981361 in *GRHL2* gene was contributed to a higher risk of NIHL (Xu et al., 2016). Additionally, the fact that the rs3735715 GG genotype had a higher NIHL risk compared with the GA genotype was also verified in another study among Chinese population (Yang Q. Y. et al., 2016).

Metabolic Glutamate Receptor 7 Gene

Metabolic Glutamate Receptor 7 Gene (GRM7) is mainly responsible for glutamate-mediated postsynaptic excitation of neurons. In order to study the effect of *GRM7* polymorphism on NIHL susceptibility, Yu et al. (2018a) selected 292 NIHL patients and 584 workers with normal hearing in a steel factory as subjects. It is found that the C allele genotype of the rs1485175 mutant of *GRM7* gene plays an important role in reducing the incidence of NIHL. Permutation test of generalized multiple dimensionality reduction (GMDR) suggested that rs1920109, rs1485175 and rs9826579 might interact with each other in the pathogenesis of NIHL.

HOX Transcript Antisense RNA

LncRNA HOTAIR is a non-coding RNA that plays a crucial role in RNA processing, gene regulation, chromatin modification, gene transcription, post-transcriptional regulation (Kalwa et al., 2016). It is involved in the alterations of oxidative stress levels, cell proliferation, cell cycle progression and apoptosis. As its expression level is always dysregulated in variety of cancers, it is considered to be used as a potential biomarker (Yang et al., 2019). In order to explore the effect of *HOTAIR* polymorphisms on the NIHL susceptibility, three tag SNPs of the *HOTAIR* (rs874945, rs4759314 and rs7958904) were genotyped in a Chinese population including 570 NIHL cases and 570 controls (Wang B. et al., 2017). The results showed that individuals with the G allele of *HOTAIR* tagSNP rs4759314 and the haplotype (rs874945, rs4759314 and rs7958904) were associated with an increased risk of NIHL.

POU Class 4 Homeobox 3

POU Class 4 Homeobox 3 (POU4F3), also known as Bm3.1 or Bm3c, is a transcription factor which is important for the maturation, differentiation and survival of inner ear hair cells. Xu et al. (2016) performed a matched case-control study to explore the relationship between SNPs in the *POU4F3* gene and susceptibility to high frequency hearing loss in a Chinese population. They revealed that when CNE > 95 dB, individuals carrying the AA genotype had an increased risk of hearing loss compared to the CC/CA genotype at SNP rs1368402. Compared to the AA/GA genotype at rs891969, the GG genotype revealed to be a risk genotype.

CONCLUDING REMARKS

As the death of hair cells in the cochlear is irreversible, and NIHL is a completely preventable disease, it is particularly important to prevent the potential hearing impairment in advance through possible screening and evaluation. The explore of NIHL susceptible genes offers an opportunity to decrease the incidence of hearing loss by risk assessment as early as infant. The incidence of NIHL would be significantly reduced by distributing the susceptible individuals away from intense noise exposure. For example, factories could assign different

employees according to their genotype of NIHL susceptible genes to avoid the occupational impairment; NIHL susceptibility screening could also be applied during conscription.

Although dozens of possible susceptibility genes related to NIHL have been screened, there is still a big gap between practical application and researches. Taylor et al. (1965) first established a linear regression model between noise exposure and high frequency hearing threshold in 1965. It was found that the distribution of NIHL susceptibility in the population showed a unimodal left bias, and there was no single peak on the right side of the main peak (susceptible area), suggesting that the susceptibility is related to many factors and is likely to be affected by multiple minor genes, which increases the difficulty of the study on susceptibility genes.

In relation to the screening of NIHL susceptibility genes, there are some limitations whether using animal research or population study. For animal research, although it has the advantages of short test cycle and easy to obtain materials, the results must be verified in the population. For population study, family analysis is the most effective method to study susceptibility genes, but medical ethics cannot expose all subjects to noise environment, so pedigree analysis cannot be used in the study, only NIHL susceptibility genes can be searched in the genome. Besides, due to many factors, such as regional diversity, ethnic differences, study sample size and gene interaction, inconsistent research conclusions is a commonplace, resulting in limited clinical reference value. Most studies have been conducted in a single population, so further analysis of the correlation between different populations is essential.

Currently, only a handful of NIHL susceptibility genes have been uncovered, and existing studies suggest that NIHL may be caused by accumulative abnormal influence of multiple genes. Further in-depth researches are needed to explore gene-gene interaction and find comprehensive and dominant susceptibility genes among numerous NIHL susceptibility genes. Although there are still great difficulties and challenges in the study of NIHL susceptibility genes, with the further research on new genetic research methods, such as next-generation DNA sequencing (NGS) and high-throughput genotyping array, more susceptibility genes related to NIHL will be found. The luminant prospect of designing of molecular probes that can be used for clinical detection of NIHL susceptible individuals is awaiting on the way.

In conclusion, genetic factor plays a vital role on the pathogenesis of NIHL. NIHL susceptible genes can be used for better identification of potential risks and prevent the occurrence of NIHL. Through the continuous screening of genetic variants in the susceptibility of NIHL, new susceptibility genes will come to light, and ideally, get into the stage of clinical application, which lays a solid foundation for the accurate screening of high-risk population and the reduction of NIHL incidence.

AUTHOR CONTRIBUTIONS

X-MC and X-MX drafted the manuscript. NY, W-WG, and S-LY collected the literature. X-MX summarized the literature. X-MC tabulated the data and drew the figures. Q-QJ revised the manuscript. Q-QJ and S-MY took responsibility for the integrity of the data and the accuracy of the manuscript. All authors contributed to the article and approved the submitted version.

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Evaluation of the Dangerous Decibels Brazil Program in Workers Exposed to Noise

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Introduction: Noise-induced hearing loss can be avoided by taking preventive measures.

Objective: To assess the effectiveness of the Brazilian version of the Dangerous Decibels® program for noise-exposed workers, using the ecological model as an educational intervention plan.

Method: Non-randomized interventional study with a quantitative, experimental trial design, conducted at a meatpacking company. The participants were divided into two groups—the first one (n = 132, divided into 6 subgroups) received the Dangerous Decibels® Brazil educational intervention (DDBEI) adapted to workers while the second group (n = 138, divided into 5 subgroups) received a conventional educational intervention (CEI). The interventions lasted 50 min. The Hearing Protection Assessment Questionnaire (HPA-5) was administered before and after the interventions. The five dimensions (attitude, behavior, knowledge, supports, and barriers) were compared using the Student's t-test for paired data (<0.05).

Results: After both the DDBEI and CEI training, workers improved significantly in barriers, supports, knowledge, attitudes, and behavior around noise. By chance, the CEI group scored lower in all measures than the DDBEI group before training, and though both groups improved, the difference was maintained after training.

Conclusion: The Brazilian version of the Dangerous Decibels® program for noise-exposed workers was effective, influencing positively the factors at different levels of the ecological model. Though the DDBEI was no more effective than the CEI, the CEI participants began at much lower levels, so the effectiveness of the DDBEI may have been underestimated.

Keywords: hearing, noise-induced hearing loss, hearing protection, knowledge, habits, attitudes

INTRODUCTION

Brazil has public and federal policies related to workers' health, and their purpose is to define the principles, guidelines, and strategies to be observed by the three spheres of the Unified Health System for the development of comprehensive care to workers' health, with emphasis on surveillance, promotion and protection of workers' health and the reduction of morbidity and mortality resulting from development models and production processes (Brasil, 2012). The federal regulatory standards relating to occupational safety and medicine have mandatory compliance by private and public companies and public agencies of direct and indirect administration, as well as by agencies of the Legislative and Judiciary Branches, which have employees governed by the Consolidation of Labor Laws. These guide health actions, including auditory health actions, in work environments.[1]

Noise-Induced Hearing Loss (NIHL) is considered the most common health problem among workers in several industrial activities worldwide and can damage health and quality of life. However, NIHL can be avoided if preventive measures are adopted (Nelson et al., 2005; Sliwinska-Kowalska and Davis, 2012; Brasil, 2020).

Therefore, agencies recommend the implementation of Hearing Loss Prevention Programs (HLPP) in the work environment (NIOSH - National Institute for Occupational Safety and Health, 1996, 2018; OSHA - Occupational Safety and Health Administration, 2002; Brasil, 2020; Conselho Federal de Fonoaudiologia – CFFa, 2021). Educational interventions are an essential part of this program. They provide workers with the chance to rethink their health and quality of life and work, generating safer, and more stimulating working conditions (Oliveira et al., 2015; Kim et al., 2016; Ramos et al., 2017).

The Ecological Model for Health Promotion, which uses more than one behavior change theory targeting individual and environmental influences, is considered more effective in health promotion interventions (Kok et al., 2008; Sallis et al., 2008; Angus et al., 2013). This model provides an opportunity to identify gaps in NIHL prevention and develop educational interventions targeted at different levels of influence on hearing preservation behavior.

The Ecological Model for Health Promotion (Mcleroy et al., 1988) is an extension of Bronfenbrenner's theory and is conceptualized by five social levels corresponding to Bronfenbrenner's levels, which include: the intrapersonal level (the individual characteristics such as knowledge, attitudes, values, and skills), the interpersonal level (social relationships including family, peers, and peer networks), the organizational level (organizational norms, policies, and support), the community level (community norms, standards, and social media), and the policy level (health promotion policies and legislation and their regulation, interpretation, and enforcement).

The MATCH - Multi-level Approach to Community Health Model (Simons-Morton et al., 2012) ecological planning model was used to adapt a classroom hearing loss prevention program named Dangerous Decibels® (DD) for use with workers (Reddy, 2014; Reddy et al., 2017). The DD program[2] was originally developed and proven effective for children in schools in Oregon and Washington (Martin et al., 2006; Griest et al., 2007) and in other countries, including Brazil (Knobel and Lima, 2013). The DD mission is to significantly reduce the prevalence of noise-induced hearing loss and tinnitus through exhibits, education, and research. The goal of the program is to improve knowledge, attitudes and behaviors regarding noise exposure and hearing protection strategies (Martin et al., 2006).

The behavioral health education pedagogical design used in Dangerous Decibels® prioritizes educational aspects linked to individual and environmental behavioral risk factors, using the health belief model, the social cognitive theory, and ecological model for health promotion as a pedagogical intervention plan. It proved effective for workers in New Zealand, promoting knowledge and change of habits, attitudes, and behaviors regarding noise and the use of hearing protection by workers (Reddy, 2014; Reddy et al., 2017). Thus, bringing a new perspective to educational interventions in the occupational context is an interactive and dynamic program that provides greater worker participation (Reddy, 2014).

There is no hearing health program for workers employing the behavioral pedagogical conception using the ecological model as a pedagogical intervention plan in Brazil. The implementation of a program using these principles would be a great contribution to the Brazilian worker. Instead, most Brazilian programs use traditional pedagogical conceptions for the educational interventions for workers. Considering the aspects addressed here, we propose to answer the following question: "Will the educational intervention proposed by the Dangerous Decibels Brazil (DDB) program prove effective when compared to conventional educational intervention?"

This study aims to evaluate the effectiveness of the educational intervention Dangerous Decibels Brazil for workers exposed to noise compared to the conventional educational intervention proposed by the company.

METHODOLOGY

Study Type and Location

The Ethics Committee of the Graduate Program in Communication Disorders at the Universidade Tuiuti do Paraná approved this study, process 2.725.935, and the company approved it. The study is a non-randomized interventional study of the experimental, quantitative type conducted in a meatpacking plant.

The company was selected because it is a local company, with its headquarters and most of its branches in the same city in the south of the country. It is part of a cooperative, being considered one of the largest food cooperatives in Brazil, formed by more than a hundred-thousand families, a total that includes forty thousand direct jobs, besides the 10,000 employees and the 65,000

[1] www.gov.br/trabalho-e-previdencia/pt-br/composicao/orgaos-especificos/secretaria-de-trabalho/inspecao/seguranca-e-saude-no-trabalho/ctpp-nrs/normas-regulamentadoras-nrs

[2] http://dangerousdecibels.org/

families of rural entrepreneurs from the 11 cooperatives that are part of its system.

As it is a company that has always sought to invest in better health conditions for its employees, the company follows the recommendations of the federal government and has health programs described in the Regulatory Norms (RN), such as RN-6 on Individual Protection Equipment, RN-7 on the Occupational Health Medical Control Program, RN-15 on Unhealthy Activities and Operations, RN-17 on Ergonomics, RN-36 on Safety and Health at Work in slaughterhouses and meat and meat processing companies. It develops actions aimed at minimizing the risks caused by noise, which ranges from 78 to 120 dB HL (depending on the sectors and locations), through improvements in the work environment, use of hearing protection equipment and awareness of its workers. The company also has an auditory conservation program.

Selection, Inclusion, and Exclusion Criteria—Participants

The sample selected was by convenience, as the researcher had access to the location and participants. This study's participants were selected during the admission selection process and invited to participate in the survey at the integration process held at the company. Initially, the number of participants was 509. However, during the 3-month interval, the intervention period, 239 participants did not remain in the company. Therefore, 270 Southern Brazilian workers of both genders participated in the study, distributed into the DDB experimental/intervention group (DDBEI: $n = 132$) and the conventional control/intervention group (CEI: $n = 138$).

Instruments

We used as instruments: (a) the Dangerous Decibels Brazil educational intervention for workers (DDBEI) and the company's conventional educational intervention (CEI); and (b) the Hearing Protection Assessment Questionnaire (HPA-5). The current study replicates the original New Zealand research that used the validated HPA-5 Questionnaire as the data collection tool (Reddy, 2014; Reddy et al., 2017). The hearing protection assessment questionnaire assessing five measures (HPA-5) is an extension of the two-measure (HPA-2) questionnaire developed and described elsewhere (Reddy et al., 2014). The HPA-5 assessed barriers and supports, knowledge, attitudes and behavioral measures toward hearing protection. The knowledge, attitudes and behavioral measures were adapted from a questionnaire used to assess the effectiveness of the school-based Dangerous Decibels Programme in the United States of America (Griest et al., 2007). The Hearing Protection Assessment Questionnaire (HPA-5) was translated and adapted to Portuguese, named *Questionário de Avaliação da Proteção Auditiva* (APA; **Supplementary Appendix**) by Bramati et al. (2021) (in press, Codas, 2022), applied to both groups before and after the educational intervention.

Educational Interventions for Workers - At this stage (3 months after the admission exam), the participants were randomly divided into two groups, where the first group received the DDBEI adapted for workers (Reddy et al., 2017) and provided by the researcher Speech Therapist, Dangerous Decibels Brazil Educator and the other half received the traditional educational intervention (TEI), provided by the health and safety team.

The DDBEI was conducted at the company's premises in a group of 132 workers, divided into 6 groups with an average of 22 workers in each group. The EIDDB intervention lasted 50 min.

The DDBEI was inspired by Reddy (2014) and Reddy et al. (2017) and reinforced key messages to improve and motivate hearing health behaviors in workers. All modules used different strategies, such as demonstrations, audio-visual resources, use of objects, worker involvement, and interaction, to convey the program's messages. It was essential to the program's objectives, especially when effective training involves strategies such as:

(a) delivery of relevant information and concepts;
(b) demonstration of knowledge, attitudes, and skills to be taught;
(c) opportunity to practice the skills learned; and
(d) facilitation of feedback between the educator and the learner/participant (Salas and Cannon-Bowers, 2001).

This study used the Dangerous Decibels® program training script (manual) developed by Reddy (2014) and Reddy et al. (2017) before the program was conducted at workplaces. It comprises completing systematic training instructions on approaching each of the program's components and how to carry them out. In addition, the script encourages educators to include or generate discussions on examples relevant to the training participants. Furthermore, a summary version of the script was developed as a series of nine cards for each module. **Figure 1**—DDBEI.

The DDBEI was conducted after cultural adaptation for Brazilian workers using mainly examples and situations that describe the work reality within the company, and was divided into nine modules proposed by Reddy (2014) and Reddy et al. (2017).

Module 1: Program objective and introduction:

The workplace DDBEI included more occupational sector-specific information than the original school-based Dangerous Decibels® program, such as the high prevalence of NIHL affecting workers and increasing economic and social costs. In addition, there was more emphasis on workplace noise control strategies, such as engineering measures, administrative measures, and individual hearing protection.

In addition to the original Dangerous Decibels® program messages of "stay away," "protect your ears" and "turn down the volume," messages in the occupational context such as "eliminate," "isolate" "minimize" were emphasized as warning signs and signs on the dangerous level of noise sources were displayed to communicate these messages.

Module 2: The physics of sound and energy (sound/energy):

This part of the original Dangerous Decibels® program was fully maintained from the school-based program to the workplace version. The objective was to involve the workers and give concrete examples that would help them understand the concept of sound energy as something that can cause harm.

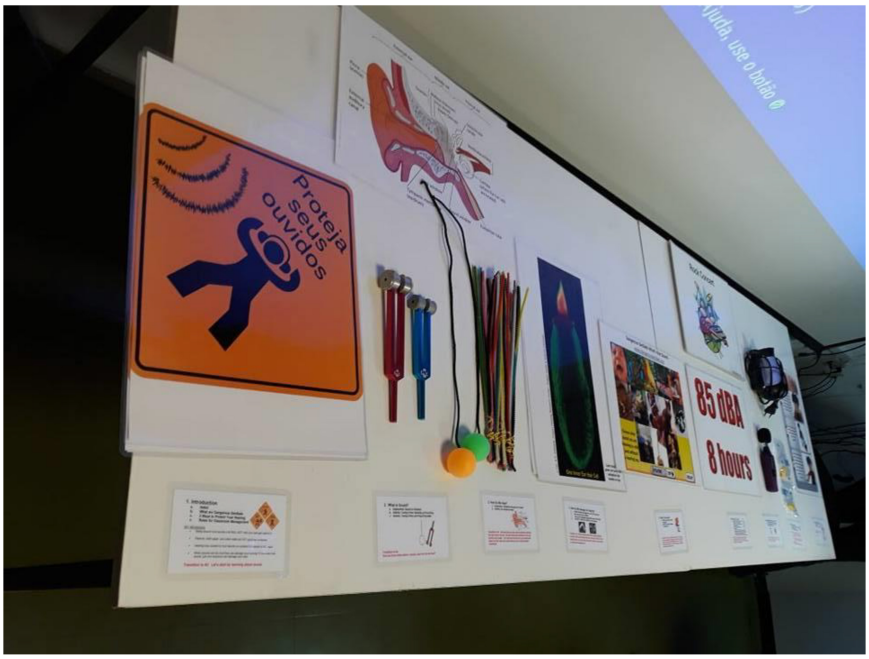

FIGURE 1 | Summary version of the script DDBEI and educational materials.

Module 3: Ear:

An ear anatomy poster was used to explain how sound waves reach the ear and provide a basic explanation of the processes occurring to make sound heard. It included understanding the physiology of auditory sensory cells (hair cells) and sound detection at a basic level. This explanation of a complicated concept in a simple, concrete form facilitated understanding.

Module 4: The hearing loss process (hearing damage):

This module demonstrates how high sound pressure levels damage the ear's hair cells. This part was based on the previous modules, describing vibrations and how the hair cells are involved in the hearing process. In addition, it helped reinforce the messages regarding the susceptibility and severity of noise dangerous to human hearing.

Module 5: The hearing loss consequences (experience/hearing loss):

Hearing loss simulation software (Huckvale, 2010) was used to demonstrate the hearing loss effects. The module emotionally and reflexively emphasized the consequences of hearing loss and its effect on life quality. Workers were encouraged to discuss how they spent time with family and friends, and the simulator was used to demonstrate how Hearing Loss can affect activities and social interactions.

Module 6: Workplace sounds loudness (sound sources/ flashcards):

The decibel scale was introduced with an emphasis on the 85 dB tolerance limit. We also discussed the concept of reducing exposure time when noise levels increase. Workers were encouraged to engage in an activity involving several *flashcards* with images of common work tools and activities. The DDBEI included examples specific to the occupational context, such as power tools and heavy machinery, along with other examples such as tractor noise, washing machine noise, and rock concerts.

Module 7: Sound measurement (experience/distance sound pressure levels):

The workers learned how to measure sound using a sound pressure level meter. Next, a drill was used as a sound source to demonstrate the noise level. Then, the concept of reducing noise exposure by moving away from the sound source was demonstrated and discussed. In addition, there was a discussion regarding machines creating different noise levels when applied to different materials such as wood, glass, or steel.

Module 8: Proper use and maintenance of hearing protection devices (HPD):

The correct method for inserting hearing protectors and ensuring adequate protection was demonstrated. The workers were encouraged to practice the correct procedure with their fellow workers. The DDBEI also emphasized the importance of correctly wearing protectors with caps and/or long hair. HPD maintenance was discussed, and workers were encouraged to seek management assistance to ensure a high HPD standard. The objective was to improve the workers' self-efficacy.

Module 9: Peer modeling and workplace hearing health promotion (experience/work environment):

The DDBEI used this component to encourage peer modeling and promote hearing health in their settings. The emphasis was on creating a working environment that takes hearing health promotion seriously. For example, the classroom program explores the hearing protection behavior of children and their friends when exposed to high noise levels during rock concerts. In addition, the work program was adapted to encourage the worker regarding their own and

their colleagues' hearing protection behavior when exposed to workplace noise.

The CEI was conducted on the company's premises, with a group of 138 workers, divided into 6 groups with an average of 23 workers in each group. The CEI lasted 50 min. It was divided into 5 modules and was performed on a single day. The CEI was carried out with a slide presentation, where aspects regarding hearing protection care were addressed:

Module 1: Program objective and introduction:

The CEI in the workplace and occupational sector-specific information relays the program's objectives, providing information on the high prevalence of NIHL affecting workers and increasing economic and social costs. In addition, there was more emphasis on workplace noise control strategies, such as engineering measures, administrative measures, and individual hearing protection.

Module 2: NIHL—Hearing Anatomy and Physiology:

This module explains how the auditory system works, using visual resources to explain the subject.

Module 3: Noise—concept and characteristics:

The decibel meter instrument was used to demonstrate the noise levels at different locations in the room, explaining its concept and characteristics.

Module 4: HLPP—Hearing Loss Prevention Program:

An oral explanation explained how the hearing loss prevention program is developed within the company, which laws refer to this program, and what role each participant should play.

Module 5: How to prevent NIHL:

In this module, the participants received information through oral explanations and visual resources on measures to reduce noise levels in the workplace, the importance of wearing hearing protectors, and awareness of the importance of each person's role in decreasing noise levels.

(b) HPA-5 Questionnaire: The Brazilian version of the HPA-5 questionnaire named *Avaliação da Proteção Auditiva* (APA) (Bramati et al., 2021, in press, Codas) was used before and after the educational intervention (DDBEI and CEI). The APA was applied to evaluate the DDBEI's effectiveness. The APA questionnaire was applied to all workers who took the admission exam (audiometry) and after, immediately after participating in the educational intervention (DDBEI and CEI). The APA assessed barriers and supports, knowledge, attitudes, and behavioral measures regarding hearing protection. Knowledge, attitudes, and behavioral measures were adapted from a questionnaire used to assess the effectiveness of the school-based Dangerous Decibels® Program in the United States (Griest et al., 2007). The scales related to knowledge, attitudes, and behavior have multiple choice questions, each of which has only one correct answer. There are five questions for the knowledge scale about sound science, hearing loss, and hearing conservation, two questions related to the attitudes measure about noise protection and hearing protection two questions about work safety behavior attitudes (questions 7 and 8), and three questions about behavior (questions 10, 20, and 21). The measures regarding barriers and supports included nine items, each describing why they (support) and would not wear (barriers) HPD when exposed to noise at work. It allowed respondents to endorse any item they identified with for each measure. The two questions related to Support are questions 9 and 11. Question 11 has four subscales in the responses (safety culture, risk recognition, behavior motivation, and safety culture). The Barriers-related question is question 12, with two subscales in the responses (justification of risk and restrictions on DPA use).

The questionnaire also included demographic items, such as gender and age. In addition, two items describe attitudes toward safety behavior at work, and one item documents HPD self-reported use.

Data Analysis

Comparisons were made separately for the five scales (attitude, behavior, knowledge, supports, and barriers) assessed using Student's *t*-test for paired data to detect significant differences in results between pre-intervention and post-intervention. All tests were considered at the 0.05 significance level.

Considering that the five scales evaluated in the pre- and post-intervention questionnaire have different numbers of items, the response scores were converted into percentages to allow comparability among them, and for attitude, behavior, and knowledge, into hit percentages, where the analysis form recommended for the Dangerous Decibels® program was followed. In addition, the percentage of marked items was considered for supports and barriers since the answers for these scales were presented as affirmative sentences.

The independent variables were Time, which had two levels (pre-training and post-training), and Training method, which also had two levels (DDBEI and CEI). In each model, training type (DDBEI or CEI) was a between-subjects factor, and the two measures (pre- and post-training) were treated as repeated measures.

Five repeated measures analyses of variance (ANOVAs) were conducted to test training effects on the five scales. The five outcome measures were: knowledge, attitudes, behavior, supports, and barriers. Each outcome measure was modeled with a separate ANOVA procedure and treated as a repeated measure over time, while the training groups were treated as independent. The interaction between Time and Training was used to test the hypothesis that the training methods differed in effectiveness. If the interaction were significant, it would mean that the outcome measure for one training group changed more than the same measure for the other training group. The homoscedasticity and normality assumptions were graphically examined for the change (from pre- to post-training) in the five scales, and all were satisfactory. We used a 0.05 alpha criterion level.

The data were verified for statistical test assumptions. Given that the repeated measures approach was used, the change in scores between pre- and post-training measures was evaluated, and visual inspection of the histogram showed approximately normal distributions. There were three scores on the knowledge scale (two in the DDBEI group and one in the CEI group) where participants scored lower after the intervention than before. However, removing these cases from the analysis did not affect the findings, so they were left for the results presented.

RESULTS

Table 1 presents the results of the participants' profiles according to the variables gender, sector, shift, position, and nationality.

Dangerous Decibels® Brazil Educational Intervention Results

Figure 2 presents the scale results for attitudes, behaviors, and knowledge in the DDBEI pre- and post-intervention questionnaire. Significant increases were observed after the intervention for all scales at $p < 0.001$.

Figure 3 presents the scale results for supports and barriers in the DDBEI pre- and post-intervention questionnaire. Significant improvements were observed pre- and post-intervention for both scales at $p < 0.001$.

Conventional Educational Intervention Results

Figure 2 presents the scale results for attitudes, behaviors, and knowledge in the CEI pre- and post-intervention questionnaire. Again, significant improvements were observed before and after the intervention for all scales at $p < 0.001$.

Figure 3 presents the scale results for supports and barriers in the CEI pre- and post-intervention questionnaire. Again, significant improvements were observed pre- and post-intervention for both scales at $p < 0.001$.

TABLE 1 | Participants' profile in the CEI group ($n = 132$) × DDBEI group ($n = 138$).

Variable	DDBEI group	CEI group	*p*
Gender	*n* (%)	*n* (%)	
Female	77 (58.3)	75 (54.3)	0.5083
Male	55 (41.7)	64 (45.7)	0.5083
Sector/average NPS			
Cutting (A B C)/89.8	103 (78.0)	104 (75.4)	0.3068
Packaging (A B)/91.6	15 (11.4)	19 (13.8)	0.2764
Scalding A/94.4	1 (0.8)	(0.8)	NA
Evisceration (A B)/89.1	10 (7.6)	8 (5.8)	0.5542
Sanitation C/91.2	3 (2.3)	4 (2.9)	NA
Tunnels A/76.9	– (0.0)	2 (1.4)	NA
Shift			
First	49 (37.1)	54 (39.1)	0.7263
Second	66 (50.0)	60 (43.5)	0.2689
Third	17 (12.9)	24 (17.4)	0.2852
Position/function			
Production operator I	96 (72.7)	83 (60.1)	0.0237*
Production operator II	30 (22.7)	40 (29.0)	0.2211
Production operator III	3 (2.3)	10 (7.2)	NA
Sanitizer I	3 (2.3)	3 (2.2)	NA
Production balancer	– (0.0)	2 (1.4)	NA
Country of birth			
Brazil	119 (90.2)	121 (87.7)	0.4972
Haiti	13 (9.8)	17 (12.3)	0.5973

*The Test for Difference of Proportions was applied at a 0.05 significance level. NA, the test is Not Applicable. * significant difference.*

Comparison Between Dangerous Decibels® Brazil Educational Intervention and Conventional Educational Intervention Interventions

Figure 2 presents the DDBEI and CEI comparisons on the attitudes, behaviors, and knowledge scales.

Figure 3 presents the comparison for supports and barriers in the pre- and post-intervention questionnaire in the DDBEI and CEI groups.

The overall effects showed an increase in all five scales after the intervention for both groups, implying that the DDBEI and CEI methods were both effective [$F(1, 268) = 179.313$, $p < 0.001$]. However, there was a pre-existing difference between the two intervention groups in which the group receiving the DDBEI scored higher on all measures (and lower on Barriers) before the intervention. Therefore, statistical tests compared the overall effects, where we observed a difference between the groups (DDBEI and CEI). However, this difference was present before and after the intervention.

No interaction between time and training was found for any of the five scales [$F(1, 268) = 0.285$, $p = 0.594$]. This means that both groups improved pre- and post-intervention similarly. Thus, both DDBEI and CEI were effective and caused equal improvement after the intervention.

DISCUSSION

The pedagogical conception of the behavioral type in DDBEI and the use of the ecological model to identify and direct hearing preservation behavior at different levels of influence contributed to the Brazilian workers' reflection on the preservation of their hearing and health when exposed to noise. The results showed that the DDBEI effectively improved several measures that positively influenced the wearing of hearing protection devices in workers. These results align with Reddy et al. (2017), where their results show a significant effect of the intervention measures over time, indicating that these measures improved significantly after the intervention.

This study observed improved motivation for healthy behaviors and habits and increased knowledge. This data is especially important since workers new to the company tend to model their protective behavior based on the behaviors of more experienced workers. Moreover, according to the Social Cognitive Theory principles, behavior is initiated, and maintained by the reciprocal influences between the person, the behavior, and the environment (Bandura, 1986). Therefore, interventions employing active training methods are more effective in reducing negative health outcomes and promoting worker safety and health (Burke et al., 2006). According to the ecological model, intrapersonal and interpersonal influences strengthen organizational norms and culture at the organizational level that supports health promotion (Reddy, 2014).

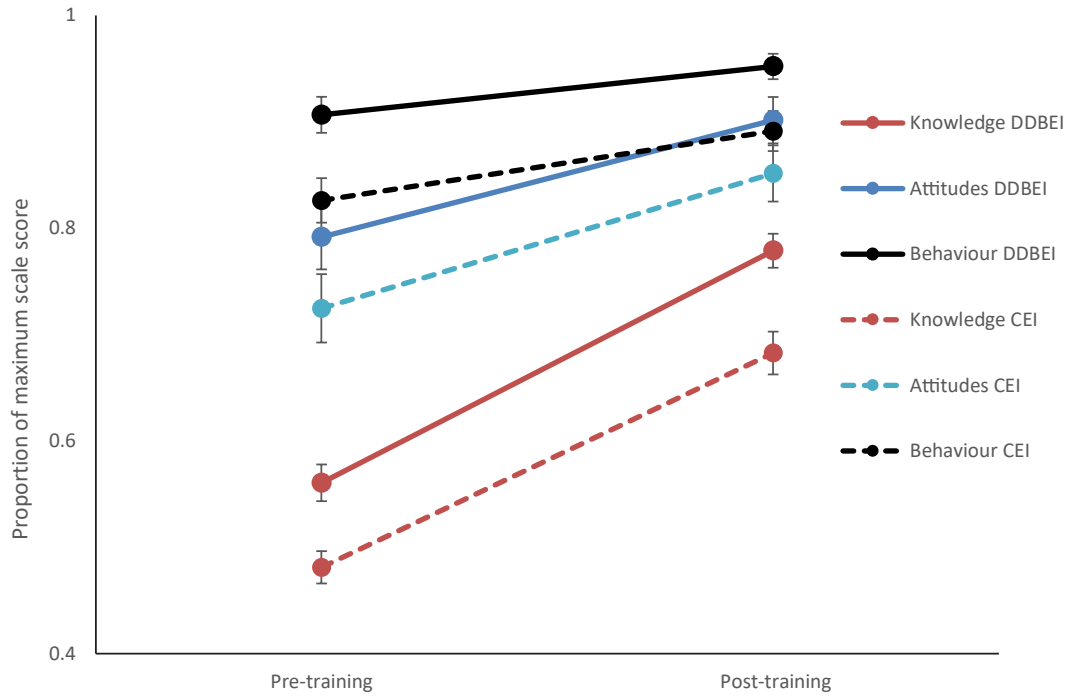

FIGURE 2 | Graph of mean Knowledge, Attitude and Behavior scores as a proportion of the maximum possible on each scale, before and after training with the Dangerous Decibels (DDBEI) and Conventional (CEI) training methods. Error bars represent one standard error of the mean.

We observed significant pre- and post-intervention differences regarding supports, demonstrating an increase in the aspects supporting the proper use of HPD. Risk recognition, behavioral motivation, and company safety culture are important aspects to consider as support and include the influences of peer modeling on hearing protection behavior at the interpersonal level. At the organizational level, employer modeling, workplace rules compliance, and training influence motivation and safety culture. These results support evidence that workers' acceptance and promotion of workplace safety and protective behavior is an HDP predictor (Edelson et al., 2009).

According to Areosa (2007), regarding risk perceptions at work, they are constructed by multiple factors, knowing that they can have a diversified capacity to influence the worker. We find that the risk perception at work is a variable phenomenon within the set of social actors. For example, a given factor can exert an extraordinary influence on one individual's behaviors, attitudes, and representations and be indifferent to another. In part, this ambiguity characterizes risk perceptions at work. Thus, heterogeneity, ambivalence, and uncertainty characterize risk perception at work. Areosa (2012) found that workers' risk perceptions in the early days at a job position may correspond to a greater perception of occupational hazards, if we consider that they make more use of HPD. It is pertinent to remember that workers' risk perceptions are absolutely "real and objective" for them, and they tend to act upon those perceptions. Therefore, integrating the different risk perceptions of workers into risk analyses is a key step toward the success of an organizational risk management program and, consequently, toward accident prevention.

Regarding the barriers related to restrictions on the use of hearing protection, we observed significant differences pre- and post-intervention, showing a decrease in barriers. It corroborates Reddy (2014), where the results show a significant intervention effect on the use of hearing protection over time, with a 26% improvement in the consistent use of hearing protection, and 44% of workers in the group reporting always using hearing protection when exposed to noise before the intervention. After the intervention and at the 8-week follow-up, 70% of the workers reported always wearing hearing protection when exposed to noise.

When comparing the DDBEI with the CEI, we observed no significant differences between the interventions. However, the workers showed significant improvements on all five scales after the two interventions. This finding applies to attitudes, knowledge, and behavior, similarly, to supports and barriers. It is worth noting that the CEI group scored lower on the scales than the pre-intervention DDBEI group. It was unexpected, and it is possible that if the two groups were homogeneous, we would have observed a difference in the result. For example, it is possible that the effectiveness of the DDBEI was concealed by the higher pre-training level of that group compared to the group trained with the CEI.

However, considering that the DDBEI was new to the company's workers who were used to passively participating in traditional educational interventions, the DDBEI was well received and accepted by the Brazilian workers and their

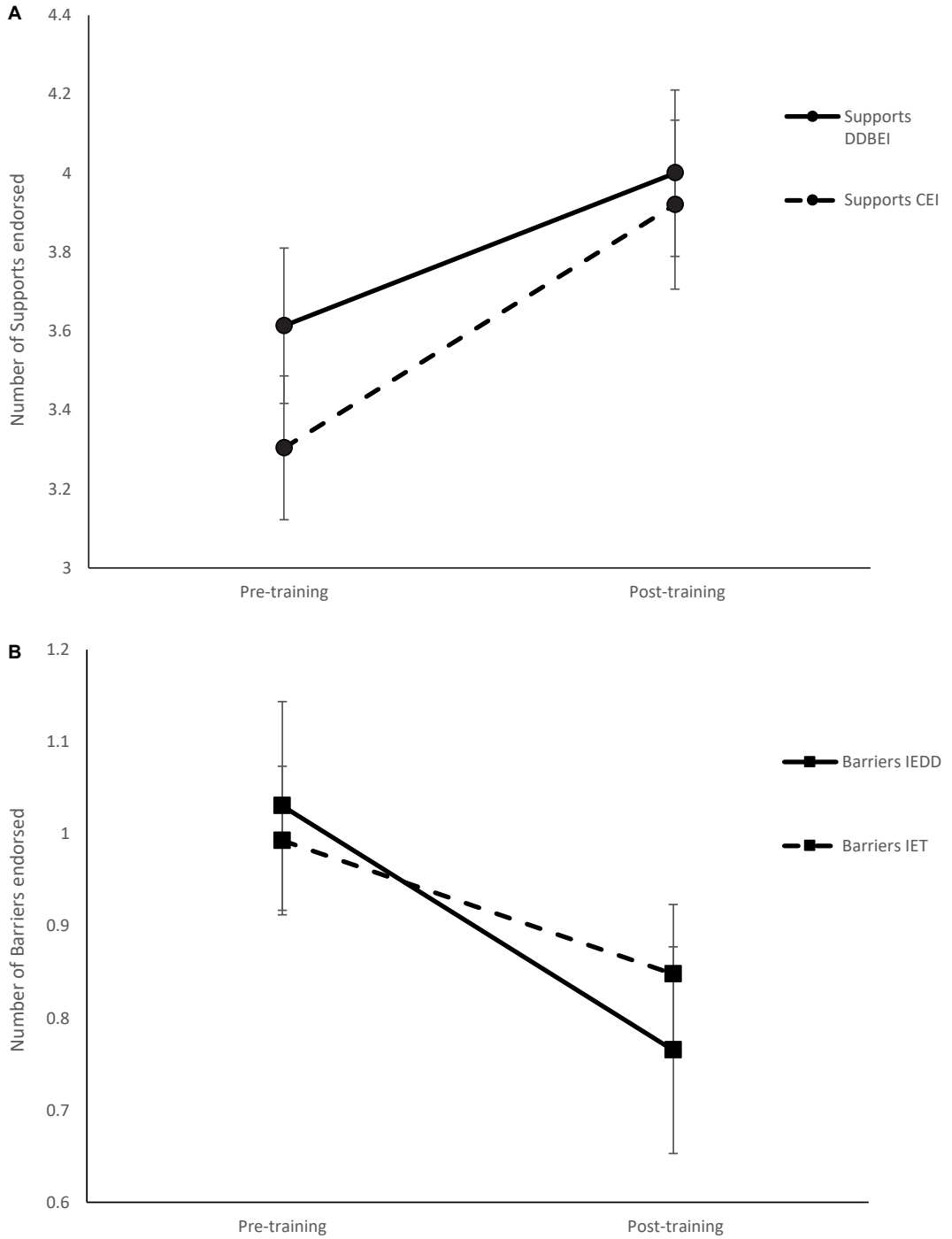

FIGURE 3 | Change in the number of Supports **(A)** and Barriers **(B)** for hearing-protective behavior before and after training with the Dangerous Decibels (DDBEI) and Conventional (CEI) training methods. Error bars represent one standard error of the mean.

managers. They appreciated the opportunity, the relevance, and the modules' content. It suggests that this program does not disrupt workplace practices and encourages hearing health promotion and the prevention culture.

The prevention culture concept is implicitly based on the safety culture concept (HSE - Health and Safety Executive, 2005). Both use a cultural approach. A safety culture aims to reduce work-related risks, while a prevention culture aims to reduce both work-related and non-work-related risks. Safety culture is mainly directed at the workplace level, while prevention culture is directed at the societal or national level. In a safety culture, the emphasis is on health protection,

while in prevention culture, it emphasizes health protection and promotion (ILO–International Labour Organization, 2014; European Commission, 2020). Most probably, the practical nature of the educational intervention helped workers understand the hearing health concepts as a relevant issue (Reddy et al., 2017).

Limitations

The study had limitations. This study was conducted with a Southern Brazilian convenience sample of workers from a meatpacking plant, not representing all Brazilian workers, making it necessary to evaluate DDBEI in other country regions. The project was conditioned to 1 year, being possible to apply the questionnaires before and after the intervention, not being possible to evaluate the follow-up after 6 months or 1 year. Finally, another factor considered important was the difference between the groups in the pre-intervention, with the DDBEI group showing a higher score on all scales. Perhaps it would be possible to identify differences between the groups if it did not happen.

Recommendations for Future Studies

We suggest applying the questionnaire at four time-points: pre-intervention, post-intervention, and at 3- and 6-month periods, so the results can be observed over time. We also suggest comparing the intervention in homogeneous groups since the pre-intervention and improving the program with educational strategies, focusing on the risk justification subscales and restrictions on the HPD use regarding the barriers scale. In addition, on the safety culture, risk recognition, and behavior motivation subscales, relating to the supports scale, so that significant results on these scales can be observed in further studies.

The survey focused primarily on three ecological model levels: intrapersonal, interpersonal, and organizational. However, there is room for research and the development of interventions targeting the community as a whole, directing future research in this scenario.

CONCLUSION

When comparing the DDBEI with the CEI, we observed no significant differences between the interventions. However, the DDBEI for workers exposed to noise in occupational settings proved effective and contributed to worker training by increasing knowledge, changing attitudes, and intrapersonal behavior, while also increasing support and reducing barriers regarding HPD use. Furthermore, the results obtained with the DDBEI for workers will contribute to developing new proposals and materials specific to the DDB program, targeted to be offered as another alternative to the NIHL Prevention Programs.

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 the Ethics Committee of the Graduate Program in Communication Disorders at the Universidade Tuiuti do Paraná approved this study, process 2.725.935. 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

AL and LB: conceptualization, data curation, investigation, and writing—original draft preparation. AL, LB, DW, and RR: methodology. CG and JM: validation. JM and DW: formal analysis. AL, LB, CG, RR, and DW: writing—review and editing. All authors contributed to the article and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnint.2022.909972/full#supplementary-material

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No effect of occupational noise exposure on auditory brainstem response and speech perception in noise

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The primary aim of this study was to investigate whether auditory brainstem response (ABR) and speech perception in noise (SPiN) were associated with occupational noise exposure in normal hearing young factory workers. Forty young adults occupationally exposed to noise and 40 non-exposed young adults (control group) from Zhejiang province in China were selected. All participants presented with normal hearing thresholds and distortion product otoacoustic emissions. Participants were evaluated with the Mandarin Bamford-Kowal-Bench (BKB) test and ABR. The latter was obtained for click stimulus at 50, 60, 70, 80, and 90 dBnHL. Peak-to-trough amplitudes and latencies for waves I and V were obtained. The ABR wave I amplitude, the wave I/V amplitude ratio, the slope of the wave I amplitude growth as a function of stimulus intensity (AMP-I_{Slope}), and the wave V latency shift with ipsilateral noise (LAT-V_{Slope}) were used as ABR outcomes. Finally, equivalent continuous average sound pressure level normalized to 8 h ($L_{Aeq.8h}$) and cumulative noise exposure (CNE) were obtained for noise-exposed participants. No significant differences between groups were found for any ABR outcomes. Noise-exposed participants exhibited worse BKB scores than control group participants. A multivariate regression model showed that 23.3% of the variance in BKB scores was explained by group category (exposed vs. non-exposed) and hearing thresholds. However, since none of the ABR outcomes exploring cochlear synaptopathy were associated with noise exposure, we cannot conclude that cochlear synaptopathy was the contributing factor for the differences between groups for BKB scores. Factors that go beyond sensory processing may explain such results, especially given socio-economic

differences between the noise-exposed and control groups. We conclude that in this sample of participants, occupational noise exposure was not associated with signs of cochlear synaptopathy as measured by ABR and BKB.

KEYWORDS

cochlear synaptopathy (CS), hidden hearing loss (HHL), occupational noise exposure, auditory brainstem response (ABR), speech perception in noise (SPiN)

Introduction

A number of studies have reported that a moderate-to-high noise exposure can induce auditory damage in experimental animals (Kujawa and Liberman, 2009; Lin et al., 2011; Furman et al., 2013; Fernandez et al., 2015; Gannouni et al., 2015; Jensen et al., 2015). While most of these studies found auditory damage after short exposures (i.e., 97–106 dB SPL for 2 h), lower exposure levels for longer duration (i.e., 70 and 85 dB SPL, 6 h/day, 3 months) have also been shown to be harmful (Gannouni et al., 2015). This auditory damage is characterized by an injury to inner hair cell (IHC) synapses (Kujawa and Liberman, 2009), with a subsequent preferential loss of low spontaneous rate (SR) auditory fibers (Furman et al., 2013; Fernandez et al., 2015). This phenomenon has been referred to as cochlear synaptopathy and has also been associated with normal aging in animals without noise exposure (Sergeyenko et al., 2013; Gleich et al., 2016). Because low-SR auditory fibers are not involved in the coding of the amplitude of low-level sounds (Ruggero, 1992; Bourien et al., 2014), an injury to such fibers does not affect hearing thresholds. In the animal model, this can be observed by a reduction in auditory brainstem response (ABR) wave I amplitude at suprathreshold levels (Kujawa and Liberman, 2009; Sergeyenko et al., 2013). Therefore, cochlear synaptopathy may be observed despite normal hearing thresholds and the integrity of outer hair cells (OHC), as measured by otoacoustic emissions (Kujawa and Liberman, 2009).

Studies investigating cochlear synaptopathy in humans *in vivo* have used behavioral and electrophysiological measures to detect auditory deficits induced by noise exposure (for a review, see Barbee et al., 2018; Leroux and Pinsonnault-Skvarenina, 2018). The ABR (at suprathreshold levels) and speech perception in noise (SPiN) tests are the most used procedures for such purposes. Regarding ABR measures, previous studies have typically investigated the amplitude of wave I. However, the wave I/V amplitude ratio (e.g., Schaette and McAlpine, 2011), the summating potential (SP)/action potential (AP) ratio from the electrocochleography (e.g., Liberman et al., 2016), the wave I amplitude growth as a function of stimulus intensity (e.g., Kujawa and Liberman, 2009), the wave V latency (e.g., Skoe and Tufts, 2018), and the shift in the latency of wave V as a function of an ipsilateral white noise masker (e.g., Mehraei et al., 2016) have been proposed for their ability to serve as biomarkers of cochlear synaptopathy. Results from different studies using these procedures with normal hearing young individuals are controversial. This is because some studies have found an association between ABR measures and/or SPiN test results and noise exposure (e.g., Liberman et al., 2016; Bramhall et al., 2017; Grant et al., 2020; Kikidis et al., 2020; Wang et al., 2021), while others have not (e.g., Fulbright et al., 2017; Grinn et al., 2017; Grose et al., 2017; Prendergast et al., 2017a,b; Yeend et al., 2017; Washnik et al., 2020). Some authors have suggested that ABR and SPiN measures may not be sensitive enough to detect this condition or that cochlear synaptopathy may not manifest in young people with normal hearing thresholds (Guest et al., 2018; Bramhall, 2021). Additionally, some authors have suggested that typical recreational noise exposure may not be sufficient to cause cochlear synaptopathy in young normal hearing individuals (Prendergast et al., 2017a; Guest et al., 2018).

Most of the previous studies have investigated samples of young adults recreationally exposed to noise. Little is known about occupational populations exposed to noise. If noise-induced cochlear synaptopathy occurs in humans, then it is likely that workers exposed to noise may develop such a condition prior to permanent threshold shifts. Indeed, it has been documented that normal hearing workers exposed to noise complain of challenges understanding speech in difficult listening situations, despite presenting with normal hearing thresholds (Soalheiro et al., 2012). Difficulties understanding speech in challenging acoustical conditions in the presence of normal hearing thresholds have been proposed as a perceptual consequence of cochlear synaptopathy (e.g., Liberman et al., 2016; Mepani et al., 2020). Thus, we hypothesize that workers exposed to noise may develop cochlear synaptopathy, and that such a condition can be detected using ABR and SPiN tasks. Identifying cochlear synaptopathy in workers exposed to noise may be key for prevention programs in this population. Accordingly, the aim of this study was to determine whether ABR results and scores for a SPiN test were associated with occupational noise exposure in young workers with normal hearing thresholds and presence of otoacoustic emissions.

Materials and methods

The Ethics Committee of the Faculty of Medicine of the University of Montreal, the Committee on the Protection of Human Subjects of SUNY Plattsburgh, and the Ethics Committee of Zhejiang Provincial Center for Disease Control and Prevention approved the study protocol. All participants signed a consent form prior to being included in the study.

Participants

Two groups of participants from Zhejiang province in China were selected. Forty male workers exposed to occupational noise (noise-exposed group) at or above 80 dBA (based on the equivalent continuous average sound pressure level normalized to 8 h, $L_{Aeq.8h}$), along with 40 male participants without occupational exposure to noise (control group), were recruited. Participants from both groups were required to be aged between 18 and 40 years and have no family history of hearing loss, history of ear surgery, use of ototoxic drugs, or neurological disorders. They all presented with normal tympanometry (middle-ear pressure and compliance readings), and hearing thresholds (in at least one ear) equal to or better than 20 dB HL across frequencies (0.5–8 kHz). They also exhibited the presence of distortion product otoacoustic emissions (DPOAEs) (at least +3 dB SNR) for the frequency range of 2–10 kHz. Additionally, extended high frequency thresholds (9–14 kHz) were measured in both groups, but they were not used as an exclusion criterion. All participants were native Mandarin speakers.

Procedures

A research team member administered a questionnaire to the participants in both groups in order to collect the following information: general demographic information (e.g., age); occupational history (e.g., factories, worksite, job description, length of employment, duration of daily noise exposure, and history of using hearing protection); and overall health status (e.g., history of ear disease or ototoxic drug exposure). Workers exposed to noise for a minimum of 2 years in the same workplace were selected from four different types of industries (furniture manufacturing, n = 6, 15%; industrial equipment manufacturing, n = 12, 30%; electric and appliances' industry, n = 16, 40%; textile industry, n = 6, 15%) located in Zhejiang province, China. Participants without occupational noise exposure (control group) were university students from the Zhejiang Chinese Medical University. Participants with a history of ear disease or other related health conditions associated with auditory disorders were not included in the sample. Additionally, participants in both groups were asked whether they had experienced significant recreational noise exposure. This means exposure to firearms, playing a musical instrument or in a band, frequent attendance to concerts or sporting events, noisy bars and and/or nightclubs, along with excessive use of listening devices at elevated volumes. Participants' responses to this question were used to make sure that they did not report significant exposure to recreational noise.

Selected participants were scheduled for an assessment session at Zhejiang Chinese Medical University (Hangzhou, China). Initially, bilateral otoscopy was carried out with the aim of excluding participants with abnormalities in the external ear canal and tympanic membrane. Hearing testing was conducted in a double-walled, soundproofed, and electrically shielded room. The better ear (based on the results of pure-tone audiometry and DPOAEs tests) was selected for the statistical analyses.

Use of hearing protection devices

The frequency of use of hearing protection devices (HPDs) in the workplace, usually slow-recovery formable earplugs, was assessed through field observations by the industrial hygienist and in the questionnaire. For those participants who had never used HPDs, the members of the research team recommended the use of appropriate HPDs after data collection. During this study, workers in the investigated factories received training on how to properly use HPDs.

Noise exposure assessment in participants occupationally exposed to noise

Shift-long noise recordings were obtained for each noise-exposed participant using an ASV5910-R digital recorder (Hangzhou Aihua Instruments Co., Hangzhou, China). The ASV5910-R digital recorder is a specialized sound recording device that can be used for precision measurements and analysis of personal noise exposure since it allows to record the waveform. The instrument uses a $^1/_4$-inch pre-polarized condenser microphone characterized by good stability, a high upper measurement limit, and wide frequency response (20 Hz – 20 kHz). The sensitivity level of the microphone is 2.24 mV/Pa, and the measurement range is 40–141 dBA. The device was worn on the worker's shoulder during the entire work shift. The recorder was calibrated before and after each sampling period with the use of a sound level calibrator (Hangzhou Aihua Instruments, AWA6221B), according to the instructions provided by the manufacturer. Before recording, a research team member confirmed with the manager of the workplace that this was the noise the workers were typically exposed to on an average working day. One full-shift recording of each participant's noise exposure was captured by the ASV5910-R at 32-bit resolution with a 48-kHz sampling rate and saved in a raw audio format (WAV file). The noise record was saved on a 32 GB micro-SD card and transferred to a portable hard disk for subsequent analysis. The equivalent continuous average sound pressure level (LEQ) normalized to 8 h ($L_{Aeq.8h}$) was obtained

for each worker. Each one presented with a $L_{Aeq.8h}$ equal to or higher than 80 dBA. In addition, a composite noise exposure index, the CNE, in dBA.year, was calculated to quantify the noise exposure for each participant. The CNE is defined as:

$$CNE = L_{Aeq.8h} + 10 \log T$$

where $L_{Aeq.8h}$ is the equivalent continuous A-weighted noise exposure level normalized to an 8-h working day, in decibels, occurring over the time interval T in years.

As can be seen in the calculation, when a noisy activity is performed for many years, the numeric contribution to the total CNE diminishes with each additional year. Therefore, the CNE considers that early exposure has contributed more to the total exposure energy because the accumulation of noise exposure over the years is logarithmic. It has been reported that noise-induced hearing loss develops most rapidly in the first 10 years and then slows with additional exposure to noise (Dobie, 2001; Zhang et al., 2020). The CNE was previously used to evaluate the risk of hearing loss in workers exposed to occupational noise (e.g., Zhao et al., 2010; Xie et al., 2016).

In addition, corrected $L_{Aeq.8h}$ ($L_{Aeq.8h}$-HPD) and CNE (CNE-HPD) were calculated by incorporating estimates of HPD use into individual noise exposure calculations. First, the attenuation of each participant's HPD was derated based on the NIOSH recommendations to compensate for known differences between laboratory-derived attenuation values and the attenuation obtained in the real world (National Institute for Occupational Safety and Health [NIOSH], 1998). To do so, the noise reduction rating (NRR) was reduced by 50% since all participants used slow-recovery formable earplugs. For example, if a participant used an HPD with an attenuation of 29 dB, the derated NRR value was 14.5 dB. This value was then subtracted from the $L_{Aeq.8h}$ of each participant. For example, if a participant presented with a $L_{Aeq.8h}$ of 90 dBA, the 14.5 dB NRR was subtracted, and a new L_{Aeq} of 75.5 dBA was obtained. Then, the $L_{Aeq.8h}$-HPD value was obtained for each participant, based on the frequency of HPD use. For example, if a participant reported using HPDs ∼25% of the time, the total unprotected exposure (75% of the total time at a L_{Aeq} without HPD; 6 h at 90 dBA in this example) and the total protected exposure (25% duration at a protected level; 2 h at 75.5 dBA in this example) were combined. Finally, a corrected CNE value (CNE-HPD) was calculated for each participant based on the $L_{Aeq.8h}$-HPD. The $L_{Aeq.8h}$-HPD is defined as:

$$L_{Aeq.8h}HPD = 10 \log \left[\frac{1}{8}\left(\left(T_{unprotected} \times 10^{L_{Aeq.8h}/10}\right) + \left(T_{protected} \times 10^{(L_{Aeq.8h} - NRR \times 50\%)/10}\right)\right)\right]$$

where $L_{Aeq.8h}$-HPD is the equivalent continuous A-weighted noise exposure level normalized to an 8-h working day and corrected for HPD attenuation, in decibels, occurring over the time interval $T_{unprotected}$ and $T_{protected}$ in hours.

Tympanometry and pure-tone audiometry

An Interacoustics Titan device (Middelfart, Denmark) was used for tympanometry. The tympanometer probe was inserted into the external auditory canal. A 1,500 ms pulsed 226 Hz probe tone was presented, and middle-ear pressure and compliance readings were recorded. Participants were excluded from the study if they were classified with results different than type A in both ears, based on Jerger's classification (Jerger, 1970): middle ear compliance < 0.2 cc or middle ear pressure < -150 daPa (decaPascals).

Air-conduction pure-tone thresholds were obtained bilaterally with an Interacoustics AC629 clinical audiometer (Middelfart, Denmark) and Sennheiser HDA 300 headphones. The Hughson-Westlake procedure described by Carhart and Jerger (1959) was used. Hearing thresholds at 0.5, 1, 2, 3, 4, 6, 8, 9, 10, 11.2, 12, and 14 kHz were obtained. Included participants presented with hearing thresholds from 0.5 to 8 kHz, equal to or better than 20 dB HL in at least one ear.

Distortion product otoacoustic emissions

Distortion product otoacoustic emissions for both ears were obtained, measured, and analyzed using an Interacoustics Titan equipment with DPOAE440 module (Middlelfart, Denmark), connected to a Lenovo laptop computer (Beijing, China). The primary frequencies selected for the evaluation were the geometric means of f_1 and f_2 at 2, 3, 4, 5, 6, 7, 8, 9, and 10 kHz, using primary levels (L1/L2) of 65/55 dB SPL and a primary ratio (f_2/f_1) of 1.22. The levels of the $2f_1$-f_2 DPOAEs and the corresponding noise floor were registered as a function of f_2. Values for DPOAEs were obtained by subtracting the noise floor from the DPOAE amplitudes. Selected participants should have exhibited presence of DPOAEs (+3 dB SNR) for each of the aforementioned frequencies in at least one ear.

Auditory brainstem response

The ABR was recorded using an Intelligent Hearing System (IHS, Smart EP model, Miami, FL, United States) connected to a Lenovo laptop (Beijing, China). Surface electrodes were placed at the vertex (Cz, non-inverting electrode) and the forehead (Fpz, ground), in accordance with the International 10–20 system of EEG recordings. In addition, an extra-tympanic electrode (Lilly TM-Wick, IHS, Miami, FL, United States) was placed in the ipsilateral external auditory canal, sitting at the tympanic membrane (inverting electrode). This placement was chosen to improve the visualization of wave I and reduce intra-subject variability (Lefler et al., 2021). The amplifier bandpass was set between 0.3 and 3 kHz. Two trials, each averaging 2,000 responses, were obtained using rarefaction click stimulus at 90, 80, 70, 60, and 50 dBnHL presented monaurally to the better ear (according to pure-tone audiometry and DPOAEs) at a rate of 11.1 stimuli/second, with ER3A insert earphones. Trials were compared to check the reproducibility of the responses. Electrode impedance was less than 5 kOhms. Responses with an

amplitude above 30 μV were automatically rejected. In addition, electrical activity/noise that was common to both electrodes (i.e., inverting and non-inverting) was canceled out by common mode rejection. At each stimulus level, when waves I and V were below the residual noise, the waveform was excluded from the analysis. The recordings were visually inspected by a group-blind experienced audiologist to identify waves I, III, and V. The peak-to-trough amplitudes for waves I and V were obtained for analysis purposes. In addition, the slope of the wave I amplitude growth as a function of stimulus intensity (μV/dB) was calculated (AMP-I_{Slope}). The AMP-I_{Slope} was computed by fitting a straight line across the conditions in which the waveforms were identifiable. All conditions in which the ABR wave I was clear were required for the linear fits. When this was not the case, the participant was excluded from the analyses. Finally, the wave I/V amplitude ratio for 90 nHL stimulus was obtained in each participant.

In addition, ABRs for rarefaction click stimulus at 80 dBnHL with ipsilateral white noise at 45, 55, 65, 75, and 85 dB SPL were obtained (using a similar method as the one described by Mehraei et al., 2016). Surface electrodes were placed at the scalp, at the vertex (Cz), the ipsilateral mastoid (A1/A2), and the forehead (Fpz, ground). Latencies for wave V with ipsilateral masking noise at each of the aforementioned intensities were obtained. The latency shift (ms/dB) was calculated by fitting a straight line across the conditions in which the waveforms were identifiable at each level of the ipsilateral masking noise (LAT-V_{Slope}). All conditions in which the ABR wave V was clear were required for the linear fits. When this was not the case, the participant was excluded from the analyses.

Mandarin Bamford-Kowal-Bench sentence test (Mandarin BKB)

Speech recognition in noise was evaluated with the Mandarin BKB (Xi et al., 2012) in the better ear (according to pure-tone audiometry and DPOAEs). Initially, one list of 10 sentences was used as a practice round. Then, two lists of 10 sentences were presented monaurally through HDA 300 headphones (Sennheiser, Germany) at 70 dB HL fixed speech level in a background of four-talker babble noise (three females and one male). For each list, SNRs varied from +21 dB to −6 dB, beginning with the most favorable SNR (+21 dB) and progressing in 3 dB steps to more difficult SNRs (+21, +18, +15, +12, +9, +6, +3, 0, −3, and −6 dB). The first sentence had 4 key words, and the remaining nine sentences each had three key words. Participants were instructed to repeat back each sentence. The number of correctly repeated key words for each list was summed, and afterward subtracted from 23.5 to obtain the SNR-50%. This represents the SNR at which a listener correctly identifies 50% of the key words. Then, an average between SNR-50% for both lists was calculated (Etymotic Research Inc, Elk Grove Village, IL, United States). A lower SNR-50% score indicates better SPiN performance.

Statistical analysis

Statistical analyses were performed with SPSS V27 (IBM Corp, 2020). First, Student *t*-tests were used to compare the noise-exposed and control groups' age, and to compare noise levels (CNE/$L_{Aeq.8h}$) between participants who reported to use HPDs and those who did not.

Second, differences in individual hearing thresholds and DPOAE amplitudes were analyzed using repeated measures ANOVAs, with individual frequency as an intra-subject factor and group as a between-subject factor. *Post hoc* Student *t*-tests with Bonferroni corrections were used to describe possible interactions and main effects. Since group differences were observed for pure-tone thresholds at 0.5, 1, and 4 kHz and for extended high frequencies at 11.2, 12, and 14 kHz, two averages were calculated for the hearing thresholds in the better ear: one average for hearing thresholds at 0.5, 1, 2, and 4 kHz (PTA_4) and another for hearing thresholds from 9 to 14 kHz (PTA_{EHF}). Also, a $DPOAE_{mean}$ was calculated by averaging the amplitude in dB SNR of DPOAEs in the better ear across all frequencies (2–10 kHz).

Third, ABR measures (waves I and V amplitudes and latencies, ABR I/V amplitude ratio, AMP-I_{Slope}, and LAT-V_{Slope}) and BKB test results were compared between groups using Student *t*-tests. An ANCOVA test was also performed for all ABR and BKB measures, controlling for hearing thresholds (PTA_4 and PTA_{EHF}). This aimed to better control for differences in the audiogram between the noise-exposed and control groups.

Pearson correlations between CNE/CNE-HPD, $L_{Aeq.8h}$/$L_{Aeq.8h}$-HPD, age, PTA_4, PTA_{EHF}, $DPOAE_{mean}$, ABR results, and SPiN were computed with the data obtained from the sample of workers exposed to noise. Finally, bivariate and multivariate regression models were constructed to independently investigate possible associations between SPiN (i.e., the dependent variable) and the independent factors of age, PTA_4, PTA_{EHF}, $DPOAE_{mean}$, and the ABR results. For the multivariate models, a backward elimination technique was used to select the remaining significant variables in the adjusted analysis, using a selection criterion of $\alpha < 0.05$.

Results

Age, noise exposure and use of hearing protection devices

The group mean age was 28.4 ± 5.3 years for noise-exposed participants and 21.1 ± 3.7 years for control group participants. Control group participants were significantly younger than noise-exposed participants [$t(78) = 7.24$, $p < 0.001$]. In the noise-exposed group ($n = 40$), the mean occupational noise exposure level for $L_{Aeq.8h}$ was 89.8 ± 5.4 dBA and the group

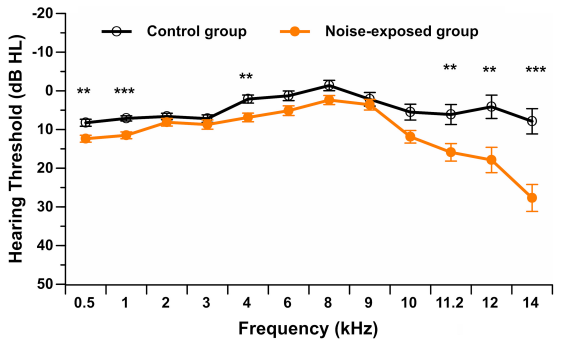

FIGURE 1
Pure-tone audiometric thresholds (in dB HL) in the better ear from 0.5 to 14 kHz in noise-exposed and control group participants. Error bars represent the standard error. $^{**}p < 0.01$; $^{***}p < 0.001$.

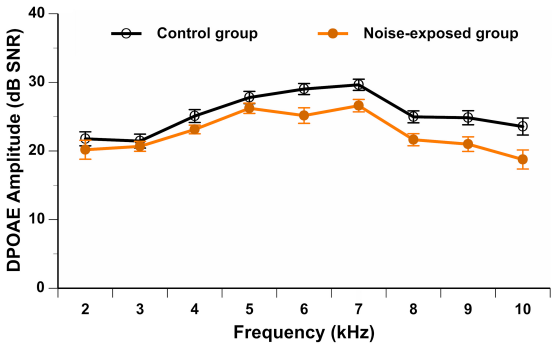

FIGURE 2
DPOAE amplitudes (in dB SNR) in the better ear from 2 to 10 kHz in noise-exposed and control group participants. Error bars represent the standard error. No significant differences between groups are observed after Bonferroni correction for multiple comparisons.

mean of CNE was 96.3 ± 5.6 units of noise exposure (dBA.year), while the $L_{Aeq.8h}$-HPD and the CNE-HPD were 76.7 ± 4.8 dBA and 82.5 ± 5.5 dBA.year respectively. Duration of exposure to noise in the workplace ranged from 2 to 18 years (mean ± SD: 6.3 ± 4.6 years).

Regarding HPDs, 75% of noise-exposed participants ($n = 30$) reported to use them in their workplace. Out of these participants, 90% ($n = 27$) reported to use them "often," while 10% ($n = 3$) reported to use them "sometimes." A significantly higher $L_{Aeq.8h}$ [$t(38) = -2.86$, $p = 0.007$] was obtained in participants who reported to use HPDs (91.1 ± 5.4 dBA) compared to those who did not report to use HPDs (85.9 ± 4.5 dBA). A similar result was obtained for CNE [$t(38) = -2.24$, $p = 0.031$], with a higher CNE in participants who reported to use HPDs (97.4 ± 5.2 dBA.year) compared to those who did not (93.0 ± 5.8 dBA.year).

Hearing thresholds and distortion product otoacoustic emissions

All participants presented with hearing thresholds from 0.5 to 8 kHz, equal to or better than 20 dB HL in the better ear. Note that this was part of the inclusion criteria. Participants also presented with normal or near-normal hearing thresholds in the contralateral ear (equal to or better than 20 dB HL). Figure 1 displays the hearing thresholds in the better ear (0.5–14 kHz) for each group of participants at all tested frequencies.

For the standard pure-tone audiometry (0.5–8 kHz), the repeated measures ANOVA showed no interaction between group and stimulus frequency [$F(6,450) = 1.35$, $p = 0.232$]. A significant main effect of group [$F(1,75) = 10.06$, $p = 0.002$] was observed. *Post hoc* *t*-tests showed that control group participants presented with a significantly lower (i.e., better) hearing threshold than noise-exposed participants at 0.5 kHz ($p = 0.002$, mean difference of 4.1 dB HL), 1 kHz ($p < 0.001$, mean difference of 4.4 dB HL) and 4 kHz ($p = 0.003$, mean difference of 4.8 dB HL) after controlling for multiple comparisons (Bonferroni correction; 0.05/7 = 0.007). Although statistically reliable, these threshold differences were small and were not clinically significant.

For extended high-frequency pure-tone audiometry (9–14 kHz), the repeated measures ANOVA showed a significant interaction between group and stimulus frequency [$F(4,304) = 9.91$, $p < 0.001$]. *Post hoc* *t*-tests with a Bonferroni correction showed that participants in the control group did not exhibit significant differences in hearing thresholds among extended high frequencies. However, noise-exposed participants presented with worse hearing thresholds at 14 kHz and better hearing thresholds at 9 kHz compared to all other extended high frequencies ($p < 0.001$). Additionally, control group participants presented with a significantly lower (i.e., better) hearing threshold than noise-exposed participants at 11.2 kHz ($p = 0.007$, mean difference of 9.8 dB HL), 12 kHz ($p = 0.003$, mean difference of 13.8 dB HL), and 14 kHz ($p < 0.001$, mean difference of 19.8 dB HL) after controlling for multiple comparisons (Bonferroni correction; 0.05/5 = 0.01).

As previously mentioned, all participants should have presented with DPOAE amplitudes equal to or better than 3 dB SNR at each tested frequency ($f2$: 2–10 kHz) in the better ear. None of the participants presented with an absence of DPOAEs in the contralateral ear (defined as an amplitude smaller than 3 dB SNR). Figure 2 displays the DPOAE amplitudes in the better ear for both groups. The repeated measures ANOVA showed no significant interaction between group and stimulus frequency [$F(9,666) = 1.68$, $p = 0.089$]. A significant main effect of group was observed [$F(1,74) = 6.36$, $p = 0.014$], with control group participants presenting with higher (i.e., better) DPOAE amplitudes than noise-exposed participants. *Post hoc*

t-tests showed a significant difference in DPOAE amplitudes between groups at 6 kHz ($p = 0.007$, mean difference of 3.4 dB SNR), 7 kHz ($p = 0.017$, mean difference of 3.0 dB SNR), 8 kHz ($p = 0.010$, mean difference of 3.3 dB SNR), 9 kHz ($p = 0.013$, mean difference of 3.9 dB SNR) and 10 kHz ($p = 0.013$, mean difference of 4.8 dB SNR). However, these differences were no longer significant after controlling for multiple comparisons (Bonferroni correction; 0.05/10 = 0.005).

Auditory brainstem response

Figure 3A displays the grand mean ABR waveform for each group of participants, which was obtained using click stimuli at 90 dBnHL. In Figures 3B,C, individual ABR waveforms for click stimulus at 90 dBnHL are displayed for noise-exposed and control participants respectively.

Peak-to-trough amplitudes (μV) and latencies (ms) for ABR wave I and wave V at each stimulus presentation level (i.e., 90, 80, 70, 60, and 50 dBnHL) were obtained for each participant (see Table 1 for a summary). Even at low stimulus levels, waves I and V were identifiable in most waveforms and the response was above the residual noise (e.g., at 50 dBnHL, $n = 67$ for wave I and $n = 76$ for wave V; at 60 dBnHL, $n = 68$ for wave I and $n = 74$ for wave V; at 70 dBnHL, $n = 78$ for waves I and V). Mean wave I amplitudes ranged from 0.27 μV at 50 dB nHL to 1.88 μV at 90 dB nHL.

No significant differences between groups were observed for the amplitudes of wave I at 50 dBnHL [$t(65) = -0.97$, $p = 0.335$], 60 dBnHL [$t(66) = 0.27$, $p = 0.786$], 70 dBnHL [$t(76) = -0.57$, $p = 0.568$], 80 dBnHL [$t(74) = 0.39$, $p = 0.700$] and 90 dBnHL [$t(78) = -0.66$, $p = 0.513$]. Similarly, no differences between groups were observed for the amplitudes of wave V at 50 dBnHL [$t(74) = -0.70$, $p = 0.487$], 60 dBnHL [$t(72) = -0.54$, $p = 0.593$], 70 dBnHL [$t(76) = -0.46$, $p = 0.650$], 80 dBnHL [$t(74) = -0.70$, $p = 0.484$] and 90 dBnHL [$t(74) = -0.96$, $p = 0.342$] (see Table 1). Wave I amplitudes for both groups at each stimulus level are also shown in Figure 4A. The ABR I/V amplitude ratio at 90 dBnHL was calculated for each participant to better control for individual variability (Figure 4B). No significant difference between noise-exposed participants and control participants was observed [$t(78) = -1.21$, $p = 0.230$]. Regarding the ABR wave I and V latencies, no significant differences were observed between groups at any stimulus levels (see Table 1).

Additionally, the ABR AMP-I_{Slope} was computed. For some participants, the ABR AMP-I_{Slope} could not be obtained because the waveform was not identified in at least one stimulus level ($n = 9$ for the noise-exposed group and $n = 9$ for the control group). No significant difference between groups was observed for the ABR AMP-I_{Slope} [$t(60) = -0.02$, $p = 0.984$] (Figures 4C,D).

Finally, the ABR LAT-V_{Slope} was obtained for each participant. Figure 5A displays the ABR wave V latency at each intensity level of the ipsilateral white noise. In Figures 5B,C, the ABR wave V latency shift as a function of ipsilateral white noise intensity (ABR LAT-V_{Slope}) is displayed for noise-exposed and control participants respectively. The response was above the residual noise for all recordings, and the wave V with ipsilateral white noise was identifiable in most waveforms. For some participants, the ABR LAT-V_{Slope} could not be calculated because the waveform was not identified for at least one intensity level of the white noise ($n = 2$ for the noise-exposed group and $n = 10$ for the control group). No significant difference between groups for the ABR LAT-V_{Slope} was observed [$t(66) = -0.66$, $p = 0.514$]. In addition to these analyses, we performed another analysis on ABR outcomes between groups controlling for PTA_4 and PTA_{EHF} (see Supplementary Table 1). No significant differences between groups were observed for any ABR outcomes.

Speech perception in noise

Noise-exposed participants presented with significantly poorer BKB results (i.e., higher SNR-50%) than control group participants [$t(73) = 3.87$, $p < 0.001$] (Figure 6), even when controlling for PTA_4 and PTA_{EHF} by using an ANCOVA [$F(1,71) = 6.55$, $p = 0.013$].

Correlations between noise exposure and auditory outcomes

A Pearson correlation matrix between CNE/CNE-HPD, $L_{Aeq.8h}$/$L_{Aeq.8h}$-HPD, hearing thresholds (PTA_4 and PTA_{EHF}), $DPOAE_{mean}$, ABR results, and BKB scores was obtained in noise-exposed participants ($n = 40$) (Table 2). First, no significant correlations were observed between CNE/$L_{Aeq.8h}$ (uncorrected and corrected for HPD use) variables and any of the auditory outcomes (PTA_4, PTA_{EHF}, $DPOAE_{mean}$, ABR and BKB results). Second, $DPOAE_{mean}$ was significantly correlated with age, PTA_{EHF}, and ABR I/V amplitude ratio. Third, the amplitude of wave I at 90 dBnHL was significantly correlated with the ABR I/V amplitude ratio and the AMP-I_{Slope}. Finally, BKB results were not correlated with the ABR measures.

Regression models

The previous analyses showed significant differences between noise-exposed workers and control group participants for BKB scores. Therefore, bivariate linear regression analyses were carried out to further examine associations between BKB scores and group category (noise exposure) along with other factors that may be associated with SPiN including both age and auditory outcomes (PTA_4, PTA_{EHF}, $DPOAE_{mean}$, and

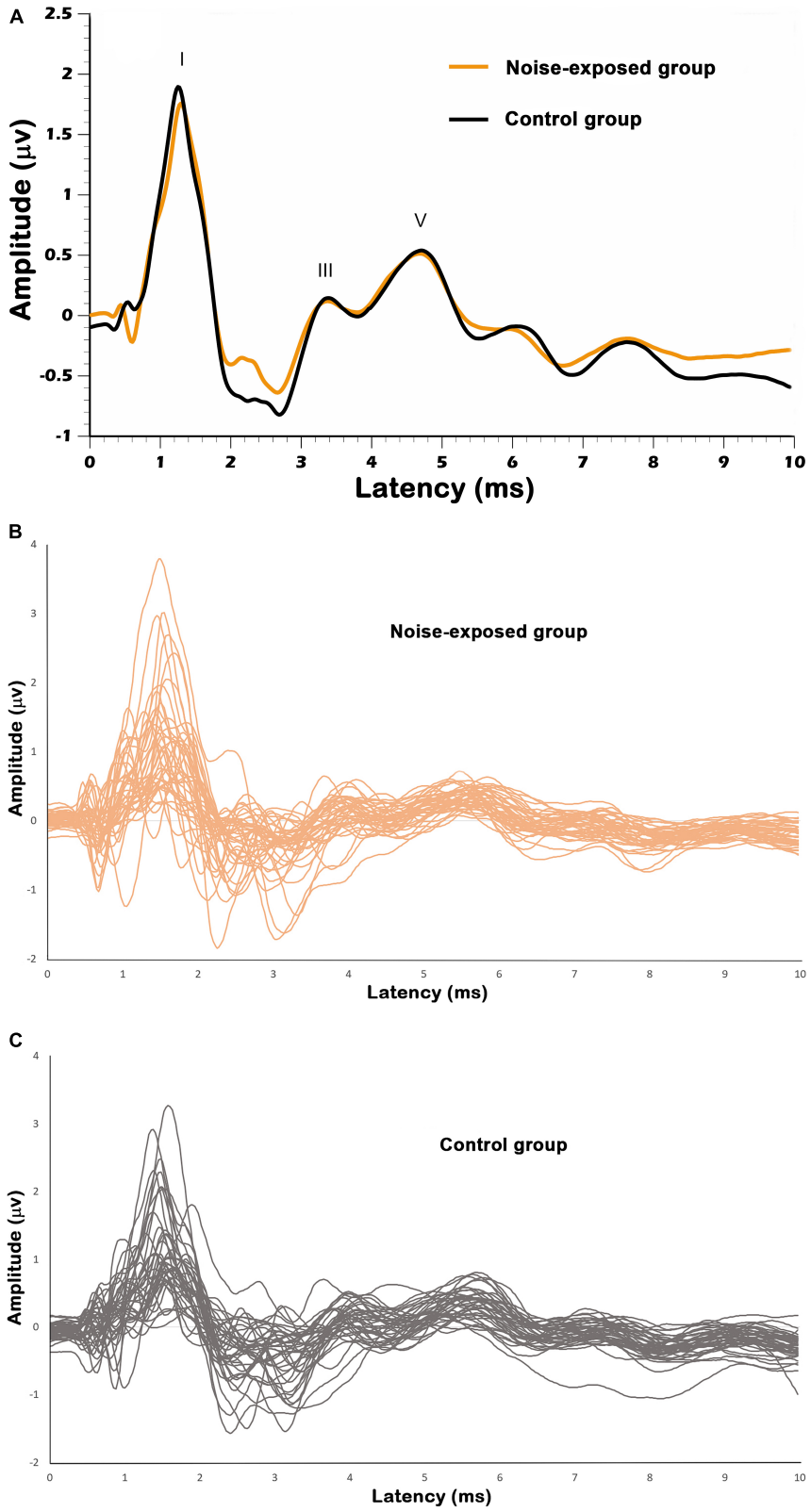

FIGURE 3
(A) Grand mean ABR triggered by click stimulus at 90 dBnHL for the noise-exposed and control group. The individual ABR waveforms are also illustrated in **(B,C)** for noise-exposed and control groups. Surface electrodes were placed at the vertex (Cz, non-inverting electrode) and the forehead (Fpz, ground), while an extra-tympanic electrode (inverting electrode) was placed sitting at the tympanic membrane. I, III, and V denote wave I, wave III, and wave V.

TABLE 1 Mean, standard deviation, and group comparisons for ABR wave I and wave V variables (amplitude and latency).

	Noise-exposed group	Control group	
ABR measures	**Mean ± SD (*n*)**	**Mean ± SD (*n*)**	***P*-value**
Amplitude (μV)			
Wave I			
90 dBnHL	1.72 ± 1.00 (*n* = 40)	1.88 ± 1.12 (*n* = 40)	0.513
80 dBnHL	1.45 ± 0.91 (*n* = 36)	1.38 ± 0.82 (*n* = 40)	0.700
70 dBnHL	0.76 ± 0.53 (*n* = 38)	0.83 ± 0.51 (*n* = 40)	0.568
60 dBnHL	0.35 ± 0.34 (*n* = 34)	0.33 ± 0.23 (*n* = 34)	0.786
50 dBnHL	0.27 ± 0.35 (*n* = 34)	0.35 ± 0.28 (*n* = 33)	0.335
Wave V			
90 dBnHL	0.51 ± 0.19 (*n* = 40)	0.54 ± 0.25 (*n* = 40)	0.521
80 dBnHL	0.40 ± 0.19 (*n* = 36)	0.42 ± 0.16 (*n* = 40)	0.484
70 dBnHL	0.32 ± 0.13 (*n* = 39)	0.34 ± 0.18 (*n* = 39)	0.650
60 dBnHL	0.24 ± 0.09 (*n* = 36)	0.26 ± 0.14 (*n* = 38)	0.593
50 dBnHL	0.21 ± 0.10 (*n* = 40)	0.22 ± 0.09 (*n* = 36)	0.487
Latency (ms)			
Wave I			
90 dBnHL	1.56 ± 0.17 (*n* = 40)	1.54 ± 0.16 (*n* = 40)	0.615
80 dBnHL	1.68 ± 0.18 (*n* = 36)	1.64 ± 0.16 (*n* = 40)	0.289
70 dBnHL	1.88 ± 0.29 (*n* = 38)	1.83 ± 0.22 (*n* = 40)	0.383
60 dBnHL	2.20 ± 0.34 (*n* = 34)	2.17 ± 0.33 (*n* = 34)	0.687
50 dBnHL	2.74 ± 0.38 (*n* = 34)	2.61 ± 0.30 (*n* = 33)	0.128
Wave V			
90 dBnHL	5.65 ± 0.24 (*n* = 40)	5.59 ± 0.23 (*n* = 40)	0.263
80 dBnHL	5.75 ± 0.22 (*n* = 36)	5.71 ± 0.22 (*n* = 40)	0.393
70 dBnHL	5.91 ± 0.26 (*n* = 39)	5.85 ± 0.23 (*n* = 39)	0.299
60 dBnHL	6.20 ± 0.36 (*n* = 36)	6.10 ± 0.27 (*n* = 38)	0.188
50 dBnHL	6.56 ± 0.39 (*n* = 40)	6.52 ± 0.27 (*n* = 36)	0.571

ABR AMP-I_{Slope}). Then, multivariate regression analyses were performed to model the association between BKB scores and the factors tested in the bivariate regression models (**Table 3**). Age, group category (noise exposure) and PTA_4 were significantly associated with BKB scores in the bivariate models. The final multivariate regression model indicated that group category (noise exposure) and PTA_4 significantly predicted 23.3% of the variability in the BKB scores.

Discussion

Auditory brainstem response outcomes

In this study, we used four ABR outcomes that may be affected by cochlear synaptopathy. Occupationally noise-exposed and control participants did not significantly differ for any of these outcomes (i.e., wave I amplitude at 90 dBnHL, wave I/V amplitude ratio at 90 dBnHL, the slope of the wave I amplitude growth as a function of stimulus intensity, and the slope of wave V latency shift as a function of ipsilateral white noise intensity). To our knowledge, this was the first study investigating all four ABR outcomes in normal hearing young adults with occupational noise exposure. The results indicate that cochlear synaptopathy was not observed in this sample of participants, or that these ABR outcomes were not sensitive enough to detect this condition in humans with the characteristics of our sample.

Previous studies in humans have extensively used the ABR in an attempt to detect cochlear synaptopathy in humans. Like this study, other studies investigating non-occupational populations exposed to noise have not found an effect of noise exposure on ABR wave I amplitude (Fulbright et al., 2017; Prendergast et al., 2017a; Ridley et al., 2018; Couth et al., 2020). Additionally, in a study with 20 normal hearing persons with occupational noise exposure, Pushpalatha and Konadath (2016) did not find an effect of noise exposure on ABR wave I amplitude. However, a reduction of ABR wave I amplitude associated with noise exposure has been found by some researchers in non-occupational samples of persons exposed to noise (Stamper and Johnson, 2015; Valderrama et al., 2018; Wang et al., 2021). In addition, a reduction in wave I amplitude was observed in a population of veterans exposed to firearms (Bramhall et al., 2017) and in a population of musicians (Kikidis et al., 2020). A number of factors, such as participants' inclusion criteria, noise exposure metrics, and participants' profiles, may explain the differences in study results. In two of these studies (Valderrama et al., 2018; Kikidis et al., 2020), researchers did not control for possible hair cell deficits (measured by hearing thresholds and DPOAEs), which could likely explain the reduced ABR wave I amplitude in the noise-exposed group.

The differences in results among the previous studies may also be explained by intersubject variability of ABR wave I amplitude due to electrode placement and head size (Bramhall, 2021). Therefore, it has been suggested that using the ABR wave I/V amplitude ratio can diminish that variability by canceling out the subject-specific factors that impact all peaks. However, when the measure was used in this research, no significant differences between groups were observed. Like this study, previous research has not found an association between noise exposure and the ABR wave I/V amplitude ratio (Guest et al., 2017). However, other authors have reported a reduced ABR wave I/V amplitude ratio associated with non-occupational noise exposure (Grose et al., 2017) or tinnitus (Schaette and McAlpine, 2011). This reduced ratio has been explained by a smaller wave I amplitude with no changes in wave V amplitude. It is important to note that some of the studies that have found an effect of noise exposure on ABR wave I and/or wave I/V amplitude ratio have included female participants. It has been reported that gender has an effect on ABR outcomes (for a

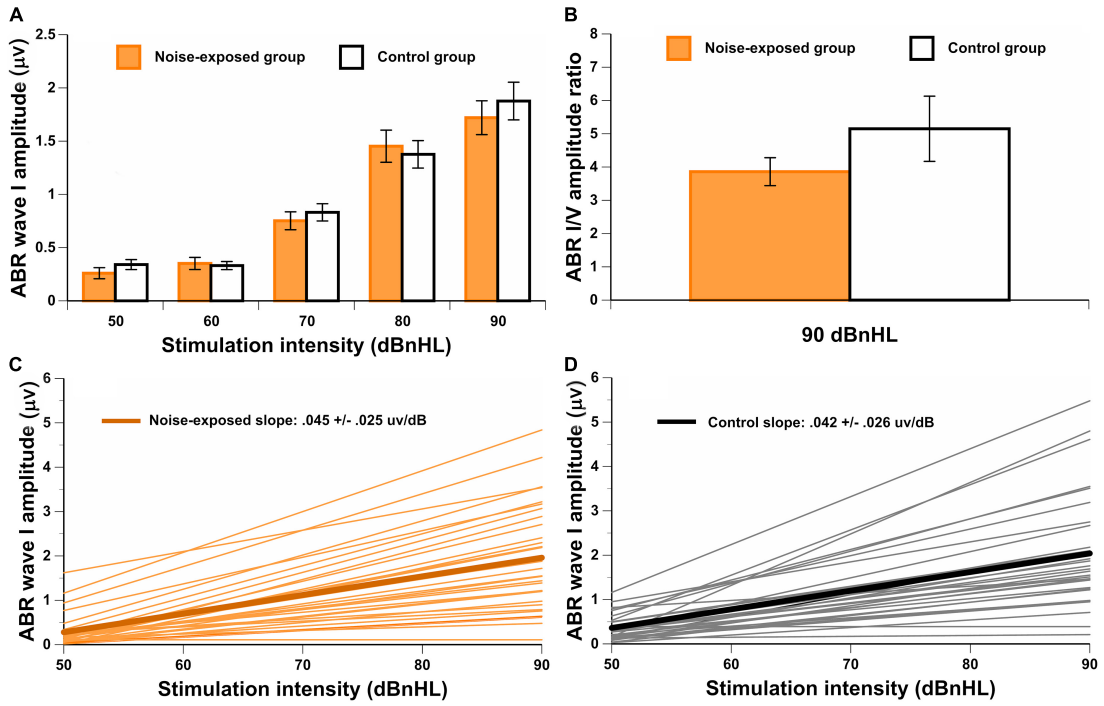

FIGURE 4
(A) ABR wave I amplitude for both groups at each stimuli level. In **(B)**, the wave I/V amplitude ratio for both groups at 90 dBnHL. The group mean and individual results for the ABR AMP-I_{Slope} are also illustrated in **(C,D)** for noise-exposed and control groups. There are no significant differences between groups.

review, see Bramhall, 2021), and that may have affected their results. In this study, we selected only male workers, with the aim of controlling for gender differences in ABR. Finally, the stimulation rate may be another explanation for the divergent results among studies. Kikidis et al. (2020) found a reduced ABR wave I amplitude and I/V amplitude ratio in musicians compared to non-musicians, and such differences were more marked at higher stimulation rates. The authors concluded that a higher stimulation rate would better allow the detection of cochlear synaptopathy. However, the reasoning of Kikidis and colleagues' rests on the assumption that low-SR fibers will be "stressed" by high presentation rates. It could be argued more cogently that high presentation rates will reduce the contribution of low-SR fibers to the response, leading to ABRs that are dominated by high-SR fibers and hence less sensitive to cochlear synaptopathy.

In this study, we also calculated the slope of ABR wave I amplitude growth as a function of stimulus intensity (AMP-I_{Slope}). We hypothesized that in the presence of cochlear synaptopathy, noise-exposed workers would present with a reduced AMP-I_{Slope} as compared to unexposed participants. This was because at low stimulation intensity, the activity of the auditory system mainly comes from medium- and high-SR fibers, which are less susceptible to noise exposure (Bourien et al., 2014; Marmel et al., 2015). As the stimulus intensity increases, the auditory system also increases the recruitment of low-SR auditory fibers, which are affected by cochlear synaptopathy (Furman et al., 2013). Thus, individuals with cochlear synaptopathy should exhibit a reduced AMP-I_{Slope} as compared to individuals who do not exhibit cochlear synaptopathy. The results of this study did not support our hypothesis, as no differences between the noise-exposed and control participants were found. Previously, Bramhall et al. (2020) found a steeper ABR wave I amplitude growth function in veterans with decreased sound tolerance. However, this finding was observed in a different population (i.e., veterans with exposure to impulse noise from firearms and with reported decreased sound tolerance) than the one investigated in this study.

Finally, we obtained the latency of ABR wave V in the presence of ipsilateral white noise at different intensities. The aim of this technique was to obtain the slope of the amount of shift of ABR wave V latency as a function of the intensity of the masker (LAT-V_{Slope}). We hypothesized that in the presence of cochlear synaptopathy, noise-exposed workers would present with a reduced LAT-V_{Slope} compared to unexposed participants. This hypothesis was supported by the results of Mehraei et al. (2016). They found that mice with histologically confirmed cochlear synaptopathy showed a smaller latency shift of wave IV (equivalent to wave V in humans) in the presence of masking

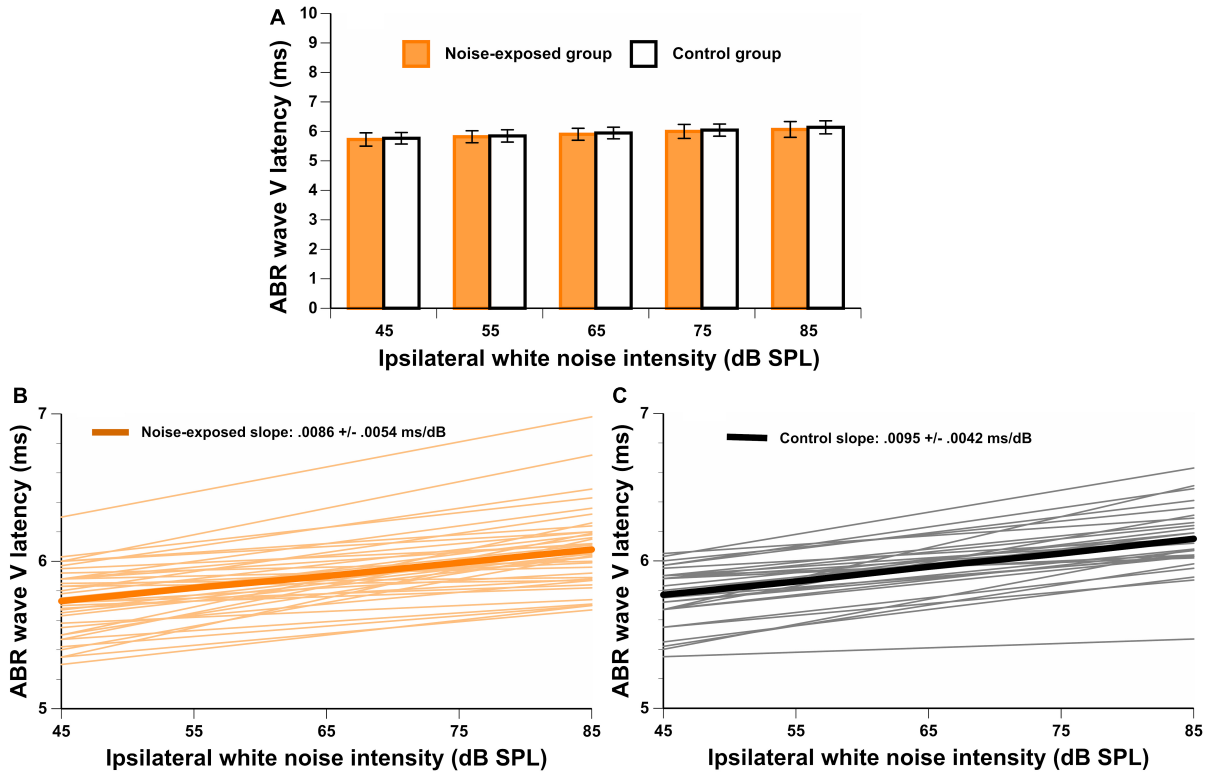

FIGURE 5
(A) Wave V latencies for a stimulation level of 80 dBnHL with different ipsilateral white noise intensities (45, 55, 65, 75, and 85 dB SPL). In **(B,C)**, the ABR LAT-V_{Slope} is illustrated for both groups. There are no significant differences between groups.

noise than control mice. In addition, in their human cohort, they found that participants with reduced wave V latency shift also displayed worse performances on a sound localization in noise task. However, since Mehraei et al. (2016) did not quantify participants' noise exposure, it is still unclear if this ABR outcome might be affected by cochlear synaptopathy in humans. In our study, we did not find significant differences between groups for this ABR outcome. To our knowledge, no other studies have used this technique to detect cochlear synaptopathy in humans exposed to noise.

In addition to group comparisons, we performed a correlation analysis with noise-exposed workers between their noise exposure levels and auditory outcomes (e.g., ABR and SPiN). Noise exposure levels (i.e., $L_{Aeq.8h}$ and CNE, corrected and uncorrected for HPD use) were not significantly correlated with these outcomes. Also, BKB scores, which showed significant differences between groups (see below), were not significantly correlated with the ABR outcomes used in this study. In addition, note that noise-exposed workers were significantly older (by around 7 years) than control participants. They also presented with significantly worse hearing thresholds at some frequencies than control participants, although these were within normal ranges. Both variables are likely to reduce ABR wave I amplitude, and yet no significant differences between groups were observed. In light of these results, we believe that cochlear synaptopathy could not be observed in this sample of workers.

Speech perception in noise

Significantly worse SPiN scores (BKB) were found for noise-exposed participants than for controls. These results were in agreement with those of some previous studies conducted on university students (Liberman et al., 2016) and construction workers (Vijayasarathy et al., 2021). For example, Liberman et al. (2016) found significantly worse results for SPiN in individuals considered at high risk to develop cochlear synaptopathy (based on their noise exposure history) than in individuals considered at low risk. However, these results were only obtained for the most challenging listening conditions (with reverberation and time-compressed speech). Furthermore, the SPiN material was presented at moderate (around 40 dB SPL) intensity, where it is unclear how much recruitment of low-SR fibers there would be. Several other studies have not found an effect of noise exposure on SPiN outcomes (e.g., Fulbright et al., 2017; Grinn et al., 2017; Grose et al., 2017; Prendergast et al., 2017b; Yeend et al., 2017; Guest et al., 2018; Smith et al., 2019).

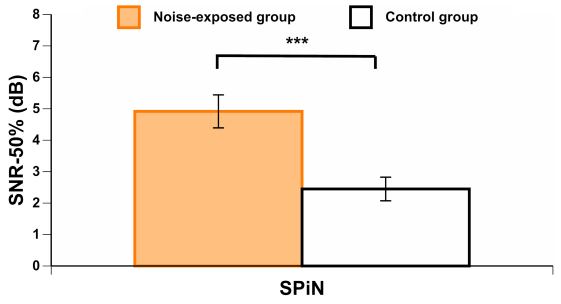

FIGURE 6
BKB scores in each group. Higher scores indicate a higher signal-to-noise ratio loss (worse SPiN performance). Error bars represent the standard error. ***$p < 0.001$.

In addition to the significant difference between groups for BKB scores, a multivariate regression model showed that 23.3% of the variance in BKB scores was explained by group category (noise-exposed vs. control) and PTA_4. Age, $DPOAE_{mean}$, PTA_{EHF}, and ABR AMP-I_{Slope} did not explain the worse SPiN scores in the noise-exposed group, since these factors were not associated with BKB scores in the regression model. Audibility has been suggested to be associated with performance on SPiN tests, although it does not fully account for the variance in SPiN scores (e.g., Anderson et al., 2011). In this study, we hypothesized that workers exposed to noise would exhibit signs of cochlear synaptopathy. However, we discard the hypothesis that cochlear synaptopathy explains the effects of group category on BKB scores. This is because, as discussed previously, no signs of cochlear synaptopathy were observed in the sample of workers exposed to noise by the use of four ABR outcomes. In addition, noise exposure levels ($L_{Aeq.8h}$ and CNE, corrected and uncorrected for HPD use) were not significantly associated with BKB scores. Therefore, we suggest that variables associated with group category other than noise exposure may explain these results. Factors that go beyond sensory processing may have been implicated. For example, factory workers are likely to present with poorer performance for working memory, attention, and language capacities than university students (control group participants). This is because in general factory workers in China have a lower educational level (Chen and Guan, 2016). It has been previously reported that both cognitive resources and language competence can influence SPiN performance (Schneider et al., 2002; Pienkowski, 2017; DiNino et al., 2022). These aspects were not explored in the present study, and thus, we cannot conclude that sensory processing was the main underlying factor that explained our results. Future studies should control for cognitive abilities when interpreting SPiN performance in individuals with occupational noise exposure. In summary, we conclude that differences between groups for BKB scores were not likely associated with cochlear synaptopathy or with another auditory deficit associated with noise exposure, but rather that such differences likely rely on non-sensory processing differences between groups.

Limitations

We identified five main limitations in the present study. First, we collected data from participants' better ears. For

TABLE 2 Correlation coefficients (Pearson) between $L_{Aeq.8h}$/$L_{Aeq.8h}$-HPD, CNE/CNE-HPD, age, hearing thresholds, DPOAEs, BKB results, and ABR measures for the noise-exposed group.

	Age	PTA_4	PTA_{EHF}	$DPOAE_{mean}$	BKB	Amp I	I/V	AMP-I_{Slope}	LAT-V_{Slope}
$L_{Aeq.8h}$	−0.178	0.060	−0.031	0.059	−0.159	0.186	0.120	0.342	0.095
$L_{Aeq.8h}$-HPD	−0.273	0.140	0.004	0.041	−0.157	0.140	0.083	0.337	0.086
CNE	0.181	0.137	−0.073	0.034	−0.137	0.285	0.182	0.342	0.177
CNE-HPD	0.106	0.254	0.012	−0.008	−0.152	0.136	0.059	0.326	0.146
Age	·	−0.072	0.200	−0.413**	−0.057	−0.019	0.063	−0.110	−0.024
PTA_4	·	·	0.044	0.149	0.266	−0.280	−0.131	−0.216	0.114
PTA_{EHF}	·	·	·	−0.425**	0.008	−0.095	−0.057	−0.071	−0.005
$DPOAE_{mean}$	·	·	·	·	−0.122	−0.236	−0.397*	−0.138	−0.082
BKB	·	·	·	·	·	−0.265	−0.021	−0.309	−0.168
Amp I	·	·	·	·	·	·	0.777***	0.960***	0.272
I/V	·	·	·	·	·	·	·	0.685***	0.161
AMP-I_{Slope}	·	·	·	·	·	·	·	·	0.327
LAT-V_{Slope}	·	·	·	·	·	·	·	·	·

$L_{Aeq.8h}$, equivalent continuous sound level for an 8 h work shift; $L_{Aeq.8h}$-HPD, equivalent continuous sound level for an 8 h work shift corrected for HPD use; CNE, cumulative noise exposure; CNE-HPD, cumulative noise exposure corrected for HPD use; Age, age of participant in years; PTA_4, pure-tone threshold average of the better ear at 0.5, 1, 2, and 4 kHz; PTA_{EHF}, pure-tone threshold average of the better ear from 9 to 14 kHz; $DPOAE_{mean}$, DPOAEs amplitudes of the better ear from 2 to 10 kHz; BKB, Mandarin Bamford-Kowal-Bench sentence test scores; Amp I, ABR wave I amplitude at 90 dBnHL (μV); I/V, amplitude ratio between ABR wave I and wave V at 90 dBnHL; AMP-I_{Slope}, slope of the ABR wave I amplitude growth as a function of stimulus intensity; LAT-V_{Slope}, shift of the ABR wave V latency with ipsilateral white noise. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

TABLE 3 Bivariate and multivariate linear regression analyses for BKB scores.

	Bivariate model			Initial multivariate model		Final multivariate model	
Characteristic	**Beta**	***P*-value**	R^2	**Beta**	***P*-value**	**Beta**	***p*-value**
Age	0.295	0.010	0.087	−0.077	0.616		
Occupational noise exposure:Exposed	0.413	<0.001	0.171	0.318	0.034	0.297	0.009
Unexposed	Ref						
$DPOAE_{mean}$	−0.270	0.019	0.073	−0.370	0.019		
PTA_4	0.422	<0.001	0.178	0.312	0.015	0.311	0.006
PTA_{EHF}	0.226	0.051	0.051	−0.206	0.177		
ABR AMP-I_{Slope}	−0.212	0.104	0.045	−0.198	0.090		
				Adjusted R^2 = 0.272		Adjusted R^2 = 0.233	

Age, age of participant in years; PTA_4, pure-tone threshold average of the better ear at 0.5, 1, 2, and 4 kHz; PTA_{EHF}, pure-tone threshold average of the better ear from 9 to 14 kHz; $DPOAE_{mean}$, DPOAEs amplitudes of the better ear from 2 to 10 kHz; ABR AMP-I_{Slope}, slope of the ABR wave I amplitude growth as a function of stimulus intensity.

some participants, data were acquired in the left ear, while for others, testing was conducted in the right ear. A recent study suggested that electrophysiological measures (i.e., ABR wave I/V amplitude ratio) are associated with SPiN performance, specifically in the left ear (Megarbane and Fuente, 2020). This could be explained by differences in aspects such as internal redundancy between the right and the left auditory pathways, with the left-ear pathway being less dominant for the processing of speech stimuli than the right-ear pathway (Lazard et al., 2012). It is generally accepted that click ABR latencies are relatively symmetrical between the right and the left ears (Rowe, 1978). However, results regarding ABR amplitudes are less clear, as some researchers have suggested bigger ABR amplitudes for right ear stimulation (Levine et al., 1988). Since we did not control for the tested ear (right versus left) when comparing results between groups, we are not certain whether possible ear asymmetries for the processing of stimuli might have affected our results. Future studies should explore the possible differences between the right and the left ear for the measurement of cochlear synaptopathy in persons occupationally exposed to noise.

Second, the SPiN test (BKB) consisted of the repetition of sentences, which relies on a higher cognitive load than the repetition of words. None of the participants had a cognitive assessment, and young university students likely have better cognitive and language abilities than young factory workers. Note that the BKB speech material was created to be understood by children aged between 4 and 5 years (Xi et al., 2012). This may have decreased the effect of language experience differences between groups in this study. However, differences in cognitive capacities between groups are not controlled by the characteristics of the verbal material in the speech test.

Third, we selected participants with normal hearing thresholds and normal DPOAE amplitudes. This procedure might have caused a selection bias, which could explain the lack of significant differences in some experimental measures and the lack of correlation between these measures and noise exposure variables ($L_{Aeq.8h}$ and CNE). We probably selected people with "tough" ears, who might not have presented evident signs of cochlear synaptopathy. This conclusion is supported by other studies that have found a difference in individual susceptibility to noise, suggesting the idea of "tough" versus "tender" ears (Cody and Robertson, 1983; Maison and Liberman, 2000; Lie et al., 2016). It is possible that individuals with "tough ears" are less susceptible to noise exposure, and will therefore not exhibit poorer hearing outcomes related to cochlear synaptopathy (e.g., ABR). In this study, we might have selected participants who did not present with cochlear synaptopathy, since normal hearing thresholds and DPOAEs were required for participation. However, we believe this to be a reasonable approach to investigating neural damage "beyond the audiogram."

Fourth, a regular use of HPDs was observed in participants with high noise exposure (>90 dBA). For participants who did not report to use HPDs, noise levels ($L_{Aeq.8h}$/CNE) were significantly lower than for participants who reported to use HPDs. Therefore, it is possible that the regular use of HPDs might have reduced noise exposure and prevented cochlear synaptopathy to develop in our sample of workers. This might explain why no differences in ABR were measured between our groups and why no correlations were observed between $L_{Aeq.8h}$/CNE and other variables used to investigate cochlear synaptopathy (i.e., ABR and SPiN). Although we incorporated HPD use into noise-exposure calculations, HPD reports by participants might not have been accurate enough to estimate the actual noise exposure.

Finally, we tried to control for significant recreational noise exposure, such as exposure to firearms, playing a musical instrument or in a band, frequent attendance to noisy bars and/or nightclubs, along with excessive use of listening devices at elevated volumes. However, since we did not measure recreational noise exposure by dosimetry, we relied

on participants' responses regarding significant noise exposure, which might have been insufficiently sensitive.

Conclusion

The sample of occupationally noise-exposed participants did not differ from control participants without occupational noise exposure for four ABR outcomes that may detect cochlear synaptopathy (i.e., wave I amplitude at 90 dBnHL, wave I/V amplitude ratio at 90 dBnHL, the slope of the wave I amplitude growth as a function of stimulus intensity, and the slope of wave V latency shift as a function of ipsilateral white noise intensity). Noise-exposed workers exhibited worse SPiN results than control group participants. However, we suggest that factors associated with non-sensory processing are likely to explain such results. The results of the present study suggest that noise exposure was not significantly associated with cochlear synaptopathy in this sample of workers. Further studies are still required to determine whether occupational noise exposure is associated with cochlear synaptopathy prior to observing changes in the audiogram.

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 the Ethics Committee of the Faculty of Medicine of the University of Montreal, the Committee on the Protection of Human Subjects of SUNY Plattsburgh, and the Ethics Committee of Zhejiang Provincial Center for Disease Control and Prevention. The patients/participants provided their written informed consent to participate in this study.

Author contributions

AP-S wrote the manuscript. WQ and AF designed the study and provided the critical revision of the manuscript. WZ and MZ performed the experiments and collected the data. AP-S, KM-D, and AF analyzed the data. All authors discussed the results and implications and commented on the manuscript at all stages.

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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.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.915211/full#supplementary-material

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Killer or helper? The mechanism underlying the role of adenylate activated kinase in sound conditioning

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Objective: To investigate whether sound conditioning influences auditory system protection by activating adenylate activated kinase (AMPK), and if such adaption protects ribbon synapses from high-intensity noise exposure.

Materials and methods: CBA mice (12 weeks old) were randomly divided into four groups (n = 24 mice per group): control, sound conditioning (SC), sound conditioning plus noise exposure (SC+NE), and noise exposure (NE). Hearing thresholds were assessed before testing, after sound conditioning, and 0, 3, 7, and 14 days after 110 dB noise exposure. Amplitudes and latencies of wave I at 90 dB intensity were assessed before test, after conditioning, and at 0 and 14 days after 110 dB noise exposure. One cochlea from each mouse was subjected to immunofluorescence staining to assess synapse numbers and AMPK activation, while the other cochlea was analyzed for phosphorylated adenylate activated kinase (p-AMPK) protein expression by western blot.

Results: There was no significant difference in auditory brainstem response (ABR) threshold between SC and control mice. The degree of hearing loss of animals in the two SC groups was significantly reduced compared to the NE group after 110 dB noise exposure. Animals in the SC group showed faster recovery to normal thresholds, and 65 dB SPL sound conditioning had a stronger auditory protection effect. After sound conditioning, the amplitude of ABR I wave in the SC group was higher than that in the control group. Immediately after noise exposure (D0), the amplitudes of ABR I wave decreased significantly in all groups; the most significant decrease was in the NE group, with amplitude in 65SC+NE group significantly higher than that in the 85SC+NE group. Wave I latency in the SC group was significantly shorter than that in the control group. At D0, latency was prolonged in the NE group compared with the control group. In contrast, there was no significant difference in latency between the 65SC+NE and 85SC+NE groups. Further, at D14, there was no significant difference between the NE and control groups, while latency remained significantly shorter in the 65SC+NE and 85SC+NE

groups compared with controls. Number of ribbon synapses in SC mice did not differ significantly from that in controls. After 110 dB noise exposure, there were significantly more ribbon synapses in the SC+NE group than the NE group. Ribbon synapses of all groups were recovered 14 days after the noise exposure, while the SC group had a shorter recovery time than the non-SC groups ($p < 0.05$). AMPK was highly activated in the SC group, and p-AMPK expression was detected; however, after 110 dB noise exposure, the strongest protein expression was detected in the NE group, followed by the SC+NE groups, and the lowest protein expression was detected in the control group.

Conclusion: Sound conditioning animals were more noise resistant and recovered hearing faster than non-SC animals. Further, 65 dB SPL SC offered better hearing protection than 85 dB SPL SC. Early AMPK activation may protect hearing by increasing ATP storage and reducing the release of large quantities of p-AMPK, which could help to inhibit synapse damage.

KEYWORDS

noise-induced hearing loss, sound conditioning, synapses, ATP-consumption, hair cell

Introduction

Noise intensity and duration of exposure determine the level of noise damage to an organism. High-intensity noise exposure damages inner hair cells (IHCs), primarily through two pathways: direct mechanical damage, in which noise can destroy the static cilia of hair cells, resulting in hair cell loss and damage to supporting cells and spiral ganglia (Kapoor et al., 2019); and biochemical reactions, which cause hair cell apoptosis or necrosis (Kurabi et al., 2017). Oxidative stress, poor energy metabolism, inflammatory reactions, glutamate buildup, and calcium overload are all known to trigger noise-induced apoptosis in IHCs (Wu J. et al., 2020). Low-intensity noise can both cause hearing loss and strengthen noise resistance in an organism, where the latter is referred to as sound conditioning. Sound conditioning is defined as exposure to low-level, non-traumatic sound stimuli, prior to high-intensity noise exposure, which does not generate permanent threshold shift (PTS), but rather reduces the transient threshold shift (TTS) or PTS triggered by subsequent high-intensity noise (Suryadevara et al., 2009; Roy et al., 2013; Chen et al., 2014; Waqas et al., 2018). Hair cell protection, activation of the hypothalamic-pituitary axis (HPA) axis, oxidative stress, control of the auditory efferent neural system, and increase of cochlear microcirculation are among the mechanisms underlying the protective impacts of sound conditioning on hearing that have been proposed to date (Cody and Johnstone, 1982; Niu et al., 2003; Harris et al., 2006; Henderson et al., 2006; Tahera et al., 2007; Zuo et al., 2008; Maison et al., 2013; Alvarado et al., 2016); however, the entire repertoire of mechanisms involved in this process is not fully understood.

Adenylate activated kinase (AMPK) is a critical cellular energy sensor that regulates cellular and systemic energy homeostasis and may be involved in the relationship between neuronal activity and energy availability, as it responds rapidly to elevated intracellular AMP/ATP ratios, and controls food intake and peripheral energy expenditure in the hypothalamus (Xue and Kahn, 2006; Winder and Thomson, 2007). AMPK protects the central nervous system in patients with ischemic stroke by reducing oxidative stress, decreasing neuroinflammation, improving mitochondrial function, and suppressing glutamate excitotoxicity (Matthews and Fuchs, 2010; Liu et al., 2014; Qiu et al., 2016). Nevertheless, massive AMPK activation causes phosphorylation of AMP-activated protein kinase (p-AMPK), which activates c-Jun N-terminal protein kinase (JNK), causing apoptosis (Weisova et al., 2011). When CBA mice were exposed to white noise at 106 dB sound pressure level (SPL) for 2 h, a single massive activation of AMPK led to a significant hearing threshold shift and a reduction in IHC ribbon synapse release, while a 30% reduction in AMPK activation induced by application of antagonists reduced the degree of hearing loss by 80%, implying that excessive AMPK activation is also a relevant mechanism underlying noise-induced hearing loss (Hill et al., 2016). These findings suggest that AMPK performs a complex and multi-targeted role in noise-induced inner ear injuries. We hypothesized that AMPK activation acts as a "two-way switch," in which activation of p-AMPK at a certain concentration causes death of inner ear cells, while below that threshold, AMPK is generally protective.

Noise levels between 55 and 100 dB SPL are proven to have protective effects, while it is difficult to produce sound conditioning effects with noise < 55 dB SPL, and levels > 100 dB SPL are likely to cause hearing loss; hence, noise intensities used in sound conditioning studies to date have generally been in the range 85–100 dB SPL (Canlon, 1997; Pukkila et al., 1997; Fan et al., 2020). In this study, the effects of sound conditioning on the auditory system were assessed by observing AMPK activation and ribbon synapse release during sound conditioning. Our findings provide a new theoretical basis for future strategies to prevent noise-induced deafness and cochlear synaptic disorder.

Materials and methods

Experimental animals and groups

Experimental animals were purchased from Spelford Biotechnology Company (Beijing, China). CBA male mice (12-weeks-old; weight, 28 ± 2 g) free of external or middle ear lesions, were used in this study. Animals were handled and treated according to the guidelines established by the Animal Care and Use Committee of the Chinese PLA General Hospital. Mice were randomly separated into the following groups (approximately 24 mice per group): Control, noise exposure (NE), 65 dB SPL sound conditioning plus noise exposure (65SC+NE), and 85 dB SPL sound conditioning plus noise exposure (85SC+NE). The study design is illustrated in **Figure 1**.

Noise exposure

To elicit threshold shifts, awake mice in separate stainless steel wire cages (15 cm × 5 cm × 5 cm) were exposed to white noise at 110 dB SPL for 2 h. A loudspeaker (YH25-19B, 25 W, 16 W, China) powered by a power producer (33220A, China) fed by the noise program was used in the sound exposure chamber. Audio editing software was used to create and equalize noise sound software files. To verify the uniformity of sound fields, a sound level meter (Model 1200; Quest Technologies, Oconomovoc, WI, United States) was calibrated at multiple positions within the sound chamber. To guarantee consistency, sound levels were measured before and after each session.

For sound conditioning, the 65SC and 85SC groups were exposed to white noise at 65 or 85 dB SPL, respectively, for 8 h per day for 7 days. After resting for 24 h, both groups were exposed to 110 dB SPL white noise for 2 h.

Auditory brainstem response threshold test

Time domain transmission (TDT) audiometry equipment (TDT, Alachua, Florida, United States) and Biosig audiometry software were used to test auditory brainstem response (ABR). Before audiometry, mice were anesthetized using 1% pentobarbital (1 mg/kg, intraperitoneal injection) and placed in a soundproof room. A recording electrode was subcutaneously implanted at the middle of the anterior margin of the auricle, and reference and recording electrodes were placed subcutaneously behind the ear. Stimulation sound level was gradually reduced in steps of 10 dB until it was no longer heard, starting at the maximum stimulation intensity (90 dB) and steadily reducing until no repetitive ABR waveform could be detected, then increased by 5 dB until a repetitive ABR waveform could be detected. ABR audiograms were performed before noise exposure, after sound conditioning and immediately (D0), and at 3 (D3), 7 (D7), and 14 (D14) days post-acoustic trauma for each individual.

In the present study, ABR wave I amplitudes and latencies evoked by 90 dB pure tone at 4, 8, and 16 kHz were collected and recorded for each group at D0 and D14 after sound conditioning. The 90 dB SPL sound that elicited wave I, as well as its initial negative (n) and subsequent positive (p) deflections, were measured. Ip–In (delay = 1.2–1.9 ms) was used to define wave I amplitude. MATLAB software was used to create an algorithm for automatically determining ABR amplitude (MathWorks, Natick, MA, United States).

Cochlear basilar membrane processing

After completion of ABR testing, mice were sacrificed, and cochleae quickly removed from the skull, perfused with 4% paraformaldehyde, and fixed overnight through the round and oval windows and the apex. Cochlea shells were decalcified in 10% EDTA for 4–6 h after fixation, and the basal turn separated under a dissection microscope in 0.01 mmol/L PBS solution. Then, the vestibular and tectorial membranes were removed.

Immunofluorescence staining

Isolated basilar membranes were punched in 0.5% Triton X-100 solution for 30 min, then blocked with 10% goat serum for 1 h at room temperature. Tissues were incubated overnight at 4°C with the following primary antibodies: monoclonal mouse anti-carboxyl-terminal binding protein 2 (CtBP2) (diluted 1:200; Abcam, Cambridge, United Kingdom); or monoclonal rabbit anti-AMPKα (T177) (diluted 1:50; Cell Signaling Technology, Boston, United States). After washing three times, specimens were incubated with secondary antibody

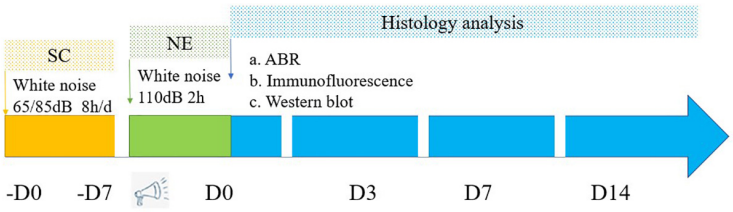

FIGURE 1
Flowchart depicts the major procedures of the experiment. SC, sound conditioning; NE, noise exposure; -D0 ~ -D7, the 65SC and 85SC groups were exposed to white noise at 65 or 85 dB SPL, respectively, for 8 h per day for 7 days; D0, after sound conditioning and immediately post-acoustic trauma; D3, after sound conditioning and at 3 day post-acoustic trauma; D7, after sound conditioning and 7 day post-trauma; D14, after sound conditioning and at 14 day post-acoustic trauma.

(Alexa Fluor 568 or Alexa Fluor 488, 1:1,000, Thermo Fischer Scientific, Waltham, MA, United States) and phalloidin (1:1,000, Thermo Fischer Scientific, Waltham, MA, United States) for 1 h at room temperature. After a final wash, cochleae were mounted on slides and stained with DAPI (Abcam, Cambridge, United Kingdom). Prepared slides were inverted onto a Zeiss confocal microscope and observed under a 63× magnification oil microscope, with a scanning layer spacing of 0.35 μm/layer. Wavelengths of 405, 488, 555, and 647 nm were used for specimen laminar sweeping, corresponding to blue, green, orange, and red under the fluorescence laser, respectively. Laminar scan was initiated when the signal appeared and terminated when the signal faded, with all pictures overlapped to generate the final result. Mean fluorescence intensity values for relevant areas were determined using the Zeiss software measurement tool and the same sized area (approximately 60 outer hair cells) selected for each photograph.

Protein extraction

One cochleae of each mouse was promptly extracted from skulls and dissected in PBS at 4°C to remove any excess tissue. Pooled tissue samples were lysed in RIPA sample buffer containing phosphatase inhibitor, protease inhibitor, and PMSF. To remove tissue debris, supernatants were centrifuged at 12,000 × g (Fresco17, Walthman, United States) for 20 min at 4°C, and protein concentrations determined using a Bio-Rad Protein Assay kit. Two cochleae from the same animal were combined for each sample.

Western blot

Western blot analysis was used to evaluate levels of p-AMPK protein expression in mice. One cochleae of each mouse was quickly removed from temporal bones after decapitation. Under an anatomical microscope (Olympus, SZX7, Tokyo, Japan), soft tissues were extracted from cochleae and homogenized in RIPA lysis buffer. An enhanced BCA Protein Assay Kit was used to assess total protein concentrations. Aliquots (25 μg) of each protein lysate were separated by 12% SDS-PAGE and transferred to polyvinylidene difluoride membranes. After incubation at room temperature for 1 h in blocking solution (5% non-fat dried milk in tris-buffered saline containing 1% Tween-20 [TBST]), membranes were washed and dried. Subsequently, blots were incubated overnight with primary antibodies (1:500, p-AMPKα, Cell Signaling Technology, Boston, United States; 1:1,000, β-actin, Abcam, Cambridge, United Kingdom), washed five times for 5 min each with PBST, then incubated with appropriate secondary antibodies at 1:1,000 for 1 h at room temperature. Then, after the membranes were washed well, immunoreactive bands were observed by ECL. X-ray film was scanned and analyzed using Image J software, and background staining density of an area with no bands subtracted from band densities, then target protein/β-actin ratios were calculated to obtain the relative expression levels of target proteins. Finally, differences were statistically analyzed, using at least three samples per group.

Statistical analysis

Statistical analyses were performed using GraphPad Prism 8 and SPSS 25.0 software. Data variability and the extent of differences between groups were used to calculate *in vivo* group sizes (n). One-way ANOVA with Tukey's multiple comparisons, repeated-measures ANOVA with *post hoc* testing, unpaired *t*-tests, one-sample t tests, and linear regression analysis were among the statistical procedures applied. Multivariate ANOVA was used for analyses of ABR thresholds, amplitudes, and wave I latencies, while one-way ANOVA was used for analysis of synapse count, p-AMPKα fluorescence intensity, and protein expression, and linear regression analyses were used to assess p-AMPK integrated density and ribbon synapse numbers. All tests were two-tailed, and $p < 0.05$ was considered significant.

Results

Sound conditioning protects the auditory system and prevents noise-induced hearing loss

Auditory brainstem response thresholds in the Control, SC, NE, and SC+NE groups were investigated at each time point (D0, D3, D7, and D14). The 65SC and 85SC groups did not differ significantly from the control group after sound conditioning (**Figure 2A** and **Table 1**).

After exposure to 110 dB SPL white noise for 2 h, the hearing thresholds of all three experimental groups were considerably higher than those of the control group ($p < 0.001$). The ABR thresholds of the NE group were markedly higher than those of the 65SC+NE and 85SC+NE groups ($p < 0.001$). Further, ABR thresholds at Click, 4 and 8 kHz were significantly lower in the 65SC+NE group than those in the 85SC+NE group ($p < 0.01$, **Figures 2B,C,E** and **Table 2**).

Following exposure to 110 dB SPL white noise, thresholds of the three groups gradually recovered on D3. The highest hearing thresholds were recorded at 4 and 24 kHz in all three animal groups. At 8 and 16 kHz, hearing thresholds in the 65SC+NE and 85SC+NE groups recovered to normal, while the NE group had significantly higher hearing thresholds than the SC+NE groups across all frequencies ($p < 0.05$) (**Figures 2B–D,F** and **Table 3**).

On D7 after exposure, the 4 kHz hearing thresholds of the three experimental groups remained significantly higher than those of the control group, while thresholds at 8, 16, and 24 kHz were no longer significantly different from those of the control

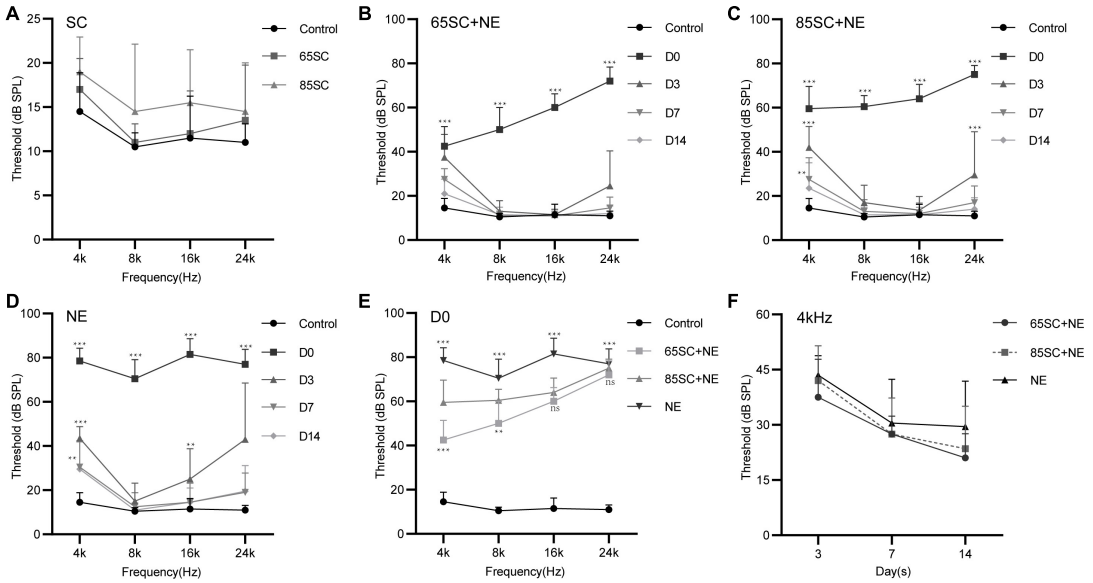

FIGURE 2
Hearing thresholds of mice after sound conditioning and changes in hearing thresholds of mice in each group after 110 dB SPL noise exposure. **(A)** ABR thresholds did not differ between SC groups and the control group after sound conditioning ($p > 0.05$). **(B)** Changes in ABR thresholds at different time points after noise exposure in the 65SC+NE group: the highest hearing threshold was at D0 ($p < 0.001$), and hearing thresholds returned to normal at D14 ($p > 0.05$). **(C)** Changes in ABR thresholds at various time points after noise exposure in the 85SC+NE group: the highest threshold was at D0 ($p < 0.001$), and hearing thresholds returned to normal at D14 ($p > 0.05$). **(D)** ABR thresholds in the NE group at each time point after noise exposure: the highest threshold was at D0 ($p < 0.001$), and hearing thresholds returned to normal at D14 except 4 kHz frequency ($p > 0.05$). **(E)** Comparison of ABR thresholds in the control and experimental groups at D0. Sound conditioning contributed to hearing protection immediately after noise exposure, and hearing protection in the 65SC+NE group was superior to that in the 85SC+NE group, primarily at 4 and 8 kHz frequencies. **(F)** The 65SC+NE, 85SC+NE, and NE groups were more seriously affected by noise at 4 kHz. $^{**}p < 0.01$, $^{***}p < 0.001$.

TABLE 1 Auditory brainstem response (ABR) thresholds for each group at the end of the sound conditioning (dB SPL).

Group	Click	4 kHz	8 kHz	16 kHz	24 kHz
Control	13.00 ± 2.58	14.50 ± 4.38	10.50 ± 1.58	11.50 ± 4.74	11.00 ± 2.11
65SC	15.50 ± 4.97	17.00 ± 3.50	11.00 ± 2.11	12.00 ± 4.83	13.50 ± 6.26
85SC	20.00 ± 5.27	19.00 ± 3.94	14.50 ± 7.62	15.50 ± 5.99	14.50 ± 5.50
P	0.075	0.058	0.417	0.064	0.308

TABLE 2 Auditory brainstem response (ABR) thresholds of each group at the moment of noise exposure (D0) (dB SPL).

Group	Click	4 kHz	8 kHz	16 kHz	24 kHz
Control	13.00 ± 2.58	14.50 ± 4.38	10.50 ± 1.58	11.50 ± 4.74	11.00 ± 2.11
65SC+NE	43.00 ± 12.06	42.50 ± 8.90	50.00 ± 10.00	60.00 ± 6.24	72.00 ± 6.32
85SC+NE	62.50 ± 7.17	59.50 ± 10.12	60.50 ± 4.97	64.00 ± 6.58	75.00 ± 4.08
NE	75.50 ± 8.32	78.50 ± 5.8	70.50 ± 8.64	81.50 ± 7.09	77.00 ± 6.75
P	<0.001***	<0.001***	<0.001***	<0.001***	<0.001***

***$p < 0.001$.

TABLE 3 Auditory brainstem response (ABR) thresholds for each group at the moment of noise exposure (D3) (dB SPL).

Group	Click	4 kHz	8 kHz	16 kHz	24 kHz
Control	13.00 ± 2.58	14.50 ± 4.38	10.50 ± 1.58	11.50 ± 4.74	11.00 ± 2.11
65SC+NE	22.00 ± 5.37	37.50 ± 10.34	13.00 ± 4.83	11.50 ± 2.42	24.50 ± 15.89
85SC+NE	21.50 ± 6.26	42.00 ± 9.49	17.00 ± 7.89	13.50 ± 6.26	29.50 ± 19.64
NE	23.00 ± 9.19	43.50 ± 5.3	15.00 ± 8.16	25.00 ± 13.74	43.00 ± 25.52
P	0.036*	<0.001***	0.201	0.014*	<0.001***

*$p < 0.05$, ***$p < 0.001$.

group ($p > 0.05$). The hearing thresholds of the NE group at 4 kHz remained higher than that of the control group ($p < 0.05$). The ABR thresholds of SC+NE group had all returned to normal levels by D14 (Figures 2B–D,F).

In animals treated by sound conditioning, hearing thresholds recovered more quickly. Hence, our data show that sound conditioning reduced subsequent high-intensity noise-induced hearing damage, with the protective effect being most pronounced in the immediate aftermath of loud noise. Further, sound conditioning with 65 dB SPL was significantly more effective in protecting hearing than conventional sound conditioning with 85 dB SPL.

After sound conditioning, ABR I wave amplitude in the 65SC group was significantly higher than that in the control group ($p < 0.01$); amplitude did not differ between the 85SC group and the control group ($p > 0.05$) (Figure 3A). Immediately after noise exposure (D0), ABR I wave amplitude decreased significantly in all groups, with the most significant decrease in the NE group. Wave I amplitude in the 65SC+NE group was significantly higher than that in the 85SC+NE group at 8 kHz ($p < 0.001$) and 16 kHz ($p < 0.01$) (Figure 3B). Further, at D14 after noise exposure, wave I recovered at all frequencies for all groups, with the 65SC+NE and 85SC+NE groups recovering to pre-exposure levels, while the NE group had not fully recovered at 8 kHz ($p < 0.01$) and 16 kHz ($p < 0.001$) (Figure 3C).

Wave I latency in the 65SC group was significantly shorter than that in the control group especially at 8 and 16 kHz ($p < 0.001$), and latency in the 85SC group was also shorter than that in the control group, except at 4 kHz ($p < 0.01$) (Figure 3D). At D0, latency was prolonged in the NE group at 4 kHz compared with the control group ($p < 0.05$). In contrast, there was no significant difference between SC+NE groups and the control group ($p > 0.05$) (Figure 3E). At D14, latency was reduced in the NE group, while it remained significantly shorter in the SC+NE groups compared with the control group ($p < 0.001$) (Figure 3F).

Sound conditioning activates adenylate activated kinase, resulting in increased expression of p-AMPKα in the cochlea

To investigate AMPK activation in the presence of noise, we used immunolabeling and western blotting analyses to assess p-AMPKα expression in cochleae from each group of mice. The fluorescence intensity of p-AMPKα increased in the SC groups compared to the control group ($p < 0.001$), and the results of western blotting revealed that p-AMPK levels increased following sound conditioning, with protein levels in the 65SC group was twice that of the control group, and in the 85SC group 2.7 times higher than those of the control group (Figures 4, 9A,A').

At D0, all groups, particularly the NE group, had increased p-AMPK expression, with fluorescence intensity values 2.7 times higher than those of the control group. Protein band gray scale values also differed significantly at approximately 9-fold those of the control group ($p < 0.001$). Fluorescence intensity values in the 65SC+NE and 85SC+NE groups did not differ significantly from one another ($p > 0.05$), and were twice that of the control group ($p < 0.001$). Protein gray scale values in the 65SC+NE

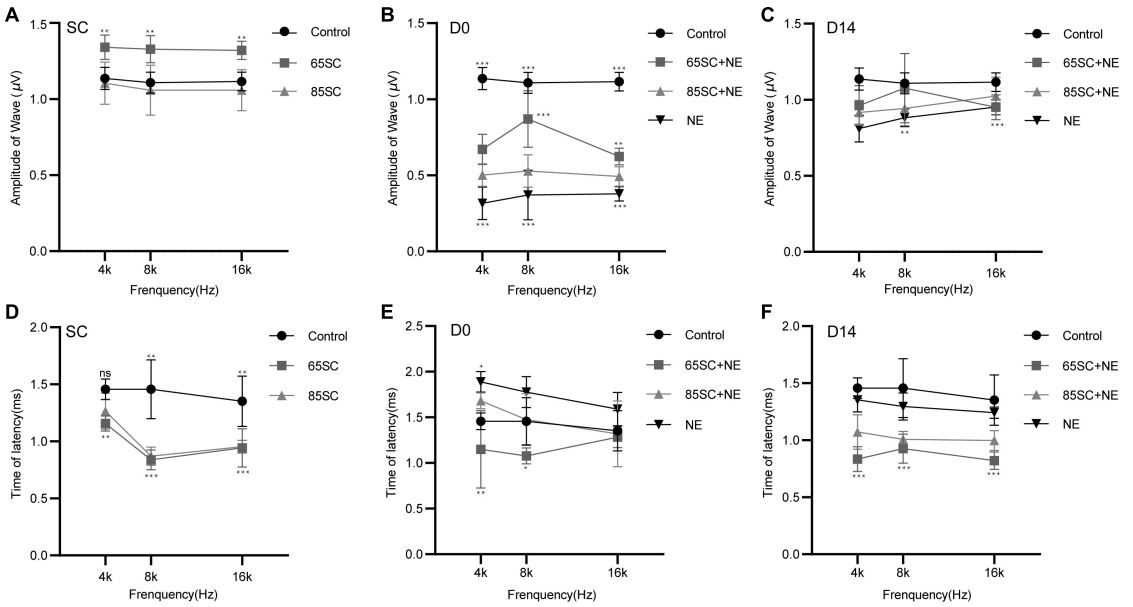

FIGURE 3
Amplitudes and Latencies of wave I at each time point after sound conditioning or noise exposure. **(A)** The amplitude of wave I in the 65SC group significantly increased than that in the control group at all frequencies after sound conditioning ($p < 0.01$); the amplitude of wave I did not differ between the 85SC group and the control group ($p > 0.05$). **(B)** ABR I wave amplitudes decreased significantly in all groups at D0. The amplitude of wave I in the NE group decreased obviously ($p < 0.001$). Comparing with 65SC+NE group, the amplitude of wave I in the 85SC+NE group was decreased at 8 kHz ($p < 0.001$) and 16 kHz ($p < 0.01$). **(C)** At D14, wave I recovered at all frequencies for all groups, with the 65SC+NE and 85SC+NE groups recovering to pre-exposure levels, while the NE group had not fully recovered at 8 kHz ($p < 0.01$) and 16 kHz ($p < 0.001$). **(D)** The latency of wave I in the 65SC group was significantly shorter than that of the control group at all frequencies after sound conditioning especially at 8 and 16 kHz ($p < 0.001$); and latency in the 85SC group was also shorter than that in the control group, except at 4 kHz ($p < 0.01$); the latency of wave I did not differ between the 65SC group and the 85SC group ($p > 0.05$). **(E)** The latency of wave I did not differ between the SC+NE groups and the control group at D0 ($p > 0.05$); and in the NE group the latency of wave I was prolonged especially at 4 kHz ($p < 0.05$). Comparing with 85SC+NE group, the latency of wave I in the 65SC+NE group was shorter at 4 kHz ($p < 0.01$) and 8 kHz ($p < 0.05$). **(F)** The latency of wave I did not differ between the NE group and the control group at D14 ($p > 0.05$); the latency of wave I in the SC+NE groups was still significantly shorter than that of the control group ($p < 0.001$). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

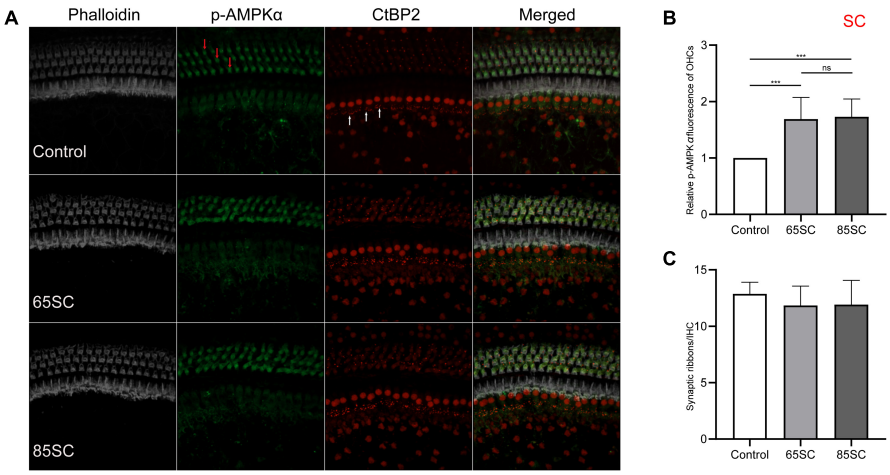

FIGURE 4
Sound conditioning activated AMPK, and did not decrease ribbon synapses. **(A)** p-AMPKα fluorescence intensity in outer hair cells (red arrows) was increased relative to the unexposed control group on exposure to 65 or 85 dB SPL white noise for 1 week; CtBP2 (white arrows) was not significantly reduced. **(B)** Quantification of p-AMPKα immunolabeling indicating that sound conditioning may activate AMPK. There was no significant difference in p-AMPKα fluorescence intensity between the 65SC and 85SC groups. **(C)** Ribbon synaptic amount did not differ significantly between sound conditioning and control group animals. ***$p < 0.001$.

group did not differ significantly from those of the control group ($p > 0.05$), but were approximately 4.2 times higher and significantly different in the 85SC+NE group compared to the control group ($p < 0.01$), around 2.8 times higher in the NE group than the 65SC+NE group ($p < 0.001$), and 2.1 times higher ($p < 0.01$) than the 85SC+NE group (**Figures 5**, **9B,B'**).

At D3, the p-AMPKα fluorescence intensity and protein gray scale values of the 65SC+NE group were no longer significantly different from those of the control group ($p > 0.05$); the fluorescence intensity of the NE group was almost twice that of the control group, while protein band gray scale values were 3.8 times that of the control group ($p < 0.001$); the fluorescence intensity of the 85SC+NE group was 2.2 times that of the control group ($p < 0.001$), and the protein gray scale value was 1.9 times higher than that of the control group ($p < 0.01$). Fluorescence intensity of p-AMPKα did not differ significantly between the NE and 85SC+NE groups ($p > 0.05$), while protein gray scale values of the NE group were about 2 times higher than that of the 85SC+NE group ($p < 0.01$). Further, fluorescence intensity of the NE group was 1.5 times greater than that of the 65SC+NE group ($p < 0.001$), and protein expression was 2.3 times higher than that of the control group ($p < 0.001$). Furthermore, fluorescence intensity of the 85SC+NE group was 1.4 times that of the 65SC+NE group ($p < 0.01$), while there was no significant difference in protein gray scale value ($p > 0.05$) (**Figures 6**, **9C,C'**).

At D7, the NE and 85SC+NE groups showed significant changes in p-AMPKα fluorescence intensity and protein gray scale values relative to the control group ($p < 0.001$), but the p-AMPKα fluorescence intensity and protein gray scale values did not differ between the 65SC+NE group and the control group ($p > 0.05$). Fluorescence intensity in the NE group was 1.2 times that of the 65SC+NE group ($p < 0.05$), while protein expression was twice that in the control group ($p < 0.001$). Compared with the 65SC+NE group, 85SC+NE fluorescence intensity was 1.25 times ($p < 0.05$) and gray scale value 1.7 times ($p < 0.01$), which were significant differences. At this time point, p-AMPKα fluorescence intensity in the 65SC+NE group did not differ significantly from that in controls ($p > 0.05$), while protein expression was 1.5 times higher, which was significantly different ($p < 0.05$) (**Figures 7**, **9D,D'**).

At D14, fluorescence intensity in the 85SC+NE group was 1.5 times that of the control group ($p < 0.01$), while that of the NE group was 1.7 times higher ($p < 0.001$); the protein gray scale values of the NE and 85SC+NE groups did not differ significantly from one another ($p > 0.05$), but were slightly higher than that of the control group ($p < 0.05$). There was no significant change in fluorescence intensity or protein expression in the 65SC+NE group relative to the 85SC+NE group (**Figures 8**, **9E,E'**).

Sound conditioning protects synapses from high intensity noise damage

The number of synapses in each specimen was counted individually in IHCs, and the differences between the control, SC, SC+NE, and NE groups assessed at each time point.

Mice in the 65 and 85 dB SPL condition groups had 11.84 ± 1.72 and 11.91 ± 2.15 synapses per IHC after 1 week of

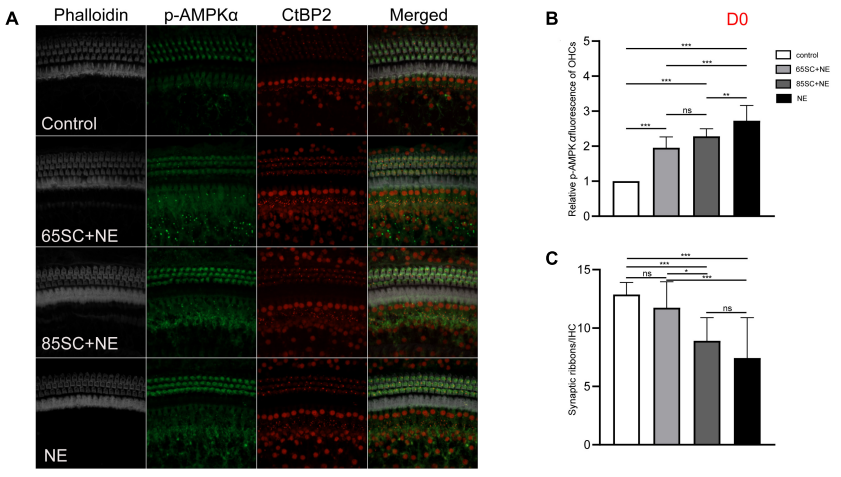

FIGURE 5
The p-AMPKα fluorescence intensity and the number of ribbon synapses changed in each group of mice after being exposed to 110 dB SPL noise at D0. **(A)** The p-AMPKα fluorescence intensity and the number of synapses of the four groups at D0. **(B)** NE group showed the highest p-AMPKα fluorescence intensity ($p < 0.001$), and the intensity of SC+NE groups were stronger than the control group ($p < 0.001$). **(C)** The number of synapses did not differ between the 65SC+NE group and the control group ($p > 0.05$), and both the 85SC+NE group and the NE group showed less synapses than the control group and 65 SC group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

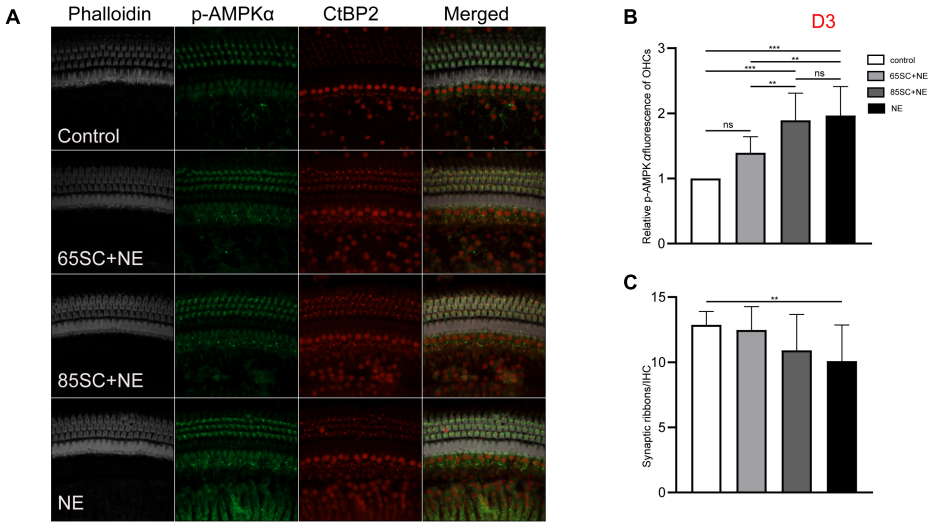

FIGURE 6
The p-AMPKα fluorescence intensity and the number of ribbon synapses restored in all groups at D3. **(A)** The p-AMPKα fluorescence intensity and the number of synapses of the four groups at D3. **(B)** The p-AMPKα fluorescence intensity in the four groups from the highest to the lowest was in the NE group, 85SC+NE group, 65SC+NE group, and control group. Among them, there was no statistical difference between the 65SC+NE group and the control group ($p > 0.05$). **(C)** The number of synapses in the NE group was lower than the control group ($p < 0.01$), and the number of synapses did not differ between the SC+NE groups and the control group ($p > 0.05$). $^{**}p < 0.01$, $^{***}p < 0.001$.

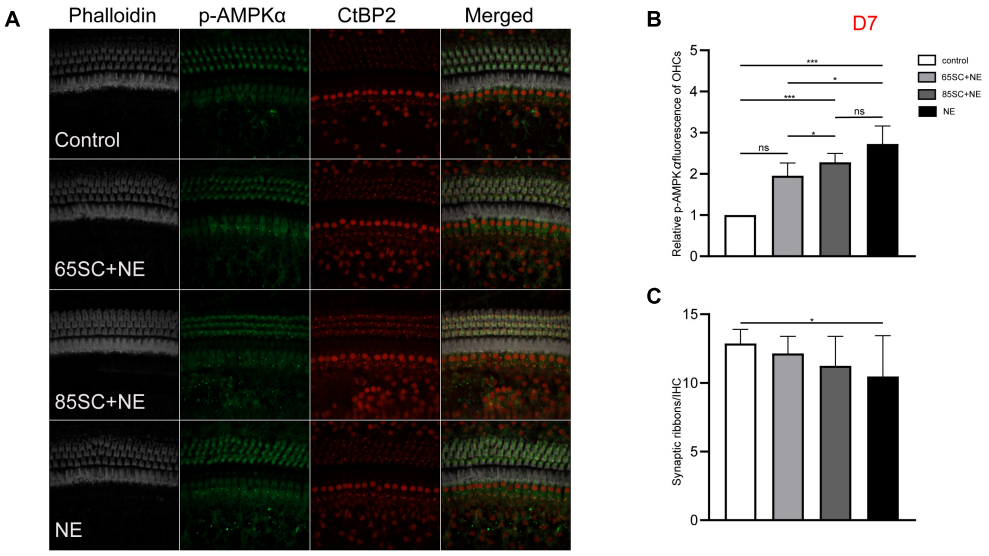

FIGURE 7
The p-AMPKα fluorescence intensity decreased and the number of ribbon synapses gradually recovered in the noise exposed groups at D7. **(A)** The p-AMPKα fluorescence intensity and the number of synapses of the four groups at D7. **(B)** There was no significant difference in p-AMPKα fluorescence intensity between the 65SC+NE group and the control group ($p > 0.05$), p-AMPKα fluorescence intensity of the NE and 85SC+NE groups was higher than of the control group ($p < 0.001$), the NE group showed greater p-AMPKα fluorescence intensity than the 65SC+NE group ($p < 0.05$). **(C)** Among the four groups, the NE group had the least number of ribbon synapses ($p < 0.05$). $^{*}p < 0.05$, $^{***}p < 0.001$.

sound conditioning, which was not significantly different from the control group (12.88 ± 1.03; $p > 0.05$) (Figures 4A,B).

After 110 dB SPL noise exposure (D0), mean numbers of synapses in the NE (7.43 ± 3.47) and 85SC+NE group (9.47 ± 1.21) groups were significantly reduced, and differed significantly from the control group ($p < 0.001$). Mean number of synapses in the 65SC+NE group (11.2 ± 42.14) did not differ significantly from the control group ($p > 0.05$), but

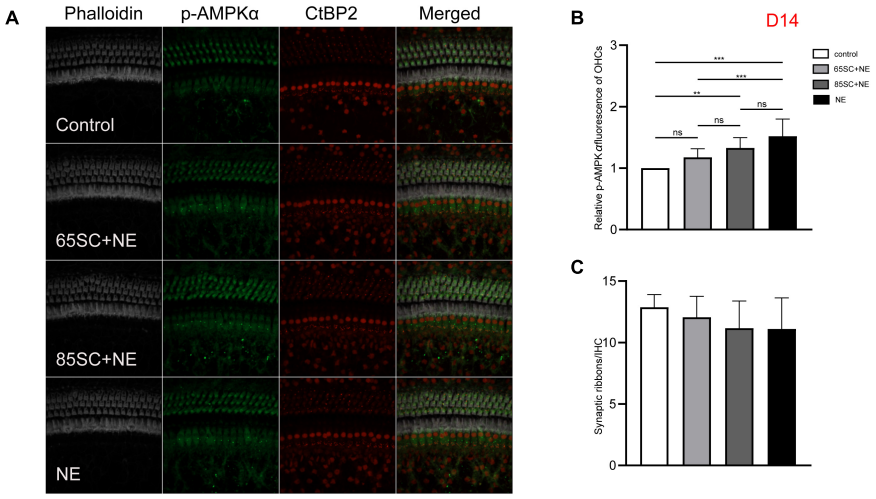

FIGURE 8
The p-AMPKα fluorescence intensity and the number of ribbon synapses returned to normal at D14. **(A)** The p-AMPKα fluorescence intensity and the number of synapses of the four groups at D14. **(B)** The p-AMPKα fluorescence intensity of the NE group was higher than that of the control ($p < 0.001$) and the 65SC+NE group ($p < 0.01$), the p-AMPKα fluorescence intensity did not differ between the 65SC+NE group and the 85SC+NE group ($p > 0.05$). **(C)** The number of synapses did not differ between the noise exposed animals and the controls ($p > 0.05$). $^{**}p < 0.01$, $^{***}p < 0.001$.

were significantly higher than in the NE group ($p < 0.001$). The 65SC+NE group had significantly more synapses than the 85SC+NE group ($p < 0.05$) (Figures 5A,B).

The number of synapses in the 85SC+NE group had recovered to normal at D3 after noise, but there remained a significant difference between numbers of synapses in the NE and control groups until D14 ($p < 0.001$) (Figure 6), when the number of synapses in the NE group recovered completely, and there was no significant difference between the groups ($p > 0.05$) (Figures 7, 8).

Activation of adenylate activated kinase did not correspond with changes in synapse number during low-intensity sound conditioning, while activated p-AMPK content and synapse number were negatively correlated during high-intensity stimulation

Adenylate activated kinase was activated and p-AMPK expression enhanced when low-intensity noise was delivered; however, the number of ribbon synapses did not change appreciably relative to the control group. At D0, the SC+NE groups had more ribbon synapses than the NE group, despite p-AMPK expression being lower in the SC+NE groups. The 65SC+NE group had the lowest p-AMPK fluorescence intensity and the most synapses, while the NE group had the highest p-AMPK fluorescence intensity and the fewest synapses, at D3, D7, and D14 (Figure 4).

After noise exposure, changes in p-AMPK and ribbon synapses in each group of animals were negatively correlated

After noise exposure, p-AMPK intensity changed in all groups of animals, with the lowest intensity in the 65SC group after sound conditioning and the highest intensity at D0 in the NE group. In contrast, the number of ribbon synapses showed the opposite trend in all groups, and correlation analysis detected a negative correlation between p-AMPK intensity and the number of ribbon synapses (Figure 10).

Discussion

Sound conditioning protects the auditory system from noise-induced hearing loss, while enabling faster recovery from transient threshold shift

Canlon et al. (1988) was the first to describe sound conditioning, using an 81 dB SPL, 1 kHz sound stimulus for conditioning. Sound conditioning has since been reported to reduce noise-induced hearing loss. Although the precise

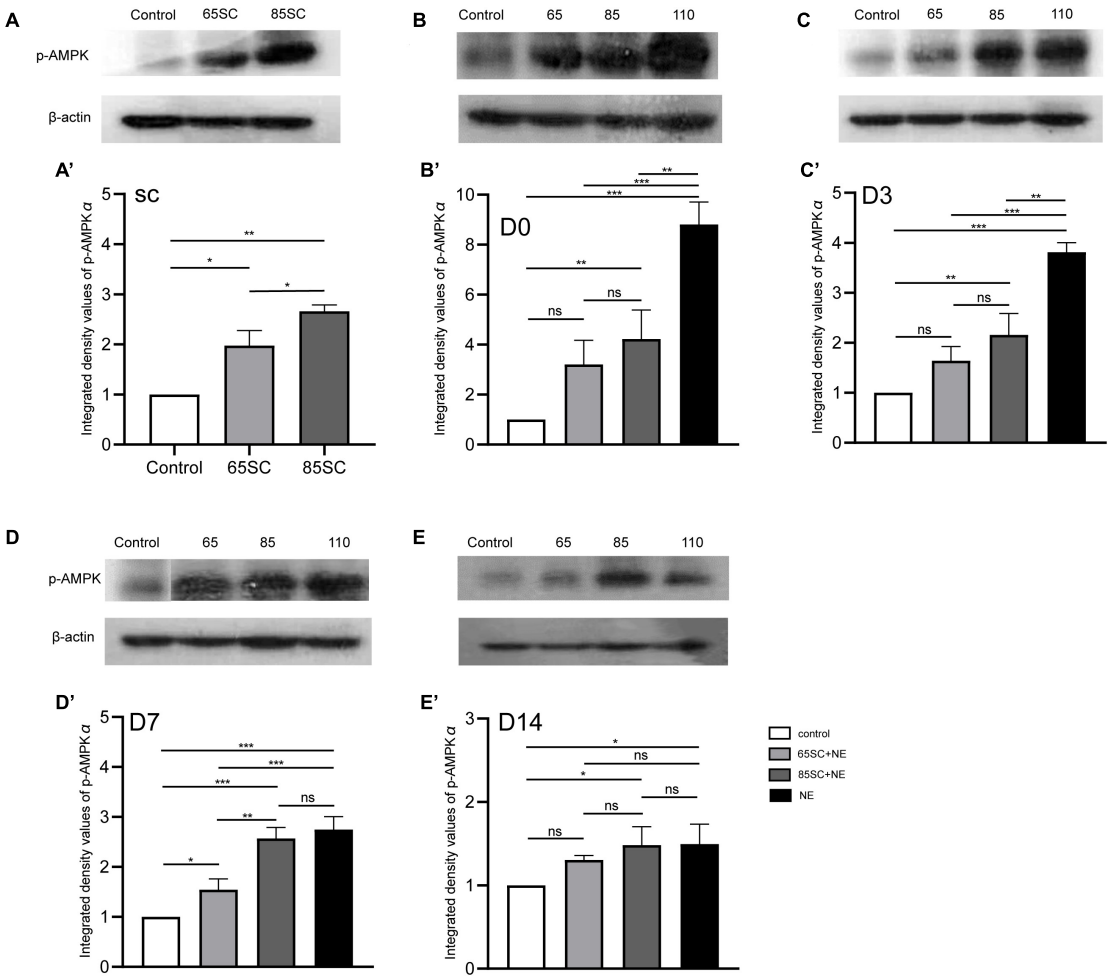

FIGURE 9
The expression of the p-AMPK protein in the cochlea of mice varied in each group at different time point. **(A,A')** p-AMPK protein expression increased in the SC groups after sound conditioning, and the 85SC group showed higher expression than the 65SC group ($p < 0.05$). **(B,B')** Among the four groups, it showed the highest p-AMPK protein expression in the NE group ($p < 0.001$), followed by the 85SC+NE group at D0 ($p < 0.01$); there was no statistical difference between the 65SC+NE group and the control group ($p > 0.05$); and the NE group's protein gray value was significantly higher than the 65SC+NE group ($p < 0.001$) and the 85SC+NE group ($p < 0.01$). **(C,C')** The expression of p-AMPK protein reduced in each experimental group at D3. **(D,D')** The p-AMPK protein level in the NE group decreased obviously at D7, but there was no significant change when comparing to the 85SC+NE group ($p > 0.05$). **(E,E')** The p-AMPK protein expression in the 85SC+NE group and NE group were higher than the control group at D14 ($p < 0.05$). *$p < 0.05$, **$p < 0.01$ ***$p < 0.001$.

mechanism involved is not completely understood, the underlying physiological adaptation responses may be as follows: 1. Sound conditioning increases the movement capacity of outer hair cells, as well as their aptitude to adapt to repeated environmental stimulation and post-stimulation fatigue, which enables the hair cells to actively screen or reduce persistent auditory stimulations, as a protective mechanism, at the ipsilateral cochlea and basilar membrane level; 2. Sound conditioning may downregulate glucocorticoid receptor expression induced by trauma, which may also protect the central components of the HPA; 3. Sound conditioning prevents reorganization of the cortical tonotopic map in cats after cochlear damage, which suggests a positive effect on both the central and peripheral auditory systems; 4. Sound conditioning increases levels of antioxidant enzymes in the cochlea, thereby enhancing free radical scavenging and protecting the organ of Corti from noise-induced damage by increasing stria vascularis levels of catalase, a hydrogen-peroxide-scavenging enzyme (Hu et al., 1997; Yamasoba et al., 1999); and 5. Induction of high heat shock protein (HSP) expression levels by sound conditioning have been demonstrated (Dechesne et al., 1992; Thompson and Neely, 1992). When HSP levels are high, animals show greater recovery from noise trauma relative to those without high HSP levels (Myers et al., 1992). There are also other theories about the underlying mechanisms, such as upgrading of calcium-buffering systems and increases in some

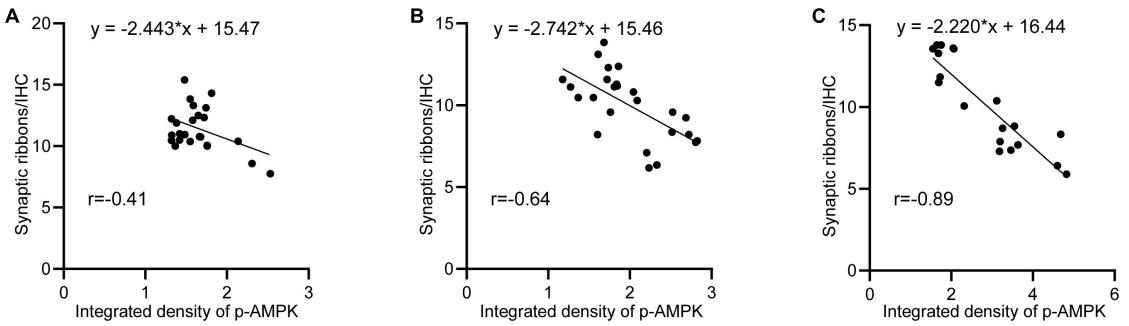

FIGURE 10
Negative correlation between p-AMPK and the amount of ribbon synapses. **(A)** Linear regression analysis of p-AMPK intensity and the number of ribbon synapses at D0, D3, D7, and D14 in the 65SC and 65SC+NE groups ($r = -0.41$, $p < 0.05$). **(B)** Linear regression analysis of p-AMPK intensity versus the number of ribbon synapses at D0, D3, D7, and D14 in the 85SC and 85SC+NE groups ($r = -0.84$, $p < 0.05$). **(C)** Linear regression analysis of p-AMPK intensity versus the number of ribbon synapses at D0, D3, D7, and D14 in the NE group ($r = -0.89$, $p < 0.001$).

neurotrophic factors, among others (Niu and Canlon, 2002). Regardless of which mechanism is most dominant, they will ultimately cause metabolic changes leading to "toughening" or resistance to noise-induced hearing loss. As demonstrated in this study, when mice underwent conditioning for 1 week, wave I amplitude, which indicates the firing potential at the site of connection between IHCs and type I spiral ganglion neurons (Moser et al., 2020), and represents synchronous evoked activity of cochlear nerve fibers in response to acoustic stimulation, was increased, indicating an active function of connections among IHCs after noise exposure. Further, conditioned animals showed mild hearing loss and faster recovery than controls.

The lowest sound intensity that induced sound conditioning was 65 dB sound pressure level

Several animal studies with noise levels ranging from 85 to 100 dB SPL, have been conducted to evaluate sound conditioning settings (Campo et al., 1991; Subramaniam et al., 1992; Dagli and Canlon, 1997). Yoshida et al. devised two sound conditioning techniques using CBA male mice: (1) Sound conditioning at 81 dB SPL, 8.0–16.0 kHz, for 1 week; and (2) 15 min of 89 dB SPL, 8.0–16.0 kHz sound conditioning, followed by 2 h exposure to the same frequency of high-intensity noise at 100 dB SPL. The experiments revealed that both sound conditioning plus noise exposure groups exhibited significantly reduced subsequent severe noise-induced compound action potential (CAP) and distortion product otoacoustic emissions (DPOAE) threshold shifts (Yoshida and Liberman, 2000).

Sheppard et al. (2018) employed a lower-intensity experimental setting, in which rats were exposed to noise at 65 dB SPL, 10–20 kHz for 5 weeks, and found no significant increase in DPOAE, but a significant increase in CAP amplitude, after a week of rest, relative to the control group. Further, after 6 weeks exposure to low-intensity noise at 18–24 kHz, 55 dB SPL, Liu et al. (2020) showed a reduction in CAP amplitude.

Discrepancies in experimental design, such as experimental animal selection and characteristics, including the frequency spectrum, sound level, and period of sound conditioning and noise blast, can easily lead to contradictory outcomes.

The sound conditioning conditions used in this experiment were 65 and 85 dB SPL white noise. White noise at 65 dB SPL, which is the lowest noise level known to cause habituation, can provide substantial protection. Relative to 85 dB SPL, 65 dB noise had a stronger beneficial effect on auditory protection, which was particularly clear in the immediate aftermath of loud noise and during the recovery period.

Mice exposed to narrow-band noise at 100 dB SPL for 2 h showed a transient increase in ABR thresholds, and when removed from the noise environment for 2 weeks, these animals showed a temporary hearing threshold shift, as ABR thresholds returned to pre-exposure levels (Kujawa and Liberman, 2009); this hypothesis has been supported by a number of studies (Liu et al., 2012; Shi et al., 2015). ABR thresholds recovered to pre-noise levels in both groups 2 weeks after high intensity noise, with the sound conditioning group recovering considerably faster than the non-sound conditioning group.

Sound conditioning activates adenylate activated kinase to protect ribbon synapses from noise damage

Normal function of the cochlear ribbon synapse, which is positioned between the IHC and type I spiral ganglia, is critical for hearing conduction. The transit, aggregation, and release of cochlear ribbon synaptic presynaptic vesicles are all dependent on the consumption of massive amounts of ATP supplied by IHC mitochondria (Griesinger et al., 2005; Matthews and Fuchs, 2010). Consequently, the ability of mitochondria to produce

enough ATP is critical for maintaining synaptic function. Strong noise exposure causes a high inward flow of calcium ions, which facilitates mitochondria-related cell death; calcium inward flow is linked to energy expenditure, which impairs mitochondrial metabolism and thus leads to apoptosis.

Oxidative stress plays an important role in the process of noise-induced hearing loss, as excessive production of reactive oxygen species can directly damage DNA, especially mitochondrial DNA (mt DNA), break C-H bonds in DNA pentose sugars, and break down nucleotides. Simultaneously, ROS can mediate direct peroxidation of unsaturated fatty acids on biological membranes and damage mitochondrial membranes, causing impaired energy metabolism (Caston and Demple, 2017). Downstream of ROS, inner ear stress-activated MAPKs (including JNK and p-AMPK) mediate cellular stress responses and inflammation through intermediate signaling protein activation (Wu F. et al., 2020), which in turn affects cell proliferation, differentiation, and apoptosis. Under hypoxic conditions in the inner ear after noise exposure, AMPK activates a ROS-dependent pathway and mediates outer hair cell apoptosis (Hill et al., 2016).

Adenylate activated kinase is a trimeric complex that is involved in cellular energy balance regulation. It is activated by noise-induced ATP decreases, leading to phosphorylation of downstream sites and restoration of energy balance by increasing catabolism (ATP generation) and decreasing anabolism (ATP utilization) (Housley et al., 2013; Carling, 2017). During loud noise exposure, a significant drop in ATP reduces the number of ribbon synapses. AMPK activation can function to adjust an organism to oxidative stress, while also inhibiting the cytotoxic response induced by glutamate. According to Hill et al. (2016) AMPK activation alone does not protect the inner ear from noise damage; instead, when p-AMPK accumulates to a certain level, it activates the JNK pathway and triggers apoptosis. These researchers found that CBA mice exposed to 98 and 106 dB SPL for hours had increased p-AMPK expression, but that ribbon synaptic release was much lower in the 106 dB SPL group than in the 98 dB group. They hypothesized that p-AMPK expression was noise-dependent, and used siAMPKa1 to block the AMPK activation site, revealing that a 30% drop in AMPK activation resulted in an 80% reduction in hearing loss (Hill et al., 2016). The findings of the current study also imply that sound conditioning may protect hearing *via* AMPK-mediated mechanisms, as follows: 1. Early activation of AMPK increases ATP reserves, which protects hair cells from death and synaptic loss caused by rapid ATP depletion during subsequent intense noise exposure. The modest dose of p-AMPK elicited by low-dose noise is insufficient to produce damage to the inner ear, which explains why 65 dB SPL sound conditioning is superior to 85 dB SPL sound conditioning. 2. As AMPK is a protein kinase, its activation is limited, and when sound conditioning causes a reduction in AMPK content or receptor, following activation of a proportion of AMPK, it cannot be activated as much as in normal animals when exposed to subsequent noise, but instead mitigates p-AMPK elevation. 3. Since AMPK activation can inhibit the cytotoxic response induced by glutamate, sound conditioning can protect inner hair ribbon synapses to mitigate the damage caused by subsequent noise-induced glutamate release.

One limitation of this study is that the role of upstream and downstream of AMPK pathway components in the sound conditioning protective mechanism was not thoroughly investigated. Further, we do not provide direct proof of the relationship between AMPK and ribbon synaptic release. As previously stated, a specific degree of activity of AMPK protects the inner ear, but once the activation product, p-AMPK, exceeds a specific concentration, it causes cochlear hair cell death and hearing disability. Future research work will aim to determine the "turning point" between cochlea protection and cochlea damage caused by AMPK activation and elucidate the role of AMPK in noise-induced deafness using antagonists.

By exposing CBA mice to low-intensity noise, we discovered that sound conditioning can activate AMPK and protect ribbon synapses from subsequent intense noise damage. We suspect that AMPK may restore some energy during sound conditioning, allowing ATP to be compensation and lowering ATP consumption under high noise. At varying noise intensities, however, the amount of AMPK activated will alter as p-AMPK levels change, resulting in diverse outcomes. This research adds to understanding of how sound conditioning protects hearing and implies that suitable experimental circumstances have potential for application in preventing hair cell loss and cochlear synaptopathy.

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the Animal Care and Use Committee of Chinese PLA General Hospital.

Author contributions

RZ participated in the analysis and interpretation of the data. LS and NY made substantial contributions to the

conception and design of the study. CM and MW performed the statistical analysis. XL and WL contributed to the acquisition of data. All authors have read and approved the final manuscript.

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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.

Publisher's note

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Epidemiological characteristics of hearing loss associated with noise temporal structure among manufacturing workers

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Objective: This study aimed to investigate the epidemiological characteristics of occupational noise-induced hearing loss (NIHL) among manufacturing workers, and to provide evidence for diagnosing and preventing occupational hearing loss caused by complex noise, which is different from Gaussian noise in temporal structure.

Methods: One thousand and fifty manufacturing workers exposed to occupational noise were recruited in a cross-sectional survey. Exposure characteristics and epidemiological distribution of hearing loss and noise exposure metrics (noise energy and kurtosis) were investigated, and the relationship between noise exposure and hearing loss was analyzed. The effects of kurtosis on hearing threshold shift across different frequencies and on NIHL development with exposure duration and noise intensity were also investigated.

Results: Each type of work had specific noise exposure metrics. Noise intensity and kurtosis were independent parameters ($r = -0.004$, $p = 0.885$). The prevalence of NIHL and the hearing threshold level had a specific distribution in different types of work. Kurtosis deepened the hearing notch at high frequencies and accelerated the formation of early hearing loss. The effect of exposure duration and noise intensity on the prevalence of high-frequency NIHL (i.e., at 3, 4, 6, and 8 kHz) for manufacturing workers increased with kurtosis in workers with noise exposure duration of less than 10 years and with $L_{Aeq.8h}$ between 80 and 90 dB(A). Male (OR = 1.557, 95%CI = 1.141–2.124), age (OR = 1.033, 95%CI = 1.014–1.052), exposure duration (OR = 1.072, 95%CI = 1.038–1.107), kurtosis (OR = 1.002, 95%CI = 1.001–1.003), and noise intensity ($L_{Aeq.8h}$; OR = 1.064, 95%CI = 1.044–1.084) were risk factors for high-frequency

NIHL. The speech-frequency NIHL (i.e., at 0.5, 1, and 2 kHz) risk of workers exposed to manufacturing noise was related to age (OR = 1.071, 95%CI = 1.043–1.100). There were no statistically significant associations between speech-frequency NIHL and sex, noise exposure duration, kurtosis, and noise intensity ($L_{Aeq.8h}$).

Conclusion: The high-frequency NIHL prevalence among manufacturing workers is associated with sex, age, exposure duration, noise intensity, and temporal structure of noise, while the speech-frequency NIHL prevalence is associated with age. Kurtosis strengthens the association of noise exposure duration and noise intensity with high-frequency hearing loss. The influence of noise temporal structure should be considered in the diagnosis and early prevention of occupational hearing loss caused by complex noise.

KEYWORDS

noise, complex noise, hearing loss, manufacturing industry, epidemiological characteristics

Introduction

Over 5% of the world's population (i. e., 430 million people) suffer from deafness and hearing loss (WHO, 2021). Exposure to occupational noise is one of the most common risks for hearing loss in China and across the world. About 16% of adult hearing loss cases are associated with occupational noise exposure (Nelson et al., 2005). Noise-induced hearing loss (NIHL) is the second leading cause of sensorineural hearing loss (Chen et al., 2019). Occupational NIHL is the most prevalent occupational disease among working-age people worldwide (Chen et al., 2020). In China, occupational NIHL ranks as the second primary occupational disease with an annual increase of approximately 20% (Zhou et al., 2020).

In terms of its temporal structure, industrial noise can be divided into Gaussian noise (i.e., steady-state, continuous noise) and non-Gaussian noise, also known as complex noise (Qiu et al., 2006). Complex noise consists of transient high-energy impulsive noise superimposed on Gaussian background noise. The characteristics of hearing loss based on noise energy are well understood. With the development of industrialization, non-Gaussian noise has been the most prevalent type of noise in working environments (Hamernik and Qiu, 2001). Researchers proposed that the equal energy hypothesis (EEH; ISO, 2013) in the existing international noise exposure standards (e.g., ISO 1999:2013) might not be adequate for complex noise evaluation (Zhang et al., 2021a). One of the risk factors that may contribute to the high incidence of occupational NIHL is considered to be the damage-risk criteria for noise exposure relying only on energy-based exposure assessment (Davis et al., 2009).

In recent years, hearing loss caused by complex noise has become a hotspot worldwide. An energy metric alone should not be adequate to predict the risk of NIHL (Davis et al., 2009). The previous animal experiments and epidemiological studies demonstrated that the temporal structure of noise was an additional metric to assess the hearing loss caused by complex noise (Qiu et al., 2006; Davis et al., 2012; Suter, 2017). Studies showed that the noise temporal structure is a risk factor for NIHL, and complex noise has a much greater impact on hearing loss than Gaussian noise (Dunn et al., 1991; Hamernik and Qiu, 2001; Zhou et al., 2020; Shi et al., 2021). Recently, evidence has shown that the temporal structure of complex noise can be expressed in the kurtosis metric (β), which is defined as the ratio of the fourth-order central moment to the squared second-order central moment of a distribution (Davis et al., 2009; Zhao et al., 2010; Davis and Clavier, 2017). For a fixed range of noise exposure level and duration, the noise-induced permanent threshold shifts (NIPTS) increased with the kurtosis of the noise (Zhang et al., 2021c).

Studies have indicated that the prevalence of NIHL increased with exposure duration, noise energy levels, sex, and age (Lie et al., 2014; Chen et al., 2019; Zhou et al., 2020; Zhang et al., 2021b). A total of 24.4% of adults had an audiometric notch in the United States, this was more common among males than females (Carroll et al., 2017). The prevalence of NIHL was higher in workers who experienced prolonged exposure and older workers in textile industries (Abraham et al., 2019). However, the epidemiological characteristics of occupational NIHL related to the kurtosis metric of complex noise have been much less explored. In this study, the noise exposure and hearing loss of workers in the textile, furniture, and general equipment manufacturing industries were investigated. We analyzed the epidemiological characteristics (especially those associated with the temporal structure of noise) of

occupational hearing loss caused by complex noise in the manufacturing industry to help provide a basis for diagnosis and early prevention of occupational hearing loss caused by complex noise.

Materials and methods

Subjects

From 2017 to 2019, we carried out a cross-sectional survey of the manufacturing industry in Zhejiang province, China. The cluster sampling method was used to recruit noise-exposed workers from four textile enterprises, six furniture manufacturers, and eight general equipment manufacturing enterprises. Each participant was asked to sign an informed consent form after being informed of the purpose of this study. The participants met the following requirements, which were determined from the noise exposure questionnaire: (1) working in the same type of work in the current factory; (2) no history of another high-level noise exposure except for the current job, including occupational and non-occupational noise exposure; (3) no co-exposure history of noise and ototoxic organic solvents or heavy metals; (4) self-reported never using ototoxicity drug; (5) never suffered from ear diseases; (6) no diabetes; (7) never had military service or shooting experience; (8) no or minimal use of hearing protection devices (HPD). The Medical Ethics Committee of Zhejiang Center for Disease Control and Prevention approved the study protocol (approval reference number: ZJCDC-T-043-R), which met the ethical requirements.

Finally, we enrolled 1,050 workers who met the study's inclusion criteria. The workers were divided into different groups by types of work, which included spinners, weavers, roller operators in the textile industry, gun nailers and carpenters in the furniture manufacturing industry, and assemblers, metal processing workers, welders, polishers, forgers, stampers, and carvers in the general equipment manufacturing industry.

Field investigation and questionnaire survey

A field investigation in workplaces was conducted to get information on devices, materials, products, production processes, the number of workers exposed to the noise, the distribution of noise sources, and measures taken to reduce the noise level of each factory. The questionnaire designed by the research team was used to conduct the face-to-face questionnaire survey of all participants by occupational hygienists. There were eight occupational hygienists in our research team, who were responsible for conducting the questionnaire, and they were trained to standardize their understanding of the questionnaire. Each worker was assisted by an occupational hygienist to complete the questionnaire. The questionnaire collected the following information from the participants: (1) general personal information and lifestyle (e.g., age, sex, smoking, and alcohol use); (2) health conditions and medical history: blood pressure, complaints of hearing impairment, history of ear diseases and hearing loss, history of other diseases (chronic diseases, traumatic brain injury, mumps, scarlet fever, measles, etc.), surgical history, and use of ototoxic drugs (gentamicin, streptomycin, clarithromycin, quinine, etc.); (3) occupational history, such as industry, factory, workshop, type of work, noise exposure duration (ED), chemical exposure at work, and HPD use, including information of current and previous work; (4) non-occupational noise exposure (e.g., frequency and duration of recreational noise exposure); (5) other information (military service or shooting behavior, family history of hearing loss, etc.).

Noise exposure measurement

The digital individual noise recorder (ASV5910-R, Hangzhou Aihua Instruments Co., Ltd., China) that can measure noise from 40 dB(A) to 141 dB(A) was used to record a shift-long personal noise exposure for each participant. The recorder uses a pre-polarized condenser microphone with a broad response frequency (20 Hz to 20 kHz) and high sensitivity level (2.24 mV/Pa). The microphone was placed on the shoulder of each participant during the whole work shift.

The A-weighted noise exposure level normalized to a nominal 8-h working day ($L_{Aeq.8h}$) and kurtosis of noise (β) were used to quantify noise exposure in this study. The MATLAB software was used to analyze the shift-long noise and obtain the $L_{Aeq.8h}$ and kurtosis. The $L_{Aeq.8h}$ level was calculated by the formula in ISO 1999 (ISO, 2013):

$$L_{\mathrm{Aeq,8h}} = L_{\mathrm{Aeq,Te}} + 10 \times \lg\left(\frac{T_e}{T_o}\right) \quad (1)$$

Where T_e is the effective duration of the working day in hours; T_0 is the reference duration (T_0 = 8 h); and $L_{Aeq,Te}$ is the L_{Aeq} for T_e. The kurtosis values were computed over consecutive 40-s time windows without overlap over the shift-long noise record using a sampling rate of 48 kHz. The mean of the kurtosis values was then calculated to be the kurtosis metric in this study.

The occupational exposure limit (OEL) of workplace noise level is 85 dB(A) in China. Then we divided noise levels into four groups according to $L_{Aeq.8h}$: <80, 80–85, 85–90, and ≥ 90 dB(A). This study set β = 10 as a boundary to distinguish complex noise from steady-state noise (Davis et al., 2009). Furthermore, we divided complex noise into two groups of 50.

Hearing loss determination

Audiometric test

Pure tone air conduction hearing threshold measurements at the speech frequencies (i.e., 0.5, 1, and 2 kHz) and the high frequencies (i.e., 3, 4, 6, and 8 kHz) at both ears were performed after excluding conductive hearing impairments by general ear examination of each participant. The participants were out of the occupational noise environment for at least 16 h before the test. The audiometric test was performed in an audiometric room of a mobile physical examination vehicle using an audiometer (Interacoustics AD629, Denmark) with an air conduction headphone (HDA300), which was calibrated by the Zhejiang Institute of Metrology according to the Chinese standard (Verification Regulation of Audiological Equipment Pure-tone Audiometers, JJG 388-2012). The NIPTS at each frequency for each participant were obtained according to Annex A of ISO 1999 (ISO, 2013). Measured hearing threshold levels (HTLs) at each frequency of each participant were adjusted by subtracting the age- and sex-specific HTL according to Table B.3 of ISO 1999 (ISO, 2013).

Definition of hearing loss

From the perspective of hearing protection, high-frequency noise-induced hearing loss (HFNIHL) was defined as adjusted HTL $\geq$ 30 dB, in either ear, at one or more of the HTLs 3 kHz, 4 kHz, and 6 kHz (Zhao et al., 2010; Chen et al., 2019; Zhou et al., 2020). Speech-frequency noise-induced hearing loss (SFNIHL) was defined as an average hearing threshold of HTL $\geq$ 26 dB in the better ear at speech frequencies of 0.5 kHz, 1 kHz, and 2 kHz (Zhou et al., 2020).

Statistical analyses

Two study staff entered the data into an Excel spreadsheet for Windows Microsoft, WA, USA for analysis using the SPSS 19.0 program. Continuous variables were expressed as mean with standard deviation (mean $\pm$ SD). A one-way analysis of variance was used to compare continuous variables among the different types of work. The Chi-square test and Fisher's exact test were used to compare the prevalence of HFNIHL (HFNIHL%) and the prevalence of SFNIHL (SFNIHL%) across different groups. We set the age of workers into six groups ($\leq$ 25, 25–30, 30–35, 35–40, 40–45, and >45 years), and also set the noise exposure duration into six groups ($\leq$3, 3–5, 5–10, 10–15, 15–20, and >20 years). The correlation between continuous variables was analyzed using the Pearson correlation method. Binary logistic regression analysis was used to analyze the odds ratio (OR) and 95% confidence interval values (CIs) of key factors affecting the HFNIHL% and SFNIHL% (as a categorical dependent variable). Differences with a $p < 0.05$ were considered statistically significant.

Results

Noise exposure and hearing loss associated with noise level and kurtosis

Table 1 shows the general information of noise exposure and hearing loss of manufacturing workers in this study. There were 1,050 participants in the present study; 751 (71.5%) of them were males. The mean age of the workers was 34.8 $\pm$ 9.8 years. The average noise exposure duration of participants was 7.3 $\pm$ 6.5 years.

Noise exposure among different types of work

The average $L_{Aeq.8h}$ among the 1,050 workers was 89.4 $\pm$ 7.6 dB(A), ranging from 61.3 dB(A) to 105.6 dB(A). A total of 785 (74.8%) of workers from the manufacturing industry were occupationally exposed to noise levels above 85 dB(A), which exceeds the OEL in China. The proportion of workers exposed to occupational noise exceeding the OEL varied by industry and type of work ($p < 0.05$), as summarized in Table 1. For industries, 85.4% of workers from the textile industry were exposed to occupational noise above 85 dB(A), followed by the furniture manufacturing industry (82.4%) and the general equipment manufacturing industry (60.6%). The types of work with a higher $L_{Aeq.8h}$ exceeding the OEL were weavers (99.3%), spinners (84.6%), gun nailers (84.4%), and polishers (81.5%; $p < 0.05$). There were statistically significant differences in kurtosis between different types of work ($p < 0.001$). The gun nailers were exposed to noise with the highest kurtosis (β = 246.4 $\pm$ 172.8), while the weavers were exposed to the lowest kurtosis (β = 8.1 $\pm$ 12.4), followed by the spinners (β = 10.4 $\pm$ 11.2; $p < 0.05$), as shown in Table 1. The correlation analysis across all the 1,050 subjects showed no correlation between $L_{Aeq.8h}$ and kurtosis ($r = -0.004$, $p = 0.885$).

Prevalence of hearing loss among different types of work

The audiometric test results showed that the average HFNIHL% and SFNIHL% among workers exposed to manufacturing noise were 64.5% and 7.4%, respectively (Table 1). Significant differences were observed in average HFNIHL% and SFNIHL% among different types of work (for HFNIHL%, $\chi^2 = 56.58$, $p < 0.001$; for SFNIHL%, $\chi^2 = 21.59$, $p = 0.028$). The polishers had the highest HFNIHL%, followed by gun nailers, carpenters, and welders, while the

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TABLE 1 Noise exposure and hearing loss among different types of work in manufacturing industries (n = 1,050).

Industry	Work type	n	Male (%)	Age (year)	ED (year)	$L_{Aeq.8h}$ [dB(A)]		Kurtosis	HFNIHL (%)	SFNIHL (%)
						Mean	≥85 (%)			
Textile		**321**	**165 (51.4)**	**34.0 ± 8.5**	**7.8 ± 6.0**	**93.6 ± 8.1**	**85.4**	**11.6 ± 13.3**	**206 (64.2)**	**18 (5.6)**
	Spinners	130	104 (80.0)	33.4 ± 8.4	9.4 ± 7.1	95.2 ± 9.2	84.6	10.4 ± 11.2	90 (69.2)	7 (5.4)
	Weavers	135	23 (17.0)	33.4 ± 8.6	6.6 ± 5.2	95.8 ± 3.3	99.3	8.1 ± 12.4	92 (68.1)	10 (7.4)
	Roller operator	56	38 (67.9)	36.9 ± 7.8	7.1 ± 3.4	84.5 ± 7.3	53.6	22.7 ± 14.0	24 (42.9)	1 (1.8)
Furniture		**318**	**303 (95.3)**	**33.6 ± 9.5**	**4.9 ± 4.9**	**88.9 ± 4.3**	**82.4**	**200.4 ± 161.7**	230 (72.3)	22 (6.9)
	Gun nailers	212	207 (97.6)	30.8 ± 8.1	4.3 ± 4.2	89.1 ± 4.4	84.4	246.4 ± 172.8	155 (73.1)	8 (3.8)
	Carpenters	106	96 (90.6)	39.2 ± 9.5	6.1 ± 5.9	88.5 ± 4.2	78.3	108.6 ± 78.5	75 (70.8)	14 (13.2)
General equipment		**411**	**283 (68.9)**	**36.3 ± 10.8**	**8.7 ± 7.5**	**86.4 ± 7.6**	**60.6**	**36.9 ± 52.5**	**241 (58.6)**	**38 (9.2)**
	Assemblers	152	61 (40.1)	34.6 ± 7.5	9.0 ± 7.0	86.9 ± 5.8	63.2	45.8 ± 77.8	96 (63.2)	10 (6.6)
	Metal processing workers	38	34 (89.5)	41.6 ± 11.0	15.4 ± 9.7	85.2 ± 6.7	42.1	34.2 ± 22.2	26 (68.4)	6 (15.8)
	Welders	34	33 (97.1)	41.0 ± 9.5	11.7 ± 8.4	89.8 ± 8.2	73.5	37.7 ± 30.4	24 (70.6)	3 (8.8)
	Polishers	27	23 (85.2)	48.3 ± 7.9	9.8 ± 4.8	91.4 ± 8.3	81.5	26.9 ± 32.3	21 (77.8)	3 (11.1)
	Forgers	42	40 (95.2)	45.5 ± 11.9	11.8 ± 6.8	85.6 ± 8.1	57.1	42.1 ± 33.2	27 (64.3)	6 (14.3)
	Stampers	64	50 (78.1)	27.0 ± 5.0	3.3 ± 3.4	86.1 ± 8.6	75.0	20.9 ± 19.3	26 (40.6)	7 (10.9)
	Carvers	54	42 (77.8)	32.1 ± 11.1	5.1 ± 5.3	82.2 ± 8.1	33.3	33.5 ± 27.7	21 (38.9)	3 (5.6)
Average		**1,050**	**751 (71.5)**	**34.8 ± 9.8**	**7.3 ± 6.5**	**89.4 ± 7.6**	**74.8**	**78.7 ± 124.9**	**677 (64.5)**	**78 (7.4)**

ED, Exposure duration; $L_{Aeq.8h}$, The 8-h equivalent continuous A-weighted sound pressure level in decibels [dB(A)]; HFNIHL, High-frequency noise-induced hearing loss; SFNIHL, Speech-frequency noise-induced hearing loss.

metal processing workers had the highest SFNIHL%, followed by forgers and carpenters.

Principal characteristics of HFNIHL and SFNIHL prevalence

Results of the Chi-square test for the HFNIHL% and SFNIHL% in different groups were listed in Table 2. Sex, age group, noise exposure duration, $L_{Aeq.8h}$, and kurtosis were all related to the HFNIHL%, while the SFNIHL% was only related to age and noise exposure duration. Male workers had a higher prevalence of HFNIHL than female workers (χ^2 = 7.99, p = 0.005). Overall, the HFNIHL% increased with age (χ^2 = 62.97, p < 0.001), although differences between some groups were not statistically significant = 62.97. The HFNIHL% of workers also increased with noise exposure duration (χ^2 = 60.14, p < 0.001), as well as $L_{Aeq.8h}$ level (χ^2 = 47.05, p < 0.001). There were differences in the HFNIHL% of different kurtosis groups, and the HFNIHL% was the highest for those exposed to noise with kurtosis >50 (χ^2 = 25.04, p < 0.001). The SFNIHL% increased with age and noise exposure duration (χ^2 = 51.86, p < 0.001; χ^2 = 13.47, p = 0.019, respectively).

The effect of kurtosis on the association of noise exposure duration and noise intensity with hearing loss

The relationship between noise exposure duration and hearing loss at different kurtosis levels

As shown in Figure 1, noise exposure duration promoted both HFNIHL and SFNIHL, but the effect of noise exposure duration on the HFNIHL% was more pronounced than that on the SFNIHL%. There was no significant difference in the SFNIHL prevalence among the noise exposure duration groups at different kurtosis levels after grouping according to the kurtosis level (for β ≤ 10, χ^2 = 4.38, p = 0.496; for β greater than 10 and less than or equal to 50, χ^2 = 9.72, p = 0.084; and for β > 50, χ^2 = 7.06, p = 0.216). There were statistical differences of the HFNIHL% between noise exposure duration groups at different kurtosis levels (for β ≤ 10, χ^2 = 39.03, p < 0.001; for β greater than 10 and less than or equal to 50, χ^2 = 37.54, p < 0.001; and for β > 50, χ^2 = 11.39, p < 0.001). The HFNIHL% of workers exposed to noise for 3 years or less was 44.9%, 39.0%, and 64.1% when β ≤ 10, 10–50, and >50, respectively.

There were significant differences in HFNIHL% between kurtosis levels when the noise exposure duration was less than 10 years. The HFNIHL% with β > 50 was always significantly higher than that of those with β ≤ 50 for workers with ED ≤10 years (for ED ≤ 3 years, χ^2 = 22.53, p < 0.001; for ED

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TABLE 2 Principal characteristics of HFNIHL and SFNIHL among workers in manufacturing industries (n = 1,050).

Factor	Group	n	HFNIHL		SFNIHL	
			n	%	n	%
Sex	Male	751	504	67.1	59	7.9
	Female	299	173	57.9	19	6.4
			$\chi^2 = 7.99, p = 0.005$		$\chi^2 = 0.70, p = 0.402$	
Age (year)	≤25	201	91	45.3	10	5.0
	25–30	226	136	60.2	9	4.0
	30–35	180	114	63.3	7	3.9
	35–40	145	101	69.7	7	4.8
	40–45	146	114	78.1	13	8.9
	>45	152	121	79.6	32	21.1
			$\chi^2 = 62.97, p < 0.001$		$\chi^2 = 51.86, p < 0.001$	
ED (year)	≤3	398	206	51.8	22	5.5
	3–5	131	88	67.2	6	4.6
	5–10	278	186	66.9	23	8.3
	10–15	133	103	77.4	12	9.0
	15–20	68	58	85.3	7	10.3
	>20	42	36	85.7	8	19.1
			$\chi^2 = 60.14, p < 0.001$		$\chi^2 = 13.47, p = 0.019$	
$L_{Aeq.8h}$ [dB(A)]	< 80	111	45	40.5	3	2.7
	80–85	154	85	55.2	9	5.8
	85–90	283	184	65.0	22	7.8
	≥90	502	363	72.3	44	8.8
			$\chi^2 = 47.05, p < 0.001$		$\chi^2 = 5.52, p = 0.138$	
Kurtosis	≤10	242	163	67.4	17	7.0
	10–50	445	250	56.2	30	6.7
	>50	363	264	72.7	31	8.5
			$\chi^2 = 25.04, p < 0.001$		$\chi^2 = 1.01, p = 0.602$	

HFNIHL, High-frequency noise-induced hearing loss; SFNIHL, Speech-frequency noise-induced hearing loss; ED, Exposure duration; $L_{Aeq.8h}$, The 8-h equivalent continuous A-weighted sound pressure level in decibels [dB(A)].

between 3 and 5 years, $\chi^2 = 8.30$, $p = 0.004$; for ED between 5 and 10 years, $\chi^2 = 4.23$, $p = 0.040$). In contrast, the HFNIHL% at different kurtosis levels were not statistically different when the noise exposure duration exceeded 10 years ($p > 0.05$). The SFNIHL% among 1,050 workers also did not differ by kurtosis level regardless of the duration of noise exposure ($p > 0.05$).

The association of noise intensity and kurtosis with hearing loss at different kurtosis levels

Overall, the HFNIHL% increased with $L_{Aeq.8h}$ levels, while the SFNIHL% showed no difference between different $L_{Aeq.8h}$ levels ($\chi^2 = 5.52$, $p = 0.138$). The influence of noise intensity on the HFNIHL% and SFNIHL% at different kurtosis levels was additionally analyzed; results were summarized in **Figure 2**. At each kurtosis level, there were statistical differences in the HFNIHL% among different $L_{Aeq.8h}$ groups (for $\beta \leq 10$, $\chi^2 = 17.96$, $p < 0.001$; for β greater than 10 and less than or equal to 50, $\chi^2 = 12.98$, $p = 0.005$; and for $\beta > 50$, $\chi^2 = 15.50$, $p = 0.001$). The HFNIHL% of workers at the same noise level increased with the kurtosis level when the $L_{Aeq.8h}$ was between 80 dB(A) and 90 dB(A) ($p < 0.05$). However, there were no statistical differences in the HFNIHL% at different kurtosis levels when workers were exposed to noise with $L_{Aeq.8h} < 80$ dB(A) and $L_{Aeq.8h} \geq 90$ dB(A) (for $L_{Aeq.8h} < 80$ dB(A), $\chi^2 = 0.10$, $p = 0.950$; for $L_{Aeq.8h} \geq 90$ dB(A), $\chi^2 = 6.01$, $p = 0.050$). At the same time, there were still no statistical differences in the SFNIHL% between $L_{Aeq.8h}$ levels after stratifying by the kurtosis level ($p > 0.05$).

NIPTS associated with noise exposure characteristics

Symmetrical and notching shape of NIPTS curves among different types of work

The mean NIPTS of the speech frequencies (19.1 ± 7.0 dB HL) was lower than that of the high frequencies (24.1 ± 13.3 dB HL; $p < 0.05$). **Figure 3A** shows the curves of the average NIPTS of manufacturing workers. The shapes of the average NIPTS curves of left and right ears almost overlapped across the speech and high frequencies, with a classic "V" shape notch. The average NIPTS increased with the test frequencies at 0.5 kHz to 4 kHz. After exhibiting the highest level of average NIPTS at 4 kHz, it then gradually decreased with the test frequencies from 4 kHz to 8 kHz. The mean NIPTS of the speech frequencies between different types of work was statistically different ($p < 0.001$), and that of the high frequencies. The NIPTS curves of different types of work were different, as shown in **Figures 3B,C,D**. The NIPTS curves of stampers, carvers, roller operators, and weavers had a shallow depth of "V", while the curves of polishers, welders, gun nailers, assemblers, and metal processing workers were a deeper "V" shape.

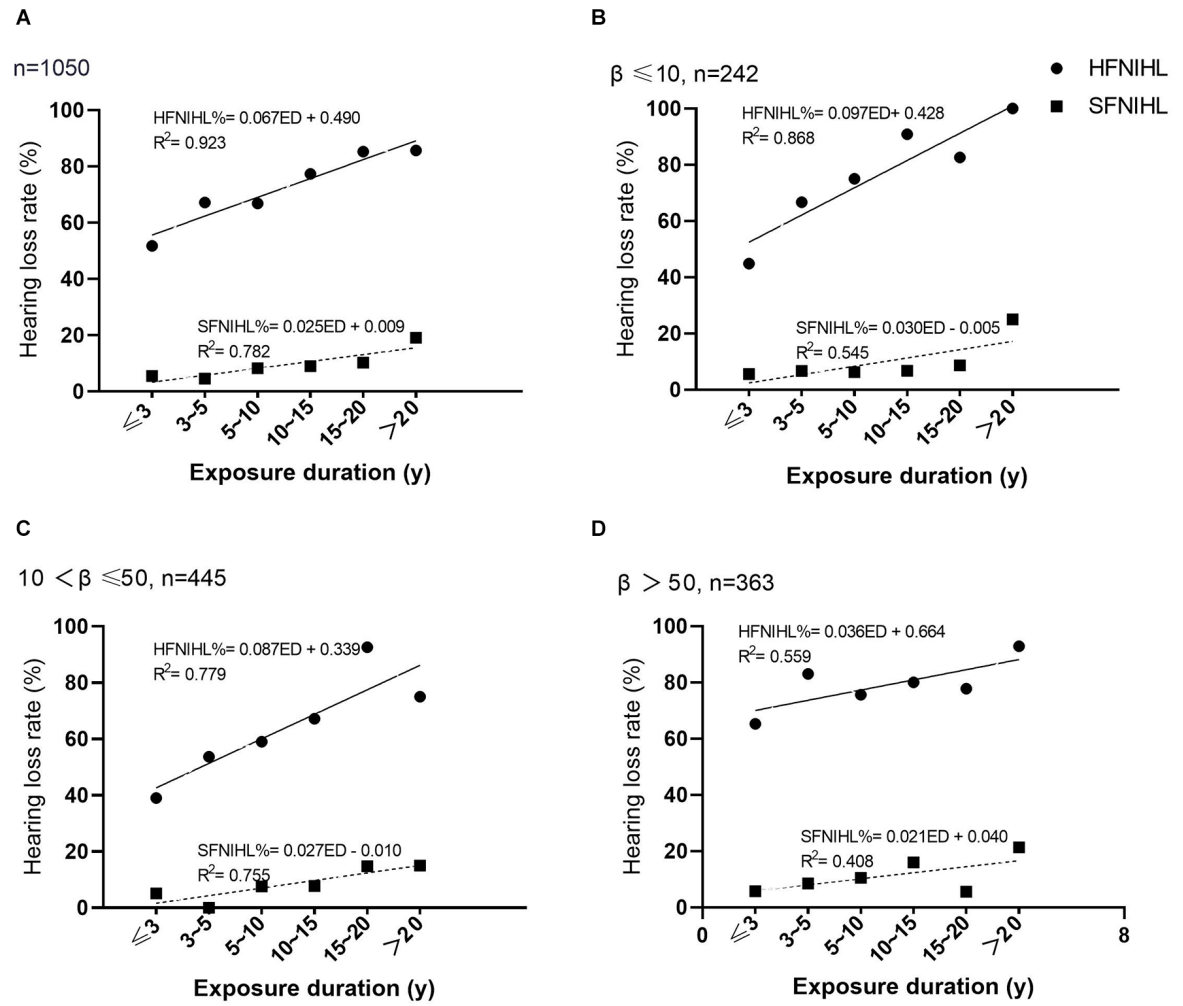

FIGURE 1
The relationship between noise exposure duration and hearing loss at different kurtosis levels. **(A)** The HFNIHL% and SFNIHL% of different noise exposure duration groups. **(B)** The HFNIHL% and SFNIHL% of different noise exposure duration groups when β ≤ 10. **(C)** The HFNIHL% and SFNIHL% of different noise exposure duration groups when β was between 10 and 50. **(D)** The HFNIHL% and SFNIHL% of different noise exposure duration groups when β > 50.

Association of average NIPTS for manufacturing workers with noise exposure duration, noise intensity, and kurtosis levels

Figure 4 displays the "V"-shaped average NIPTS curves of manufacturing workers at different levels of noise exposure duration, $L_{Aeq.8h}$, and kurtosis. The average NIPTS of speech frequencies did not show a significant trend of increasing with noise exposure duration and $L_{Aeq.8h}$ ($p > 0.05$). The depth of the "V" shape notch at 4 kHz in the NIPTS curves deepened with the noise exposure duration when the exposure duration was within 15 years but did not gradually deepen with the exposure duration when the ED > 15 years (Figure 4A). The notch depth of the average NIPTS curves deepened gradually with $L_{Aeq.8h}$ levels, especially for frequencies of 3 kHz to 6 kHz ($p < 0.001$; Figure 4B). Figure 4C illustrated that the high-frequency "V"-shaped hearing valley of curves of workers with β > 50 was significantly deeper than that of workers exposed to noise with β ≤ 50 ($p < 0.05$). However, there was no difference in the shift of speech frequency hearing threshold at different kurtosis levels ($p > 0.05$).

The influence of kurtosis on the association of average NIPTS for manufacturing workers with noise exposure duration and noise intensity

Figures 5A,B shows that the V-shaped dips of the curve (i.e., hearing threshold shift at 3 kHz, 4 kHz, and 6 kHz) were generally deeper when β > 50 than those exposed to noise with β ≤ 50 for workers with ED ≤ 10 years. However, this effect

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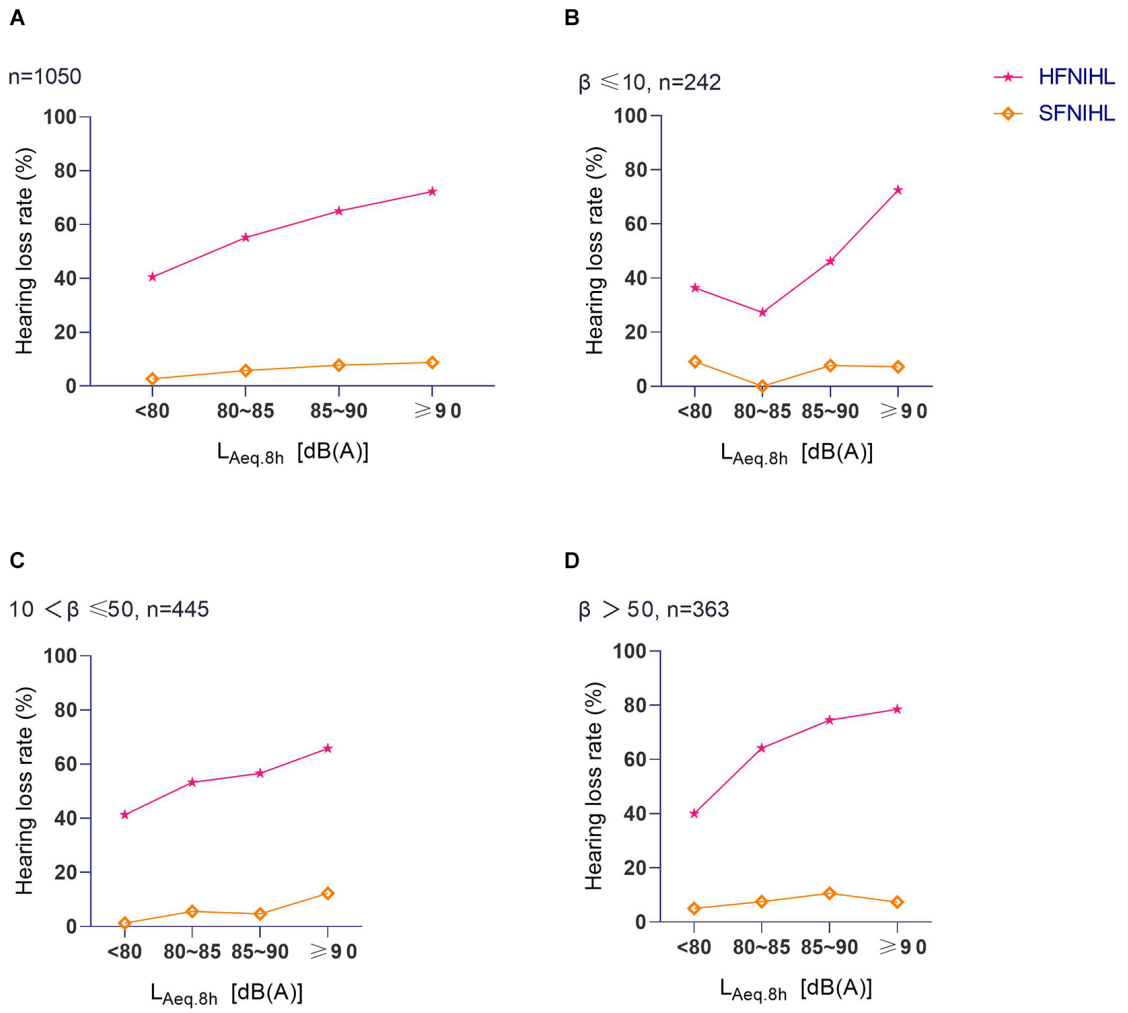

FIGURE 2
The relationship between $L_{Aeq.8h}$ and hearing loss at different kurtosis levels. **(A)** The HFNIHL% and SFNIHL% of different $L_{Aeq.8h}$ groups. **(B)** The HFNIHL% and SFNIHL% of different $L_{Aeq.8h}$ groups when $\beta \leq 10$. **(C)** The HFNIHL% and SFNIHL% of different $L_{Aeq.8h}$groups when β was between 10 and 50. **(D)** The HFNIHL% and SFNIHL% of different $L_{Aeq.8h}$ groups when $\beta > 50$.

was not shown among workers with ED > 10 years. This result suggested that the effect of kurtosis on the association of noise exposure duration and mean NIPTS was more pronounced for workers with ED ≤ 10 years than those with ED > 10 years. Furthermore, the notch of NIPTS curves at high frequencies for workers exposed to noise with $L_{Aeq.8h}$ level between 80 dB(A) to 90 dB(A) was significantly deeper than that of workers exposed to noise with $\beta \leq 50$, as shown in Figures 5C,D. This effect was not shown among workers exposed to noise with $L_{Aeq.8h} < 80$ dB(A) or $L_{Aeq.8h} \geq 90$ dB(A). It indicates that the effect of kurtosis on the association of noise intensity and the average NIPTS in workers exposed to noise with $L_{Aeq.8h}$ between 80 dB(A) to 90 dB(A) was more significant than in those exposed to noise with $L_{Aeq.8h} < 80$ dB(A) or $L_{Aeq.8h} \geq 90$ dB(A).

Binary logistic regression analysis of the association between key factors and the HFNIHL and SFNIHL prevalence

As demonstrated in Table 3, after controlling for the influence of other factors, the HFNIHL% of workers exposed to manufacturing noise was related to sex, age, noise exposure duration, kurtosis, and noise intensity ($L_{Aeq.8h}$). Male workers had a 55.7% higher risk of HFNIHL than female workers (OR = 1.557, 95%CI = 1.141–2.124). The risk of HFNIHL increased with age (OR = 1.033, 95%CI = 1.014–1.052) and noise exposure duration (OR = 1.072, 95%CI = 1.038–1.107). Both kurtosis and noise intensity contributed to an increase risk of HFNIHL (for kurtosis, OR = 1.002, 95%CI = 1.001–1.003; for $L_{Aeq.8h}$, OR = 1.064, 95% CI = 1.044–1.084).

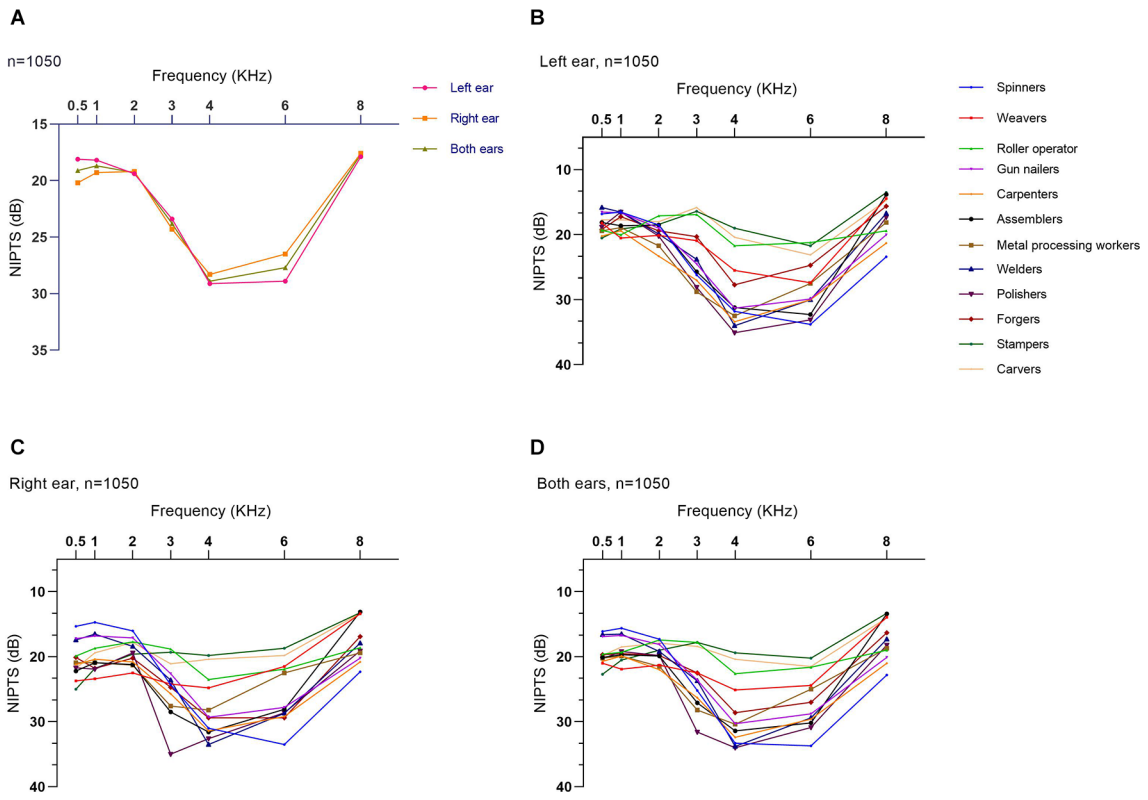

FIGURE 3
Symmetrical and notching shape of average NIPTS curves for manufacturing workers. **(A)** Average NIPTS curves of the left ear, right ear, and both ears of manufacturing workers. **(B)** Average NIPTS curves of the left ears of different types of work. **(C)** Average NIPTS curves of the right ears of different types of work. **(D)** Average NIPTS curves of both ears of different types of work.

Unlike the HFNIHL, after controlling other factors, the SFNIHL risk of workers exposed to manufacturing noise was only related to age (OR = 1.071, 95%CI = 1.043–1.100). There were no statistical associations between the SFNIHL and sex, noise exposure duration, kurtosis, and noise intensity ($L_{Aeq.8h}$; $p > 0.05$).

Discussion

As a type of progressive sensorineural hearing loss, NIHL has been a global public health problem for a long time (Basner et al., 2014). Hearing loss caused by occupational noise exposure in the workplace is a worldwide health problem. In China, 67.56% of the diagnosed cases of occupational otolaryngological and stomatological diseases were from the manufacturing industry in 2020 (Zheng et al., 2021). The equal energy hypothesis, which has been the basis of the noise evaluation metric (L_{Aeq}; ISO, 2013), implies that hearing loss is independent of the temporal characteristics of noise. Nonetheless, many industrial noise environments are non-Gaussian noise (Zhou et al., 2020). Studies have revealed that complex noise exposure could cause a greater risk of NIHL than Gaussian noise (Zhao et al., 2010; Goley et al., 2011; Suter, 2017; Zhang et al., 2021b). The energy metric of noise alone does not apply to the assessment of hearing loss caused by non-Gaussian noise in the workplace and the temporal metric of noise should be considered to be a supplemental indicator of NIHL assessment (Davis et al., 2009, 2012; Seixas et al., 2012; Xie et al., 2016; Zhang et al., 2022).

This study investigated the epidemiological characteristics of hearing loss due to kurtosis-based noise exposure in manufacturing workers. **Table 1** showed that manufacturing workers occupationally exposed to noise with an average $L_{Aeq.8h}$ of 89.4 ± 7.6 dB(A), 74.8% of them were exposed to noise exceeding the OEL of 85 dB(A). Our findings were consistent with those of other studies. In South Korea, more than 90% of workplace noise levels exceeded 85 dB(A) (Kim, 2010). Zhang et al. (2021b) investigated noise exposure levels of workers in six Chinese manufacturing industries, in which 77.6% of the workers were exposed to noise levels higher than 85 dB(A). The noise intensity metric ($L_{Aeq.8h}$) and the noise temporal metric (kurtosis) were distributed differently in different types of work in this study. The weavers, spinners, and gun nailers were exposed to higher $L_{Aeq.8h}$ levels than other types of work, while

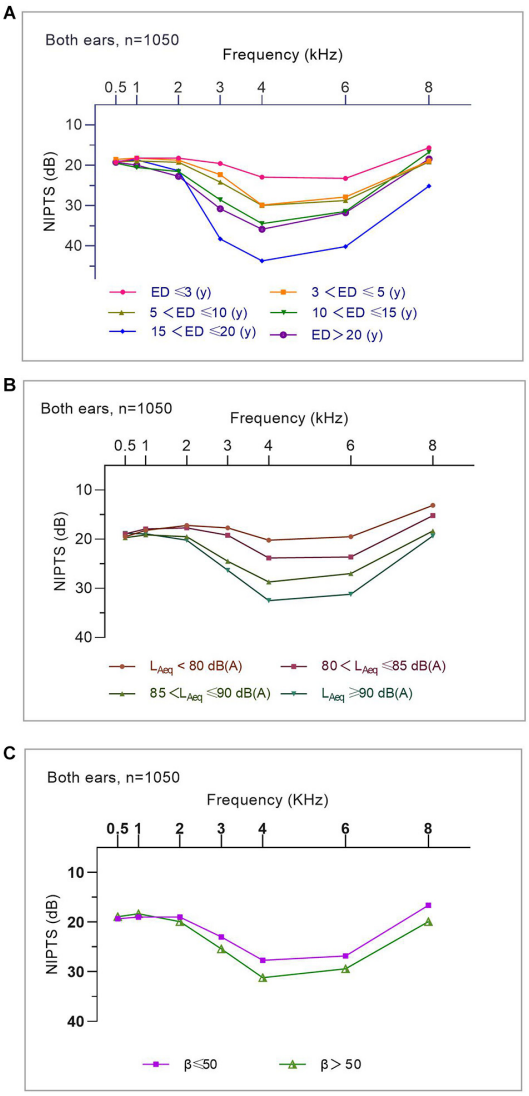

FIGURE 4
The association of average NIPTS curves for manufacturing workers with noise exposure duration, noise intensity, and kurtosis levels. **(A)** Average NIPTS curves of workers with different noise exposure duration. **(B)** Average NIPTS curves of workers exposed to noise at different $L_{Aeq.8h}$ levels. **(C)** Average NIPTS curves of workers exposed to noise at different kurtosis levels.

gun nailers and weavers were exposed to the highest and lowest noise kurtosis levels, respectively. These results indicated that the noise intensity and kurtosis were independent parameters, which was supported by the result of the correlation analysis. Similar results have been found in previous studies (Chen et al., 2019; Zhou et al., 2021).

The prevalence of hearing loss among workers remains high due to the high level of noise exposure. In the United States, approximately 15% of workers have experienced NIHL (Shargorodsky et al., 2010). A meta-analysis study found that the occupational NIHL prevalence in China was 21.3%, of which 30.2% and 9.0% accounted for the prevalence of HFNIHL and SFNIHL, respectively (Zhou et al., 2020). Likewise, the audiometric test results of this study indicated that the average HFNIHL% (64.5%) was much higher than the average SFNIHL% (7.4%) for workers occupationally exposed to manufacturing noise. Significant differences were observed in the average HFNIHL% and SFNIHL% among different types of work. Meanwhile, although both the mean NIPTS for speech frequencies (19.1 ± 7.0 dBHL) and high frequencies (24.1 ± 13.3 dB HL) were within the normal limits, workers of different types of work had their own unique NIPTS curves, which has been revealed in previous studies (Chen et al., 2019; Zhang et al., 2021b).

The average age of participants in this study was 34.8 ± 9.8 years. Age-related hearing loss, defined as a progressive, bilateral, symmetrical age-related sensorineural hearing loss, is a complex disorder that results from the cumulative effects of aging on the auditory system (Bowl and Dawson, 2019). The effect of age on hearing loss is most pronounced at the higher frequencies and a lifetime of noise overexposure also significantly worsens age-related hearing loss (Wu et al., 2020). In this study, the risk of HFNIHL and SFNIHL both increased with age. In addition, male workers experienced a higher risk of HFNIHL than female workers. Some studies reported similar results that age and sex were risk factors for NIHL, even though the hearing thresholds were already adjusted by age and sex based on Annex B Table B.3 in the ISO 1999 (ISO, 2013; Lie et al., 2014; Bolm-Audorff et al., 2020; Zhang et al., 2021b).

The prevalence of NIHL increased with exposure duration, especially during the first 10 years of noise exposure (Bauer et al., 1991; Zhou et al., 2020). The average noise exposure duration of manufacturing workers recruited in this study was 7.3 ± 6.5 years. After controlling other risk factors, the odds of the HFNIHL increased 7.2% with noise exposure duration as shown in **Table 3**. A cross-sectional study in eastern Saudi Arabia revealed that noise exposure is the primary cause of hearing loss (Ahmed et al., 2001). The prevalence of HFNIHL was associated with both noise intensity and its temporary structure as detected in the binary logistic regression results, which were supported by previous studies. Chen et al. (2019) studied the prevalence and determinants of NIHL among workers in the automotive industry and found the prevalence of NIHL increased with the increasing noise energy levels including $L_{Aeq.8h}$. Zhang et al. (2021b) also found the $L_{Aeq.8h}$ has the highest contribution to NIHL.

In recent years, researchers realized that in addition to the noise intensity, the temporal metric plays an important role in leading NIHL. A meta-analysis study in China found the overall weighted OR for complex noise was 1.95, which demonstrated that exposure to complex noise could lead to greater hearing loss than exposure to Gaussian noise (Zhou et al., 2020). Other epidemiological studies have also suggested that the kurtosis

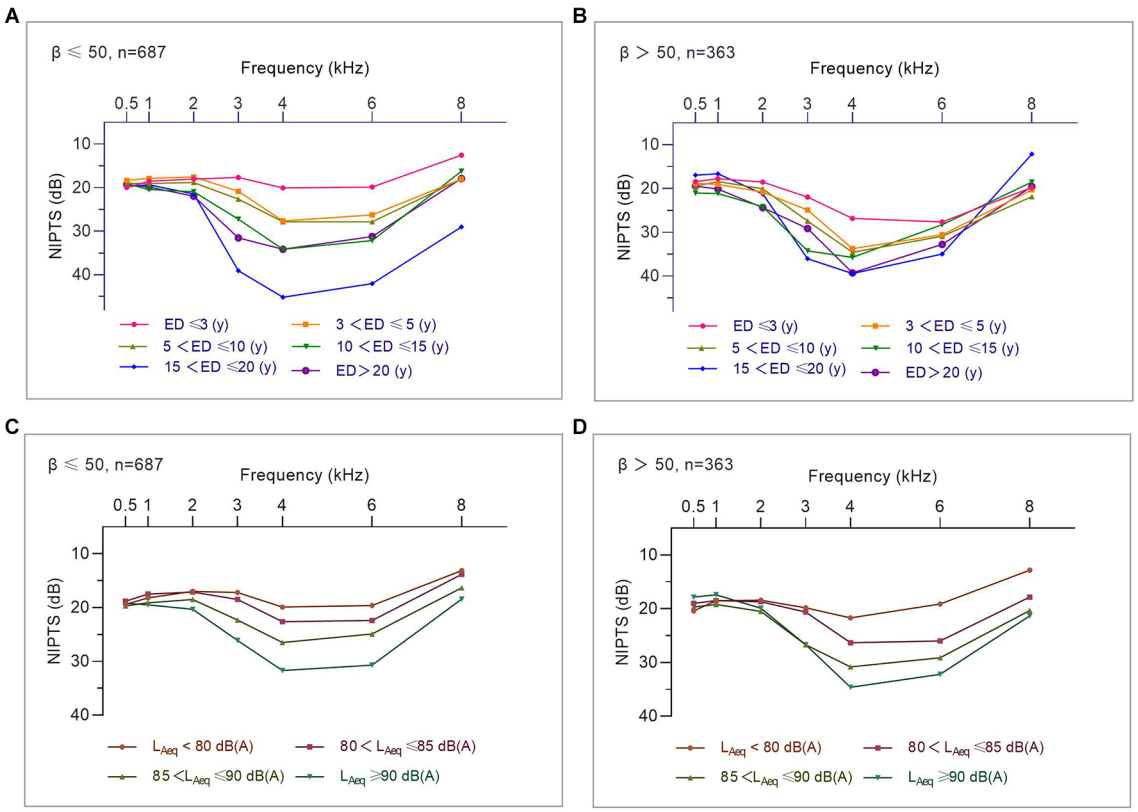

FIGURE 5
The influence of kurtosis on the association of average NIPTS curves for manufacturing workers with noise exposure duration and noise intensity. **(A)** Average NIPTS curves of workers with different noise exposure duration when $\beta \leq 50$. **(B)** Average NIPTS curves of workers with different noise exposure duration when $\beta > 50$. **(C)** Average NIPTS curves of workers exposed to noise at different $L_{Aeq.8h}$ levels when $\beta \leq 50$. **(D)** Average NIPTS curves of workers exposed to noise at different $L_{Aeq.8h}$levels when $\beta > 50$.

metric should be considered when evaluating noise exposure and the risk and cause of NIHL (Qiu et al., 2006; Xie et al., 2016; Shi et al., 2021; Zhang et al., 2021a,b). Our findings indicated that kurtosis was an independent risk factor for the HFNIHL% and it could make the NPTS curve of manufacturing workers a deeper V-shape.

The results of this study further uncovered the effect of kurtosis on the association of exposure duration and noise intensity with NIHL under certain conditions. Kurtosis was able to deepen the hearing notch at high frequencies and accelerate the formation of early hearing loss. The HFNIHL% increased with kurtosis level ($\beta > 50$ vs. $\beta \leq 50$) when the noise exposure duration was within 10 years. Conversely, the kurtosis did not affect the relationship between the HFNIHL% and noise exposure duration when ED >10 years. A similar result was obtained from another study, which suggested that ISO 1999 underestimated the noise exposure duration of NIPTS by less than or equal to 10 years (Zhang et al., 2021c). The present study also identified that the HFNIHL% of workers exposed to noise at the same intensity level increased with the kurtosis level when the $L_{Aeq.8h}$ was between 80 and 90 dB(A). However, the effect of $L_{Aeq.8h}$ on HFNIHL was not affected by kurtosis when workers were occupationally exposed to noise with $L_{Aeq.8h}$ < 80 dB(A) or ≥ 90 dB(A). Even if the $L_{Aeq.8h}$ levels meet the OEL, the HFNIHL risk for workers exposed to high kurtosis noise may still be unacceptable, especially those exposed to noise with $L_{Aeq.8h}$at 80–85 dB(A). These results suggested that the OEL of 85 dB(A) regardless of the kurtosis of noise should be reconsidered. It was consistent with the results of previous studies. Zhang et al. suggested the uncertainty of the OEL of 85 dB(A) might be related to noise exposure with a complex temporal structure, for the NIHL% of workers exposed to noise with $L_{Aeq.8h}$ level of 80–85 dB(A) with a high kurtosis ($\beta > 100$) was significantly higher than those exposed to noise at the same level of $L_{Aeq.8h}$ with $\beta < 100$ (Zhang et al., 2021b).

This study had several limitations. The number of participants in some types of work recruited in this study may result in limited numbers of certain categories after grouping by variables, which may affect the statistical efficiency of some analyses. Therefore, we grouped kurtosis less than some similar studies to reduce the impact of this limitation, and the results

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TABLE 3 Binary logistic regression analysis showing association between key factors and the prevalence of HFNIHL and SFNIHL among workers in manufacturing industries (n = 1,050).

Factor	HFNIHL					SFNIHL				
	B	SE	Wald χ^2	p	OR (95% CI)	B	SE	Wald χ^2	p	OR (95% CI)
Male	0.443	0.158	7.808	0.005	1.557 (1.141–2.124)	0.123	0.292	0.179	0.673	1.131 (0.639–2.003)
Age (year)	0.032	0.009	12.39	0.004	1.033 (1.014–1.052)	0.069	0.014	25.750	<0.001	1.071 (1.043–1.100)
ED (year)	0.069	0.016	17.934	<0.001	1.072 (1.038–1.107)	0.004	0.018	0.053	0.818	1.004 (0.969–1.041)
Kurtosis	0.002	0.006	10.088	0.002	1.002 (1.001–1.003)	0.001	0.001	0.911	0.340	1.001 (0.999–1.003)
$L_{Aeq.8h}$ [dB(A)]	0.062	0.009	42.28	<0.001	1.064 (1.044–1.084)	0.026	0.018	2.145	0.143	1.026 (0.991–1.062)

HFNIHL, High-frequency noise-induced hearing loss; SFNIHL, Speech-frequency noise-induced hearing loss; SE: Standard error; OR, Odds ratio; CI, Confidence interval; ED, Exposure duration; $L_{Aeq.8h}$, The 8-h equivalent continuous A-weighted sound pressure level in decibels [dB(A)].

can still basically draw its influence on hearing loss and its risk factors. Additionally, the majority of participants of this study were young men, whose exposure duration might be shorter than elder workers. As a result, the representativeness of the sample in the manufacturing industry might be insufficient. Another limitation of this study was that it included only a limited number of industries and types of work, which may be slightly under-represented in the broad range of noise types in different manufacturing industries. More participants from various industries including more types of work should be recruited in future studies to improve representation.

Conclusion

The results of this study indicated that: (1) the HFNIHL among manufacturing workers is associated with sex, age, noise exposure duration, $L_{Aeq.8h}$,and kurtosis, while the SFNIHL is associated with age; (2) the kurtosis strengthens the association of noise exposure duration and noise intensity with hearing loss among workers exposed to noise with $L_{Aeq.8h}$ between 80 and 90 dB(A) or with ED less than10 years; (3) an acoustic energy metric is necessary but not sufficient to evaluate the risk of NIHL; (4) the temporal structure of noise such as kurtosis is an additional metric should be considered when evaluating the risk of NIHL by complex noise. These findings would be better replicated using data from a larger sample of workers exposed to a wide range of noise types to provide more information on NIHL in future studies.

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 The Medical Ethics Committee of Zhejiang Center for Disease Control and Prevention. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LZ, XR, and TW: investigation, formal analysis and writing—original draft. HX: methodology and investigation. YH: investigation and data curation. ZS, JX, and JZ: formal analysis and visualization. PX, FW, and YZ: investigation, data curation, and formal analysis. MZ: conceptualization,

funding acquisition, writing—review and editing. HZ: funding acquisition, writing—review and editing, and supervision. All authors contributed to the article and approved the submitted version.

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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.

Publisher's note

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The hunt for hidden hearing loss in humans: From preclinical studies to effective interventions

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Many individuals experience hearing problems that are *hidden* under a normal audiogram. This not only impacts on individual sufferers, but also on clinicians who can offer little in the way of support. Animal studies using invasive methodologies have developed solid evidence for a range of pathologies underlying this *hidden* hearing loss (HHL), including cochlear synaptopathy, auditory nerve demyelination, elevated central gain, and neural mal-adaptation. Despite progress in pre-clinical models, evidence supporting the existence of HHL in humans remains inconclusive, and clinicians lack any non-invasive biomarkers sensitive to HHL, as well as a standardized protocol to manage hearing problems in the absence of elevated hearing thresholds. Here, we review animal models of HHL as well as the ongoing research for tools with which to diagnose and manage hearing difficulties associated with HHL. We also discuss new research opportunities facilitated by recent methodological tools that may overcome a series of barriers that have hampered meaningful progress in diagnosing and treating of HHL.

KEYWORDS

speech-in-noise hearing difficulties, cochlear synaptopathy, central gain, demyelination, noise-induced hearing loss, noise exposure, hearing aids, hearables

Introduction

The World Health Organization (WHO), in its "2021 World Report on Hearing," estimates that half the global population is at risk of developing hearing loss due to unsafe listening practices (World Health Organization, 2021), including exposure to loud sounds at work and during social activities. Up to 1/3 of the workforce is regularly exposed to damaging levels of loud sounds (Schneider, 2005), and more than half of people aged 12–35 regularly expose themselves to sound levels that pose a risk to hearing

either from personal listening devices or by attending loud venues such as nightclubs (Sliwinska-Kowalska and Zaborowski, 2017).

Early signs of hearing loss usually involve difficulties understanding speech in noisy environments, often with no discernible change in hearing thresholds (Lopez-Poveda, 2014; Bramhall et al., 2019). This form of hearing problem is widely referred to as hidden hearing loss (HHL, Schaette and McAlpine, 2011)—*hidden* because it is not possible to diagnose using best-practice clinical tools, such as the audiogram (Bramhall et al., 2019). In fact, one in ten patients who visit a hearing clinic reporting speech-in-noise difficulties remain untreated because the nature of their hearing difficulties cannot be determined (Pryce and Wainwright, 2008; Tremblay et al., 2015; Parthasarathy et al., 2020).

It is now well accepted that hearing loss negatively impacts mental health, behavior, and quality of life, and increases the risk of social isolation, anxiety and depression (Pryce and Wainwright, 2008; Tremblay et al., 2015). Alarmingly, hearing loss in midlife represents the single largest modifiable risk factor for a later dementia diagnosis (Ford et al., 2018; Livingston et al., 2020). Similar assessments of the impacts of HHL on broad health outcomes are now underway. Using *design thinking* methodologies based on online surveys and semi-structured interviews Mealings et al. (2020) reported unmet needs from individuals experiencing HHL and from the clinicians who treat them. They showed that individuals with HHL report that hearing difficulties severely impacted their quality of life, leading them to expend more effort in, and receive less enjoyment from everyday conversations. The same people also reported that missing information in conversations provoked frustration and anxiety associated with potentially misinterpreting what was said. These hearing problems led them to significantly curtail their social encounters. Clinicians reported that they had insufficient training or resources to support such individuals, and that they lacked confidence when recommending treatment options. The main reason for this lack of confidence was the absence of any sensitive measure with which to diagnose HHL; and no uniform or standardized, evidence-based protocol to diagnose and treat their patients.

Considering the potentially high incidence of HHL (Pryce and Wainwright, 2008; Tremblay et al., 2015), its impacts on every-day communication (Ford et al., 2018; Livingston et al., 2020; Mealings et al., 2020), the absence of standardized clinical protocols (Bramhall et al., 2019; Mealings et al., 2020), and the high risk for progression to more severe hearing difficulties (Schneider, 2005; World Health Organization, 2021), there is an urgent need to improve the diagnosis of HHL and to offer solutions to clinicians and their patients.

Here, we review specific highlights of the state-of-the-art relative to the diagnosis and management of HHL (sections "Diagnosing *hidden* hearing loss" and "Intervention strategies for *hidden* hearing loss," respectively), and discuss perspectives and forthcoming trends enabled by emerging methodological tools and outcomes—some of them developed by our own laboratories (section "Discussion"). For clarity, this paper uses the term "HHL" according to the definition provided by the WHO—*<<the condition where an individual experiences common symptoms associated with noise-related auditory damage, such as difficulty in hearing in noise, and that is undetectable on pure-tone audiometry>>* (World Health Organization, 2021).

Diagnosing hidden hearing loss

Neurophysiological pathologies in animal models

Neurodegeneration induced by aging and over-exposure to loud sounds is considered a contributing factor in those who struggle to understand speech, particularly in environments with high levels of background noise. Evidence suggests that at least four neurophysiological pathologies impair the encoding of sounds without elevating hearing thresholds. These, likely related, and potentially interactive, pathologies are cochlear synaptopathy, auditory nerve demyelination, elevated neural gain in the central nervous system, and impaired neural adaptation.

The concept of *cochlear synaptopathy* was first posited by Kujawa and Liberman (2009), who reported that mice experiencing a single exposure to octave band noise (8—16 kHz) at 100 dB sound pressure level (SPL) for 2 h showed an acute and irreversible loss of synaptic ribbons (specialized structures in cochlear sensory hair cells responsible for the release of neurotransmitter required to generate action potentials in afferent auditory nerve fibers) and a subsequent degeneration of these fibers in the absence of any obvious damage to the sensory hair cells. The general tenet of these findings—since replicated in a range of mammalian species, including guinea pigs (Lin et al., 2011; Furman et al., 2013), rats (Bing et al., 2015; Niwa et al., 2016), mice (Chambers et al., 2016; Maison et al., 2016), gerbils (Bourien et al., 2014; Gleich et al., 2016), and rhesus monkeys (Valero et al., 2017)—is that high-threshold auditory nerve fibers (i.e., those with high threshold for sound-evoked activity) are more vulnerable to the damaging effects of loud sounds (noise exposure) than are low-threshold fibers (Furman et al., 2013; Liberman et al., 2015). In addition to over-stimulation, cochlear synaptopathy is also thought to be the primary neural degeneration in age-related hearing loss (Sergeyenko et al., 2013; Kujawa and Liberman, 2015). These findings are consistent with the notion that both aging and noise exposure impact directly the neural encoding of sounds at suprathreshold levels, and suggest that cochlear synaptopathy underlies difficulties understanding speech in noise in individuals with otherwise normal audiograms.

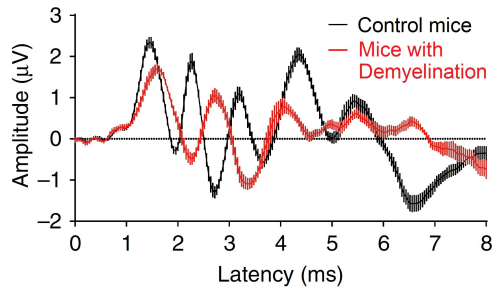

FIGURE 1
Effect of demyelination on the mice auditory brainstem response. Figure adapted from Wan and Corfas (2017).

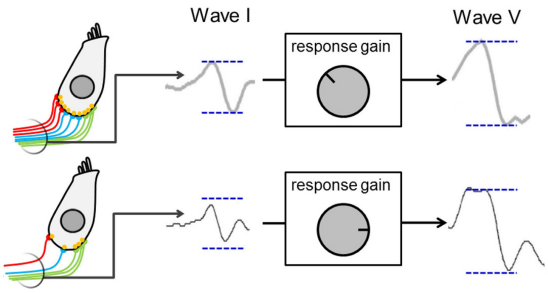

FIGURE 2
Schematic presenting a model for elevated central gain—decreased auditory nerve activity resulting from cochlear synaptopathy leads to a lower wave I amplitude and to activation of central-gain mechanism that increase neural sensitivity and restore wave V amplitude at the level of the midbrain. Figure adapted from Schaette and McAlpine (2011).

A second potential contributing factor to HHL within the inner ear is *auditory nerve demyelination*, a pathology that results from an inefficient repair that follows a loss of cochlear Schwann cells in peripheral terminals of Type I spiral ganglion neurons (Wan and Corfas, 2017). Auditory nerve demyelination occurs independent of noise exposure and is therefore potentially additive to the effects of cochlear synaptopathy. The morphology of sound-evoked auditory brainstem responses (ABRs, **Figure 1**) suggests that demyelination reduces the neural synchrony of the auditory nerve, evident as a reduction in the amplitude and an increase in latency of ABR wave I, and an increase in neural transmission time from cochlea to the cochlear nucleus, assessed in terms of the difference in latency between the first two peaks of the ABR (Wan and Corfas, 2017). Impaired processing following demyelination might also be expected to impact the precise timing required for successful spatial hearing (Stange-Marten et al., 2017), leading to impaired speech-in-noise performance (Swaminathan et al., 2016). Interestingly, the apparently permanent nature of this pathology might also explain hearing problems that arise in those who suffer acute demyelinating diseases such as Guillain-Barré syndrome (Nelson et al., 1988; Takazawa et al., 2012).

Beyond direct effects on auditory nerve fibers, *in vivo* studies have shown that reduced cochlear output arising from cochlear synaptopathy triggers a series of changes in neural processing in later stages of the auditory system that may explain some of the reported manifestations of HHL in humans (Schaette and McAlpine, 2011; Bakay et al., 2018; Resnik and Polley, 2021); specifically, elevated central-gain and mal-adaptation to unfolding sound environments.

Elevated central gain refers to a (potentially homeostatic) increase in neural sensitivity (or activity) in the central auditory system, arising as early as the cochlear nucleus in the brainstem (Schaette and Kempter, 2006), and evident in the midbrain nucleus of the inferior colliculus (Schaette and McAlpine, 2011; Auerbach et al., 2014; Hesse et al., 2016; Monaghan et al., 2020) and auditory cortex (Resnik and Polley, 2021). **Figure 2** presents a schematic model of the central-gain hypothesis at the level of the midbrain (Schaette and McAlpine, 2011), in which elevated central gain arises from reduction in excitatory that generates a, potentially compensatory, change in the balance of excitatory and inhibitory neural activity in an attempt to restore the neural representation of sound following some form of cochlear insult, e.g., denervation through cochlear synaptopathy (Schaette and McAlpine, 2011; Auerbach et al., 2014). Whilst this compensatory mechanism helps restore sounds detection in quiet, it impairs the neural representation of speech and impacts temporal processing of sounds in background noise (Chambers et al., 2016; Monaghan et al., 2020; Resnik and Polley, 2021). Interestingly, Hesse et al. (2016) showed that elevated central gain was more pronounced in animals with synaptopathy (exposed to 100 dB SPL noise) than in animals with a permanent increase in hearing threshold (exposed to 105 dB SPL noise), thus suggesting a non-monotonic relationship between subtle cochlear damage and elevated central gain. In addition, elevated central gain may contribute to pathologies such as tinnitus or hyperacusis (Schaette and McAlpine, 2011; Auerbach et al., 2014; Hesse et al., 2016). It is worth noting that the minimal requirement for elevated neural gain may simply be a reduction in sensory input brought about by reduced sound levels or conductive forms of hearing loss (Maslin et al., 2013; Munro and Turtle, 2013; Parry et al., 2019).

Neural mal-adaptation refers to the inability of neurons along the auditory pathway to adapt their response to loud acoustic environments to optimize the neural encoding of information in those environments (Dean et al., 2005)—potentially critical for understanding speech in noise. Dean et al. (2008) demonstrated that neurons in the auditory midbrain of guinea pigs adapt their firing pattern to the mean sound level of the background with the consequence that sensitivity to those sound levels improves over time. This form of neural adaptation, evident in the responses of auditory nerve fibers (Wen et al., 2009), is expanded by the level of auditory

cortex (Watkins and Barbour, 2008), and is altered in HHL. Specifically, Bakay et al. (2018) found that the ability of midbrain neurons to adapt to loud sound environments was impaired in mice with noise-induced synaptopathy, relative to control mice with no prior noise exposure. This supports the view that hearing-in-noise difficulties in humans might arise from suboptimal neural adaptation to loud sound environments.

Candidate measures of hidden hearing loss

An important methodological challenge to diagnosing the pathologies that underlie HHL in living humans is the lack of potential biomarkers [i.e., biological marker—an externally measurable representation of a specific condition or pathology (Strimbu and Travel, 2010)] of inner ear physiology and anatomy that mirror invasive methodologies in *in-vivo* animal preparations such as immunostaining or serial-section electron microscopy (Viana et al., 2015). To this end, current diagnostic tools for assessing HHL continue to rely on non-invasive methodologies commonly employed in assessing hearing function.

The most widely reported measure is the amplitude of the click-evoked wave I of the ABR, measured at suprathreshold sound levels. Kujawa and Liberman (2009) reported that the suprathreshold increase in magnitude of ABR wave I with increasing sound intensity was correlated with the number of intact synapses in the auditory nerve following noise injury in rodents, with a lower rate of increase associated with evidence of cochlear synaptopathy. In human listeners, the ratio of the amplitude of waves I and V of the click-evoked ABR has been proposed as an indicator of elevated central gain—a relative measure within the individual that is intended to reduce inter-subject variability and is based on the hypothesis that cochlear synaptopathy generates a reduced amplitude wave I and a compensatory increase in wave V amplitude in audiometrically normal individuals with tinnitus (for whom the term HHL was originally coined; Schaette and McAlpine, 2011). Further, Mehraei et al. (2016) reported that, relative to control animals, noise-exposed mice showed a shorter shift in latency of ABR wave IV (equivalent to wave V in humans) with increasing levels of masking noise—a result consistent with the selective loss of high-threshold auditory nerve fibers expected in individuals with HHL (Bourien et al., 2014). Together, the data are consistent with the relative magnitude of ABR waves being a potential biomarker of HHL.

Liberman et al. (2016) hypothesized that the ratio of amplitude of the summating potential and the compound action potential (SP/AP) amplitude ratio might also represent a biomarker sensitive to HHL. Since cochlear synaptopathy affects the auditory nerve synapses but leaves the cochlear sensory hair cells intact (Kujawa and Liberman, 2009), higher scores of this indicator are expected to be associated with cochlear synaptopathy. Consistent with their hypothesis, Liberman et al. (2016) found that the amplitude ratio of the SP/AP was higher individuals at high risk for ear damage, characterized by normal hearing thresholds up to 8 kHz but elevated thresholds over the extended, high-frequency range (up to 16 kHz). However, counter to their hypothesis, the greater amplitude ratio of the SP/AP in the high-risk group was associated with a higher magnitude SP, rather than a reduction in the magnitude of the AP, making it difficult to interpret in terms of potential synaptopathy. Wan and Corfas (2017) showed that, relative to controls, animals with confirmed auditory nerve demyelination showed similar SP amplitudes but reduced AP amplitudes, with a concomitant increase in the SP/AP ratio. While these results potentially support this measure acting as biomarker for HHL, the fact that the SP is generated by multiple sources, not only inner-hair cells, but also outer-hair cells and even the auditory nerve (Durrant et al., 1998; Pappa et al., 2019; Lutz et al., 2022), renders its use as a biomarker for HHL unlikely.

The envelope following response (EFR) is an auditory steady-state response (i.e., a periodic neurophysiological response resulting from the sum of several overlapping auditory evoked potentials—usually analyzed in the frequency domain; Valderrama, 2022) evoked by an amplitude-modulated tone, and has been used as a physiological measure of the temporal representation of suprathreshold sounds in the auditory brain (Bharadwaj et al., 2014). EFR amplitudes appear smaller in mice with noise-induced synaptopathy, relative to unexposed control mice (Shaheen et al., 2015). Consistent with this, Bharadwaj et al. (2015) reported a significant correlation between the slope of the EFR magnitude as a function of modulation depth and amplitude-modulation detection threshold—a behavioral measure of temporal coding—in normal-hearing young adults. Further, Parthasarathy et al. (2020) found that the combination of ASSR to frequency modulation, pupillometry measures, and a behavioral measure based on a frequency-modulation (FM) detection task accounted for 78% of the speech-perception variability in adults with hearing thresholds in the normal range. However, the relationship of this measure of FM detection to HHL remains complex since, over the near-normal hearing range, sensitivity to slow-FM (a proposed metric for HHL) is correlated with place-coding fidelity (i.e., variations in the cochlear place of stimulation), a likely consequence of "standard" hearing loss arising from damage to cochlear hair cells, rather than retro-cochlear damage (Whiteford et al., 2020).

Vander Ghinst et al. (2021) used magnetoencephalography to record cortical responses to the envelope of running speech in multi-talker background noise, and found that individuals with normal audiograms but difficulties understanding speech-in-noise showed reduced cortical tracking of speech, relative to control individuals who did not have hearing difficulties. This is consistent with the degraded neural representation

of speech in background noise observed in noise-exposed animals (Monaghan et al., 2020). Vander Ghinst et al. (2021) also found that human listeners with hearing difficulties showed an increased functional connectivity between auditory cortices and brain areas involved in semantic and attention processes, consistent with Yeend et al. (2017) who reported that selective attention was a significant predictor of speech-in-noise problems in many individuals with presumed HHL.

Finally, the middle-ear muscle reflex (MEMR) has been proposed as a potential biomarker of cochlear synaptopathy due to its strong dependence on the integrity of high-threshold auditory nerve afferent fibers (Liberman, 1988; Kobler et al., 1992). Loud sounds contract the stapedius muscle, stiffening the ossicular chain and tilting the stapes away from the cochlea. This elicits a bilateral increase in middle-ear impedance that can be assessed by measuring otoacoustic emissions (Boothalingam and Goodman, 2021). Valero et al. (2016, 2018) found that MEMR thresholds were elevated, and suprathreshold amplitudes attenuated in noise-exposed mice, relative to unexposed animals. Consistent with these data, Wojtczak et al. (2017) showed that normal or near-normal hearing individuals with tinnitus presented a significantly weaker MEMR strength, compared to individuals without tinnitus. However, these results were not replicated by Guest et al. (2019), who found no association between the MEMR threshold and tinnitus, speech-in-noise hearing performance or noise exposure history in individuals with normal audiograms.

Sensitivity of candidate measures

Despite the large number of non-invasive candidate measures potentially sensitive to HHL in humans, there is no consensus view that the neurophysiological pathologies evident in animal models of HHL are evident in humans or that these represent the underlying cause of speech-in-noise hearing difficulties reported by individuals with normal audiograms (Kobel et al., 2017; Barbee et al., 2018; Bramhall et al., 2019; Kohrman et al., 2020; Bramhall, 2021).

A possible argument explaining the differences in outcomes across studies and null results across the literature is that the human auditory structures are less susceptible to the adverse effects of noise exposure than in rodents—variations in inter-species susceptibility were reported by Valero et al. (2017), who needed around 20 dB higher noise level to induce a similar degree of cochlear synaptopathy in primates compared to rodents—and therefore, it could be the case that the actual noise-induced neurophysiological damage in humans is minimal. Another possible explanation is that the existing measures (mostly relying on ABR and EFR measures) are not sensitive enough to the neurophysiological damage associated with HHL, and that large inter-subject variability in these measures prevents their use in selectively diagnosing underlying neurophysiological pathologies at the individual level (Valderrama et al., 2018; Bramhall et al., 2019). In fact, current measures based on ABR, EFR and MEMR are affected by several extraneous factors, such as hair-cell loss in basal regions of the cochlea (Don and Eggeront, 1978; Yeend et al., 2017), ear canal effects that add variability to the auditory stimulus presented in testing, even if an insert earphone is used (Souza et al., 2014), and individual variance in the spectral component of MEMR measurements—which could compromise sensitivity when a tone probe is used to measure the MEMR (Bharadwaj et al., 2019). Further, considering that noise exposure accelerates the effects of aging (Fernandez et al., 2015), it is possible that young adults with a history of noise exposure have not yet developed substantial degradation of inner-hair cell synapses or demyelination. This would explain the negative results reported by several studies conducted in young adults (Prendergast et al., 2016; Fullbright et al., 2017; Grinn et al., 2017; Guest et al., 2019). It should also be noted that regardless of the metric, estimates of noise-exposure history are unvalidated and largely subjective, and range from estimates made over recent years to estimated noise-exposure history over the lifetime (Valderrama et al., 2018; Bramhall et al., 2019).

Intervention strategies for hidden hearing loss

Interventions strategies for HHL can be classified in two categories: assistive listening devices that improve the hearing experience of their users, and emerging therapeutic interventions aimed at restoring the neurophysiological damage.

Assistive listening devices

In the absence of any definitive objective measure or diagnostic for HHL in humans, researchers and clinicians continue to rely on questionnaires and surveys, to ascertain the hearing difficulties associated with HHL and to suggest treatment options. Koerner et al. (2020), for example, reported that, in addition to counseling patients with tactics that improve communication in noisy venues, around 23% of surveyed audiologists (n = 157) used mild-gain hearing aids as their preferred rehabilitation strategy, even though little-to-no research has been conducted to evaluate the efficacy of these technologies in adults with hearing difficulties but normal audiograms. In fact, to date, only two studies have investigated the use of a mild-gain hearing aid for this population (Roup et al., 2018; Singh and Doherty, 2020). These studies showed that while mild-gain hearing aids helped people with HHL reduce their hearing-in-noise handicap to some extent, only 3 out 17 participants in Roup et al. (2018), and

2 from 10 participants in Singh and Doherty (2020), reported being willing to continue using the devices in noisy listening situations. These studies suggest that whilst mild-gain hearing aids might potentially reduce the hearing-in-noise handicap of individuals with normal hearing, these technologies remain suboptimal for most of them. Another possible explanation for the low uptake of such technologies might be related to the effects of compression and amplification algorithms—a neurophysiological study conducted in hearing-impaired gerbils showed that although these algorithms help improve sound perception, they fail to restore the selectivity of neural responses to different speech sounds (Armstrong et al., 2022).

Therapeutic interventions for synaptopathy

If synaptopathy represents a primary lesion in HHL, it makes sense to target the inner ear with therapeutics that might ameliorate its effects or reverse it altogether. Neurotrophins are a family of proteins that participate in the development and growth of neurons (Reichardt, 2006), and have been used to investigate the regeneration of the neurophysiological damage associated with cochlear synaptopathy. Wise et al. (2005) found in drug-induced deaf guinea pigs, that spiral ganglion cells regenerated peripheral axons of auditory nerve fibers toward their target inner hair cell following a cochlear perfusion of neurotrophin-3. Further, Suzuki et al. (2016) reported that round-window delivery of neurotrophin-3 24 h following exposure to a synaptopathic noise insult regenerated a significant proportion of the lost synaptic connections in mice, and led to the recovery of the suprathreshold amplitude of the ABR wave I. In a more complete form of hearing loss, gene transfer into the inner ear of guinea pigs deafened with gentamicin and implanted with cochlear implants demonstrated the capacity not only to grow neurites back toward potential targets using neurotrophins, but to use the electrode contacts within the ear to steer the therapy toward the desired location (Pinyon et al., 2019). These results support that a therapeutic intervention based on neurotrophins has the potential to prevent, decelerate or restore the adverse effects of cochlear synaptopathy in humans.

Discussion

Toward sensitive diagnostic biomarkers of hidden hearing loss in humans

Despite animal studies provide solid models of neurophysiological pathologies plausibly involved in HHL, research efforts inspired by Kujawa and Liberman's (2009) seminal study of synaptopathy have, to date, failed to identify non-invasive biomarkers of HHL in humans appropriate for diagnostic purposes. Here, we discuss considerations and promising research opportunities provided by emerging methodological tools that seek to overcome barriers to the identification of non-invasive biomarkers of HHL.

One factor in the failure to identify HHL in human listeners is the aim to "hunt for pure HHL" which likely exists only rarely, if at all, beyond experimental laboratory settings. If cochlear synaptopathy precedes damage to outer hair cells (Kujawa and Liberman, 2009), most incidents of HHL are likely comorbid with "standard" audiometric hearing loss, especially given the near decade delay between individuals (or their associated others) noticing they might have hearing problems and seeking professional help (Simpson et al., 2019). To this end, one group of listeners for whom HHL is almost certainly an issue are those with near-normal thresholds or mild hearing loss.

Another barrier to developing biomarkers for HHL is a continued focus on cochlear synaptopathy, ignoring the role of other pathologies that might also underlie speech-in-noise difficulties reported by individuals with normal audiograms. Future efforts might usefully focus on developing novel non-invasive biomarkers that also target auditory nerve demyelination, central gain, and mal-adaptation. An example of such a biomarker might be an objective metric of performance in binaural listening tasks such as the *interaural phase modulation—following response* (IPM-FR, Undurraga et al., 2016). Since the neural encoding of small interaural time differences requires exquisite temporal precision in the activity of the auditory nerve from both ears (Stange-Marten et al., 2017), problems arising from demyelination might be expected to degrade this measure (Resnik and Rubinstein, 2021). Indeed, Bernstein and Trahiotis (2016) reported that individuals with highly sensitive hearing thresholds at 4 kHz (better than 7.5 dB hearing level) but reporting problems listening in noise performed worse in a binaural behavioral task, suggesting that early signs of hearing loss might be associated with deficits in binaural listening. Further, a study conducted on 23 normal-hearing listeners demonstrated a strong correlation between the amplitude of the IPM-FR and the ability to understand speech in noise as a function of interaural-time differences resulting from the spatial location of the speaker, both at individual and group levels, supporting the potential sensitivity of this measure to speech-in-noise hearing difficulties expected in individuals with HHL (Undurraga et al., 2020).

Additionally, new biomarkers could be retrieved from the *full-range* auditory evoked response (see **Figure 3**; de la Torre et al., 2020)—this response applies a latency-dependent filtering which, combined with the representation of the signal in the logarithmic time scale, enables the representation of all the components of the auditory pathway, from cochlea to cortex. This novel representation of transient auditory evoked potentials not only provides standard metrics such as the amplitude of wave I [appropriate to study synaptopathy

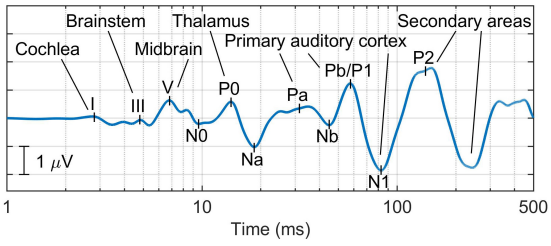

FIGURE 3
Example of the *full-range* auditory evoked response, which provides a comprehensive representation of all the components of the auditory pathway—from the cochlea to the cortex (de la Torre et al., 2020).

(Kujawa and Liberman, 2009)], the waves I-III interpeak latency [since demyelination impairs the neural transmission time in the auditory nerve, longer values in this metric could be associated with demyelination this pathology (Wan and Corfas, 2017)], and the waves I-V amplitude ratio—an index of elevated central gain in the midbrain (Schaette and McAlpine, 2011); but also novel relative measures between central and peripherical components such as the ratio of the amplitude of wave I to P1 to assess the presence of elevated cortical gain proposed by Resnik and Polley (2021).

The potential sensitivity of these measures to diagnosing problems listening in noise is supported by recent data from our own research. **Figure 4** presents speech-in-noise hearing performance measured *via* the high-cue (HC) condition of the Listening in Spatialized Noise test (LiSN, Cameron and Dillon, 2008) on a cohort of 64 individuals with normal audiograms reporting different degrees of hearing-in-noise difficulties, who were categorized according to whether they had elevated central gain and their brainstem neural transmission time (measured *via* the ABR waves I-V amplitude ratio and interpeak latency, respectively) (Valderrama et al., 2018). These data demonstrate that in individuals with elevated central gain, those with longer brainstem neural transmission times showed impaired speech-in-noise performance, demonstrating an interaction between neural conduction times and elevated central gain. Consequently, it is reasonable to hypothesize that biomarkers associated with elevated central gain and neural transmission times might help characterize speech-in-noise intelligibility difficulties in individuals with normal audiograms.

A second barrier is the technical limitation imposed by standard processing methods that average several segments of the electroencephalogram to increase the signal-to-noise ratio (SNR) of the auditory evoked response. These traditional methods impose important constraints on the experimental design to meet the requirement of the inter-stimulus interval being longer than the duration of the evoked response so that the estimation of one response is not affected by adjacent responses (Valderrama et al., 2012). Overcoming this problem requires signal-processing algorithms that enable deconvolution of overlapping auditory evoked potentials. Some examples of these algorithms are iterative randomized stimulation and averaging (IRSA, Valderrama et al., 2014, 2016; de la Torre et al., 2019) and subspace-constrained least squares deconvolution (SC-LS, de la Torre et al., 2022). Importantly, IRSA enables the recording of the full-range response evoked by the fine structure of natural speech (Valderrama et al., 2019), and therefore provides a novel measure that may help advance knowledge in how the human auditory system encodes speech in challenging listening scenarios—a critical step to characterize HHL with objective biomarkers.

Another barrier to understanding HHL is the use of non-invasive methodologies in human research—which presents a *validation* problem (Plack et al., 2016) because it is difficult to assess *pre-mortem* human neural structures and confirm that a diagnostic tool is sensitive to a certain pathology. As a substitute, pathologies and biomarkers might be simulated in computational models of the auditory system. Examples of this approach include validation of the wave I of the ABR as a biomarker for cochlear synaptopathy (Verhulst et al., 2016), simulation of demyelination on the neural encoding of interaural time differences (Resnik and Rubinstein, 2021), characterization of the combined effect of synaptopathy and demyelination on the compound action potential (Budak et al., 2021), and simulation of the effect of different hearing damage mechanisms on speech-in-noise perception (Haro et al., 2020). Simulating pathologies in a computational model provides a controlled environment with the opportunity to validate the sensitivity of novel biomarkers to the target pathologies.

An important consideration is that even if a specific measure is found to be sensitive to HHL at group level (e.g., presenting statistically significant differences between the distributions observed on an *experimental* group with HHL and a *control* group with no hearing problems), the large inter-subject variability typically observed in neurophysiological measures such as metrics derived from the ABR or the EFR might prevent their use for diagnostic purposes (see **Figure 4**). One approach that has been reported to overcome this problem is the use of relative measures such as amplitude ratios, inter-peak latencies, or the slope of growth functions. The use of relative measures will likely rule out individual effects that add variability to specific measures, e.g., head size, ear canal shape, the individual anatomy of cochlear mechanics (Bharadwaj et al., 2019). A second approach is to analyze multiple biomarkers targeting different neurophysiological pathologies, through a comprehensive test battery of electrophysiological, behavioral, cognitive, and psychoacoustic measures, and use machine learning to estimate the magnitude of hearing damage associated with HHL. Machine learning approaches have been used to predict noise-induced hearing impairment in individuals exposed to complex industrial noise (Yanxia et al., 2019), and could provide links between neurophysiological pathologies

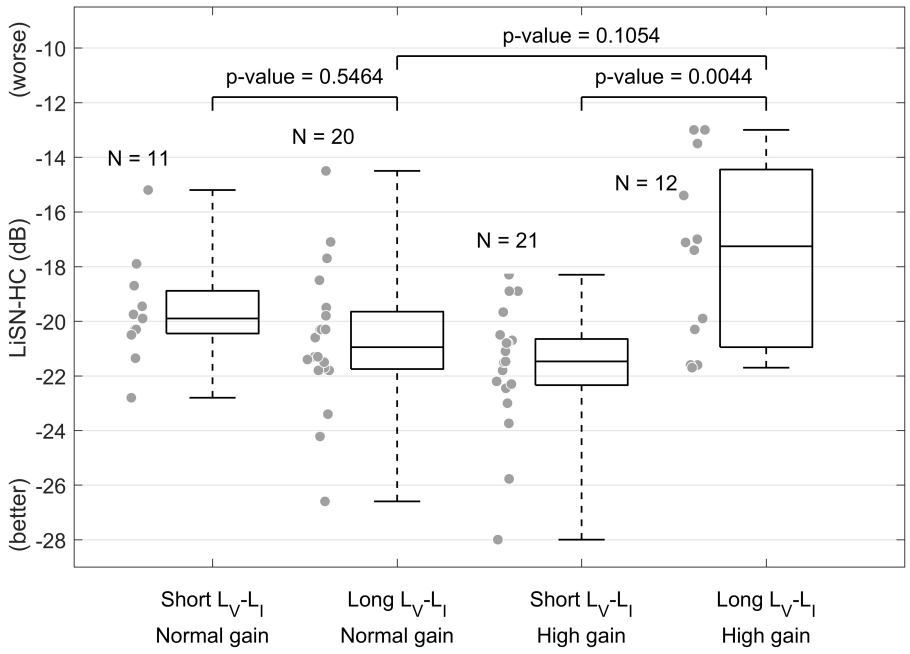

FIGURE 4
Effect of elevated central gain and brainstem neural transmission time on speech-in-noise intelligibility (Valderrama et al., 2018). This figure shows that when central gain at the level of the midbrain is elevated (high gain), individuals with longer brainstem neural transmission time (measured *via* the ABR waves I-V interpeak latency) presented worse speech-in-noise hearing performance.

and perceptual difficulties, essential to developing a sensitive diagnostic tool for HHL.

A common problem faced by most investigations of HHL in humans is the validity of estimates of noise-exposure history, as these rely heavily on subjective questionnaires and self-reported measures. In this respect, future investigations could benefit from emerging technologies such as portable noise-exposure dosimeters embedded in wearables like smart watches to generate more reliable measures of noise exposure. Further, access to individualized metrics of noise exposure background may also benefit from *citizen science* or crowd-sourcing of data, an ideal means of identifying individuals at risk of HHL, tailoring strategies to prevent hearing loss, and engaging beneficiaries of future therapies and interventions for HHL. An example of this approach is the Apple Hearing Study (Apple Inc., Cuppertino, CA)—a large-scale national study conducted in the United States that uses mobile applications on the Apple Watch to assess the intensity of environmental sounds and cardiovascular metrics in order to understand the impacts of being exposed to loud sounds on hearing and cardiovascular health (Neitzel et al., 2022).

A final consideration relies on the methodologies used to measure the MEMR—a potential biomarker for HHL in humans (Valero et al., 2016, 2018; Wojtczak et al., 2017). Although standard clinical measures of the MEMR employ pure tones (typically at 226 Hz or 1000 Hz) to evoke the reflex (Schairer et al., 2013), Bharadwaj et al. (2019) suggested that individual variations in the middle-ear anatomy may influence the frequency spectrum and magnitude of MEMR measures. This might explain the null results reported by Guest et al. (2019) when they investigated the relationship between MEMR and tinnitus, speech-in-noise performance, and noise-exposure background. Wideband probe stimuli such as chirps or clicks could be used as probe stimuli to overcome this problem and increase sensitivity. Novel MEMR methodologies based on click-evoked otoacoustic emissions such as the one developed by Boothalingam et al. (2021) could play an important role in the differential diagnosis of HHL.

On the search of optimal management strategies

While therapeutic interventions may eventually prevent the start, delay the progression of, or even reverse, the impairment of age- and noise-induced HHL, it will likely be some time before clinicians can administer an efficient drug to treat patients with HHL. This means that there is an urgent need to develop and standardize a non-pharmacologic solution that improves the hearing experience of individuals with HHL.

An immediate approach to help HHL patients to deal with their hearing difficulties could be to provide them with training on coping strategies typically used by people with hearing loss, e.g., mobile- and web-based applications that provide

lipread training. Pang et al. (2019) identified that the most commonly reported coping strategies used by HHL individuals were non-verbal cues such as lip reading, gestures and facial expressions; moving closer or tilting toward the speaker; moving to quieter locations; concentrating harder in conversations; avoiding noisy places; and whenever possible, reducing the level of noise ambience, e.g., by turning down the television volume. Appropriate counseling about these coping strategies in clinical appointments could provide practical guidelines to HHL patients.

Edwards (2020) proposed a model anticipating which technologies would be preferred to attend the hearing needs of different segments of the broad spectrum of people with hearing difficulties. This model predicted that individuals who self-perceive hearing difficulties but do not have a measurable hearing loss are potential candidates for *hearables*—technologies that use directionality and smart audio processing to attenuate the effect of background noise and enhance the hearing experience of their users. In fact, a study conducted by the authors and their research teams showed that a significant proportion of individuals with speech-in-noise intelligibility difficulties but normal or near normal audiograms reported to be *ready and willing* to trial hearables in acoustically challenging situations such as cafeterias and noisy restaurants (Mealings et al., 2020). To this end, future research might usefully assess the value of hearables in meeting the unique hearing needs of individuals with HHL.

In order to validate the value of these technologies as an intervention for listening problems associated with HHL, clinicians need to know (i) to what extent these devices improve the hearing experience of their users [this will help clinicians manage the expectations of their patients]; (ii) what are the listening scenarios in which devices perform best/worse [this will help them provide adequate counseling on the capabilities of the devices]; (iii) what proportion of users benefit when using these devices in acoustically challenging situations [this will provide an estimation of the success rate of this intervention]; (iv) what are the unique features that characterize those who do benefit from these technologies [this will help clinicians anticipate which patients would benefit the most]; (v) how close do these listening devices match a prescription target [this will ensure users receive optimal audibility and that their hearing is not compromised as a consequence of any possible over-amplification]; and (vi) what are the main barriers that would discourage users from using the devices (e.g., cost, stigma, comfort, battery life) [this will help clinicians provide informed recommendations to their patients, and may also inspire technology manufacturers in the development of the next-generation products that will close the gap between the technology features and the users' *gains* and *pains*, thus eventually increasing the adoption rate of these technologies]. Addressing these questions will likely lead to the development of clinical-management guidelines for HHL that could be standardized globally.

Unlike traditional research methods—largely based on laboratory-based measures of hearing and speech-in-noise intelligibility, novel methodologies based on *ecologically-momentary assessment* (EMA, Timmer et al., 2018) have the potential to capture difficult-to-assess factors such as user satisfaction, emotional state and perceived hearing benefit from listening technologies. In contrast to traditional questionnaires, which are usually applied at the completion of a study, EMA tools increase reliability and reduce recall bias by enabling users to provide real-time feedback of their hearing experience in those listening settings in which they experience difficulties. In addition, EMA tools can record acoustic features of the sound environment such as the A-weighted sound level and reverberance, which would help respond to some of the research questions mentioned above. Further, future research methodologies might also consider conducting a randomized control trial, including a control group fitted with an *acoustically transparent* device (i.e., a hearing device that does not apply any gain or compression) to account for any possible placebo effect derived from device placement within the ear (Dawes et al., 2013).

The use of assistive devices such as hearables to improve the listening experience of people with HHL involves providing users with a mild gain (i.e., 5–10 dB insertion gain) that may compensate for some degree of hearing loss, and provides an acoustic advantage in noisy environments thanks to the directionality of their microphones and noise-reduction algorithms. Future research endeavors might investigate whether the acoustic benefit of these devices increases by incorporating advanced signal-processing features that have been proven successful in hearing aids and cochlear implants, including adaptive selection of the device output levels to optimally fit an individual's hearing dynamic range (Blamey, 2005), smart algorithms based on contralateral inhibition that enhance binaural cues (Lopez-Poveda et al., 2022), and the use of effective voice activity detection algorithms that provide an enhanced SNR to the listener in situations with background noise (Ramirez et al., 2004; de la Torre et al., 2006; Liu and Demosthenous, 2021).

Other avenues to explore include novel interventions based on attenuating (rather than amplifying) high-intensity sounds. The intention of this apparently counter-intuitive approach is to shift input sounds to a level range in which individuals with HHL are expected to have optimal sensitivity. Recent experimental animal findings [see **Figure 5**, adapted from Monaghan et al. (2020)] suggest that loss of, or damage to, high-threshold auditory nerve fibers resulting from cochlear synaptopathy leads to a saturation of the spiking probability in neurons of the inferior colliculus (**Figure 5A**). Elevation of central gain (**Figure 5B**) in response to reduced sensory

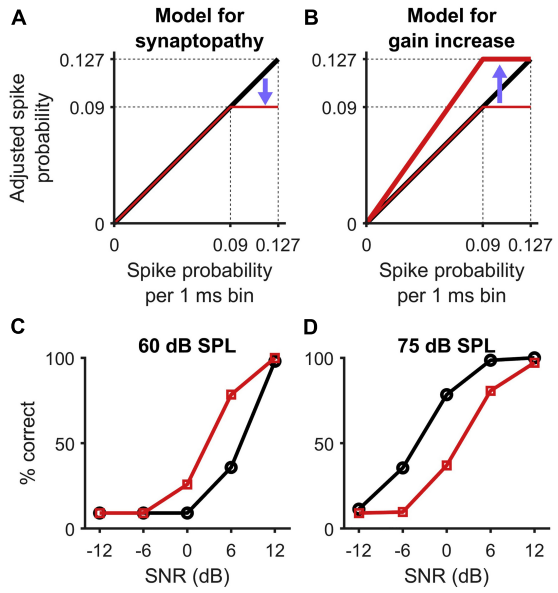

FIGURE 5
Figure adapted from Monaghan et al. (2020) presenting a model for synaptopathy and central gain activation. **(A)** The loss of high-threshold auditory nerve fibers in cochlear synaptopathy saturates the spiking probability of neurons in the inferior colliculus at supra-threshold level. **(B)** Central gain activation presents a multiplicative increase of the neurons sensitivity to restore the maximum (non-synaptopathic) spike probability. **(C,D)** As a consequence, the slope of the spike probability function increases in mid-levels, which leads to better discriminability from the HHL model (squares) than from the control model (circles) at 60 dB SPL, but reduced discriminability at 75 dB SPL.

input seeks to restore the maximum (non-synaptopathic) spike probability, with the consequence that the slope of the spike-probability function increases for mid-level sounds, leading to better discriminability in the HHL model relative to that in the control model at 60 dB SPL, but not at 75 dB SPL (**Figures 5C,D**). Based on this model, an assistive listening device based on an attenuator could potentially help individuals with HHL communicate better in noisy and loud environments.

Finally, it could be the case that a *one-size-fits-all* solution is not appropriate to reducing listening difficulties associated with HHL, and that different solutions are required for different segments of the HHL population. In this regard, the objective determination of the site of neurophysiological lesion might lead to the development of different strategies tailored to individual listeners. For example, technologies based on directionality or background-noise reduction might improve the hearing experience of individuals with synaptopathy; technologies that enhance binaural-hearing cues such as binaural-weighted subtraction (Lopez-Poveda et al., 2022) could help individuals with auditory nerve demyelination problems; and cognitive training programs could benefit individuals with intact peripherical neural structures but with selective-attention difficulties. The use of objective methods that provide measures from both peripherical and central neural stations such as the full-range auditory evoked potential (de la Torre et al., 2019) could help identify neural structures presenting abnormal activity patterns.

Authors contributions

JV conceived and wrote the manuscript. AT and DM provided the conceptual framework, assisted in the literature review, and participated in the writing of the manuscript. DM proofread the English of the manuscript. All authors read and approved the final manuscript.

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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.

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Noise exposure in early adulthood causes age-dependent and brain region-specific impairments in cognitive function

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Hearing loss is a chronic health condition that affects millions of people worldwide. In addition to age-related hearing impairment, excessive noise exposure is a leading cause of hearing loss. Beyond the devastating effects of hearing impairment itself, epidemiological studies have identified hearing loss as a major risk factor for age-related cognitive decline, including dementia. At present, we currently lack a full understanding of the brain regions and underlying molecular changes that are responsible for mediating the link between hearing loss and cognitive impairment across aging. In the present study, we exposed 6-month-old rats to an occupational-like noise (100 dB SPL, 4 h/day × 30 days) or sham exposure and investigated both hippocampal-dependent (i.e., spatial learning and memory, assessed using the Morris water maze) and striatal-dependent (i.e., visuomotor associative learning, assessed using an operant-conditioning task) cognitive function across aging at 7, 10, and 13 months of age. We also investigated brain region-specific changes in microglial expression following noise/sham exposure in order to assess the potential contribution of this cell type to noise-induced cognitive impairments. Consistent with human studies, the occupational-like noise exposure resulted in high-frequency hearing loss, evidenced by a significant increase in hearing thresholds at 20 kHz. Ultimately, our results suggest that not all higher-level cognitive tasks or their associated brain regions appear to be equally susceptible to noise-induced deficits during aging, as the occupational-like noise exposure caused an age-dependent deficit in spatial but not visuomotor associative learning, as well as altered microglial expression in the hippocampus but not the striatum. Interestingly, we found no significant relationships between spatial learning ability and the level of hearing loss or altered microglial density in the hippocampus following noise exposure, suggesting that other changes in the brain likely contribute to hippocampal-dependent cognitive dysfunction following noise exposure.

Lastly, we found that a subset of younger animals also showed noise-induced deficits in spatial learning; findings which suggest that noise exposure may represent an increased risk for cognitive impairment in vulnerable subjects. Overall, our findings highlight that even a mild occupational-like noise exposure earlier in adulthood can have long lasting implications for cognitive function later in life.

KEYWORDS

noise exposure, cognitive impairment, aging, microglia, hippocampus, striatum

Introduction

Hearing loss is a chronic health condition affecting over 400 million people worldwide, with one of the leading causes being excessive exposure to loud noise from environmental (e.g., city traffic), recreational (e.g., loud music) and occupational insults (e.g., industry workers; military personnel) (Clark, 1991; Lie et al., 2016; World Health Organization [WHO], 2021). Given that recent large-scale epidemiological studies have identified hearing loss as a major risk factor for age-related cognitive decline, including dementia, there is a critical need to better understand the adverse effects of noise-induced hearing loss on the brain (Lin, 2011; Lin et al., 2011a,b, 2012; Gurgel et al., 2014; Fritze et al., 2016; Livingston et al., 2020). Based on recent studies in humans as well as more invasive studies in animal models, there is evidence that the negative effects of noise exposure are not restricted to the auditory system itself (Manikandan et al., 2006; Goble et al., 2009; Kraus et al., 2010; Basner et al., 2014; Li et al., 2014; Cui et al., 2015; Irgens-Hansen et al., 2015; Ruvalcaba-Delgadillo et al., 2015; Su et al., 2018; Cheng et al., 2019; Hayes et al., 2019; Stone et al., 2020; Ke et al., 2021; Osbrink et al., 2021; Molina et al., 2022). At present, however, we currently lack a full understanding of the brain regions and underlying molecular changes that are responsible for mediating the link between hearing loss and cognitive impairment across aging.

The vast majority of animal studies that have investigated noise-induced cognitive impairment have focused on characterizing deficits in hippocampal-dependent spatial learning and memory performance using the Morris water maze task shortly after noise exposure (Cui et al., 2009; Cheng et al., 2011; Chengzhi et al., 2011; Liu et al., 2016; Wang et al., 2016; Wieczerzak et al., 2021). Consequently, much less is known about whether other brain regions subserving cognitive processing are similarly vulnerable to noise-induced hearing loss, and by extension, it is unclear whether noise exposure in early adulthood differentially impacts specific cognitive domains later in life. As aging is associated with an increased reliance on the striatum to help compensate for hippocampal dysfunction (Bohbot et al., 2012; Rodgers et al., 2012; Wiener et al., 2013; Zhong and Moffat, 2018), it is important to determine how noise exposure affects the short- and long-term function of the striatum—a brain region that normally subserves stimulus-response habit-learning and goal-directed actions (McDonald et al., 2007; Delotterie et al., 2015). To date, only a few animal studies have investigated the effect of noise exposure on the striatum. For example, noise exposure can alter striatal neurotransmitter systems (e.g., dopamine, serotonin, acetylcholine, glutamate) (Ravindran et al., 2005; Sembulingam et al., 2005; Samson et al., 2006; Kazi and Oommen, 2014), and we recently found that in the weeks following intense noise exposure, rats not only exhibited deficits in hippocampal-dependent learning and memory, but also demonstrated impaired performance on a simple visual cue discrimination task largely reliant on the striatum (Wieczerzak et al., 2021). Whether more complex striatal-dependent cognitive functions, such as visuomotor associative learning, are also impaired by noise exposure has yet to be investigated. Furthermore, it is still not fully understood if hippocampal- and/or striatal-dependent cognitive impairments persist (or become exacerbated) in the months following noise exposure; findings which could go on to have significant implications for an acceleration of age-related cognitive decline.

It is well-established that noise exposure can induce inflammation in the peripheral and central auditory pathway (Henderson et al., 2006; Tornabene et al., 2006; Fuentes-Santamaria et al., 2017). In particular, noise exposure has been shown to cause an increase in the expression of microglia in both the auditory brainstem and cortex (Baizer et al., 2015; Fuentes-Santamaria et al., 2017; Wang et al., 2019; Zhuang et al., 2020). Microglia are the resident immune cells of the central nervous system and have numerous ramifications through which they search for environmental stressors [for review, see Kettenmann et al. (2011)]. Upon encountering an insult, microglia become activated and undergo rapid morphological changes, characterized by shorter ramifications and a larger soma, to provide neuroprotective effects. If this state persists, as commonly seen in aging and neurological disorders, chronically activated microglia are hypothesized to play a neurotoxic role, releasing proinflammatory cytokines, and contributing to neural degradation (Block et al., 2007). Of concern, alterations in microglial expression have also

been observed in the hippocampus of noise-exposed rodents and these immune cells have been theorized to underly the cognitive impairments commonly seen following noise exposure (Zhuang et al., 2020; Li et al., 2021). However, no studies have examined if noise-induced alterations in microglial expression occur in other cognitive brain regions, such as the striatum, further contributing to impairments in cognitive function.

Previous investigations into the link between hearing loss and cognitive impairment in animal models have utilized methodological approaches that may limit their translatability for noise-induced hearing loss in humans. For example, many of these experimental studies have employed intense noise exposures, in excess of 120 dB SPL, that cause severe hearing loss or deafness; an approach that does not closely resemble the most common forms of noise-induced hearing loss resulting from environmental, recreational, and occupational settings (Kraus et al., 2010; Tao et al., 2015; Liu et al., 2016, 2018; Zhuang et al., 2020; Li et al., 2021). Furthermore, many studies have investigated cognitive function in the immediate days or weeks following noise exposure (Cui et al., 2009; Cheng et al., 2011; Wang et al., 2016), highlighting the need to determine if noise-induced cognitive impairments persist across aging. Thus, in the present study we exposed 6-month-old rats to an occupational-like noise exposure (100 dB SPL, 4 h/day × 30 days) and assessed both hippocampal- (i.e., spatial learning and memory) and striatal-dependent (i.e., visuomotor associative learning) cognitive function at 7, 10, and 13 months of age. We also investigated the brain region-specific changes in microglial expression following noise exposure in order to assess the potential contribution of this cell type to noise-induced cognitive impairments. Ultimately, our results suggest that not all higher-level cognitive tasks or their associated brain regions appear to be equally susceptible to noise-induced deficits during aging, as the occupational-like noise exposure caused an age-dependent deficit in spatial but not visuomotor associative learning, as well as altered microglial expression in the hippocampus but not the striatum. Furthermore, we found that a subset of younger animals also showed noise-induced deficits in spatial learning; findings which suggest that noise exposure may represent an increased risk for cognitive impairment in vulnerable subjects.

Experimental procedures

Subjects and experimental design

The present study utilized Fischer 344 rats, a rodent model commonly used to study cognitive performance across the lifespan (vanderStaay and Blokland, 1996; LaSarge et al., 2007; Bizon et al., 2009). Of importance, compared to young-adult (i.e., 6-month-old) rats, age-related impairments in spatial learning and memory performance have been shown to occur by 12 months of age (i.e., older-adulthood) in this strain (Bizon et al., 2009). Thus, in order to assess both shorter and longer-term effects of noise exposure in early adulthood on cognitive function across aging, male and female Fischer 344 rats underwent noise or sham exposures at 6 months of age, with separate cohorts of rats then undergoing cognitive-behavioral testing at either 7 (Sham: 9 male, 8 female; Noise: 9 male, 12 female), 10 (Sham: 9 male, 8 female; Noise: 9 male, 9 female), or 13 (Sham: 10 male, 8 female; Noise: 10 male, 9 female) months of age (**Figure 1**). To ensure litter effects were not a confounding variable in our results, animals from each litter were distributed equally between treatment and age groups. Hearing sensitivity was assessed before noise or sham exposure and immediately before tissue collection. Experimenters were blinded to the identity of the animal treatment groups and age for the collection and analysis of all data. All rats used in this study were housed in facilities maintained by Animal Care and Veterinary Services on a 12-h light/dark cycle with food and water *ad libitum* (unless stated otherwise). Experimental procedures were approved by the Animal Care Committee at Western University and in compliance with the guidelines established by the Canadian Council on Animal Care.

Hearing assessment

Hearing sensitivity was measured using an auditory brainstem response (ABR) protocol. Rats were anesthetized with isoflurane (4% induction, 2% maintenance), placed in a sound-attenuating chamber (ENV-017M; Med Associates, St. Albans, VT, USA), and maintained at a temperature of ∼37°C using a homeothermic heating pad (507220F; Harvard Apparatus, Holliston, MA, USA). Subdermal electrodes (27 gauge; Rochester Electro-Medical, Lutz, FL, USA) were positioned at the vertex (active electrode), over the right mastoid process (reference electrode), and on the midback (ground electrode).

Auditory stimuli consisting of 2 tones (4 and 20 kHz; 5 ms duration and 1 ms rise/fall time) were generated using a Tucker-Davis Technologies (TDT) RZ6 processing module with a 100 kHz sampling rate (TDT, Alachua, FL, USA) and calibrated with custom MATLAB software (The Mathworks, Natick, MA, USA) using a 1/4-inch microphone (2530; Larson Davis, Depew, NY, USA) and preamplifier (2221; Larson Davis). The auditory stimuli were delivered by a speaker (MF1; TDT) positioned 10 cm from the animal's right ear while the left ear was occluded with a custom foam earplug. All stimuli were presented 1,000 times (21 times/s) at decreasing intensities from 90 to 10 dB sound pressure level (SPL) in 10 dB steps. Near threshold, successive steps were decreased to 5 dB SPL, and each sound level was presented twice to best determine the ABR threshold using the criteria of a just noticeable deflection of

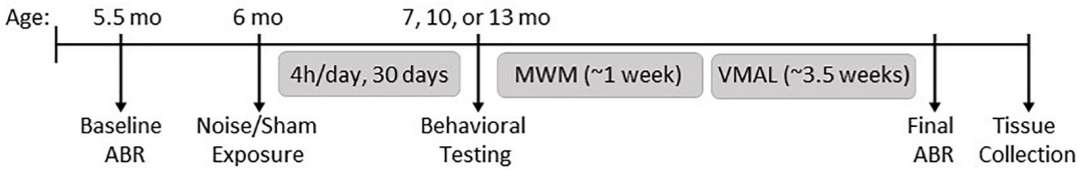

FIGURE 1
Experimental timeline. Rats underwent a baseline hearing test measured using the auditory brainstem response (ABR) at 5.5 months of age, followed by noise or sham exposure at 6 months of age (100 dB SPL; 4 h/day, 30 days). Behavioral testing began at 7 months (immediately following noise exposure), 10 or 13 months of age, and included the Morris water maze (MWM) and visuomotor associative learning tasks (VMAL). Upon completion of behavioral testing, a final hearing test was performed, and rats were then sacrificed for tissue collection.

the averaged electrical activity within the 10-ms time window. Sound-evoked responses were acquired using a low-impedance headstage (RA4LI; TDT), preamplified and digitized (RA16SD Medusa preamp; TDT) and sent to an RZ6 processing module *via* a fiber optic cable. The signal was filtered (300–3,000 Hz) and averaged using BioSig software (TDT).

Noise exposure

In an effort to replicate the intensity of repetitive noise exposures commonly experienced by individuals working in noisy environments (i.e., an occupational-like noise exposure) rats underwent noise (100 dB SPL white noise) or sham (speakers off) exposures for 4 h/day for 30 consecutive days. Both noise and sham exposures were carried out in a sound-attenuating booth (MDL 4872 S; WhisperRoom, Inc., Knoxville, TN, USA) containing a wire shelving unit (Nexel Industries Inc., Port Washington, NY, USA) from which 20 equally spaced speakers (4 speakers per row, 5 rows; PDS122; Pyle USA, Brooklyn, NY, USA) were suspended. During the exposures, animals were placed individually into modified home cages, with each cage placed directly underneath one of the suspended speakers. White noise (1–20 kHz) was generated (Audacity, version 2.3.2), amplified (XLS1000, Crown) and distributed (SP-160-10V; Specialty AV) to the speakers and calibrated to 100 dB SPL with a sound level meter (WS1361C; Koolertron) placed at the level of the rat's ears within the cage. Rats underwent noise or sham exposures at 6 months of age, with the placement of each rat rotated throughout the booth over the 30-day exposure period to ensure that all animals had equivalent exposures.

Morris water maze

Following noise or sham exposures, separate cohorts of rats were assessed for spatial learning and reference memory performance using the Morris water maze at either 7, 10, or 13 months of age. The maze consisted of a circular pool (144 cm diameter) filled with room-temperature water (22–23°C) that was dyed with black non-toxic acrylic paint. The pool was divided into four quadrants and given north (N), south (S), east (E), and west (W) designations to define specific points around the circular perimeter. The protocol occurred over a total of 8 days, where rats underwent 2 days of habituation, followed by 5 days of spatial learning trials, and then 1 day consisting of the probe test and visual cue trials.

On the first 2 days of the protocol, rats were habituated to the pool and the presence of a submerged platform located in the center of the pool (12 cm in diameter; 3 cm below the surface of the water). On both habituation days, rats were placed directly on the platform for 30 s. On the second day of habituation, after spending 30 s on the platform, the rats were removed, immediately placed in the north position of the pool, and allowed to swim to the platform. For the subsequent days of spatial learning and probe trials, spatial cues were placed at the northwest (red square), southwest (green triangle), northeast (white star), and southeast (yellow plus-sign) corners of the pool, and the platform was positioned in the center of the southwest quadrant. Rats underwent spatial learning trials for 5 consecutive days, with four 90-s trials (1-h inter-trial interval) per day to assess their ability to locate the submerged platform over time. On each day, rats were released facing the pool wall in the northwest (NW), north (N), northeast (NE), and east (E) positions for trials 1–4, respectively. If the rat did not find the platform within the 90-s maximum trial duration, it was cued to the platform by the experimenter, and allowed to rest on the platform for 30 s to observe its location with respect to the spatial cues. Throughout testing, each rat's time to locate the hidden platform and their average swim speed were collected using ANYmaze tracking software (v6.33, 64-bit; 267 Stoelting Company, Wood Dale, IL, USA) and a webcam (C930e; Logitech, Switzerland) mounted on the ceiling above the maze.

On the last day of testing, the platform was removed for the probe trial, during which the rats were placed in the NNE position and allowed to swim for the full 90 s. Reference memory was assessed by the time required for the rat to recall the location of the platform region (15 cm diameter region centered on where the platform was previously placed). One hour after the completion of the probe test, visual cue trials were conducted to

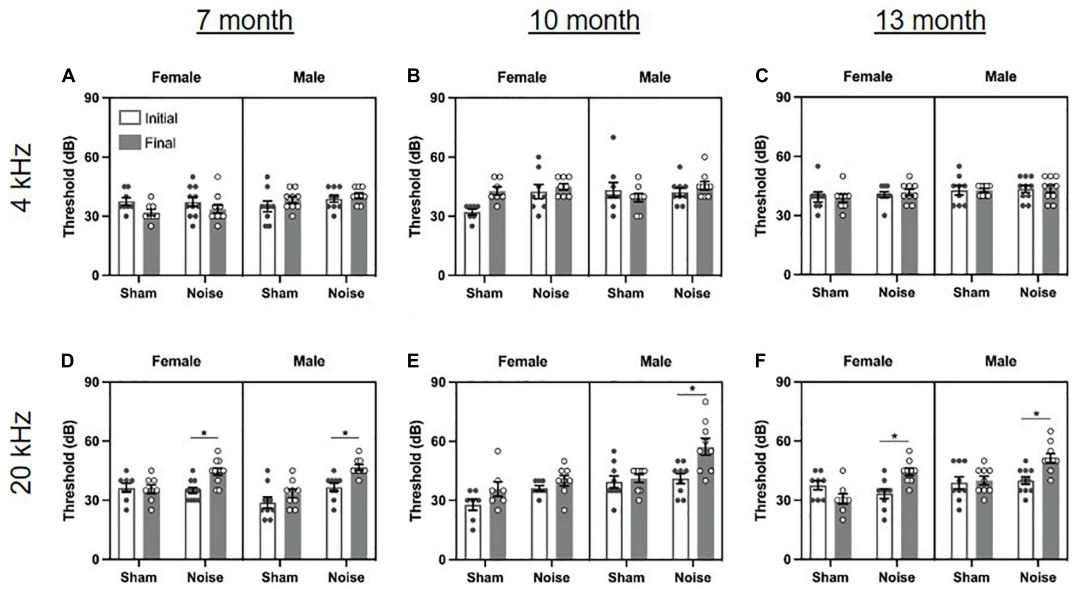

FIGURE 2
Noise exposure caused an age- and sex-dependent mild, high-frequency hearing loss. **(A–C)** The auditory brainstem response (ABR) revealed no changes in hearing sensitivity at the 4 kHz stimulus following noise or sham exposure at 7, 10, or 13 months of age. **(D,F)** Noise-exposed rats had significantly increased hearing thresholds compared to baseline at the 20 kHz stimulus at 7 and 13 months of age, regardless of sex (*$p_{bonf} < 0.05$). **(E)** At 10 months of age, noise-exposed male rats, but not female rats, had significantly increased hearing thresholds at the 20 kHz stimulus (*$p_{bonf} < 0.05$). n = 7-month (Sham: 9 male, 8 female; Noise: 9 male, 11 female), 10-month (Sham: 9 male, 7 female; Noise: 9 male, 8 female), and 13-month (Sham: 9 male, 8 female; Noise: 10 male, 9 female). Data represent group mean ± SEM.

identify any potential differences in visual acuity and/or swim speed between the sham and noise-exposed rats, as these factors can confound the interpretation of learning trial and probe test data. The spatial cues were removed from around the pool, and the location of the platform was now indicated using a marker (flag) positioned directly on the platform and protruding from the surface of the water. Rats underwent four trials (1-h inter-trial interval) in which they were placed in the north, west, south, and east start positions of the pool with the cued-platform located in the southeast, northeast, southwest, and northwest quadrants, respectively.

Visuomotor associative learning

Following the completion of the 8-day Morris water maze task, visuomotor associative learning (VMAL) was assessed in male rats in a standard modular test chamber (ENV-008CT; Med Associates) housed in a sound-attenuating box (29″ W by 23.5″ H by 23.5″ D, Med Associates). The behavioral chamber was illuminated by a house light on the back wall, whereas the front wall contained a center nose poke, a left feeder trough and a right feeder trough; each equipped with an infrared detector. For presentation of visual stimuli, an LED (ENV-229M; Med Associates) was located above the center nose poke and delivered either a steady light stimulus (1-s duration) or a flashing light stimulus (5 times/s, 1-s duration). Stimulus delivery, nose-poke responses and food rewards were controlled and monitored using custom behavioral protocols running in MATLAB (Epsych Toolbox1) which was interfaced with real-time processing hardware (RZ6; TDT).

Before beginning the visuomotor associative learning task, rats were food-restricted, weighed daily and maintained at >85% of their free-feeding body mass. They were then habituated to receiving sucrose pellets upon initiation of a trial by performing a nose poke in the center port. Once rats reached ∼150 trials during their daily 30-min session (∼6-day training period), the testing phase began. During the testing phase, one of two visual stimuli was presented upon the initiation of a trial by a nose poke in the center port. Over 18 consecutive days, rats learned to access the left feeder trough when the steady light stimulus was presented, and the right feeder trough when the flashing light stimulus was presented. The rats performed ∼150 trials per day. Custom MATLAB scripts were used to obtain the rats' overall performance (percent accuracy) and time taken to respond correctly to a given stimulus (reaction time) for each day of the visuomotor associative learning task.

Immunohistochemistry

Following the final ABR hearing assessment, noise-exposed and sham rats were deeply anesthetized with isoflurane and

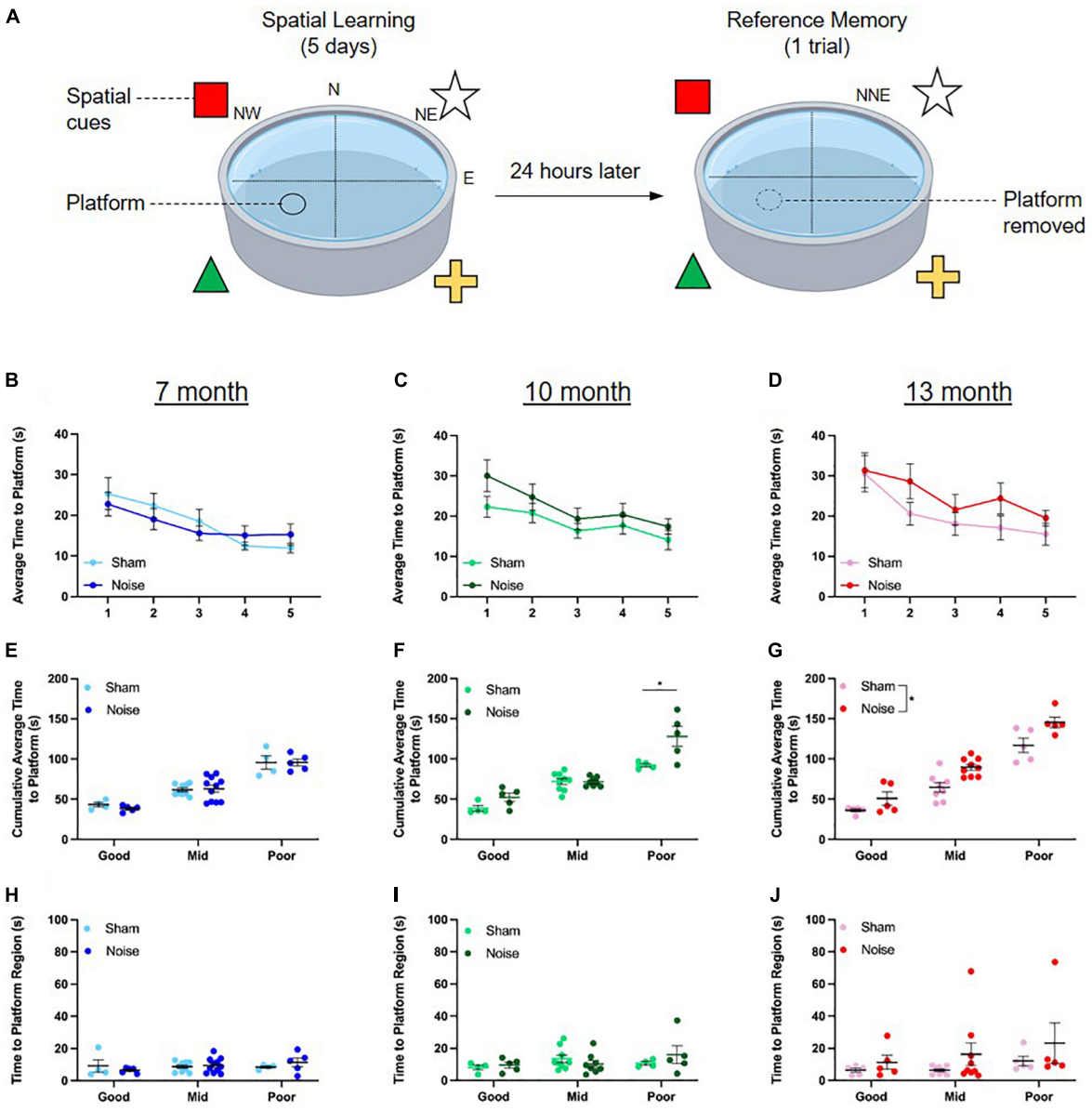

FIGURE 3
Hippocampal-dependent spatial learning was impaired in noise-exposed 13-month-old rats and a subset of noise-exposed 10-month-old rats. **(A)** During the spatial learning component of the Morris water maze task, rats underwent 4 trials/day for 5 days, utilizing extra-maze visual cues placed on the wall to locate a submerged platform in the pool. On each day, rats were released facing the pool wall in the NW, N, NE, and E positions for trials 1–4, respectively. On the 6th day, rats were placed in the NNE location and underwent one trial to assess their reference memory during which the platform was removed. **(B–D)** Rats demonstrated spatial learning, as evidenced by improved performance on the task over the 5 days of testing. There were no significant differences in the learning curves between noise- and sham-exposed rats at any age. **(E,F)** While noise exposure had no effect on the cumulative time to reach the platform at 7 months of age, spatial learning was impaired in the poorly performing noise-exposed rats at 10 months of age (*$p_{bonf} < 0.05$). **(G)** By 13 months of age, noise exposure impaired spatial learning, regardless of performance type (significant main effect of treatment, *$p < 0.05$). **(H–J)** At all ages, reference memory was unaffected by noise exposure. n = 7-month (Sham: 9 male, 8 female; Noise: 9 male, 12 female), 10-month (Sham: 9 male, 8 female; Noise: 9 male, 9 female), and 13-month (Sham: 10 male, 8 female; Noise: 10 male, 9 female). Data represent group mean ± SEM.

perfused through the heart with saline followed by 4% paraformaldehyde. Following perfusion, brains were removed and post-fixed in 4% paraformaldehyde overnight at 4°C. The following day, tissue was cryoprotected in 15% followed by 30% sucrose in phosphate buffer (PB, 0.1 M) until the tissue sank. Brain tissue was then cut into 30 μm thick coronal sections using a cryostat and stored in storage solution (30% ethylene glycol, 30% glycerol in 0.1 M PBS) at −20°C until further processing.

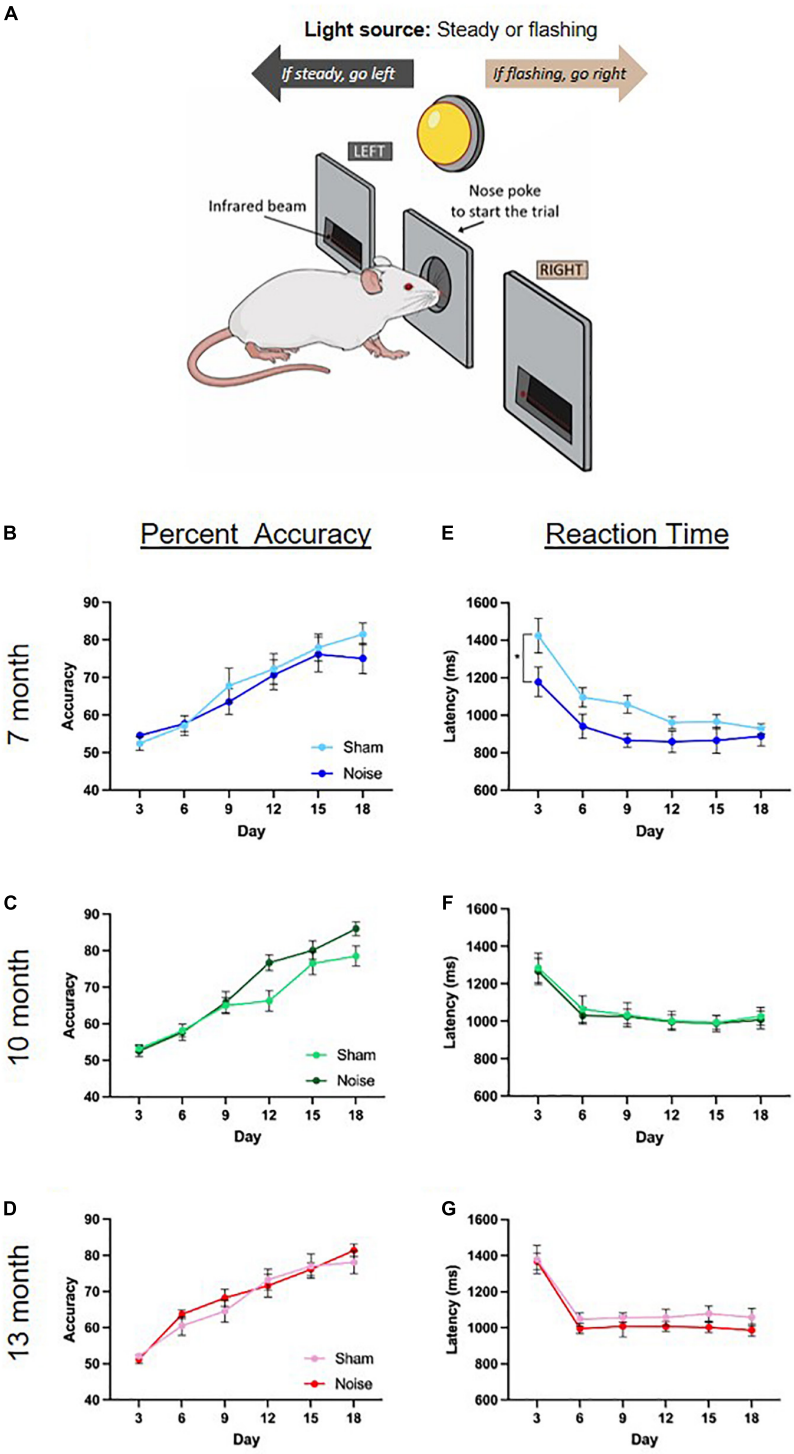

FIGURE 4
Noise-exposed rats demonstrated no significant deficits in performance accuracy on the visuomotor associative learning task, but exhibited a faster reaction time in the month post-noise exposure. **(A)** Rats were trained on an operant conditioning-based visuomotor associative learning task. Following the initiation of a trial caused by a noise poke in the center port, rats learned to access the left feeder trough when the steady light stimulus was presented, and the right feeder trough when the flashing light stimulus was presented. **(B–D)** Rats at each age demonstrated learning over the 18 days of the task, with no significant differences in percent accuracy between noise- or sham-exposed rats at any age. **(E–G)** Noise-exposed rats at 7 months of age showed a significantly faster reaction time than their sham-exposed counterparts, an effect that did not persist at 10 or 13 months of age (significant main effect of treatment, $*p < 0.05$). n = 7-month (Sham: 9 male; Noise: 9 male), 10-month (Sham: 9 male; Noise: 9 male), and 13-month (Sham: 10 male; Noise: 10 male). Data represent group mean ± SEM.

Immunolabeling was carried out on free-floating tissue sections. For every immunolabeling session, noise-exposed and sham tissue sections were processed in parallel using the same solutions. Tissue sections were initially removed from storage solution and rinsed in PBS. Tissue sections were washed in PBS for 30 min at room temperature between each of the following incubation steps. For deactivation of endogenous peroxidase, sections were treated with 1% H_2O_2 (Thermo Fisher Scientific, Waltham, MA, USA) in PBS for 10 min. Next, the sections were pre-treated with blocking solution containing 2.5% normal horse serum (VECTS2000; Vector Laboratories, Newark, CA, USA) and 0.2% Triton X-100 (X100; MilliporeSigma, Burlington, MA, USA) in PBS for 1 h. Sections were then incubated with primary antibody against Iba1 (1:1000; 019-19741; Wako Chemicals, Richmond, VA, USA) in blocking solution overnight at 4°C. Next, sections were incubated with biotinylated secondary antibody (1:500; anti-rabbit IgG, BA1100; Vector Laboratories) in blocking solution for 2 h at room temperature. Sections were processed using ABC kits (32020; Thermo Fisher Scientific) and labeling was visualized using diaminobenzidine (DAB). Immunolabeled sections were mounted on Fisher Superfrost polarized slides and dried overnight. Slides were then dehydrated in increasing concentrations of alcohol, cleared in xylene, and sealed using DPX (1005790507; MilliporeSigma).

Microglial Iba1 expression was visualized in coronal sections of the hippocampus (bregma −3.00 mm, CA1 subregion), auditory cortex (bregma −5.50 mm), and dorsal striatum (bregma 0.00 and 1.00 mm). All images were acquired using a digital camera on an upright brightfield microscope (Nikon Eclipse Ni-E, Nikon DS Fi2 color camera, NIS Elements Imaging; Mississauga, ON, USA), wherein white balance was automated using an off-tissue reference point and all settings (light intensity, exposure, aperture) were kept constant for all micrographs. Quantification of Iba1 expression was carried out using ImageJ software (version 1.53r). To assess microglial expression we quantified Iba1-positive cell morphology and density using soma-to-cell-size ratios and cell counts per micrograph, respectively. Stacked micrographs were acquired at 40× (5–8 z-plane images per stack), with four to eight randomly selected images taken within each region of interest. As previously described (Hovens et al., 2015), microglial soma area was defined as the spherical soma region of Iba1-positive microglia, while the microglial cell size was defined as the area formed by the connection of the outermost points of each dendritic process of Iba1-positive microglia. Using the polygon trace tool in ImageJ, microglial soma and cell size areas were measured for two randomly selected microglia in each 40× micrograph. Using the same 40× stacked micrographs, microglial cell number was assessed by manually counting the number of Iba1-positive microglia per micrograph. The microglial cell number for each rat was averaged among the separate 40× stacked micrographs and divided by the area of the image to obtain microglial density (cells per millimeter squared) in each region of interest. All imaging and analysis were carried out by an experimenter blinded to each rat's treatment group and age.

Data presentation and statistics

Statistical analyses were conducted using SPSS software (Version 27; IBM Corporation, Armonk, NY, USA), and included two-way or three-way analysis of variance (ANOVA) at each time point of age (see section "Results" for the details of each specific comparison). If Mauchly's test of sphericity was violated within the repeated-measures ANOVA, the Greenhouse-Geisser correction was used. The level of significance was set at $a = 0.05$ and when appropriate, Bonferroni's *post-hoc* corrections were used. GraphPad Prism (Version 9; Software Incorporated, La Jolla, CA, USA) was used to plot the graphs and BioRender (Biorender.com) was used for methodology schematics. Data are presented as mean values ± standard error of the mean (SEM).

Results

Noise exposure resulted in an age- and sex-dependent mild, high-frequency hearing loss

To assess the effect of the occupational-like noise exposure on hearing sensitivity, the ABR thresholds before and after noise/sham exposure were evaluated at each of the tonal stimuli presented (4 kHz, 20 kHz) (**Figure 2**). At each time point of age, a three-way ANOVA (treatment × sex × time) for each stimulus type was performed. As expected, there were no significant differences in hearing sensitivity for rats following the sham exposure. Although the noise-exposed rats showed no changes in hearing sensitivity for the 4 kHz stimulus (**Figures 2A–C**), they experienced high-frequency hearing loss, as evidenced by significantly increased hearing thresholds for the 20 kHz stimulus for 7-month [treatment × time interaction: $F_{(1,33)} = 10.543$, $p = 0.003$; $p_{bonf} < 0.001$; **Figure 2D**] and 13-month-old rats [treatment × time interaction: $F_{(1,32)} = 21.929$, $p < 0.001$; $p_{bonf} < 0.001$; **Figure 2F**], regardless of sex. Furthermore, 10-month-old males [treatment × sex × time interaction: $F_{(1,29)} = 8.975$, $p = 0.006$; $p_{bonf} < 0.001$], but not 10-month-old females, had significantly increased hearing thresholds at the 20 kHz stimulus (**Figure 2E**). Taken together, these data show that the occupational-like noise exposure protocol induced an age- and sex-dependent mild, high-frequency hearing loss. These results are consistent with previous studies in human and animal subjects which have also found age and sex-dependent effects of noise

exposure on hearing thresholds (Szanto and Ionescu, 1983; Milon et al., 2018; Nolan, 2020) and suggest that female rats may demonstrate resiliency or some recovery of hearing sensitivity (e.g., 10-month-old females, **Figure 2E**) following noise exposure that is not observed in male rats.

Hippocampal-dependent spatial learning was impaired in noise-exposed 13-month-old rats and a subset of noise-exposed 10-month-old rats

To determine the effect of noise exposure on spatial learning in the Morris water maze, each rat's time to platform was averaged across the four learning trials for each of the 5 days of testing. The first trial on Day 1 of testing was omitted for each rat as this represents their first time navigating the pool and is not indicative of spatial learning. A three-way ANOVA (treatment × sex × day) was performed at each time point of age. As shown in the learning curves in **Figures 3B–D**, rats at each age demonstrated spatial learning, as evidenced by improved performance on the task over the 5 days of testing [significant main effect of day for all ages; 7 month: $F_{(4,136)} = 6.784$, $p < 0.0001$; 10-month: $F_{(4,124)} = 5.609$, $p < 0.0001$; 13-month: $F_{(4,132)} = 6.544$, $p < 0.0001$]. However, no significant differences were found between the learning curves of sham and noise-exposed rats at 7, 10, or 13 months of age. In addition to examining daily performance on the task, spatial learning was quantified by calculating each rat's cumulative time to reach the hidden platform on the learning trials. Consistent with a past study which investigated the effect of age on spatial cognition (Young et al., 2010) and in an effort to discern whether some rats were more vulnerable to noise-induced cognitive deficits than others, the rats within each experimental group (i.e., sham or noise-exposed rats at 7, 10, or 13 months of age) were further classified as *good* (top 25th percentile), *mid* (middle 50th percentile), or *poor* (lowest 25th percentile) performers based on their cumulative time to reach the platform (**Figures 3E–G**). Three-way ANOVAs (treatment × sex × performer type) were conducted separately for each time point of age, and the collective results show that the effect of noise exposure varied by age and performance classification. Although noise exposure had no effect on the cumulative time to reach the hidden platform at 7 months of age (**Figure 3E**), it significantly impaired spatial learning in the poorest performing rats at 10 months of age [treatment × performer type interaction: $F_{(2,26)} = 3.798$, $p = 0.036$; $p_{bonf} < 0.001$; **Figure 3F**]. By 13 months of age, noise exposure significantly impaired spatial learning, whereby noise-exposed rats took significantly longer to reach the hidden platform compared to their sham-exposed counterparts, regardless of performer type [main effect of treatment: $F_{(1,25)} = 12.632$, $p = 0.002$; **Figure 3G**]. Carrying forward each animal's performer type classification from the spatial learning analysis, spatial reference memory was assessed using the time it took for rats to first reach the previous location of the hidden platform during the probe test session. Three-way ANOVAs (treatment × sex × performer type) were conducted and revealed no significant differences at any age, indicating that spatial reference memory was unaffected by noise exposure (**Figures 3H–J**). The collective results from the Morris water maze show that spatial learning was more susceptible to noise exposure than spatial reference memory, and that while learning was impaired across all performers in the 13-month age group, only a subset of poorly performing rats were vulnerable to noise exposure in the 10-month age group.

Noise-exposed rats demonstrated no significant deficits in performance accuracy on the visuomotor associative learning task, but exhibited a faster reaction time in the month post-noise exposure

To determine the effect of noise exposure on visuomotor associative learning, male rats' overall performance (percent accuracy) and time taken to respond correctly to a given stimulus (reaction time) were collected over an 18-day period. For both metrics, 3-day averages were calculated to obtain a total of six time points over the 18-day period (days 3, 6, 9, 12, 15, 18). To first characterize the rats' rate of learning on the task, separate two-way ANOVAs (treatment × day) were conducted for each age group. As shown in the learning curves in **Figures 4B–D**, rats at each age demonstrated an ability to learn the task, as evidenced by increasing percent accuracy over 18 days of testing [significant main effect of day for all ages; 7 month: $F_{(2.65,42.31)} = 37.240$, $p < 0.001$; 10-month: $F_{(5,80)} = 82.403$, $p < 0.001$; 13-month: $F_{(3.11,55.92)} = 65.587$, $p < 0.001$]. There were no significant differences in these learning curves between noise- and sham-exposed animals at 7, 10, or 13 months of age. Next, the effect of noise exposure on reaction time was assessed at each time point of age using two-way ANOVAs (treatment × day). Noise-exposed rats at 7 months of age showed a significantly faster reaction time than their sham-exposed counterparts [main effect of treatment: $F_{(1,16)} = 6.315$, $p = 0.023$; **Figure 4E**], whereas there were no significant differences found in the 10-month and 13-month-old rats (**Figures 4F,G**). Taken together, the results from the 18-day visuomotor associative learning task show no effect of occupational-like noise exposure on the rats' overall accuracy, but a faster reaction time in the first few weeks post-noise exposure.

Noise exposure altered microglial density in the hippocampus, but not the auditory cortex or striatum

Previous studies have implicated a role for altered microglial expression in noise-induced brain pathology in the auditory pathway (Baizer et al., 2015; Fuentes-Santamaria et al., 2017; Wang et al., 2019) and hippocampus (Cui et al., 2015; Zhuang et al., 2020; Li et al., 2021). To determine if noise exposure alters the profile of microglia in a brain region-specific manner across aging, we carried out immunolabeling of the microglial protein ionized calcium binding adaptor molecule 1 (Iba1) and quantified its expression in the auditory cortex, hippocampus, and striatum using two metrics (**Figure 5**). First, as microglia transition from a ramified shape with branched processes to a non-ramified amoeboid morphology upon activation (Kettenmann et al., 2011), we characterized the soma-to-cell-size ratio of Iba1 expressing cells. Using this metric, activated microglia with a non-ramified amoeboid shape have a higher ratio than ramified microglia. Second, cell counts in the auditory cortex, hippocampus and striatum determined if the overall density of Iba1-positive cells was affected by noise exposure. Our analysis of the hippocampus focused specifically on the CA1 region given its crucial role in spatial learning performance (Moser et al., 1994; Okada et al., 2003) as well as its implication in mediating age-related spatial learning impairments (Nicholson et al., 2004; Tombaugh et al., 2005; Burger et al., 2007). Separate three-way ANOVAs (treatment × sex × brain region) were conducted for these metrics, ultimately revealing no differences between treatment groups in soma-to-cell-size ratio at any age (**Figures 5C–E**). However, there was a significant reduction in the number of Iba1 positive cells within the hippocampal CA1 region of noise-exposed rats at 13 months of age [treatment × brain region interaction: $F_{(2,34)} = 3.730$, $p = 0.034$; $p_{bonf} < 0.05$; **Figure 5H**], with no change in density observed in the other brain regions at any age (**Figures 5F,G**). Overall, noise exposure altered the profile of microglia expression in the hippocampus at 13 months of age, characterized by a decrease in microglial density; a noise-induced effect that was not observed in the auditory cortex or striatum.

There were no significant correlations between the degree of hearing loss, spatial learning impairments, and hippocampal microglial density in the 13-month-old rats

In addition to investigating the effects of noise exposure on cognitive impairment and microglial expression, we were also interested in assessing whether spatial learning deficits or microglial density were directly associated with the degree of hearing loss. Separate linear regressions were performed for sham- and noise-exposed animals at 13 months of age and revealed that hearing loss did not predict the extent of hippocampal-dependent cognitive impairment (Sham: $R^2 = 0.004$, $p = 0.809$; Noise: $R^2 = 0.001$, $p = 0.876$; **Figures 6A,D**) or microglial density (Sham: $R^2 = 0.041$, $p = 0.575$; Noise: $R^2 = 0.056$, $p = 0.483$; **Figures 6B,E**). Furthermore, at the same time point, we sought to explore if spatial learning deficits were dependent upon hippocampal microglial density in the noise- vs. sham-exposed rats. A linear regression revealed no significant relationship between the two variables, indicating that the noise-induced microglial changes did not predict the extent of cognitive impairment (Sham: $R^2 = 0.045$, $p = 0.558$; Noise: $R^2 = 0.077$, $p = 0.408$; **Figures 6C,F**).

Discussion

The behavioral consequences of occupational-like noise exposure varied depending on the cognitive domain and time post-noise exposure

In the present study, we investigated if occupational-like noise exposure causes differential deficits in cognitive tasks primarily reliant on the hippocampus vs. striatum, and whether these deficits persist (or become exacerbated) in the months post-exposure. In addition to studying hippocampal-dependent spatial learning using the Morris water maze, we conducted the first investigation into the effects of noise exposure on visuomotor associative learning, a cognitive ability which is known to rely on striatal function. Based on our past work that found a noise-induced deficit in stimulus-response habit-learning (Wieczerzak et al., 2021), we predicted that noise exposure would also cause impairments in a more complex visuomotor associative learning task. While there were no noise-induced deficits in performance accuracy on this striatal-dependent task in any age group, we did observe an unexpected decrease in reaction time (i.e., faster responses) immediately post-exposure in the 7-month-old rats; a finding that may be related to altered dopaminergic signaling in the striatum. Support for this suggestion is derived from a past study which found a direct relationship between faster reaction times in rats trained on a lever-pressing task and an increased number of D_2 dopamine receptors in their striatum (Macrae et al., 1988), as well as previous studies that reported increased striatal dopamine levels following an occupational-like noise exposure (Ravindran et al., 2005; Samson et al., 2006). Given that the noise-induced decrease in reaction time was short-lived (i.e., it was not observed in the 10- and 13-month-old rats), it would be worthwhile for future studies to examine the

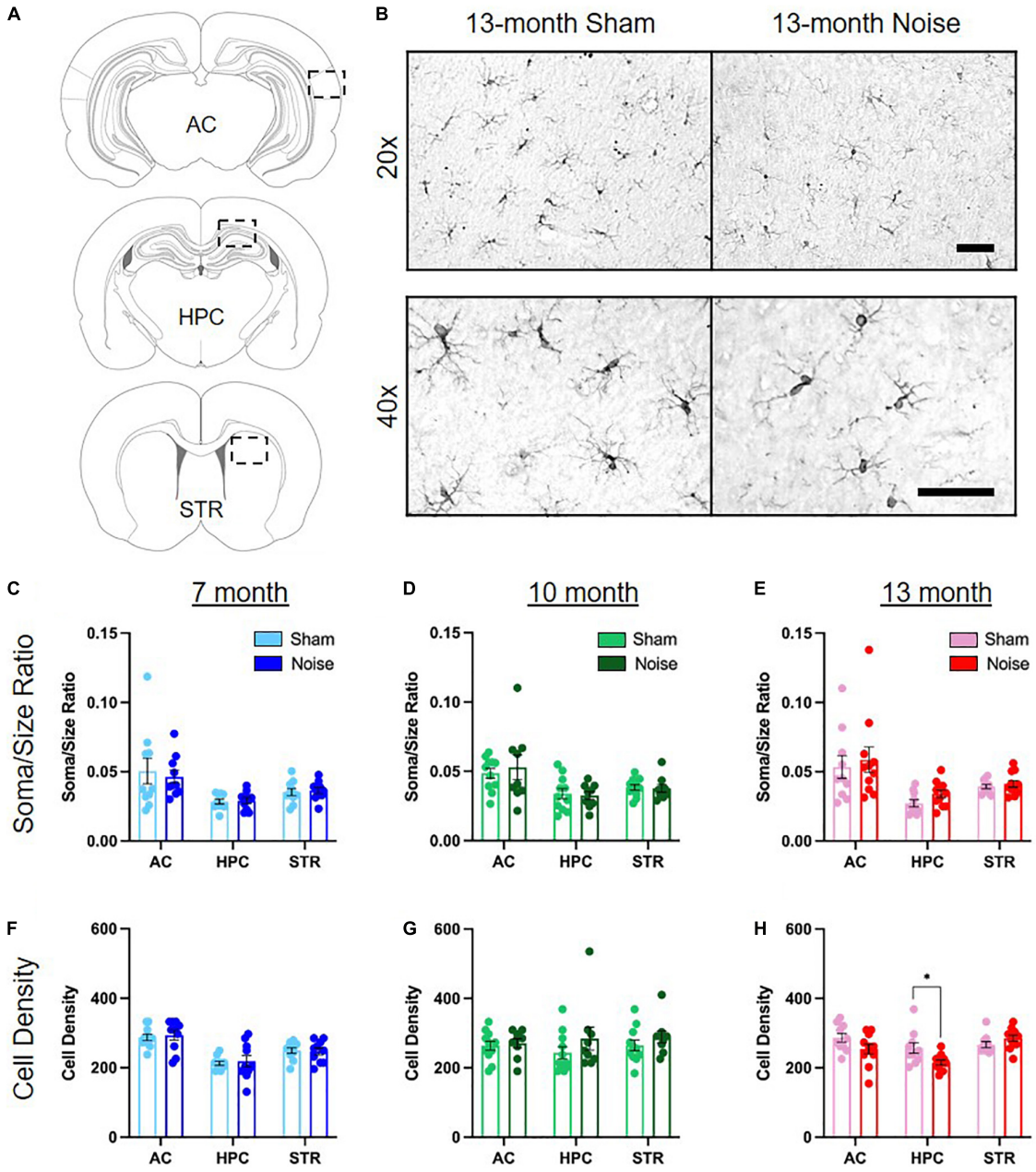

FIGURE 5
Noise exposure altered microglial density in the hippocampus, but not the auditory cortex or striatum. **(A)** Coronal section and outline for the auditory cortex (AC), hippocampal CA1 (HPC), and striatum (STR) regions of interest. **(B)** Representative 20× (top row) and 40× (bottom row) images of Iba1-positive microglia in the hippocampal CA1 of 13-month sham- and noise-exposed rats. Further quantification was performed on 40× images. See **Supplementary Figure 1** for additional images. Scale bar = 50 μm. **(C–E)** There was no difference in Iba-1-positive soma/size ratios between sham- and noise-exposed rats in the auditory cortex, hippocampus, or striatum at any age. **(F,G)** Microglial cell density was not affected by noise exposure in any brain region in 7- and 10-month-old animals. **(H)** There was a significant decrease in microglial cell density in the hippocampus in noise-exposed rats at 13 months of age, but not in the auditory cortex or striatum (*$p_{bonf} < 0.05$). n = 7-month (Sham: 4 male, 6 female; Noise: 5 male, 5 female), 10-month (Sham: 6 male, 5 female; Noise: 4 male, 5 female), and 13-month (Sham: 6 male, 4 female; Noise: 6 male, 5 female). Data represent group mean ± SEM.

interplay between noise exposure, reaction time and dopamine signaling in the striatum. Finally, we suggest that our finding of faster reaction times in noise-exposed rats performing a *visual*-based associative learning task could have implications for *auditory*-based tasks that rely on reaction time as a metric of noise-induced hypersensitivity to sound (Radziwon et al., 2019). Indeed, due to potential alterations in striatal dopaminergic signaling, noise-exposed rats may show faster reaction times

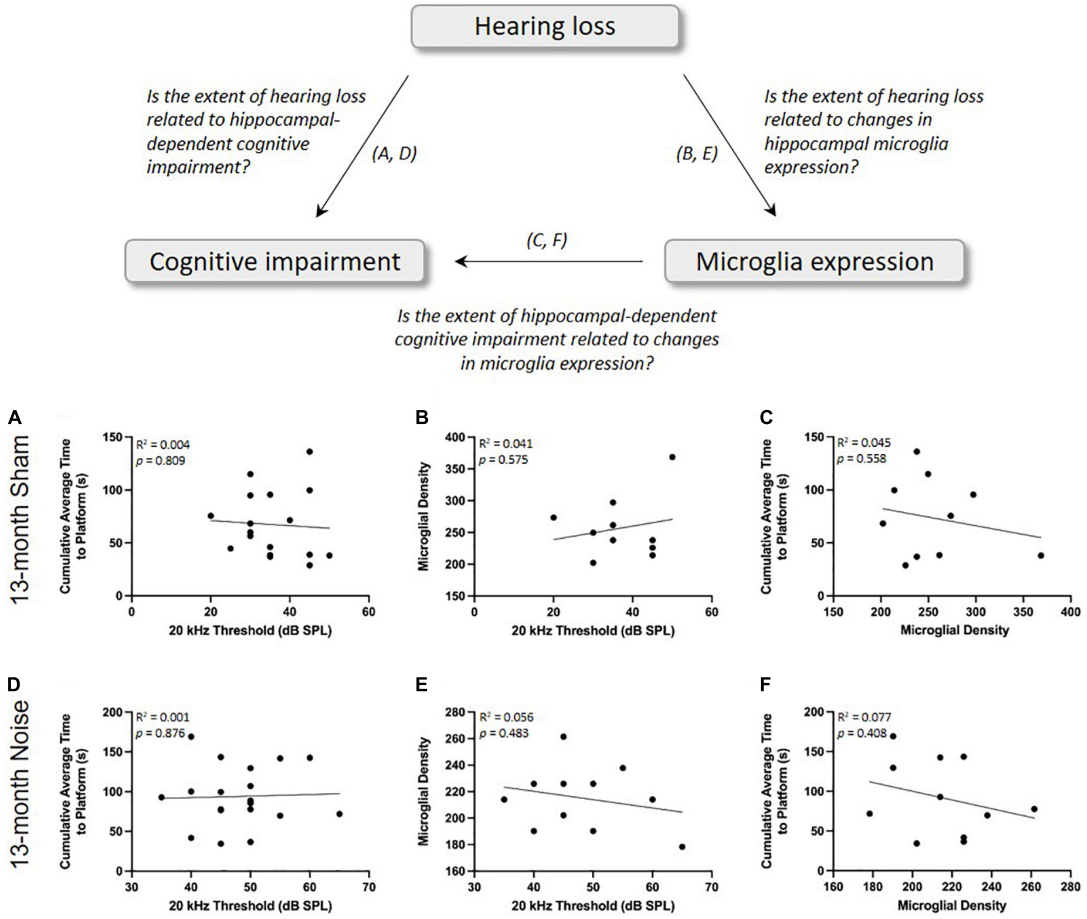

FIGURE 6
There were no significant correlations between the degree of hearing loss, spatial learning impairments, and hippocampal microglial density in the 13-month-old rats. As determined by a linear regression, the extent of hearing loss was not a significant predictor of spatial learning ability **(A,D)** or hippocampal microglial density **(B,E)** for sham- or noise-exposed rats. **(C,F)** Hippocampal microglial density was also not correlated with spatial learning ability in sham- or noise-exposed rats.

regardless of the sensory modality being tested, thereby calling into question the utility of reaction time as a specific measure of altered auditory processing post-noise exposure.

In contrast to the lack of impairment in visuomotor associative learning, the occupational-like noise exposure led to deficits in spatial learning that emerged in an age-dependent manner, such that 13-month-old rats demonstrated impaired performance on the Morris water maze several months after the cessation of the noise exposure. Importantly, these results confirm that noise exposure in early adulthood can indeed exacerbate age-related cognitive decline, as spatial learning was not impaired in the younger 7-month-old rats. As previous studies have reported deficits in spatial learning soon after noise exposure (Cui et al., 2009; Cheng et al., 2011; Chengzhi et al., 2011; Liu et al., 2016; Wang et al., 2016; Wieczerzak et al., 2021), our novel findings highlight the relevance of also considering the protracted consequences of noise exposure on cognition. In the present study, we also investigated whether some rats were more vulnerable to noise-induced cognitive deficits than others, as this could influence their trajectory for age-related cognitive decline. To that end, we performed a quartile-split of the Morris water maze data and observed a deficit in spatial learning in a subset of the 10-month-old rats—those that were already performing poorly compared to their age group (**Figure 3F**). Thus, it seems that noise exposure was most detrimental to those rats that were prone to cognitive impairment, suggesting that noise exposure may represent an increased risk for age-related deficits in an already-vulnerable group of subjects.

Past studies on humans and animal models have reported that the susceptibility to noise-induced hearing loss can vary considerably amongst individuals, even when exposed to the same noise exposure (Taylor et al., 1965; Mulrow et al., 1990; Seidman and Standring, 2010). With this in mind, we examined if the poorly performing 10-month-old rats experienced a greater degree of noise-induced hearing loss than their better-performing counterparts, and we determined whether the

overall results of spatial learning in the 13-month-old noise-exposed rats could be predicted by their hearing thresholds. Ultimately, the poorly performing 10-month-old rats had similar post-exposure 20 kHz hearing thresholds as the good-performers (poor: 35–50 dB SPL; good: 45–80 dB SPL), and we found no relationship between the noise-exposed rats' 20 kHz hearing thresholds and their spatial learning ability as assessed by the cumulative average time to reach the hidden platform in the Morris water maze (**Figure 6D**). Collectively, these findings could have significant translational implications as researchers and clinicians would not likely be able to reliably predict who may go on to experience cognitive impairments later in life based simply on the degree of hearing loss they experience due to noise exposure earlier in adulthood.

Noise exposure in early adulthood caused age-dependent and brain region-specific changes in microglial expression

Microglia have a diverse set of functions within the brain, contributing not only to the neuroinflammatory response during cellular damage, but also helping to maintain homeostasis under resting conditions by regulating synaptic plasticity (Kettenmann et al., 2011). Thus, either excessive activation of microglia or a loss of microglial activity can have devastating effects on neural function. Given their important role, it is not surprising that altered microglial expression has been implicated in mediating aspects of noise-induced pathophysiology within the brain, potentially contributing to auditory and cognitive dysfunction. For example, we and others have previously shown altered microglial expression in the cochlear nucleus following noise exposure, implicating their involvement in noise-induced damage repair and plasticity (Baizer et al., 2015; Fuentes-Santamaria et al., 2017). Similarly, within the auditory cortex, an increase in microglial expression soon after exposure to an intense noise has been proposed as a contributing factor to the aberrant neural plasticity underlying generation of the phantom auditory perception tinnitus (Wang et al., 2019). In contrast, another study using a similar intense noise exposure (Zhuang et al., 2020), as well as the present study using an occupational-like noise exposure, observed no such change in microglial expression in the auditory cortex. Taken together, these findings suggest that the relationship between noise exposure and microglia status can vary across the levels of the central auditory system; however, more research is required to uncover the diverse functions microglia may play in subcortical and cortical regions of the auditory pathway following noise exposure.

While it is well-established that the effects of noise exposure extend beyond the central auditory system, it was unclear if noise-induced changes in microglial expression are brain region-specific, or whether these changes contribute to the profile of cognitive impairment observed post-noise exposure. In the present study, we carried out the first investigation of potential noise-induced alterations in microglial expression within the striatum. In light of the lack of cognitive impairments that we observed in our striatal-dependent visuomotor associative learning task post-noise exposure, it is perhaps not surprising that we did not observe changes in microglial expression within the striatum at any age; results that suggest that the striatum is seemingly resilient to the effects of noise exposure. In contrast, our findings of noise-induced microglial changes in the hippocampus extend the results of previous studies which suggest that this brain region appears to be particularly vulnerable to noise exposure (Cui et al., 2009, 2015; Kraus et al., 2010; Cheng et al., 2011, 2016; Li et al., 2014; Tao et al., 2015; Liu et al., 2016; Wang et al., 2016; Hayes et al., 2019). Most notably, our finding of reduced microglial cell density in the hippocampus of 13-month-old noise-exposed rats is consistent with a previous study which also documented a long-term decrease in microglial cell number, albeit after a much more intense noise exposure (Zhuang et al., 2020). That we observed this alteration in microglial expression long after an occupational-like noise exposure, further emphasizes just how sensitive the hippocampus appears to be to auditory insults. Moreover, the fact that our occupational-like noise exposure and the intense noise exposure used by Zhuang et al. (2020) both caused protracted changes in microglial expression in the hippocampus raises two important questions: (1) Does the degree of hearing loss induced by the noise exposure predict the extent of microglial changes in the hippocampus? and (2) Do these changes in microglial expression contribute to the extent of cognitive impairment?

First, to investigate the potential relationship between hearing loss and the extent of microglial changes in the hippocampus, we performed a linear regression on the 13-month-old rats' 20 kHz hearing threshold and the cell density of microglia in their CA1 hippocampal region. For both the sham- and noise-exposed rats, we found no significant correlation (**Figures 6B,E**); findings that were perhaps not too surprising given that, compared to the 13-month-old rats, the 7- and 10-month-old rats showed the same degree of noise-induced hearing loss (**Figure 2**), yet they did not show any changes in their hippocampal microglial cell density (**Figures 5F,G**). These time-dependent changes in microglia warrant additional consideration, as ours is the first study to report that it took several months, not just days/weeks, following the noise exposure before the decrease in hippocampal microglial cell density emerged. Second, as past studies have suggested that noise-induced changes in microglial expression cause hippocampal dysfunction (e.g., impaired neurogenesis) (Zhuang et al., 2020; Li et al., 2021), we investigated whether noise-induced changes in microglial expression contributed to the extent of cognitive impairment

following our occupational-like noise exposure. To that end, we performed a linear regression between hippocampal microglial density and the 13-month-old rats' cumulative average time to reach the hidden platform in the Morris water maze. Ultimately, as there was no significant relationship between these variables (see **Figures 6C,F**), we suggest that the decrease in microglial density did not predict the noise-exposed rats' impaired spatial learning ability. In further support of this conclusion, we also found that the poorly performing 10-month-old rats did not show a tendency for a decrease in hippocampal microglial density compared to their sham-exposed counterparts.

In considering our collective findings in which reduced hippocampal microglial density was not correlated with the level of hearing loss or performance on the Morris water maze, we suggest that caution be taken when attempting to ascribe noise-induced hearing loss associated deficits in spatial learning to altered microglial expression, as our findings do not support that conclusion. Instead, it is possible that occupational-like noise exposure, regardless of the degree of associated hearing loss, primes the hippocampus for an accelerated age-related phenotype characterized by the co-occurrence of a reduced microglial density and impaired spatial learning. Support for our suggestion of an accelerated age-related phenotype post-noise exposure comes from past studies that have independently documented that aging results in a decline in microglial cell number (both resting and activated microglia) (Hayakawa et al., 2007; Cerbai et al., 2012) along with other molecular changes (Nicholson et al., 2004; Tombaugh et al., 2005; Burger et al., 2007) specifically within the CA1 in aged animal models, as well as age-related impairments in hippocampal-dependent behavioral tasks (Rosenzweig and Barnes, 2003; Shukitt-Hale et al., 2004; Bizon et al., 2009); the two outcomes observed in our 13-month-old noise-exposed rats. In light of our findings, we predict that had we extended the later time-point of our study, we may have observed cognitive impairment and reduced microglial density in the hippocampus in these older sham-exposed rats, thereby confirming that the outcomes in the 13-month-old noise-exposed rats were indeed due to an earlier-onset of an aged phenotype.

Future directions

The novel findings of the present study not only confirm that there is a complex relationship between noise exposure, hearing loss and cognitive decline, but they also identify worthwhile future directions for preclinical studies. For example, given our finding that a subset of younger animals showed noise-induced deficits in spatial learning, suggesting that noise exposure may represent an increased risk for cognitive impairment in vulnerable subjects, additional studies utilizing animal models of genetic susceptibility to age-related cognitive impairments are warranted (Paciello et al., 2021). Furthermore, in addition to tracking the longer-term consequences of noise exposure on aging animals (i.e., beyond the 13-month time-point investigated in the present study), future studies should also consider investigating the effects of continuous noise exposure on age-related cognitive decline using daily exposures across the lifespan. This approach would more closely resemble the daily noise exposures experienced by individuals working in noisy environments and allow for researchers to investigate whether prolonged noise exposure acts cumulatively to worsen cognition, or whether adaptive mechanisms allow aged animals to compensate through greater reliance on multiple cognitive domains. Related to the possibility of cognitive compensation, it is worth restating that aging is normally associated with an increased reliance on the striatum to help compensate for hippocampal dysfunction (for review, see Zhong and Moffat, 2018). With this in mind, it is reasonable to question whether the differential behavioral effects observed in the noise-exposed 13-month old rats (i.e., impaired spatial learning; unaffected visuomotor associative learning) were the result of an interplay between an age-related reliance on striatal processing coupled with an increased vulnerability of the hippocampus to noise exposure. Ultimately, to further investigate the relationship between noise exposure and cognitive compensation in aging, future studies could investigate noise-induced hippocampal- vs. striatal-dependent cognitive impairment and neuropathology in animals with a genetic susceptibility to age-related cognitive decline.

In addition to the aforementioned studies, researchers could also consider conducting mechanistic studies to better understand the interplay between the intensity and duration of noise exposure, the profile of altered microglial expression, and the presence/absence of cognitive impairment. For example, future studies could combine noise exposures with pharmacological manipulations (e.g., PLX3397) that disrupt the microglia population; an approach that proved successful at revealing how microglia mediate noise-induced plasticity in the auditory cortex (Wang et al., 2019). Ultimately, this mechanistic approach could help to confirm whether noise-induced changes in microglial expression play a causal role in the short- and/or long-term consequences of noise exposure on hippocampal function (e.g., neurogenesis; synaptic plasticity) and cognitive abilities (e.g., spatial learning; reference memory; episodic-like memory). Furthermore, our novel finding that decreased microglia density was not predictive of the extent of hippocampal-dependent cognitive impairment in the noise-exposed rats suggests that other molecular mechanisms likely underlie how noise exposure may accelerate aging in the hippocampus. For example, it is well known that alterations in glutamatergic neurotransmission related to NMDA receptors can result in spatial learning and memory impairments (for review, see Riedel et al., 2003) and that NMDA receptor

dysfunction is commonly seen in the brain across aging (Magnusson, 1998; Sonntag et al., 2000; Zhao et al., 2009). Of particular interest, past studies have shown that chronic noise exposure results in decreased levels of the NR2B subunit of the NMDA receptor in the hippocampus (Cui et al., 2009, 2013), suggesting that future studies should explore whether alterations in hippocampal glutamatergic signaling contribute to noise-induced spatial learning deficits across aging.

Conclusion

In the present study, we investigated the effects of occupational-like noise exposure on cognitive function and microglial expression across aging in a rodent model. Our results highlight that not all higher-level cognitive tasks or their associated brain regions appear to be equally susceptible to noise-induced deficits during aging, with the hippocampus demonstrating greater vulnerability compared to the striatum. Furthermore, our finding of noise-induced cognitive deficits in a subset of younger animals, combined with the apparent lack of a relationship between the degree of hearing loss with cognitive function, highlights the need for future studies to investigate the factors, beyond hearing loss, that make some subjects more susceptible to cognitive dysfunction following noise exposure. Overall, our findings suggest that even a mild occupational-like noise exposure earlier in adulthood can have long lasting implications for cognitive function later in life; a finding with significant clinical implications given the high prevalence of noise exposure across common environmental, recreational, and occupational settings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This animal study was reviewed and approved by the Animal Care Committee at Western University and in compliance with the guidelines established by the Canadian Council on Animal Care.

Author contributions

BA, SH, and SW: project conceptualization. SP, CD, SB, SH, and AS: data collection and analysis. SH, BA, SP, and SW: manuscript writing and editing. All authors interpreted the data.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2022.1001686/full#supplementary-material

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Sound thoughts: How understanding the teenage brain may help us look after their ears

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Neuroscience's study of brain structures and their function provides understanding of the biological underpinnings of behavior, including factors that may assist or act as barriers for programs designed to bring about behavioral change. This understanding can benefit how disease prevention and health promotion campaigns are developed and disseminated for greater effectiveness. Increasingly, public health campaigns have harnessed an understanding of neurobiology (and its complex interactions with social contexts and emotional and behavioral development of adolescents) and applied this knowledge to health promotion campaigns to enhance changed attitudes toward disease prevention and encourage healthy lifestyle choices (Bradshaw et al., 2012; Hall, 2016; Suleiman and Dahl, 2017; Pei et al., 2019).

While not an exhaustive list, some of the health focus areas that have incorporated neuroscience in their health promotion strategies include substance use (through understanding the strong association between substance use in adolescents and high levels of sensation seeking; Crawford et al., 2003), sexual health (*via* improved understanding of developing decision-making skills and the role of emotion and social influences; Ballonoff Suleiman and Brindis, 2014), and dietary health (by taking into account the neurobiological needs of safety and non-judgement; Debenham et al., 2022).

Together, these examples illustrate the importance of using our growing neuroscience knowledge about the susceptibility of risk taking, sensation seeking, and neuroplasticity in adolescence. These influences and their dynamic interplay with health conditions during this developmental phase make it an ideal time to implement positive, health-protective behavior. The consideration of neurobiological factors has been effective in increasing receptiveness to prevention messages that result in maximum engagement with young people and may be applicable across a range of health contexts and disciplines (Michie et al., 2011; Bradshaw et al., 2012; Meinke and Martin, 2017; Pei et al., 2019). One, as yet unexplored, area that may benefit from consideration is hearing health, in particular, the prevention of noise induced hearing loss (NIHL).

The impacts of hearing loss are well-documented to have far-reaching consequences that extend beyond listening and communication difficulties, impacting on personal, societal, and global levels if left untreated (Reed et al., 2019; Sheppard et al., 2020). The biological processes of how and when noise affects hearing is known—the risk of NIHL is based on the duration, frequency, and intensity of the noise exposure, regardless of the source (Clark and Bohne, 1999; Zhao et al., 2010), and so most prevention efforts are targeted at reducing the volume of sounds (i.e., reducing the risk at the source)

to which people are exposed because it is more feasible to implement. Lowering volumes reduces the risk of the source (a higher target for hierarchy of control) whereas changes to duration can be limited by the nature of the activity (for example, duration of concerts, physical fitness class is less amenable to change). Most countries have regulatory requirements and governance around occupational noise exposure as part of health and safety controls. However, managing recreational noise exposure is less straightforward.

Occupational noise regulations apply regardless of the noise source (machinery or music) however, these are only directed at employees rather than attendees. Even then questions have been raised as to the effectiveness of these for music venues, and adherence by such workplaces to the regulations; (Barlow and Castilla-Sanchez, 2012; Kelly et al., 2012; World Health Organization, 2022).

For non-occupational exposures, it is difficult to develop regulations that take into account the wide range of possible high-volume recreational activities and individual variation in participation.

As a result, recreational noise exposure remains highly dependent on the choices made by individuals about what activities they participate in, for how long, and whether they choose to take any precautions to reduce the risk to their hearing.

As individual behavior remains a major determiner of the risk to hearing from noise, prevention activities aim to motivate and encourage engagement with noise-reduction. The cumulative nature of NIHL means that there is merit in focussing attention on the noise exposure behaviors of young people, with WHO estimates that more than one billion young people (aged 12–35 years) are at risk of hearing loss due to recreational exposure to loud sounds (2022). Recreational NIHL is preventable, its consequences are as detrimental comparative to occupational NIHL, and it should be made a public health priority (Murphy et al., 2018; Pienkowski, 2021), However, noise-induced hearing loss may not be detectable or treatable during adolescence given the cumulative nature of hearing damage (Williams and Carter, 2017). Thus, this opinion piece foscusses on prevention efforts that aim to reduce the risks to hearing over time.

Adolescence is a period marked by physical change and neural development and one which also encompasses identity formation and social growth that extends from 10 to 24 years (Sawyer et al., 2018). This formative time in the life course is also associated with skill learning, exploration, and risk-taking behaviors that could promote wellbeing (such as relationship building, shifts in sociocultural perspectives, and greater peer and societal engagement). Yet, adolescents may also be vulnerable to risk-taking and sensation seeking and forming negative behavioral patterns can also lead to adverse outcomes that heighten health risks (such as substance use and engaging in risky behaviors, among others) which could increase the burden of disease in later decades of life (Suleiman and Dahl, 2017; Patton et al., 2018; Pei et al., 2019). There has, therefore, been an increasing global focus on health of adolescents (currently the largest population in human history) recognizing that appropriate health investments are needed to ensure that future generations can thrive (Patton et al., 2018).

Historically, NIHL efforts focus on hearing health education and awareness building, but there is evidence to suggest that these have been limited in their effectiveness to change behavior, shift cultural norms, or improve rates of using hearing protective devices (Weichbold and Zorowka, 2007; Vogel et al., 2008; Widén, 2013; Gilles, 2014; Keppler et al., 2015; Steinberg, 2015). Prevention of noise-induced hearing loss for adolescence should aim to set up good habits to listen safely well into future when noise risks often start to increase. As the biggest risk is often from noise exposure that is specifically sought out in leisure activities, efforts aiming to reduce rather than avoid or ban noise might be most feasible. Thus, the challenge for this population is to foster or promote a positive habit to seek out sound safely that could facilitate safe sensation-seeking. Whilst it may be possible to educate adolescents to avoid extremely loud situations where acoustic shock symptoms are obvious and immediate signs of damage, much of prevention work is targeted at more subtly risky situations where damage may occur unnoticed.

Progressively, there has been greater attention on utilizing theoretical underpinnings grafted from behavior change principles to assist researchers and clinicians in better understanding hearing health behavior change. Through this, we can broaden how we conceptualize young people's attitudes, beliefs, intentions, and motivations tailor interventions that promote hearing health behavior changes (Coulson et al., 2016). But in addition to behavior change and health promotion models, our increasing understanding of neurobiology during adolescence may provide further dimensions to how to promote and foster healthy hearing behaviors. In particular, it is worth considering two significant factors associated with adolescence—sensation seeking and social influence.

Brain areas responsible for processing reward sensitivity have shown to be hyperactivated in young adults engaging in risk-taking behaviors (Telzer et al., 2014; Qu et al., 2015). This sensation-seeking appeal is further positively reinforced by peer influence. The quality of peer relationships is crucial as it can have a positive buffering effect (that serves as a protective behavior), or increase stress, thus increasing risk-taking behaviors that negatively impact health (Galván, 2013).

Such mechanisms may explain the commonly seen disconnect between knowledge of noise-exposure risks and preventative action. Despite awareness of risks, research has frequently shown individuals choosing to participate in noisy activities, and/or declining opportunities to mitigate that risk through hearing protection activities. Social norms have been implicated in young people's decisions about personal music player listening behaviors (Gilliver et al., 2012) and rejection of earplugs at music venues (Beach and Gilliver, 2019).

Sensation-seeking, too, has been noted as an important factor for music-listening. An investigation of young people (18–25 years) by Welch and Fremaux (2017) found that enjoyment of loud sounds at music venues was related to enabling positive physical and social experiences. The physical sensation of loud music heightens emotions and arousal state, masks negative emotions, and removes inhibitions. To this end, loud music and sounds promote intimacy and social identity and further emphasize the desire to adopt or adhere to social norms.

Taken together, this has implications when considering interventions that target recreational NIHL during adolescence. If the neurobiology of adolescents and young adults is dominated by social bonds, peer influence, and heightened risk-taking behaviors, then current approaches to hearing conservation campaigns that simply aim to provide knowledge and educate the harmful effects of loud noise stemming from recreational activities are incongruent with the needs of the target audience.

A transdisciplinary approach is necessary to develop health promotion programs. Researchers, public health policy decision-makers, health practitioners, and the education system are required to work collaboratively to harness the ways in which neurobiology interacts with socio-contextual factors to order to inform effective health campaigns that are meaningful and age-appropriate (Beach, 2017; Meinke et al., 2017; Beach and Gilliver, 2019). Neuroscience-informed approaches to tackling adolescent health issues should be seen as complementary to existing behavioral and more traditional approaches to disease prevention and health promotion. The premise of this innovative approach has shown to be successful at informing and educating young people, and de-stigmatizing health conditions while promoting tolerance and understanding of (neuro)biological limitations of the adolescent brain (O'Connor and Joffe, 2013).

Summary and future directions/discussions

Adolescence is a formative period in life with a dual nature of vulnerability to risks and adaptability as an opportunity to form life-changing, health-promoting habits. With the rising number of young people at risk of recreational NIHL, there is a need for more effective ways to address this issue. The known social aspect of recreational noise experiences and the power of sensation-seeking of the adolescent brain warrants further exploration and consideration in NIHL prevention campaigns.

The application and contributions of neuroscience to inform future NIHL prevention programs for this age group will make a novel, meaningful, and innovative pursuit. It also presents an opportunity for neuroscientists who research adolescent health behaviors to explore an—as yet uncharted area of research.

Neuroscience-informed approaches to reducing recreational NIHL for young people are required to meet the needs of the developing adolescent brain. Designing age appropriate NIHL campaigns that take these factors into account may assist to increase the likelihood that interventions are efficacious and cost-effective.

Author contributions

JP and MG contributed to the conception and subsequent preparation of this manuscript. Both authors contributed to the article and approved the submitted version.

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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.

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Using an appetitive operant conditioning paradigm to screen rats for tinnitus induced by intense sound exposure: Experimental considerations and interpretation

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In an effort to help elucidate the neural mechanisms underlying tinnitus in humans, researchers have often relied on animal models; a preclinical approach which ultimately required that behavioral paradigms be designed to reliably screen animals for tinnitus. Previously, we developed a two-alternative forced-choice (2AFC) paradigm for rats that allowed for the simultaneous recording of neural activity at the very moments when they were reporting the presence/absence of tinnitus. Because we first validated our paradigm in rats experiencing transient tinnitus following a high-dose of sodium salicylate, the present study now sought to evaluate its utility to screen for tinnitus caused by intense sound exposure; a common tinnitus-inducer in humans. More specifically, through a series of experimental protocols, we aimed to (1) conduct sham experiments to ensure that the paradigm was able to correctly classify control rats as not having tinnitus, (2) confirm the time course over which the behavioral testing could reliably be performed post-exposure to assess chronic tinnitus, and (3) determine if the paradigm was sensitive to the variable outcomes often observed after intense sound exposure (e.g., hearing loss with our without tinnitus). Ultimately, in accordance with our predictions, the 2AFC paradigm was indeed resistant to false-positive screening of rats for intense sound-induced tinnitus, and it was able to reveal variable tinnitus and hearing loss profiles in individual rats following intense sound exposure. Taken together, the present study documents the utility of our appetitive operant conditioning paradigm to assess acute and chronic sound-induced tinnitus in rats. Finally, based on our findings, we discuss important experimental considerations that will help ensure that our paradigm is able to provide a suitable platform for future investigations into the neural basis of tinnitus.

KEYWORDS

tinnitus, animal models, two-alternative forced-choice behavior, intense sound exposure, hearing loss

1. Introduction

Tinnitus is the subjective perception of a phantom sound that is often described as a ringing or buzzing sensation in the ears. In the majority of cases, tinnitus is experienced temporarily, with the phantom auditory perception fading within a few minutes or hours (Henry et al., 2005). However, for as many as 10–15% of the general population, tinnitus is experienced chronically, with 1% of the population having severely debilitating forms of tinnitus that negatively impact their daily lives (Heller, 2003). Despite decades of research, there is still no widely accepted treatment available that readily suppresses tinnitus, in part because the underlying neural mechanisms responsible for this phantom perception remain elusive. Further insight into the pathophysiology of tinnitus is expected to rely heavily on animal studies involving neural recordings; an approach which first requires that researchers be able to reliably screen animals for the presence/absence of tinnitus. For a behavioral paradigm to be most effective, it should be able to (1) screen for both acute and chronic tinnitus, (2) closely reflect the human condition, (3) be able to account for the presence of hearing loss associated with tinnitus induction methods (e.g., hearing loss associated with noise exposure), and (4) allow for individual comparisons to address variability amongst tinnitus sufferers (Hayes et al., 2014).

Many of the existing behavioral paradigms used to screen animals for tinnitus are based on one of three general methods: shock avoidance (Jastreboff et al., 1988; Bauer et al., 1999; Bauer and Brozoski, 2001; Heffner and Harrington, 2002; Guitton et al., 2003; Rüttiger et al., 2003; Lobarinas et al., 2004; Yang et al., 2011; Pace et al., 2016; Jones and May, 2017; Zuo et al., 2017), appetitive two-choice operant conditioning (Sederholm and Swedberg, 2013; Stolzberg et al., 2013), or gap prepulse inhibition of the acoustic startle response (GPIAS; Turner et al., 2006). As noted in recent review articles on the topic (Hayes et al., 2014; Galazyuk and Brozoski, 2020), although each of these paradigms has its advantages, there are also notable challenges that can detract from their effectiveness as a screening tool for tinnitus. For example, some traditional shock avoidance paradigms present the issue of behavioral extinction, which precludes the ability to study persistent forms of tinnitus. Additionally, appetitive two-choice operant conditioning models can be limited by the extensive period required to train the animals prior to actually performing tinnitus screenings. Consequently, the GPIAS paradigm—which does not require overt training—quickly became the most popular behavioral method used to screen animals for tinnitus due to its high-throughput nature. However, recent studies have highlighted the need to be cautious when interpreting GPIAS results due to the potential confound of screening hearing-impaired animals for gap detection deficits using a metric reliant on their acoustic startle reflex (Longenecker and Galazyuk, 2011, 2012, 2016; Lobarinas et al., 2013; Longenecker et al., 2018), as well as the fact that the GPIAS paradigm and similar gap detection tasks have yet to convincingly identify tinnitus in human subjects (Campolo et al., 2013; Fournier and Hébert, 2013; Boyen et al., 2015).

Preclinical investigations into the neural basis of tinnitus can benefit from combining a behavioral screening with simultaneous neurophysiological recordings at the very moments when the animals are attending to their tinnitus; an approach consistent with human testing. To achieve our goal of recording neural activity as rats actively reported behavioral evidence of tinnitus, we previously designed a two-alternative forced-choice (2AFC) appetitive conditioning paradigm that required rats to categorize whether they were hearing either steady narrowband noises (NBNs), an amplitude-modulated (AM) broadband noise, or quiet (Stolzberg et al., 2013). As we were motivated to design our 2AFC task to be compatible with recording tinnitus-related cortical oscillations—the synchronized neural activity that has been suggested to underlie phantom perception (Weisz et al., 2005, 2007a,b; for review see Adjamian, 2014)—the rats were trained to poke their nose in a central port and hold relatively still for several seconds while attending to the stimulus condition (NBNs, AM or quiet) that was being presented on a given trial. During the quiet trials, this holding period would provide a sufficient epoch to accurately record the low-frequency oscillations implicated in tinnitus pathophysiology (Adjamian, 2014). Following the holding period, a cue light signaled to the trained rats to nose-poke in one feeder trough for NBNs and the other feeder trough for both AM noise and quiet trials. Thus, trained rats would go on to screen positive for tinnitus if they incorrectly identified quiet trials as though they were hearing a steady NBN; findings consistent with humans who report tinnitus as the perception of persistent sound during quiet conditions. The paradigm included the AM noise trials to ensure that rats with tinnitus continued to have reason to select both feeder troughs throughout the session, regardless of whether they made correct or incorrect choices during the quiet trials. To validate the effectiveness of the paradigm, rats were exposed to a high dose of sodium salicylate, which is known to reliably induce transient tinnitus in rodents and humans (Mongan et al., 1973; Jastreboff et al., 1988; Cazals, 2000; Guitton et al., 2005; Lobarinas et al., 2006). As predicted, rats that were able to correctly identify the quiet trials during a control session (saline injection), now went on to screen positive for tinnitus in the hours following sodium salicylate injection because they reported hearing steady NBN during a significant number of quiet trials (Stolzberg et al., 2013). Furthermore, the electrophysiological recordings made during the behavioral testing revealed that the aberrant cortical oscillations observed in rats experiencing salicylate-induced tinnitus largely paralleled the findings reported in tinnitus patients (Weisz et al., 2005).

Although our above-mentioned 2AFC behavioral paradigm showed great promise as a way to reveal the neural changes associated with salicylate-induced tinnitus, its capacity to reliably screen rats for tinnitus caused by intense sound exposure would need further evaluation. Thus, in the present study, we assessed the utility of our 2AFC behavioral paradigm to screen for intense sound-induced tinnitus by (1) conducting sham experiments to ensure that the paradigm was able to correctly classify control rats as *not* having tinnitus, (2) confirming the time course over which the behavioral testing could reliably be performed post-exposure to assess chronic tinnitus, and (3) considering if the paradigm was sensitive to the variable outcomes often observed after intense sound exposure (e.g., subjects with considerable hearing loss but without tinnitus, versus those with tinnitus and only limited hearing loss). In the first experimental series, we exposed rats to intense sound or sham conditions for 15 min, and immediately screened them for acute tinnitus using our 2AFC behavioral paradigm, with the expectation that no rats should falsely-screen positive for tinnitus post-sham exposure, yet all rats would show behavioral evidence of acute tinnitus immediately after the 15-min sound exposure. Next, in preparation

Abbreviations: 2AFC, two-alternative forced-choice; ABR, auditory brainstem response; AM, amplitude-modulated; NBN, narrowband noise.

to study persistent tinnitus, pilot experiments were conducted on a separate cohort of control rats to determine how many weeks could elapse as well as how many test sessions could be performed repeatedly before the behavioral paradigm failed to accurately report the *absence* of tinnitus. Based on these findings, we then used our 2AFC behavioral paradigm to screen for tinnitus that persisted one week after intense sound exposure, with the expectation that there would be considerable variability across animals, such that not all rats would show behavioral evidence of chronic tinnitus nor have the same degree of permanent hearing impairment. Overall, the present study documents the utility of our appetitive operant conditioning paradigm to assess acute and chronic sound-induced tinnitus in rats; an important step in moving toward using this paradigm to simultaneously record neural activity during the behavioral screening for tinnitus induced by intense sound exposure.

2. Materials and methods

A total of 23 adult male Sprague-Dawley rats (Charles River Laboratories, Inc., Wilmington, MA), separated into three experimental series, were used in the present study. All rats (60 days old at the onset of training), were housed in a 12-h light-dark cycle with water *ad libitum*. All experimental procedures were approved by the University of Western Ontario Animal Care and Use Committee and were in accordance with guidelines established by the Canadian Council of Animal Care.

2.1. Behavioral apparatus

The behavioral apparatus consisted of a standard modular test chamber (ENV-008CT; Med Associates Inc., St. Albans, VT, USA) housed in a sound-attenuating box (29" W by 23.5" H by 23.5" D; Med Associates Inc.). The front wall of the behavioral chamber included a center port with two stainless steel feeder troughs positioned on either side; each fitted with an infrared (IR) beam used to detect nose-pokes. Each feeder trough was attached to a food pellet dispenser located behind the behavioral chamber. A house light was located on the back wall to illuminate the chamber, and a white light-emitting diode (LED) was located directly above the center nose-poke, which served as a GO cue during behavioral training. Real-time processing hardware (RZ6 and BH-32, Tucker Davis Technologies, Alachua, FL) were interfaced with the test chamber. Custom behavioral protocols running in Matlab (EPsych Toolbox, dstolz.github.io/epsych/) monitored the nose poke responses, and controlled the presentation of the auditory stimuli, as well as the positive reinforcement (i.e., food pellet delivery) and punishment (i.e., the inability to begin the next trial during a 15-s timeout period, indicated by turning off the house light).

Acoustic stimuli were programmed to play from a speaker (FT28D; Fostex, Tokyo, Japan) mounted on the ceiling of the behavioral chamber. There were three types of acoustic stimuli used in the paradigm: quiet (speaker off), amplitude-modulated noise (AM; broadband noise, 100% modulation, 5 Hz), or one of five narrowband noises (NBN; 1/8th octave band, center frequencies at 8, 12, 16, 20, or 24 kHz). One of the acoustic stimuli conditions was always present in the behavioral chamber regardless of trial initiation by the rat. AM and NBN stimuli were calibrated using TDT hardware (RPvdsEx, RZ6 module; TDT) and custom MATLAB software (Mathworks) to ∼75 dB sound pressure level (SPL) using a 1/4" microphone (2530, Larson-Davis, Depew, NY, USA) and pre-amplifier (2221, Larson-Davis).

2.2. Behavioral training

Prior to commencing behavioral training, rats were food restricted to ∼85% of free-feeding weight to encourage exploration in the behavioral boxes. Rats were trained 30 min per day, and 6 days per week. Behavioral training progressed through a series of steps described in **Supplementary Table 1**. Initial training sessions (Phase 1) required rats to nose-poke a center port (detected by interruption of the center IR beam) to trigger a GO cue (flash of LED) (**Figure 1**). Upon removing its nose from the center port, the rat was immediately reinforced with a food pellet (Bio-Serv, Frenchtown, NJ, USA) which was dropped into the appropriate feeder trough associated with the acoustic stimulus playing from the overhead speaker; i.e., left feeder trough for 16 kHz NBN, and right feeder trough for quiet. If the rat then nose-poked the correct feeder trough within 5 s of the initial pellet delivery (detected by the interruption of the trough IR beam), the rat was given a second food pellet to further reinforce the stimulus association. During a 30-min training session, trial type (16 kHz NBN or quiet) was distributed evenly and presented in a randomized order. As rats became more proficient at the task, the cue delay (time required to trigger the GO cue) was progressively increased from 500 to 2,500 ms.

Upon learning to frequently nose poke the center port (typically after 2 to 3 days), rats were then trained on a new protocol (Phase 2A) where the initial pellet reinforcement was removed and pellet delivery was provided only if the rat poked its nose in the correct feeder trough in response to the given acoustic stimulus. Rats received 100% reward rates, and throughout all phases of training, incorrect feeder trough responses were punished with a 15-s timeout during which time the next trial could not be initiated. Rats remained on Phase 2A until they could correctly associate feeder troughs with the given acoustic stimuli with >92% accuracy for at least three consecutive days (typically after two weeks).

Once rats could correctly distinguish quiet trials from 16 kHz NBN trials, a new protocol (Phase 2B) was introduced where rats were trained to nose poke the right trough for quiet trials, and the left trough for all NBNs (8, 12, 16, 20, or 24 kHz). Rats continued to receive 100% reward rates for correct responses. Trial type (NBN or quiet) was distributed evenly and presented in a randomized order. Upon learning the correct feeder trough associations for at least five consecutive days at >92% accuracy (typically after two weeks), rats were trained on a new protocol (Phase 2C) where the left feeder trough represented all NBN trials, and the right feeder trough represented quiet and AM trials. During a 30-min training session, 50% of trials were NBN, 30% of trials were AM, and 20% of trials were quiet; trials were presented in a randomized order according to criteria provided by Gellermann (1933). Rats continued to receive 100% reward rates for correct responses, and timeouts for incorrect responses. Once rats learned the correct feeder trough associations for all three stimulus types (typically after 1 month), reward rates were progressively lowered to 70% until the rats were able to consistently achieve a > 92% hit-rate during each training session. Using this strategy, daily behavioral performance was highly

consistent across all trial types (see **Figure 5A** from Stolzberg et al., 2013).

2.3. Behavioral testing

To screen for behavioral evidence of tinnitus, trained rats were run on a *testing protocol* in which the previously described training protocol was modified such that responses during quiet trials were no longer rewarded nor punished, in an effort to avoid biasing test day results. Rats experiencing tinnitus were expected to perceive a steady phantom sound during quiet conditions, and as such, they would more frequently respond to the left (NBN) feeder trough (previously an incorrect response) during quiet trials, rather than the right (quiet and AM) feeder trough (previously a correct response; **Figure 1**). During testing, reward rates were increased from 70 to 90% for NBN and AM noise trials to compensate for the lack of food pellets delivered during quiet trials. As a result, the overall reward rate was similar to that of the final training protocol.

Prior to screening rats for chronic tinnitus following intense sound exposure, we carried out pilot experiments in a cohort of animals in order to determine the appropriate time course for running the testing protocol after tinnitus induction. We determined the number of days (i.e., one or two weeks) that rats could refrain from daily training and still perform the behavioral task to criteria when subsequently run on the testing protocol, as well as how many testing days in a row they could be run on the testing protocol. These control experiments allowed us to select an appropriate time point for assessing the presence of chronic tinnitus in the absence of a confounding influence of increased durations between training and testing days. Following the completion of the pilot experiments, a time point of one week was selected as the duration between intense sound exposure and the assessment of chronic tinnitus.

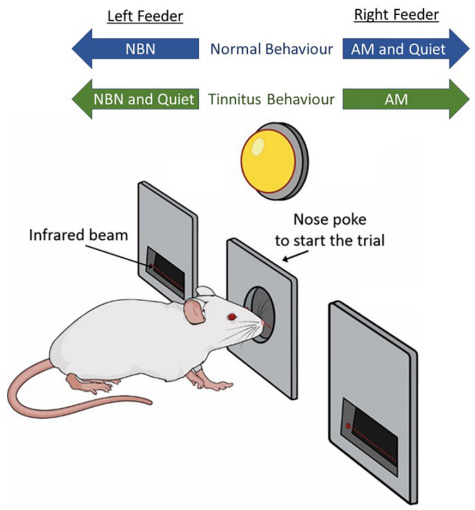

FIGURE 1
Schematic representation of behavioral paradigm. Rats hold their nose in a center port until an LED flashes, which serves as a GO cue. They are then trained to access the left feeder trough during narrowband noise (NBN) trials, and the right feeder trough for amplitude-modulated (AM) broadband noise and Quiet trials. Following tinnitus induction via intense sound exposure, rats experiencing tinnitus are expected to respond to the NBN (left) feeder trough during Quiet trials, indicating they perceived a steady phantom sound during quiet conditions.

2.4. Intense sound exposures

In the first experimental series, following three consecutive days of normal behavioral training at hit-rates of > 92% accuracy, trained rats (n = 10) were placed in a sound-attenuating chamber and subjected to either a 15-min sham exposure (quiet, speaker off), or a 15-min sound exposure (bilateral, 12 kHz tone, 110 dB SPL) from a super tweeter (T90A; Fostex) positioned above the home cage. Immediately after the exposure, rats were placed in the behavioral box and run on the aforementioned testing protocol for 120 to 130 trials. Between the sham and sound exposures, rats were given a minimum of five standard training days, during which time they had to consistently perform with >92% accuracy.

In a separate experimental series, trained rats (n = 10) were used to identify the presence of chronic tinnitus induced by intense sound. Following three consecutive days of training in which the rats demonstrated hit-rates of > 92% accuracy, they were anaesthetized with an intraperitoneal injection of ketamine (80 mg/kg) and xylazine (5 mg/kg). Once the rat's pedal reflex was absent, it was placed on a homeothermic heating pad (maintained core temperature at ~37°C; model 507220F; Harvard Apparatus) in a sound-attenuating chamber (29" W by 23.5" H by 23.5" D; Med Associates Inc.) and given a 60-min sham exposure (quiet, speaker off). Supplemental doses of ketamine/xylazine were administered intramuscularly as needed. Following the 60-min exposure, anaesthesia was reversed using an intraperitoneal injection of atipamezole hydrochloride (1 mg/kg), and the rat was returned to its home cage for recovery. Rats were not trained for the six days following the sham exposure. One week after the sham exposure, rats were run on the aforementioned testing protocol. Rats were given a minimum of five standard training days following the 60-min sham exposure test session before being prepped for the 60-min sound exposure. Once each rat had demonstrated three consecutive days of normal training at > 92% accuracy after their post-sham testing, they were again anaesthetized and placed in the sound-attenuating chamber. This time, rats were given a 60-min sound exposure (bilateral, 12 kHz tone, 120 dB SPL) from a super tweeter (T90A; Fostex) placed directly in front of their head, 5 cm from the pinna of the ears. The exposure was generated with TDT software and hardware (RPvdsEx, RZ6 module; TDT). Following the exposure, rats were administered an intraperitoneal injection of atipamezole hydrochloride (1 mg/kg) and returned to their home cage. Similar to the 60-min sham exposure, rats were not trained for the six days following the sound exposure. One week later (on Day 7), rats performed the testing protocol to screen for behavioral evidence of chronic tinnitus.

It is possible that during the initial training phases of the 2AFC task (i.e., well before the subsequent testing protocol to screen for behavioral evidence of tinnitus), the rats experienced some brain plasticity associated with first learning the rules of the behavioral protocol. However, because any learning-induced plasticity would have occurred in both the sham and tinnitus-induced rats and it would have taken place long before they underwent sham or tinnitus induction exposures, it is not expected that learning-induced plasticity would interfere with tinnitus pathophysiology while animals are run on the testing protocol of the 2AFC task.

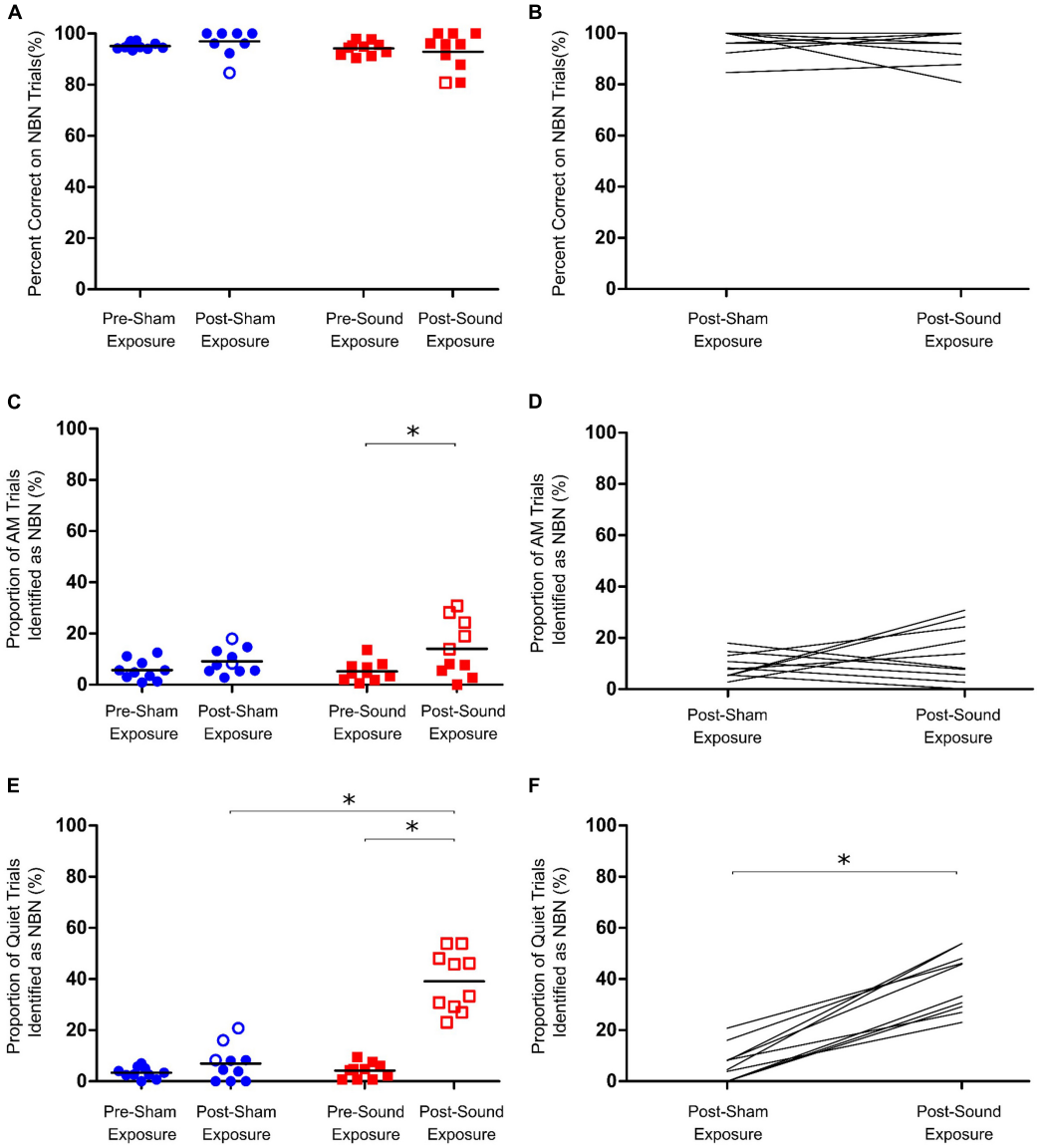

FIGURE 2
Assessment of acute tinnitus induced by intense sound exposure. **(A)** Following sham and sound exposures, rats could still accurately identify lower frequency narrowband noise (NBN) stimuli. **(B)** No change in NBN performance was observed between post-sham and post-sound exposure conditions. **(C)** AM trial performance was unaffected by sham exposure; however, at the group level, a significant increase in misidentification of AM trials as NBN was observed post- sound exposure. **(D)** No change in AM performance was observed between post-sham and post-sound exposure conditions. **(E)** Following sham exposures, rats could still correctly identify quiet stimuli. In contrast, following intense sound exposures, all rats mistakenly identified significantly more Quiet trials as NBN, indicative of tinnitus-like behavior. **(F)** On average, rats mistakenly identified significantly more Quiet trials as NBN following sound exposure than they did following sham exposure. Statistical analyses included a two-way repeated measures ANOVA (time x exposure), followed by *post hoc* paired t-tests with Bonferroni corrections, $^{*}p < 0.01$, $n = 10$. Open symbols in panels **(A,C,E)** represent individual rats with z-scores exceeding the one-tailed criterion for significance (see section "2. Materials and methods" for details). In panels **(B,D,F)**, each line represents an individual rat's performance on NBN **(B)** AM **(D)** or Quiet **(F)** trials.

2.5. Detection of hearing thresholds using auditory brainstem responses

At the conclusion of behavioral testing, hearing thresholds of rats were determined using the auditory brainstem response (ABR) to verify the extent of hearing loss in the week following the 60-min sound exposure. Rats were again anaesthetized with intraperitoneal injections of ketamine (80 mg/kg) and xylazine (5 mg/kg) and placed on a homeothermic heating pad (maintained core temperature at ~37°C) in a sound-attenuating chamber (29" W by 23.5" H by 23.5" D; Med Associates Inc.). Once their pedal reflex was absent, subdermal electrodes (27G; Rochester Electro-Medical, Lutz, FL, USA) were positioned at the vertex (active electrode), over the right mastoid (reference electrode), and on the mid-back (ground electrode). Electrodes were connected to a low-impedance headstage (RA4LI; TDT), and auditory-evoked activity was preamplified and digitized (RA16SD Medusa preamplifier; TDT) prior to being sent to an RZ6 module (TDT) via a fiber optic cable. Signals were bandpass filtered (300–3,000 Hz) and averaged using BioSig software (TDT). Briefly, acoustic stimuli consisted of a click (0.1 ms), 4 kHz tone, and 20 kHz tone (5 ms duration, 1 ms rise/fall time) presented from a speaker positioned 10 cm from the rat's exposed right ear (the left

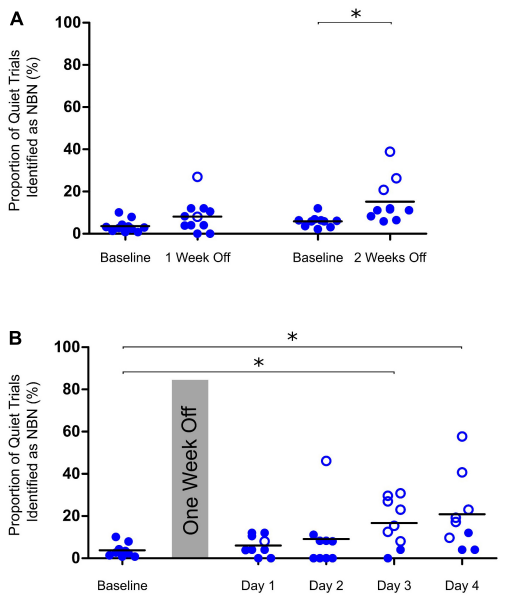

FIGURE 3
Determining appropriate time points for testing of chronic tinnitus. **(A)** Performance on Quiet trials remained consistent after control rats were given one week off between training and testing on the behavioral paradigm. After two weeks off, a significant increase in the misidentification of Quiet trials (indicating a false-positive screening of tinnitus) was observed [two-tailed paired t-test on baseline vs two weeks off, $t(9) = 2.7$, $^*p < 0.05$, $n = 10$]. **(B)** After one week off between training and testing, control rats could be tested up to two days in a row before a significant increase in misidentification of Quiet trials was observed on days 3 and 4 of repeat testing (One-way ANOVA, $F_{4,40} = 3.39$, $^*p < 0.05$, $n = 9$). Open symbols in panels **(A,B)** represent individual rats with z-scores exceeding the one-tailed criterion for significance (see section "2. Materials and methods" for details).

ear was occluded with a custom foam ear plug). Stimuli were each presented 1,000 times (21 times per second) at decreasing sound intensities from 90 to 10 dB SPL in 5 to 10 dB steps. Close to ABR threshold, stimuli were repeated in order to confirm an accurate threshold judgement using the criteria of just noticeable deflection of the averaged electrical activity within the 10 ms window (Popelar et al., 2008; Schormans et al., 2017, 2018). All acoustic stimuli were calibrated using a 1/4" microphone (2530; Larson-Davis), a pre-amplifier (2221; Larson-Davis), and custom MATLAB software (Mathworks).

2.6. Statistical analysis and data presentation

As in our previous publication (Stolzberg et al., 2013), tinnitus-like behavior was defined as a significant decrease in responses to the correct feeder trough during quiet trials on testing day compared to the response rate on quiet trials during the preceding 5 baseline days. Similarly, performance on AM and lower frequency (8–12 kHz) NBN trials was also monitored and compared to baseline performance. Lower frequency NBN trials were selected for evaluation under the assumption that they would be less likely to be affected by hearing loss following the high frequency sound exposures used to induce tinnitus. To evaluate behavioral performance on the individual level, we calculated z-scores for each rat based on its performance over the preceding 5 baseline days for each acoustic stimuli separately, and used a one-tailed criterion of $p < 0.01$ to indicate a significant change in z-score for each stimulus (Heffner, 2011; Stolzberg et al., 2013). Statistical analyses were also conducted on group behavioral data using either a two-way repeated measures analysis of variance (ANOVA), one-way repeated measures ANOVA, or paired t-test, depending on the comparison of interest (see "3. Results" section for the details of each specific comparison). All statistical comparisons used an alpha value of 0.05. When a two-way ANOVA was used, *post hoc* testing was performed with Bonferroni post-tests to correct for multiple comparisons. When a one-way ANOVA was used, *post hoc* testing was performed with Dunnett's post-tests to compare back to baseline. Sigma Stat 3.5 was used for all statistical analyses and BioRender (Biorender.com) was used for methodology schematics. All results are presented as mean ± standard error of the mean (SEM).

3. Results

3.1. Acute tinnitus induced by brief yet intense sound exposure

To determine the ability of the 2AFC behavioral paradigm to screen for acute tinnitus in the minutes following intense sound exposure, rats underwent behavioral training to distinguish between quiet, AM noise, and NBN stimuli. Once trained, they were given 15-min sham and sound exposures immediately prior to behavioral testing to determine if either exposure resulted in behavioral performance consistent with the presence of tinnitus. Tinnitus-positive behavior was scored as a shift in the response to quiet stimuli from the right trough (previously trained to be a correct response) to the left trough (previously trained to be associated with NBNs; see **Figure 1**). Performance on AM noise and NBN trials was also monitored.

Following 15-min sham and sound exposures, rats were still able to correctly identify > 90% of lower frequency NBN trials, and demonstrated no significant change in NBN performance from baseline regardless of exposure type (**Figures 2A, B**). Similarly, although a significant increase in the number of AM trials identified as NBN was observed post-sound exposure, the rats still correctly identified > 85% of the AM trials (Two-way RM ANOVA, significant main effect for time, $F_{1,9} = 12.902$, $p < 0.01$) (**Figures 2C, D**). Taken together, these results demonstrate that the rats maintained good performance on both NBN and AM trials following sham and sound exposures.

As expected, following the 15-min sham exposure, the rats correctly identified the quiet trials, whereas the 15-min sound exposure caused all rats to demonstrate tinnitus-positive behavior by shifting their responses for quiet stimuli to the left (NBN) trough (Two-way RM ANOVA, significant interaction for time and exposure, $F_{1,9} = 64.573$, $p < 0.001$) (**Figures 2E, F**). These results are consistent with studies conducted in human subjects in which a brief exposure to loud noise results in the immediate onset of acute tinnitus (Loeb and Smith, 1967; Atherley et al., 1968). On average, sound-exposed rats mistakenly identified 39.1 ± 3.7% of quiet trials as NBN during behavioral testing, whereas the same rats only misidentified 7.0 ± 2.2% of quiet trials following the sham exposure.

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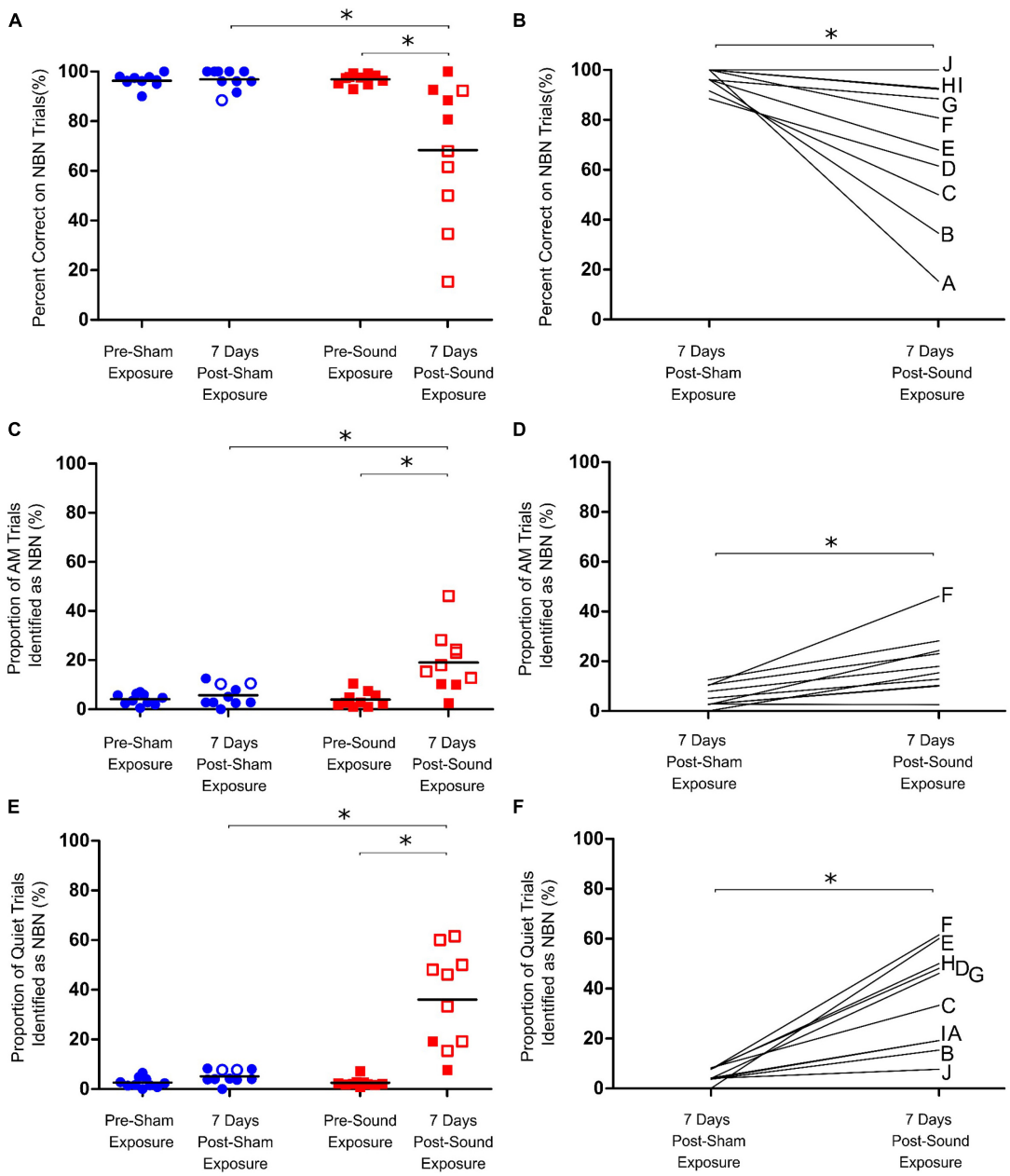

FIGURE 4
Assessment of chronic tinnitus induced by intense sound exposure. **(A)** Following sham exposure rats could still accurately identify lower frequency narrowband noise (NBN) stimuli. Following sound exposure, however, a significant drop in NBN performance was observed for the group average. **(B)** Post-sound exposure, rats misidentified significantly more NBN trials compared to post-sham, with a wide range in NBN performance observed post-sound exposure. **(C)** AM trial performance was unaffected by sham exposure; however, a significant increase in misidentification of AM trials as NBN was observed post-sound exposure for the group average. **(D)** Post-sound exposure, rats misidentified significantly more AM trials compared to post-sham. **(E)** Following sham exposures, rats could still correctly identify quiet stimuli. In contrast, following sound exposures, not all rats screened positive for tinnitus-like behavior by demonstrating an increase in the percentage of Quiet trials misidentified as NBN (i.e., closed vs. open red squares). **(F)** As a group, the rats mistakenly identified significantly more quiet trials as NBN following sound exposure than they did following sham exposure. Statistical analyses included a two-way repeated measures ANOVA (time x exposure), followed by *post hoc* paired t-tests with Bonferroni corrections, $^*p < 0.01$, $n = 10$. Open symbols in panels **(A,C,E)** represent individual rats with z-scores exceeding the one-tailed criterion for significance (see Methods for details). In panels **(B,D,F)**, each line represents an individual rat's performance on NBN **(B)**, AM **(D)** or Quiet **(F)** trials, with each rat identified by a separate letter on the right-edge of the graphs.

3.2. Chronic tinnitus induced by a 60-min intense sound exposure

In order to determine whether the 2AFC behavioral paradigm could be used to detect the presence of chronic tinnitus induced by a 60-min intense sound exposure, we first carried out pilot experiments to identify an appropriate time point post-exposure in which we could run animals on the testing protocol. As shown in Figure 3A, performance on quiet trials remained consistent when rats had one full week off between training and testing on the behavioral paradigm [one-tailed paired t-test on baseline vs one week off, $t(9) = 1.687$, $p > 0.05$]. However, when rats were given two full weeks off between

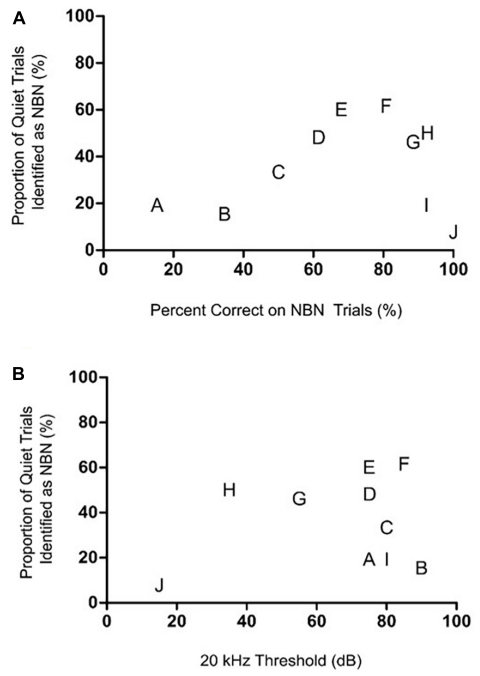

FIGURE 5
The rats' degree of high frequency hearing loss or their ability to detect the steady NBN failed to predict their Quiet trial performance during the chronic tinnitus assessment. **(A)** Relationship between NBN and Quiet trial performance post-sound exposure during the chronic tinnitus assessment. When each rat's post-sound exposure performance on NBN trials was plotted versus their Quiet trial performance, no correlation was observed, demonstrating that a rat's Quiet trial performance was independent of its NBN trial performance [$r(8) = 0.42$, $p > 0.05$]. **(B)** Relationship between the post-sound exposure 20 kHz hearing threshold and Quiet trial performance during the screening for chronic sound-induced tinnitus. No correlation was observed between hearing thresholds at 20 kHz post-sound exposure and performance on Quiet trials [$r(8) = 0.28$, $p > 0.05$]. In both graphs, each rat is identified by a separate letter which corresponds to its designation in Figure 4, as this allows for visual comparisons to be made across these correlative analyses and the sham versus sound exposure results in Figure 4.

training and testing, their misidentification of quiet trials significantly increased, indicative of a false-positive screening of tinnitus in some of the animals [one-tailed paired t-test on baseline vs. two weeks off, $t(9) = 2.7$, $p < 0.05$]. Similarly, after having one week off from training, rats could only be run on the testing protocol up to two days in a row before a significant increase in the misidentification of quiet trials occurred [One-way ANOVA, $F_{4,32} = 2.701$, $p < 0.05$] (Figure 3B). Based on these results, we opted to assess our rats for the presence of chronic tinnitus one week after the intense sound exposure without running them on the testing protocol multiple days in a row.

A separate cohort of rats ($n = 10$) were trained on the behavioral paradigm to distinguish between quiet, AM noise and NBN stimuli, and were subsequently given 60-min sham and sound exposures. As expected from our pilot testing, rats could still reliably identify AM, NBN, and quiet trials post-sham exposure, even after one week off between the sham exposure and testing day (Figures 4A, C, E). Following the sound exposure, a significant increase in the number of misidentified AM trials was observed; however, the rats were still able to correctly identify > 80% of AM trials (Two-way RM ANOVA, significant interaction for time and exposure, $F_{1,9} = 12.798$, $p < 0.01$) (Figures 4C, D). Furthermore, the sound exposure caused a significant decrease in NBN performance (68.4 ± 8.8% correct post-sound vs. 96.9 ± 1.3% correct post-sham; Two-way RM ANOVA, significant interaction for time and exposure, $F_{1,9} = 10.434$, $p < 0.01$) (Figures 4A, B). Not surprisingly, a wide range in NBN performance was observed across animals post-sound exposure, with 5 out of the 10 rats scoring > 80% correct, and the remaining 5 out of 10 rats scoring between 15 and 68% correct post-sound exposure. In addition to the changes in NBN performance post-sound exposure, there was a significant increase in the number of quiet trials misidentified as NBN, indicative of tinnitus-positive behavior (36.1 ± 5.9% misidentified post-sound vs. 5.1 ± 0.8% misidentified post-sham; Two-way RM ANOVA, significant interaction for time and exposure, $F_{1,9} = 27.875$, $p < 0.01$) (Figures 4E, F). Unlike the results from the first experimental series in which all rats screened positive for acute tinnitus by way of a significant increase in the number of misidentified quiet trials immediately after the 15-min sound exposure, not all rats demonstrated evidence of chronic tinnitus one week after the 60-min sound exposure.

When each rat's performance on NBN trials was plotted versus their quiet trial performance, no significant relationship was observed, indicating that an animal's performance on NBN trials was not predictive of their performance on quiet trials (Figure 5A). This point is highlighted by the fact that rats which demonstrated the greatest decline in NBN performance post-sound exposure did not demonstrate the greatest increase in misidentified quiet trials (e.g., Rat A in Figures 4B, F, 5). Similarly, some of the rats that demonstrated the greatest increase in misidentified quiet trials post-sound exposure still scored > 80% correct on NBN trials (e.g., Rats H and G in Figures 4B, F, 5). This demonstrates that the changes in behavioral performance following the intense sound exposure were specific to the quiet condition, and were not related to a change in overall behavioral performance. As expected, rats exposed to intense sound (12 kHz tone, 120 dB SPL, 1 h) had a high-frequency hearing loss, characterized by an average post-sound exposure threshold of 66.5 ± 7.6 dB at 20 kHz, and by thresholds of 31.5 ± 2.6 dB and 40.5 ± 1.4 dB for the 4 kHz and click stimuli, respectively. Similar to the results depicted in Figure 5A, which plots each rat's performance on NBN trials versus their performance on quiet trials, no clear relationship was observed between the level of high-frequency hearing loss for each rat and their performance on quiet trials post-sound exposure (Figure 5B). For example, Rats I and J both screened negative for tinnitus—as evidenced by no significant change in their quiet trial performance post-sound exposure (Figure 4E; filled red squares)—but these rats had dramatically different high frequency hearing thresholds resulting from the same sound exposure (Figure 5B).

4. Discussion

A reliable behavioral paradigm is essential when using animal models to investigate the neural mechanisms underlying tinnitus. In our previous publication (Stolzberg et al., 2013), we reported a novel two-alternative categorization task optimized for identifying acute drug-induced tinnitus with simultaneous recordings of neural activity in behaving rats. Here, we provide further validation of our previously established paradigm in its effectiveness at assessing rats for acute and chronic sound induced tinnitus. As discussed

in detail below, validation of our paradigm is supported by: (1) its resistance to false-positive screening of rats for intense sound-induced tinnitus, and (2) its ability to screen individual animals in order to identify the variabilities in tinnitus development and hearing loss following intense sound exposure. In light of these findings, our paradigm would be useful for investigations into the efficacy of novel therapeutics for tinnitus, as well as studies seeking to uncover the putative neural mechanisms of tinnitus. However, several considerations and limitations of the paradigm should be addressed when using our model for studying chronic sound-induced tinnitus, as discussed in detail below.

4.1. Is the 2AFC behavioral paradigm resistant to falsely-screening rats for tinnitus?

When validating an animal behavioral paradigm, it is important to consider whether false-positive screenings can occur when assessing the presence of tinnitus. To that end, we carried out a number of important control experiments in order to confirm that the behavioral screening following intense sound exposure was indeed representative of tinnitus and not the result of a separate confounding factor. To validate the use of our paradigm for the assessment of acute tinnitus, rats received 15-min sham and sound exposures immediately prior to behavioral testing. Sham exposures were not expected to cause tinnitus in rats, and this was indeed reflected in our findings, as there were no significant group differences in performance on the quiet, AM, or NBN trials following sham exposure (blue symbols in **Figures 2A, C, E**). Similar behavioral profiles were observed in our previous study when rats were given systemic injections of saline as a sham condition (Stolzberg et al., 2013). Furthermore, when performance of individual animals was assessed, only a few rats had positive z-scores for quiet trial performance post-sham exposure, demonstrating that the possibility of false positive results for our paradigm are low.

Before assessing chronic tinnitus, we first determined the appropriate time series following intense sound exposure in which animals should be run on the testing protocol, given the potential for task performance to be influenced by repetitive test sessions or a long layoff following sound exposure. As shown in **Figures 3A, B**, when control rats were given more than one week off between training and testing, or they were run on the testing protocol more than two days in a row, their performance on quiet trials began to falsely indicate the presence of tinnitus. With these results in mind, we determined that the best time to assess the presence of chronic tinnitus was one week post-exposure. These findings highlight the importance of selecting appropriate testing days when evaluating the presence of chronic tinnitus. Similar to the results obtained when screening rats for acute tinnitus with our paradigm, and consistent with our pilot testing, sham exposures had no significant effect on group performance of the quiet, AM, or NBN trials one week later (**Figures 4A, C, E**). Importantly, these extensive sham experiments allowed us to be confident that the paradigm was able to correctly classify control rats as *not* having tinnitus. Moreover, the consistency of the results following sham exposures emphasizes the robustness of our behavioral paradigm in its resistance to false indications of acute or chronic tinnitus; a criterion that is essential for any successful behavioral model of tinnitus.

4.2. Variable outcomes following intense sound exposure: The relationship between hearing loss and chronic tinnitus?

A challenge of studying sound-induced tinnitus, regardless of the behavioral paradigm used, is the potential for considerable variability in outcomes across animals; findings which may arise due to differing degrees of hearing loss induced by a given sound exposure. Furthermore, the requirement of different animal cohorts for control and experimental series has been a considerable drawback of previously established shock avoidance tinnitus models, as it is well-known that tinnitus in humans is highly variable at the level of the individual. Thus, we considered the utility of our paradigm to screen for chronic tinnitus in individual sound-exposed rats that experienced variable levels of permanent hearing impairment.

Following the intense sound exposure, not all rats demonstrated evidence of chronic sound-induced tinnitus. Consistent with our results, it is well-established that not all subjects exposed to the same level of excessive sound will develop tinnitus. For example, previous behavioral work by Brozoski et al. (2007) showed that a one-hour exposure to 120 dB SPL band-limited noise did not induce tinnitus-like behavior equally in all rodents. Variable tinnitus behavioral profiles were also observed in individual rats following noise exposure using the behavioral paradigm developed by Sederholm and Swedberg (2013), as well as the one developed by Heffner and Harrington (2002). Moreover, human studies have revealed that of the number of returning war veterans surveyed who were exposed to blast trauma (a severe form of noise exposure), only 49% of them went on to develop tinnitus (Cave et al., 2007). Thus, in the present study, it was not surprising that not all rats showed behavioral evidence of chronic tinnitus in the week following intense sound exposure.

In addition to the variable outcome of chronic tinnitus induction, we also observed variability in NBN performance one week following sound exposure (**Figures 4A, B**). The post-exposure decline in NBN performance led us to postulate that some rats likely developed a hearing loss that prevented them from perceiving NBNs, and as such, they mistakenly probed the feeder trough associated with the quiet stimulus during NBN trials. Not surprisingly, variable levels of high-frequency hearing loss were also observed following sound exposure. These results are consistent with previous findings in other models of noise-induced hearing loss in which considerable inter-animal variability was observed following exposure to the same acoustic trauma (Cody and Robertson, 1983; Mulders et al., 2011).

It is often suggested that a strong link exists between hearing loss and the presence of tinnitus, as the majority of patients who suffer from tinnitus have some degree of measurable hearing impairment (Axelsson and Ringdahl, 1989; Henry et al., 2014). That said, some tinnitus sufferers are suspected of having "hidden hearing loss"; i.e., while they have normal audiometric hearing thresholds, they still have cochlear damage characterized by a reduction in sound-evoked activity of their auditory nerve fibers (Schaette and McAlpine, 2011). In the present study, although we did not design our sound exposure protocol to cause hidden hearing loss, we did observe varying degrees of high-frequency hearing impairment in the rats that screened positive for tinnitus. Moreover, similar to Heffner and Harrington (2002) who used a shock avoidance behavioral paradigm to identify tinnitus following varying durations of sound exposure, we also observed that rats with similar degrees of hearing loss did

not all screen positive for tinnitus. As discussed above, this finding is not surprising, as many individuals with hearing loss do not experience tinnitus. Finally, we also observed varying hearing loss in rats that screened negative for tinnitus following sound exposure; e.g., one of the "no tinnitus" rats had limited high frequency hearing impairment (ABR threshold: 15 dB SPL at 20 kHz), whereas another rat had a severe hearing loss (ABR threshold: 80 dB SPL at 20 kHz). Looking forward, we envision using our 2AFC task coupled with simultaneous neural recordings to screen for tinnitus in rats with hidden hearing loss, as this would ultimately provide an effective platform to test theories derived from recent computational modeling studies and review articles that consider the relationship between (hidden) hearing loss and tinnitus (Schaette and McAlpine, 2011; Schaette and Kempter, 2012; Zeng, 2020).

4.3. Experimental considerations

The results of the present study highlight the importance of a number of experimental considerations to take into account when using our 2AFC paradigm to screen rodents for sound-induced tinnitus. First, extensive pilot testing and sham exposure experiments demonstrate that our paradigm is limited by the number of times that individual animals can be screened for the presence of chronic tinnitus post-sound exposure (**Figures 3A, B**). Based on these findings, we recommend that rodents are only run on the testing protocol up to two days in a row post-sound exposure, and that animals are given no more than one week off between sound exposure and the testing protocol. It is important to note that repeatedly running rats on the testing protocol, in which they are no longer punished or rewarded for their quiet trial performance, may lead to random selection of either food trough over time during quiet trials. However, our sham and pilot testing clearly demonstrate that the misidentification of quiet trials as NBN when the animals are first run on the testing protocol post-sound exposure reflects the presence of tinnitus and not a false-positive screening due to random probing of the food troughs.

A major benefit of our 2AFC paradigm is the ability to screen individual animals for the presence of tinnitus, as opposed to solely analyzing group behavioral performance; an important feature given that not all sound-exposed animals may develop tinnitus. However, it is important to determine an appropriate method of identifying which animals have tinnitus based on their behavioral performance. In the present study, we adopted a z-score analysis similar to that used in previous tinnitus publications (Heffner, 2011; Stolzberg et al., 2013), and classified tinnitus-like behavior as a significant decrease in responses to the correct feeder trough during quiet trials on the post- sham or sound testing day compared to the response rate on quiet trials during the preceding 5 baseline days. When using this approach, the "floor effect" must be considered, by which holding animals to a very high baseline performance criteria (i.e., 92% in the present study) can result in a very minor change in behavioral performance post-sham or sound exposure leading to a significant z-score from baseline. For example, in **Figure 4E**, two rats showed significant z-scores during quiet trial performance post-sham exposure (open blue circles) despite a very minor change in their behavioral performance from pre-sham baseline testing. Potential ways to address this concern in the future would be to hold animals to a less strict performance criterion (i.e., ∼85%) at baseline, or to set a standard threshold in performance across rats so that any individual rat whose performance crosses the threshold will be classified as having tinnitus.

Additional considerations should be made when identifying cases in which it may be inappropriate to include an animal for analysis of sound-induced tinnitus based on their behavioral performance. For example, if an animal shows a dramatic change in performance post-sham exposure, we would first suggest that the sham exposure and testing be repeated. If the animal still demonstrates a significant change in performance following the second sham exposure, we would recommend that the animal be excluded from further analysis (i.e., the animal should not go on to be sound exposed, and then tested for sound-induced tinnitus). Furthermore, if an animal shows an overall change in performance on all trial types (NBN, AM, and quiet) post-sound exposure, suggesting that the rat is no longer performing the task appropriately, we would recommend that the animal be excluded from analysis. Related to this, particular attention should be given to identifying animals that display a right or left side bias during testing (i.e., animals that go to the left feeder trough for the majority of trials, regardless of trial type). For example, Rat F in **Figure 4** falls into this category by demonstrating significant impairments across all trial types post-exposure during testing for chronic tinnitus (the rat displays a potential left side bias by choosing the left feeder trough for all trial types, including AM trials). Similarly, Rat A in **Figure 4** may display a right side bias post-exposure, as evidenced by selection of the right feeder trough for all trial types. While the need for animal exclusion is quite rare when using the present paradigm, it is an important factor to consider in order to avoid falsely-screening animals for the presence of tinnitus. Potential ways to address this concern in the future would be to set a standard threshold in performance across rats for NBN and AM trials so that any individual rat that demonstrates significantly impaired performance on these trial types should be considered as having a potential side bias, and their performance on quiet trials should be interpreted with caution. Finally, in an effort to limit potential side biases that may emerge due to the rats' difficulty hearing the task stimuli, another experimental consideration could be to adjust the stimulus intensities for each rat according to its ABR-confirmed sensation level rather than a set 75 dB SPL, as this customization could better accommodate inter-animal variability in hearing loss.

Given that we first used our 2AFC task to screen rats for salicylate-induced tinnitus (Stolzberg et al., 2013), it is worth comparing those findings with the present study in which acute tinnitus was induced by brief yet intense sound exposure. Consistent with the behavioral profile observed following salicylate administration, the rats exposed to a 12 kHz tone at 110 dB SPL for 15 min showed no change in performance during the NBN trials, but did show a significant increase in the number of misidentified quiet trials; findings indicative of the presence of tinnitus in both models. In the present study, we also observed a slight, but significant increase in the misidentification of AM trials for some rats following sound exposure. This is likely due to the presence of hearing loss which could interfere with the processing of temporal cues (Tyler et al., 1982; Henderson et al., 1984; Radziwon et al., 2019). Interestingly, we did not see this change in AM performance following salicylate exposure in our previous study (Stolzberg et al., 2013), perhaps because of the disparate effects that salicylate and noise exposure have on the auditory periphery (Henderson et al., 2006; Stolzberg et al., 2012).

4.4. Future directions

Now that we have confirmed the validity of our paradigm and its resistance to false-positives, we can envision future studies using this screening tool to investigate novel therapeutics for tinnitus prevention, as well as the pathophysiology of tinnitus. For example, because our behavioral paradigm was sensitive enough to reveal animals with differing post-exposure profiles (i.e., not all rats had tinnitus), future studies could expose groups of rats to loud sound, followed by administration of either a therapeutic-of-interest or a vehicle-control, and ultimately determine the proportion of rats in each group that go on to screen positive for tinnitus. As there is currently no widely accepted drug treatment for tinnitus prevention, and many clinical trials seeking to alleviate chronic tinnitus have found that only a subset of subjects within the treatment group experience benefit (Allman et al., 2016), it will be worthwhile for future animal models to consider the ratio of "responders" versus "non-responders" following a given intervention.

One of the major advantages of using animal models to investigate the neural basis of tinnitus is the potential to perform longitudinal studies in which a given animal's brain activity can be compared before versus after induction of tinnitus via intense sound exposure. In addition to such within-subject comparisons, efforts to contrast the electrophysiological recordings in tinnitus-positive versus tinnitus-negative rats that showed similar hearing loss profiles post-exposure would be expected to provide valuable insight into the neural correlates of tinnitus, as this comparison would be freed from issues related to hearing loss alone. This comparative approach could also be strengthened by including a complementary electrophysiological investigation between groups of animals that both screened positive for tinnitus yet differed in their degree of hearing loss, as this would help to unravel the seemingly complex relationship between the effect of hearing loss and/or tinnitus on brain plasticity. Related to the varying degrees of hearing loss observed in tinnitus patients, computational studies have attempted to model various neural mechanisms thought to underlie tinnitus, such as changes in lateral inhibition (Gerken, 1996), gain adaptation (Parra and Pearlmutter, 2007), homeostatic plasticity (Schaette and Kempter, 2006, 2009; Chrostowski et al., 2011), as well as increased central noise and variance (Zeng, 2013, 2020). Given that many of these computational models describe an increase in spontaneous firing rates as a neural correlate of tinnitus (reviewed by Schaette and Kempter, 2012), it would be worthwhile for future studies to use our 2AFC task (with its emphasis on a having rats attend to their tinnitus during actual quiet trials) to assess whether the rate and/or synchronization of the spontaneous spiking activity in a given trial does indeed predict whether a rat will go on to report that it perceives a steady sound (i.e., tinnitus).

Overall, the aforementioned examples of within-subject as well as between-subject comparisons are well-suited to the 2AFC behavioral paradigm, as we have previously confirmed that it is possible to simultaneously record neural activity at the very moments when the rats are being screened for behavioral evidence of tinnitus (Stolzberg et al., 2013). Related to this important feature, we foresee future studies being able to use this behavioral paradigm in combination with advanced techniques for real-time manipulation (e.g., optogenetics; chemogenetics) or monitoring (e.g., genetically-encoded calcium indicators) of cell/circuit-specific activity underlying sensory perception. Ultimately, given the ever-increasing number of transgenic rat models available, it is reasonable to propose that these techniques could assist in uncovering the neural signature of tinnitus, and we suggest that our behavioral paradigm could offer a suitable platform for such investigations.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

This animal study was reviewed and approved by the University of Western Ontario Animal Care and Use Committee and all procedures were in accordance with guidelines established by the Canadian Council of Animal Care.

Author contributions

SH, KB, MT, and AS: data collection and analysis. All authors: project conceptualization, data interpretation, and manuscript writing and editing.

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Conflict of interest

MT was employed by Audifon GmbH & Co. KG.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnins.2023.1001619/full#supplementary-material

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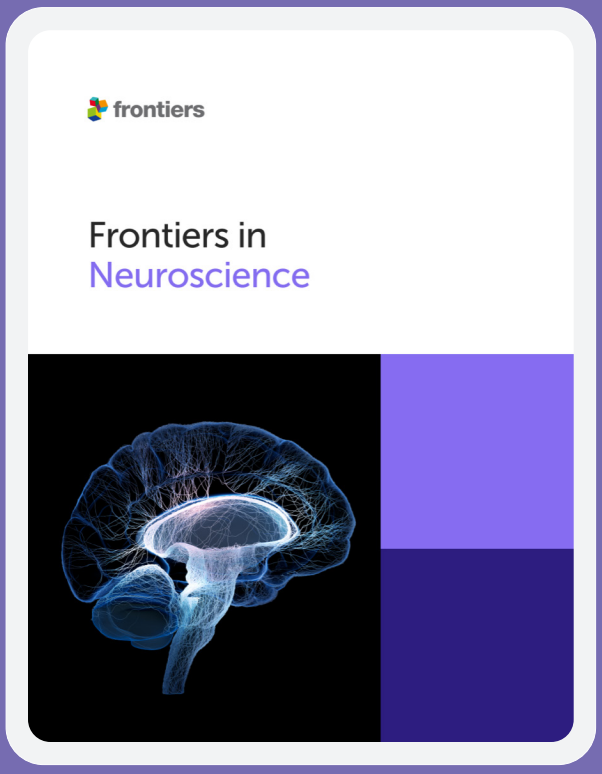

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