Serum prestin level may increase following music exposure that induces temporary threshold shifts: a pilot study.

Eleftheria Iliadou[1]*, Christopher J. Plack[2,3], Konstantinos Pastiadis[1,4], Athanasios Bibas[1]

[1] First Department of Otorhinolaryngology and Head and Neck Surgery, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece; [2] Division of Psychology, Communication and Human Neuroscience, Manchester, UK; [3] Department of Psychology, Lancaster University, Lancaster, UK School of Music Studies, [4] Aristotle University of Thessaloniki, Thessaloniki, Greece

Running Head:

Blood prestin after loud music exposure. Iliadou et al

Financial disclosures/conflicts of interest:

The research was funded by the Special Account of Research Grants of the National & Kapodistrian University of Athens. Author CP was supported by the NIHR Manchester Biomedical Research Centre (NIHR203308) and by the Medical Research Council, UK (MR/V01272X/1). The Authors declare that there is no conflict of interest, financial, or otherwise.

The data and code that support the findings of this study are available in:

https://osf.io/nuw6d/?view_only=dbef375ddf51471093553f27f041764e

All correspondence should be addressed to:

Eleftheria Iliadou, School of Medicine, National and Kapodistrian University of Athens, 1st University ENT Clinic, Vasilissis Sofias Av. 114, 11527, Athens, Greece, Tel. 0030 2132088330, email: iliadoue@med.uoa.gr

Acknowledgments

We would like to thank the many who helped with the completion of our study. These are the staff of the personnel of the Immunology and Serology Laboratory who consulted and supported the researchers with the handling and storage of blood samples, and Professor Skarlatos G. Dedos (Zoology Lab, Department of Biology, NKUA) who trained and supported the first author with the conduction of the ELISA.

Author Contributions

EI: Conceptualization, Data curation, Formal analysis; Investigation; Methodology; Project administration; Writing - original draft; Writing - review & editing. CJP: Formal analysis, Writing - review & editing, Supervision, KP: Audio material development, Writing - review & editing. AB: Conceptualization, Funding acquisition, Formal analysis, Project administration, Writing-review & editing, Supervision.

Serum prestin level may increase following music exposure that induces temporary threshold shifts: a pilot study.

Abstract

Objectives: To determine if blood prestin level changes after exposure to music at high sound pressure levels, and if this change is associated with temporary threshold shift (TTS) and/or changes in distortion product (DP) amplitude.

Design: Participants were exposed to pop-rock music at 100 dBA for 15 minutes monaurally through headphones. Pure tone audiometry, DP amplitude, and blood prestin level were measured before and after exposure.

Results: Fourteen adults [nine women; age range: 20-54 years, median age=31 (IQR=6.75)] with normal hearing were included in the study. Mean prestin level increased shortly after exposure to music, then returned to baseline within 1 week, although this trend was not observed in all participants. All participants presented TTS or a decrease in DP amplitude in at least one frequency after music exposure. There was a statistically significant average threshold elevation at 4 minutes post-exposure. Statistically significant DP amplitude shifts were observed at 4 and 6 kHz, 2 minutes following exposure. Mean baseline serum prestin level [mean: 140.00 pg/ml, 95% CI: (125.92, 154.07)] progressively increased following music exposure, reaching a maximum at 2 hours [mean: 158.29 pg/ml, 95% CI: (130.42, 186.66)] and returned to pre-exposure level at 1 week [mean: 139.18 pg/ml, 95% CI: (114.69, 163.68)]. However, after correction for multiple comparisons, mean prestin level showed no statistically significant increase from baseline at any timepoint. No correlation between maximum blood prestin level change and average TTS or DPOAE amplitude shift was found. However, in an exploratory analysis, TTS at 6 kHz (the frequency at which maximum TTS occurred) decreased significantly as baseline blood prestin level increased.

Conclusions: The results suggest that blood prestin level may change after exposure to music at high sound pressure levels, although statistical significance was not reached in this relatively small sample after correction. Baseline serum prestin level may also predict the degree of TTS. These findings thus suggest that the role of baseline serum prestin level as a proxy marker of cochlear susceptibility to intense music exposure should be further explored.

Keywords: word; music, temporary threshold shift, prestin, biomarker, pure tone audiometry, distortion product otoacoustic emissions

Introduction

Overexposure to hazardous levels of noise and music is one of the primary causes of sensorineural hearing loss (Di Stadio et al., 2018; Śliwińska-Kowalska & Zaborowski, 2017). Music-induced hearing loss may affect a wide range of people: people attending music events or using personal listening devices (Kähäri et al., 2011; Welch & Fremaux, 2017), music students and tutors, and musicians and professionals of the music industry (Di Stadio et al., 2018). It has been estimated recently that 1.1 billion teenagers and young adults are at risk of hearing loss due to overexposure to recreational noise (le Clercq et al., 2016). Although, due to ethical constrictions, studies on the permanent effects of intense music should be strictly observational, temporary - and thus reversible - changes, can be assessed at an experimental level. Although some authors have expressed their concerns on the conduction of interventional temporary threshold shift (TTS) studies (Henderson et al., 2006; Themann et al., 2015; Verbeek, 2015), assessing temporary hearing changes after exposure to intense noise or music under conditions safe for the participants may be the only time-efficient way to investigate the mechanisms of noise damage in humans reliably. To date, the most assessed effects of noise overexposure are temporary threshold elevation in pure tone audiometry (PTA), TTS, and the temporary decrease in distortion product otoacoustic emission (DPOAE) amplitude. Both outcomes have been studied previously in observational and experimental settings (Keppler et al., 2010; Kil et al., 2017; Kraaijenga et al., 2018; Le Prell et al., 2012). Although these temporary changes may relate to different pathophysiological mechanisms than the permanent ones, such as permanent threshold shift (PTS), temporary changes have been proven to serve as a useful proxy for permanent changes (Ryan et al., 2016). Repeated episodes of TTS seem to be associated with permanent hearing disorders in both rodents and humans (Wang & Ren, 2012; Ryan et al, 2016), TTS may be indicative of hazardous music overexposure capable of creating permanent changes (Kil et al, 2017), and particular otoprotective agents may have an effect on both TTS and PTS (Kil et al, 2017; Pourbakht & Yamasoba, 2003). However, TTS as an outcome measure in clinical studies may present high variability. Previously published human research has indicated that comparable exposures can result in varying level of TTS or impact distinct frequencies (Kil et al., 2017; Le Prell et al., 2011; 2016). Combining TTS with another outcome measure would add value to the findings of clinical studies. Otoacoustic emissions (especially DPOAEs) have also been proven valuable for the detection of temporary or permanent noise-induced hearing changes (Davis et al., 2005; Engdahl, 1996; Kramer et al., 2006). Previous evidence has established that DPOAEs elicited with unequal f2/f1 levels are considerably more sensitive to reductions in emission levels induced by brief exposure to high levels of sound (Sutton et al., 1994).

Apart from TTS and decrease of DPOAE amplitude, a newly proposed sensorineural hearing loss marker has emerged (Hana & Bawi, 2018; Parham, 2015; Parham et al., 2019; Solis-Angeles et al., 2021). Prestin is an outer hair cell (OHC) protein, responsible for electromotility (Liberman et al., 2002), which can be detected in the blood by means of enzyme-linked immunosorbent assay (ELISA). It has also been found in cardiomyocytes, where it is also considered to be responsible for the amplification of cardiac motor functions (Zhang et al., 2021). The way prestin exits the cochlea and enters blood circulation remains unknown. The fact that prestin molecules have been found in phagosomes of supportive cells, suggests the involvement of phagocytosis (Abrashkin et al., 2006). It is also possible that, due to its small size, prestin may cross the labyrinthine-blood barrier with no involvement of other cellular mechanisms. In humans, blood prestin level was found to be lower in individuals with sensorineural hearing loss due to chronic exposure to lead, and acutely increased after a dose of more than 80 mg of cisplatin (Jalali et al., 2022; Solis-Angeles et al., 2021). Moreover, blood prestin level has previously shown to have a weak negative correlation with age ($r = -0.350$, in 72 adults, 18-82 years old; (Parker et al., 2022a), and a moderate one with average daily noise exposure levels ($L_{Aeq,8h}$(dB), measured by means of noise dosimeters for 3 weeks in 30 adults, 18-24 years old, $r = -0.455$) (Parker et al., 2022b). This decline in serum prestin level has been linked with two hypotheses: 1. The Hidden OHC Damage Hypothesis: a lower number of intact OHCs with normal prestin production and turnover results in lower prestin level in circulation; 2. The Environmental Downregulation Hypothesis, according to which prestin expression is downregulated as part of the natural dynamics of OHCs responding to loud environmental conditions and the decreased need for cochlear amplification in loud environments (Parker et al., 2022b). In addition to these chronic changes, blood prestin level may vary after a single exposure; in a rodent study serum prestin level increased immediately after 2 hours exposure to intense noise and then decreased to near or below baseline at 14 days. (Parham et al., 2019). However, this temporal pattern has never been assessed in humans.

The study presented here is the first experimental study to evaluate blood prestin level in adults who have been exposed to music, and their association with other markers of temporary cochlear dysfunction (TTS and decrease of Distortion Product (DP) amplitudes). Based on previous evidence showing a temporal variation in blood prestin level after noise exposure, we hypothesized that:

H1. Prestin concentration changes over time immediately after exposure to high-level music.

Moreover, and to confirm that our music exposure paradigm causes temporary cochlear dysfunction, we hypothesized that:

H2a. PTA threshold (average for higher frequencies; 10 and 12.5 kHz) is different in adults with normal hearing before and after music exposure.

H2b. PTA threshold (average for lower frequencies; 1-8 kHz) is different in adults with normal hearing before and after music exposure.

H3. Otoacoustic emissions amplitude is different in adults with normal hearing before and after music exposure.

Finally, to explore any possible correlation between serum prestin level and degree of cochlear dysfunction evident in TTS, we hypothesized that:

H4a. The degree of average pure tone TTS for higher frequencies immediately after music exposure is a predictor of the maximum serum prestin level shift after music exposure, adjusted for age, gender and self-reported lifetime noise exposure.

H4b. The degree of average pure tone TTS for lower frequencies immediately after music exposure is correlated to maximum serum prestin level shift after music exposure, adjusted for age, gender and NESI score.

Maximum serum prestin was used here instead of serum prestin level at a specific time point since we did not expect that TTS / DPOAE changes would have a similar time course to the blood prestin change after music exposure. However, we did expect that the maximum TTS and maximum serum prestin level would both be representative of the cochlear changes that were induced by our music exposure paradigm.

To assess their time course of the blood and hearing variables, measurements were made at multiple timepoints before and after exposure. The hypotheses, methodology, and primary statistical design were pre-registered (https://osf.io/nuw6d/?view_only=dbef375ddf51471093553f27f041764e).

Materials and methods

Study Design

This was an experimental, longitudinal study which involved multiple measurements of the concentration of blood prestin in adults after exposure to intense music.

Setting

The study was conducted at the 1st University Department of Otolaryngology and Head and Neck Surgery of the General Hospital “Hippokrateion”, Athens, Greece. Recruitment started in November 2021 and was completed in May 2022.

Participants

Prior to their enrolment to the study, all candidates underwent screening procedures: medical and hearing history, otoscopy, tympanometry and PTA, according to the British Society of Audiology guidelines (British Society of Audiology, 2018).

Inclusion Criteria: Absence of self-reported current or previous hearing issues, speech perception loss, tinnitus, or other hearing disorders; no observable ear abnormalities during otoscopy; normal tympanometry results [middle ear pressure values between -140 and +40 daPa, peak compensated static acoustic admittance between 0.3 and 1.8 ml, and acoustic equivalent volume (Vea) between 0.8 and 2.1 cm (Le Prell et al., 2012)]; no air-bone gap greater than 10 dB in PTA; and symmetrical pure tone thresholds of 25 dB HL or lower for frequencies ranging from 0.5 to 8 kHz in both ears. Symmetry in pure tone thresholds was defined as a threshold difference between the two ears < 25 dB at any of the tested frequencies.

Exclusion criteria: Other causes that may lead to OHC dysfunction (ototoxic substances, such as aminoglycoside antibiotics, macrolide antibiotics, salicylates, chemotherapeutic agents such as cisplatin, loop diuretics, antimalarials, non-steroidal anti-inflammatory drugs, acetaminophen and aspirin in high doses, and quinine, or radiotherapy) have been linked to sensorineural hearing loss of varied degree and changes in blood prestin level (Naples et al., 2018; Shi et al., 2021; Solis-Angeles et al., 2021; Yang et al., 2014). Candidates exposed to these substances or radiotherapy within the past 12 months were excluded. Moreover, to avoid exceeding the weekly permissible noise exposure limits, participation of otherwise eligible candidates who were exposed to hazardous noise within the preceding 72 hours was postponed to a new date.

Individuals meeting the criteria for inclusion underwent detailed explanations of the study's purpose and procedures. They were then requested to provide both oral and written consent for participation.

Audio Material

The audio material was previously developed and validated for its safety and efficiency (Iliadou et al., 2023, in press). It consisted of a 30-minute compilation of 2 to 3-minute excerpts from digitally modified pop songs (.wav files). We used a low-moderate nonuniform compression scheme for the audio material which avoided over-compressing (Réveillac, 2017). The nonuniform compression scheme comprised of a 3:1 compression of peak levels (Leq,1s > -6 dB_{max}) and a 2:1 compression over the rest (the lowest parts) of the dynamic range, for each music track, with appropriate makeup gain value (again, applied individually on each track). Thus, we achieved a roughly constant average level between tracks, and at the same time, we avoided severe distortions due to clipping. Finally, the mastering level of the whole audio material was adjusted to obtain an average level of 100 dBA, measured on a BK4128 Head and Torso Simulator (HATS) with FOCAL Spirit Professional headphones [mean = 99.68 dBA, median = 99.73, SD = 2.29, IQR=3.38 (08.12-101.5)]. The calibrations of the BK4128 HATS microphones were performed utilizing a BK4228 pistonphone calibrator. Sampling of the BK4128 output was carried out at a constant rate of 44.1 kHz through a National Instruments USB-6251 along with LabView 2010 software. The voltage readings were then transformed into sound pressure level (SPL) measurements using the sensitivity values derived from the microphone calibration. Following this, the complete duration of the sampled audio content underwent analysis by determining the Leq SPL within successive 1-second intervals. All statistical metrics pertaining to the audio content were subsequently computed from these intervals. [Supplementary Material 1 shows the evolution of instantaneous SPL of the 15-minute-long audio material; Supplementary Material 2 shows the distribution of SPLs; Supplementary Material 3 shows the cumulative distribution of SPL; Supplementary Material 4 shows the main statistics of the SPL distribution; and Supplementary Material 5 shows the 95th, 50th and 5th percentiles of the 1/3-octave long term average spectrum of the audio material.]

Clinical assessment and sessions

Entry to the study: All candidates were recruited by the 1st University Otolaryngology and Head and Neck Surgery Department in Hippokrateion Hospital and underwent medical and hearing loss history taking, physical examination, otomicroscopy, tympanometry, and PTA. Those who fulfilled the inclusion criteria received oral and written explanations of the purpose and procedures of the study. If they agreed to take part, they were asked to sign the relevant consent form.

Music exposure: Participants were exposed to the audio material at 100 dBA for 15 minutes, to the left ear. As in the calibration of music levels, the music exposure was done using the same laptop computer, with the VLC Player software and the same headphones (FOCAL Spirit Professional). The contralateral (right) ear was sealed with the headphones earcup. The procedure took place in a sound-treated room, in which the average ambient noise levels (dB SPL) were 500Hz: 35dB, 1kHz: 29dB, 2kHz: 32dB, 4kHz: 33dB, 8kHz: 30dB.

Pre- and post-exposure assessments: Before music exposure, participants' demographics were collected (age, gender). Their lifetime noise exposure was evaluated with the help of a structured interview (noise-exposure structured interview or NESI). The NESI was developed for use in auditory research. It consists of a structured interview that helps the subject recall and self-report their lifetime noisy activities. NESI comes with tools for estimating the sound levels of all exposure activities, taking into account the effect of hearing protection whenever applicable. An energy-based means of combining the reported information (recreational, occupational, and exposure to firearms) is also supplied. Lifetime exposure is expressed in “NESI units”. One NESI unit is equivalent to one working year (2080 hours) of exposure at 90 dBA (Guest et al., 2018).

For both the TDH-39 and HDA300 headphones, the audiometer was calibrated before the exposure and testing phase of the research, according to the NKUA 1st University ENT clinic’s equipment maintenance procedures following the specifications and guidelines of ISO 389-1:1998 “Acoustics — Reference zero for the calibration of audiometric equipment — Part 1: Reference equivalent threshold sound pressure levels for pure tones and supra-aural earphones”, using a BK4153 Artificial Ear.

In order to avoid any time delays and keep consistency between the end of music exposure and the audiometric testing schedule, the audiometric testing was taking place in the same sound-treated room as the music exposure. The ambient noise levels were the ones reported in the Music Exposure section. These levels met the Maximum Permissible Ambient Noise Levels specifications of ANSI/ASA S3.1:1999 (R2018) for pure-tone audiometry with TDH-39 headphones in the range of 500Hz-8kHz, with an exception at 500Hz and 1kHz. However, 500Hz was not used in the PTA or DPOAE testing. As for the deviation from the ANSI/ASA S3.1:1999 specifications at 1kHz, it allowed detection of PTA thresholds down to 3dBHL which was considered clinically acceptable (Chung, 2023). Additionally, the PTA thresholds that were subsequently measured did not fall any higher than the normal HL range.

DPOAEs for the left ear were measured before and at 2 minutes post-music exposure using Titan by Interacoustics, in the aforementioned sound-treated room. The same probe was used for all measurements of the same participant. The frequency ratio of the primary tones, f2/f1, was 1.22, and their levels were 65 and 55 dB SPL, respectively. Maximum residual noise was set to 30 dB SPL. The geometric mean of the pair was swept from 8 to 1 kHz, with measurements conducted at two points per octave. Data collection was terminated after three such sweeps (Guest et al., 2019). The whole procedure lasted 1 minute. Collected endpoints included DP amplitude shift from baseline, per frequency. DPOAEs were measured again at 2 and 4-8 hours immediately before PTA and extended high-frequency PTA (EHF-PTA).

PTA and EHF-PTA were performed before and post-music exposure at three time points (4 minutes, 2 hours, 4-8 hours), immediately after DPOAE measurement. Only the exposed (left) ear was tested. Tested frequencies followed the following order: 1, 3, 4, 6, 8, 10, and 12.5 kHz. The signal level was varied in a 6-dB down, 2-dB up manner (modified Hughson-Westlake PTA; Kil et al., 2017); Each tone lasted approximately 3 seconds. Following a satisfactory positive response by the participant, the level of the tone was reduced in 6-dB steps until no further response occurred. Then the level was increased in 2-dB steps until a response occurred. After the first response using an ascending approach, the tone was decreased by 6 dB and another ascending 2-dB series was initiated. This sequence continued until the participant responded at the same level on two out of two, three or four (i.e., 50 % or more) responses on the ascent. This level was considered the hearing threshold level for that particular frequency. Since conductive hearing loss was an exclusion criterion for our study, only air conduction data were collected. The whole procedure was performed with an Affinity audiometer (EN 60645-1, ANSI S3.6, Interacoustics) using TDH39 (for ≤ 8 kHz) and HDA 300 (for > 8 kHz) headphones. The duration of the PTA procedure was approximately 3 minutes. Collected endpoints included PTA thresholds per frequency (dB).

All audiometric testing was conducted by the first author, who is an Otolaryngologist, trained by a Senior Audiologist. and with considerable experience in audiometric measurement and procedures.

Finally, blood sampling for prestin measurement by means of ELISA was performed before and after music exposure at different time points (baseline and then at 20 minutes, 2 hours, 4-8 hours, 24 hours and 1 week following music exposure, immediately after the completion of PTA, EHF-PTA, and DPOAE measurements). Serum samples were collected in Serum Separator Tubes (SST). The samples were then centrifuged for 15 minutes at 1000 g within 2 hours of collection. The resulting supernatant was stored in a –80°C refrigerator until time of assay. ELISA was performed in the Department of Biology of the National Kapodistrian University of Athens, and the samples were stored in ice during transport. Prestin level was measured using the Human Prestin (SLC26A5) MBS282125 ELISA Kit (MyBioSource, San Diego, California) as described in the manufacturer’s instruction manual. All samples were assayed in duplicates, as recommended by the manufacturer. The selected kit has a detection range of 15.6 pg/mL – 1000 pg/ml, high sensitivity, and excellent specificity. The optical density was measured at 450 nm and 540 nm FlexStation 3 Multi-Mode Microplate Reader, in ELISA mode. Collected data include serum prestin concentration in pg/ml. The pre- and post-exposure procedures are detailed in Figure 1.

Statistical analysis

R was used for the statistical analysis (R core team, 2023). To test hypotheses 1, 2a, and 2b, a two-level linear mixed effect (LME) models was constructed to account for repeated measurements of serum Prestin levels, EHF-PTA threshold (average for higher frequencies; 10 and 12.5 kHz), and PTA threshold (average for lower frequencies: 1-8 kHz) respectively, at different time points within the same participant. Time was modelled as a fixed factor and Participant as a random factor (random intercept) to account for individual differences in Prestin level for each participant before exposure.

To test hypothesis 3, a two-level linear mixed effect models was constructed to account for repeated measurements of OAE levels at the tested frequencies, at different time points within the same participant. Time and Frequency were modelled as fixed factors and Participant as a random factor (random intercept) to account for individual differences in OAE levels for each participant before exposure.

In the above LME models, deviations from homoscedasticity or normality were assessed by visual inspection of residual plots, and the Shapiro-Wilk test. Analysis of variance tables (using the Kenward–Rogers method for estimating degrees of freedom), marginal means and significance testing of their differences were calculated via the lmerTest package (Kuznetsova et al., 2017). Dunnett's correction was used for controlling the family-wise error rate.

To test hypotheses 4a and 4b, simple linear regression models were fit to explore the average PTA threshold shifts for lower and higher frequencies following music exposure as predictors for the maximum prestin serum level observed. Age, gender and NESI were used as covariates.

After reviewing and analyzing the data, the following exploratory post-hoc analyses were also performed: 1) qualitative descriptive analysis on the post-exposure prestin response among participants (responders and non-responders) 2) analysis of mean TTS per frequency, before and at 4 minutes after music exposure (the only time point when TTS was observed) with a simple linear regression model. 3) Baseline serum prestin level as a predictor of the magnitude of TTS at 6 kHz (the frequency where maximum PTA threshold shift occurred).

Deviations from the original pre-registered statistical analysis plan

Our sample size deviated from that in the pre-registration. Our initial sample size calculation was based on the statistical model required for the primary hypothesis: At the time this was one-way ANOVA, repeated measures, within-subject factor, to detect a medium effect size (Cohen's f) of 0.25 with a significance level of 0.008 (0.05 Bonferroni corrected for six hypotheses), and 80% power (1 – beta). Based on this, the required sample size was estimated to be 27 participants. Due to resource and time constraints, we decided to stop recruiting in May 2022 and proceed with the analysis of our data in the context of preliminary data.

ANOVA was initially planned for the analysis of hypotheses 1 (one-way repeated measures), 2a (one-way), 2b (one-way) and 3 (two-ways). Due to partially missing data, linear mixed effect models were used instead to maximize the use of available data and at the same time account for both repeated measures and random effects of individual subjects.

Finally, we initially considered repeating pure tone audiometry (PTA) and Distortion Product Otoacoustic Emissions (DPOAEs) at five time points (2 minutes, 2 hours, 4-8 hours, 24 hours and 7 days). During the study, we decided that we should be measuring PTA and DPOAEs only until they return to baseline. All our participants' PTA and DPOAE values had returned to baseline level by 4-8 hours post-music exposure.

Results

Participants

Fourteen participants were included in the preliminary study [nine women; age range: 20-54 years, median age=31 years (IQR=6.75 years)].

H1. Prestin concentration before and after music exposure

Out of possible 84 serum samples (14 participants, one session before and five sessions after music exposure), we managed to gather 81, since three sessions were missed by the participants. We were able to measure prestin level in 77 of them (15 samples at baseline and at 20 minutes, 11 at 2 hours, 13 at 4-8 hours, 10 at 24 hours and nine at 7 days post-exposure). Haemolysis prevented accurate prestin measurements in four of them, while four measurements with values > 450 pg/ml were considered outliers (> 3 standard deviations from the mean) and were not included in the analysis.

Serum prestin level at baseline [mean: 140.00 pg/ml, 95% CI: (125.92, 154.07)] increased gradually following music exposure, reached a maximum at 2 hours [mean: 158.29 pg/ml, 95% CI: (130.42, 186.66)], and returned to pre-exposure level at 1 week [mean: 139.18 pg/ml, 95% CI: (114.69, 163.68)] (Figure 2). According to the LME model, the overall Time effect was not statistically significant, although the serum level at 4-8 hours was significantly different to the baseline [b = 18.40; $t_{(48.66)}$ = 2.61; 95% CI: (4.26, 32.55); p = 0.012, uncorrected]. All other differences are non-significant. Using Dunnett's correction to control for multiple comparisons (for five contrasts comparing with the baseline) the difference of the marginal mean at 4-8 hours and the baseline was 18.40 pg/ml, and was not statistically significant [$t_{(54.3)}$ = 2.47; 95% CI: (3.78, 33.02); p = 0.069, corrected].

Post-hoc analysis of post-exposure prestin response among participants (responders and non-responders): Although all participants experienced a TTS in PTA and a temporary reduction in DP amplitude in the initial (up to 5 minutes) post-exposure measurements, only four of the 14 participants (three with baseline values > 150 pg/ml) showed a sharp increase in prestin level (arbitrarily defined as > 50% increase from baseline) 2–8 hours following exposure, followed by decreased values in the subsequent measurements (Figure 3). Moreover, four participants (with baseline values < 125 pg/ml) showed no change in serum prestin level following exposure to music.

H2. Average PTA thresholds for standard and extended high frequency audiometry before and after music exposure

EHF-PTA thresholds: There was no statistically significant elevation of the average EHF-PTA threshold in (≥ 10 kHz) at any time point.

Standard PTA thresholds: The overall Time effect was statistically significant in the model of PTA threshold in standard audiometry ($\leq$ 8 kHz) ($F_{(3,\ 36.33)} = 7.28$; $p < 0.001$). More specifically there was a statistically significant average threshold elevation at 4 minutes post-exposure [$b = 3.31$, $t_{(36.14)} = 3.51$, 95% CI (1.41, 5.20), $p = 0.001$, uncorrected]. Using Dunnett's correction to control for multiple comparisons (for three contrasts comparing with the baseline) the difference of the marginal mean at 4 minutes and the baseline was 3.31 dB and was statistically significant [$t_{(39.2)} = 3.36$; 95% CI: (1.34, 5.271); $p = 0.005$]. The threshold elevation at 2 and 4-8 hours post-exposure was statistically not significant (Figure 4).

Post-hoc analysis of mean PTA threshold shift per frequency: Since TTS was observed at 4 minutes post-exposure, it was further explored which frequencies were most affected, by using a simple linear regression model.

Frequency was significant in the PTA threshold shift model at 4 minutes following music exposure ($F_{(6, 91)} = 5.13$, $p <0.001$; Figure 5). The maximum TTS of 9.57 dB was observed at 6 kHz [$t_{(91)} = 6.03$, 95% CI: (6.42, 12.72), $p < 0.001$]. The following statistically significant TTSs were also observed: 3.57 dB at 3 kHz [$t_{(91)}=2.25$, 95% CI: (0.42, 6.72), $p = 0.027$], 3.71 dB at 4 kHz [$t_{(91)} = 2.34$, 95% CI: (0.57, 6.86), $p = 0.021$], and 3.29 dB at 8 kHz [$t_{(91)} = 2.07$, 95% CI: (0.14, 6.44), $p = 0.041$].

H3. Distortion product otoacoustic emissions amplitudes before and after music exposure

Both Time and Frequency effects were significant in the model ($F_{(4, 379.65)} = 2.90$, $p = 0.021$; and $F_{(6, 377.97)} = 55.90$, $p <0.001$ respectively). According to the LME model, the DP amplitude levels (across frequencies) at 2 minutes were significantly different to the baseline [$b = -2.15$; $t_{(377.97)} = -2.93$; 95% CI: (-0.71, -3.59); $p = 0.004$, uncorrected]. Using Dunnett's correction to control for multiple comparisons (for four contrasts comparing with the baseline) the difference of the marginal means at 2 minutes following music exposure and the baseline was -2.15 dB [$t_{(388)} = -2.89$, 95% CI: (-0.32, -3.98), $p = 0.015$]. There was no statistically significant shift at any later time point, with levels returning to pre-exposure levels, on average.

At 2 minutes following music exposure, the maximum DP amplitude shift of -4.07

dB was observed at 6 kHz [($t_{(91)}$= -3.54; 95% CI: (-1.79, -6.35); $p < 0.001$]. A statistically significant shift of -2.52 dB was also observed at 4 kHz [($t_{(91)}$ = -2.20, 95% CI: (-0.24, -4.80), p = 0.031)]. The DP amplitude shifts observed are presented in Figure 6 and Supplementary Material 6.

Both of the above models deviated from normality. Although linear mixed effect models are robust when distributional assumptions are objectively violated and the β parameter estimates are considered unbiased (Knief & Forstmeier, 2021; Schielzeth et al., 2020; Verbeke, 2000), robust linear mixed models were also fitted that do not make assumptions on normality to estimate 95% confidence intervals and marginal means. The results of the robust linear mixed models are comparable to the estimates of the mixed effect models.

H4. Average TTS at lower and higher frequencies as a predictor of maximum serum prestin shift

Neither average temporal threshold shifts at lower ($F_{(4,9)}$ = 0.75, p = 0.582) nor at higher frequencies ($F_{(4,8)}$ = 0.47, p = 0.758) was a significant predictor of maximum serum prestin level shift.

Exploratory post-hoc analysis

Baseline serum prestin level as a predictor of the magnitude of TTS at 6 kHz: Since the maximum PTA threshold shift occurred at 6 kHz, it was further explored as to whether baseline serum level could serve as a predictor of the magnitude of this shift, and thus justify the use of the latter as a possible biomarker of cochlear susceptibility to short-term and therefore long-term cochlear damage.

A fixed effect linear model was used to explore baseline serum prestin as a predictor of PTA threshold shift at 6 kHz (where the maximum TTS was observed), with age as a covariate. Baseline serum prestin level effects were significant (Adjusted $R^2 = 0.401$, $F_{(2,10)} = 5.02$, $p = 0.031$). More specifically, the model showed a significant decrease of TTS with increasing prestin concentrations at baseline [$\beta = -0.24$; $t_{(10)} = -3.15$; 95% CI: (-0.36, -0.12); $p = 0.010$; Figure 7]. In contrast, baseline serum prestin was not a significant predictor of DP amplitude shift at 6 kHz (where the max DP amplitude shift was observed).

Discussion

In our study, 14 participants were exposed to high-level music for a short period of time, in a setting that has been previously shown to create reliably and safely temporary cochlear dysfunction in a similar population (TTS and decrease of DP amplitude; Iliadou et al., 2023, in press). Statistically significant TTSs were observed at 3, 4, 6 and 8 kHz, while statistically significant DP amplitude shifts were recorded at 4 and 6 kHz. Our findings are consistent with previous studies showing that TTS and DP amplitude shift is more prominent in the 3-6 kHz region (Engdahl, 1996; Le Prell et al., 2012; Moshammer et al., 2015).

We hypothesized that serum prestin level of adults with normal hearing would change after our music exposure paradigm, and that this change would correlate with changes in PTA. Our hypothesis was based on previous evidence showing that in rodents, prestin concentration has been found to increase immediately after noise exposure (acute exposure) and then return back to near baseline 2 weeks later (Parham et al., 2019). Moreover, in human studies, subjects with higher daily noise exposure levels (chronic exposure) were found to have lower prestin level (Parker et al., 2022b).

In our study, after exposure to the audio material, participants' blood prestin level were measured at several time points after their exposure by means of ELISA. The fact that our patients did not have any heart conditions, and the only intervention they underwent was the exposure to high-level music, gives us confidence that observed changes in blood prestin level were not due to temporary changes in other systems where prestin is being expressed, such as the heart. Serum prestin increased soon after music exposure, reaching its maximum concentration at 2-8 hours, and then decreased back to near the pre-exposure baseline by 24 hours post-exposure. According to the model, a mean increase of 11.38 pg/ml and 18.40 pg/ml is expected in serum prestin, at 2 and 4-8 hours respectively. However, none of the changes were statistically significant after correction, so should be regarded as trends only. Blood prestin level returned to baseline level in all participants with available data at day 7 following exposure.

This time course of change of blood prestin level is in agreement with previous rodent studies showing a post-exposure initial increase and then decrease to baseline. In a recent Wistar rats study by Parham et al. (2019), prestin level were measured at 4 hours, 24 hours, 48 hours, 72 hours, 7 days, and 14 days after 2 hours of exposure to noise of 110 and 120 dB SPL. After an initial peak of prestin concentration immediately after the noise trauma (4 hours), prestin level returned to near or below baseline by day 14 post-exposure. The degree of its increase was shown to be correlated to the animal's degree of hearing loss, affected cochlear region, and degree of recovery. Our findings suggest that serum prestin could serve as the first non-auditory test to assess noise-induced cochlear insult, although further investigation is warranted. Moreover, although no statistically significant relation between TTS or DP amplitude shift (maximum or on average) and prestin level change was found, prestin level at baseline was a good predictor of TTS at 6 kHz (where the maximum TTS shift was observed in our sample): an elevation of the mean baseline prestin level by 43.48 pg/ml corresponded to a mean reduction in TTS at 6 kHz of 10 dB. This exploratory finding may show some utility of baseline level as a predictor of subclinical cochlear dysfunction or susceptibility to noise, and it warrants further investigation.

Regarding the generalizability and safety of our paradigm, the audio material used in our paradigm consists or pop commercial music, and its duration and intensity mimics regular listening habits at fitness clubs, nightclubs, at live music events, and while using personal listening devices (Dudarewicz et al., 2015; Kähäri et al., 2011; Schmidt et al., 2011; Tronstad & Gelderblom, 2016) making our lab findings comparable to real-world exposure conditions. Confirming the presence of temporary cochlear dysfunction by means of TTS or DPOAEs was considered important for confirming that our music exposure is capable of creating temporary cochlear changes. The selected music exposure paradigm was successful in the current study, since all participants presented TTS and decrease of DP amplitude in at least one frequency. Music was presented monaurally through headphones at sound levels compliant with Greek regulations (Y.A. Y2/Οικ. 15438/2001, 2001), and these levels were lower than the standards established by the National Institute for Occupational Safety & Health (NIOSH) or the World Health Organization for exposures lasting 15 minutes (Śliwińska-Kowalska & Zaborowski, 2017; WHO, 2022). It is important to note that NIOSH standards and the permitted daily noise "dose" are designed to minimize the risks associated with repetitive noise exposure over 40 years of working five days a week, rather than a single exposure, as in our experiment. Furthermore, NIOSH standards pertain to sound levels in open environments, whereas in our study, music was delivered through headphones, resulting in lower sound levels compared to open environments. We conservatively estimate that the delivered-to-the ear sound levels are 5 dB lower than the free field levels (Shaw, 1966), which practically means that participants were exposed for 15 minutes to free-field equivalent noise of 95 dBA (less than 1/3 of the maximum permissible dose in terms of energy). Regarding, the WHO guidelines for safe listening, the weekly exposure limit of 1.6 Pa^2 h (Pascal squared hours) per 7 days is recommended as the reference exposure. This limit is equivalent to 80 dBA for 40 hours a week and translates to a maximum exposure of 101 dBA for 18.75 minutes within a week (using a 3 dB exchange rate). This exposure is more than the 95 dBA free field equivalent for 15 minutes that we have used in our paradigm. The overall exposure is consistent with the guidelines, provided that our participants had not been exposed to loud environments where they would need to raise their voice to be heard from a 4 feet (1.2 m) distance for the following 7 days and had no high exposure during the preceding 72 hours (as per our instructions to the participants). In previously published paradigms participants were exposed to music for 4 hours at coupler levels of 97-100 dBA (Le Prell et al., 2012, 2016) and for 2 hours at 92.5 to 102.8 dBA (free field, mean exposure levels = 98.1 dBA; Kramer et al., 2006). As our primary aim was to evaluate the effectiveness of our paradigm in inducing TTSs, which does not necessitate exposure to and potential harm to both ears, we exclusively considered monaural exposure. The choice of monaural noise/music delivery aligns with the practices of numerous previous studies (Attias et al., 2004; Bhagat & Davis, 2008; Keppler et al., 2010; Quaranta et al., 2003; 2004). The safety of our study protocol (in terms of pure tone thresholds and DP amplitudes) is supported by the fact that, despite all participants exhibiting measurable and consistent temporary changes in their auditory function, none of them experienced nor reported any lasting hearing disorders such as PTSs.

Regarding the risk of music-induced cochlear synaptopathy in our sample, this was also considered before designing and conducting our study. Prior animal studies utilizing cochlear functional assessments and confocal imaging have demonstrated that exposure to noise levels capable of causing temporary pure tone threshold increases of approximately 40–50 dB can lead to rapid and lasting synaptic deficiencies, as well as reduced evoked potential amplitude (Kujawa &

Liberman, 2009, 2015). However, it has been established that significantly higher noise levels would be required to induce cochlear synaptopathy in primates compared to rodents (Valero et al., 2017). In a recent commentary discussing the rationale for modifying current regulations governing occupational noise exposure based on research findings related to noise-induced cochlear neuropathy in rodents, the authors conclude that these findings cannot be directly extrapolated to humans. They suggest that humans appear to be less susceptible to TTSs and likely cochlear synaptopathy (Dobie & Humes, 2017). Moreover, numerous studies attempting to identify signs of cochlear synaptopathy in humans have yielded high heterogeneity in their results (Bramhall et al., 2019). In those studies, noise exposures were of much greater energy than those used in our research (Bramhall et al., 2019; Wang et al., 2021). Nevertheless, if, in the future, any clinical tests are validated as sensitive to cochlear synaptopathy and neurodegeneration in humans, they should be incorporated into both pre- and post-exposure assessments to ensure the preservation of synaptic and neural integrity.

Limitations

The main limitation of this study is the small sample size, which reduces the power of our analysis, increases the chance of type 2 error, and limits the generalization of our findings. The lack of statistical significance after correction may be a consequence of the small sample size. Additionally, if we had used a one-tailed test, the prestin effect would have survived correction; the literature suggests a unidirectional effect with prestin level increasing following acute noise exposure in rodents (Parham et al., 2019). The fact that only some participants responded to music exposure with an elevation in serum prestin while others did not, should also be considered in the statistical analysis plan when replicating this study.

Another limitation is the limited range of age in our sample. Recently, blood prestin was shown to be significantly lower in people over the age of 50 years when compared to younger adults (Parker et al., 2022a). In the present study, the sample was small and consisted mainly of young adults with normal hearing, so our findings cannot be generalized for older adults. Moreover, one of our outcome measures, DP amplitude, seems to deteriorate with age (Lonsbury-Martin et al., 1991). This limitation was overcome by adjusting for age in the analysis of our findings. Finally, although the ELISA kit used in this study has an excellent specificity and high sensitivity, ELISA itself may be affected by multiple technical factors (refrigerated transport and storage, numerous preparation, and wash steps of the assay, haemolysis of the samples). In order to address them, all procedures were duplicated and followed the ELISA kit manual guide, while samples with high haemolysis were not included in the analysis (Ni et al., 2021).

Despite the aforementioned limitations, we consider the results of this analysis useful for better understanding prestin's behaviour after music exposure, and for optimizing the design of future studies. The effect size estimated here and observed drop-out rate may be used in obtaining a more accurate sample size calculation. Moreover, it seems that prestin level show no change from baseline at 20 minutes or 7 days after the music exposure at the level and durations tested in the present study.

Conclusions

Our study is the first human study assessing the changes of blood prestin concentration after brief exposure to intense music, and its correlation to TTS and DP amplitude decrease. Our findings suggest that serum prestin level may potentially show detectable change after music overexposure, and could thus serve in the future as a useful and relatively easy-to-perform test to indicate noise- or music-induced temporary cochlear dysfunction. Moreover, baseline prestin may predict the degree of TTS, providing individuals with a pre-exposure measure of their vulnerability to intense music. Further studies with larger samples are required to confirm these findings. Studies that measure blood prestin level changes after exposure to other types of sound (noise, real-life sounds, firearms) - taking into account factors that have been associated with vulnerability to noise, such as genetics, or melanin levels - are also warranted (Lin et al., 2012).

Disclosure statement: No conflict of interest to declare. The research was funded by the Special Account of Research Grants of the National & Kapodistrian University of Athens. Author CP was supported by the NIHR Manchester Biomedical Research Centre (NIHR203308) and by the Medical Research Council, UK (MR/V01272X/1). The Authors declare that there is no conflict of interest, financial, or otherwise.

Ethics approval: Study protocol was approved by the Institutional Scientific Review Board of Hippokrateion General Hospital (Ε.Σ.62/10-9-2021).

References

Abrashkin, K. A., Izumikawa, M., Miyazawa, T., Wang, C.-H., Crumling, M. A., Swiderski, D. L., Beyer, L. A., Gong, T.-W. L., & Raphael, Y. (2006). The fate of outer hair cells after acoustic or ototoxic insults. Hearing Research, 218(1–2), 20–29. https://doi.org/10.1016/j.heares.2006.04.001

British Society of Audiology. (2018). Pure tone air and bone conduction threshold audiometry with and without masking—British Society of Audiology. https://www.thebsa.org.uk/resources/pure-tone-air-bone-conduction-threshold-audiometry-without-masking/

Chung, K. (2023). Calibration matters: II. Measurement of ambient noise in test rooms/areas. Journal of Communication Disorders, 101, 106293. https://doi.org/10.1016/j.jcomdis.2022.106293

Davis, B., Qiu, W., & Hamernik, R. P. (2005). Sensitivity of Distortion Product Otoacoustic Emissions in Noise-Exposed Chinchillas. Journal of the American Academy of Audiology, 16(2), 69–78. https://doi.org/10.3766/jaaa.16.2.2

Di Stadio, A., Dipietro, L., Ricci, G., Della Volpe, A., Minni, A., Greco, A., de Vincentiis, M., & Ralli, M. (2018). Hearing Loss, Tinnitus, Hyperacusis, and Diplacusis in Professional Musicians: A Systematic Review. International Journal of Environmental Research and Public Health, 15(10), 2120. https://doi.org/10.3390/ijerph15102120

Dudarewicz, A., Pawlaczyk-Łuszczyńska, M., Zamojska-Daniszewska, M., & Zaborowski, K. (2015). Exposure to excessive sounds during orchestra rehearsals and temporary hearing changes in hearing among musicians. Medycyna Pracy, 66(4), 479–486. https://doi.org/10.13075/mp.5893.00163

Engdahl, B. (1996). Effects of noise and exercise on distortion product otoacoustic emissions. Hearing Research, 93(1–2), 72–82. https://doi.org/10.1016/0378-5955(95)00197-2

Guest, H., Dewey, R. S., Plack, C. J., Couth, S., Prendergast, G., Bakay, W., & Hall, D. A. (2018). The Noise Exposure Structured Interview (NESI): An Instrument for the Comprehensive Estimation of Lifetime Noise Exposure. Trends in Hearing, 22. https://doi.org/10.1177/2331216518803213

Guest, H., Munro, K. J., Prendergast, G., & Plack, C. J. (2019). Reliability and interrelations of seven proxy measures of cochlear synaptopathy. Hearing Research, 375, 34–43. https://doi.org/10.1016/j.heares.2019.01.018

Hana, R., & Bawi, B. (2018). Prestin, otolin-1 regulation, and human 8-oxoG DNA glycosylase 1 gene polymorphisms in noise-induced hearing loss. Ibnosina Journal of Medicine and Biomedical Sciences, 10(2), 60. https://doi.org/10.4103/ijmbs.ijmbs_4_18

Y.A. Y2/Oικ. 15438/2001, Pub. L. No. 1346/B` 17.10.2001, 1346/B` 17.10.2001 (2001). https://www.elinyae.gr/sites/default/files/2019-07/1346B_01.pdf

Henderson, D., Bielefeld, E. C., Harris, K. C., & Hu, B. H. (2006). The role of oxidative stress in noise-induced hearing loss. Ear and Hearing, 27(1), 1–19. https://doi.org/10.1097/01.aud.0000191942.36672.f3

Jalali, M. M., Saedi, H. S., & Saadat, F. (2022). Effect of cisplatin chemotherapy on the inner ear function and serum prestin concentration. European Archives of Oto-Rhino-Laryngology, 279(6), 2783–2789. https://doi.org/10.1007/s00405-021-06967-3

Kähäri, K. R., Aslund, T., & Olsson, J. (2011). Preferred sound levels of portable music players and listening habits among adults: A field study. Noise & Health, 13(50), 9–15. https://doi.org/10.4103/1463-1741.73994

Keppler, H., Dhooge, I., Maes, L., D'haenens, W., Bockstael, A., Philips, B., Swinnen, F., & Vinck, B. (2010). Short-term Auditory Effects of Listening to an MP3 Player. Archives of Otolaryngology–Head & Neck Surgery, 136(6), 538. https://doi.org/10.1001/archoto.2010.84

Kil, J., Lobarinas, E., Spankovich, C., Griffiths, S. K., Antonelli, P. J., Lynch, E. D., & Le Prell, C. G. (2017). Safety and efficacy of ebselen for the prevention of noise-induced hearing loss: A randomised, double-blind, placebo-controlled, phase 2 trial. Lancet (London, England), 390(10098), 969–979. https://doi.org/10.1016/S0140-6736(17)31791-9

Knief, U., & Forstmeier, W. (2021). Violating the normality assumption may be the lesser of two evils. Behavior Research Methods, 53(6), 2576–2590. https://doi.org/10.3758/s13428-021-01587-5

Kraaijenga, V. J. C., van Munster, J. J. C. M., & van Zanten, G. A. (2018). Association of Behavior With Noise-Induced Hearing Loss Among Attendees of an Outdoor Music Festival. JAMA Otolaryngology-- Head & Neck Surgery, 144(6), 490–497. https://doi.org/10.1001/jamaoto.2018.0272

Kramer, S., Dreisbach, L., Lockwood, J., Baldwin, K., Kopke, R., Scranton, S., & O'Leary, M. (2006). Efficacy of the antioxidant N-acetylcysteine (NAC) in protecting ears exposed to loud music. Journal of the American Academy of Audiology, 17(4), 265–278. https://doi.org/10.3766/jaaa.17.4.5

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in Linear Mixed Effects Models. Journal of Statistical Software, 82, 1–26. https://doi.org/10.18637/jss.v082.i13

le Clercq, C. M. P., van Ingen, G., Ruytjens, L., & van der Schroeff, M. P. (2016). Music-induced Hearing Loss in Children, Adolescents, and Young Adults: A Systematic Review and Meta-analysis. Otology & Neurotology, 37(9), 1208–1216. https://doi.org/10.1097/MAO.0000000000001163

Le Prell, C. G., Dell, S., Hensley, B., Hall, J. W., Campbell, K. C. M., Antonelli, P. J., Green, G. E., Miller, J. M., & Guire, K. (2012). Digital music exposure reliably induces temporary threshold shift (TTS) in normal hearing human subjects. Ear and Hearing, 33(6), e44–e58. https://doi.org/10.1097/AUD.0b013e31825f9d89

Le Prell, C. G., Fulbright, A., Spankovich, C., Griffiths, S. K., Lobarinas, E., Campbell, K. C. M., Antonelli, P. J., Green, G. E., Guire, K., & Miller, J. M. (2016). Dietary supplement comprised of β-carotene, vitamin C, vitamin E, and magnesium: Failure to prevent music-induced temporary threshold shift. Audiology and Neurotology. Extra, 6(2), 20–39. https://doi.org/10.1159/000446600

Liberman, M. C., Gao, J., He, D. Z. Z., Wu, X., Jia, S., & Zuo, J. (2002). Prestin is required for electromotility of the outer hair cell and for the cochlear amplifier. Nature, 419(6904), 300–304. https://doi.org/10.1038/nature01059

Lin, F. R., Maas, P., Chien, W., Carey, J. P., Ferrucci, L., & Thorpe, R. (2012). Association of Skin Color, Race/Ethnicity, and Hearing Loss Among Adults in the USA. Journal of the Association for Research in Otolaryngology, 13(1), 109–117. https://doi.org/10.1007/s10162-011-0298-8

Lonsbury-Martin, B. L., Cutler, W. M., & Martin, G. K. (1991). Evidence for the influence of aging on distortion-product otoacoustic emissions in humans. The Journal of the Acoustical Society of America, 89(4 Pt 1), 1749–1759. https://doi.org/10.1121/1.401009

Moshammer, H., Kundi, M., Wallner, P., Herbst, A., Feuerstein, A., & Hutter, H.-P. (2015). Early prognosis of noise-induced hearing loss. Occupational and Environmental Medicine, 72(2), 85–89. https://doi.org/10.1136/oemed-2014-102200

Naples, J., Cox, R., Bonaiuto, G., & Parham, K. (2018). Prestin as an Otologic Biomarker of Cisplatin Ototoxicity in a Guinea Pig Model. Otolaryngology–Head and Neck Surgery, 158(3), 541–546. https://doi.org/10.1177/0194599817742093

Ni, J., Zhu, W., Wang, Y., Wei, X., Li, J., Peng, L., Zhang, K., & Bai, B. (2021). A Reference chart for clinical biochemical tests of hemolyzed serum samples. Journal of Clinical Laboratory Analysis, 35(1), e23561. https://doi.org/10.1002/jcla.23561

Parham, K. (2015). Prestin as a biochemical marker for early detection of acquired sensorineural hearing loss. Medical Hypotheses, 85(2), 130–133. https://doi.org/10.1016/j.mehy.2015.04.015

Parham, K., Sohal, M., Petremann, M., Romanet, C., Broussy, A., Tran Van Ba, C., & Dyhrfjeld-Johnsen, J. (2019). Noise-induced trauma produces a temporal pattern of change in blood levels of the outer hair cell biomarker prestin. Hearing Research, 371, 98–104. https://doi.org/10.1016/j.heares.2018.11.013

Parker, A., Parham, K., & Skoe, E. (2022a). Age-related declines to serum prestin levels in humans. Hearing Research, 426, 108640. https://doi.org/10.1016/j.heares.2022.108640

Parker, A., Parham, K., & Skoe, E. (2022b). Noise exposure levels predict blood levels of the inner ear protein prestin. Scientific Reports, 12(1), 1154. https://doi.org/10.1038/s41598-022-05131-z

Réveillac. (2017). Musical Sound Effects: Analog and Digital Sound Processing | Wiley.

Ryan, Kujawa, S. G., Hammill, T., Le Prell, C., & Kil, J. (2016). Temporary and Permanent Noise-induced Threshold Shifts: A Review of Basic and Clinical Observations. Otology & Neurotology, 37(8), e271–e275. https://doi.org/10.1097/MAO.0000000000001071

Schielzeth, H., Dingemanse, N. J., Nakagawa, S., Westneat, D. F., Allegue, H., Teplitsky, C., Réale, D., Dochtermann, N. A., Garamszegi, L. Z., & Araya-Ajoy, Y. G. (2020). Robustness of linear mixed-effects models to violations of distributional assumptions. Methods in Ecology and Evolution, 11(9), 1141–1152. https://doi.org/10.1111/2041-210X.13434

Schmidt, J. H., Pedersen, E. R., Juhl, P. M., Christensen-Dalsgaard, J., Andersen, T. D., Poulsen, T., & Bælum, J. (2011). Sound Exposure of Symphony Orchestra Musicians. The Annals of Occupational Hygiene, 55(8), 893–905. https://doi.org/10.1093/annhyg/mer055

Shaw. (1966). Earcanal pressure generated by circumaural and supraaural earphones—PubMed. J Acoust Soc Am 39, 471–479.

Shi, W., Hou, X., Bao, X., Hou, W., Jiang, X., Ma, L., Jiang, X., & Dong, L. (2021). Mechanism and Protection of Radiotherapy Induced Sensorineural Hearing Loss for Head and Neck Cancer. BioMed Research International, 2021, 3548706. https://doi.org/10.1155/2021/3548706

Śliwińska-Kowalska, M., & Zaborowski, K. (2017). WHO Environmental Noise Guidelines for the European Region: A Systematic Review on Environmental Noise and Permanent Hearing Loss and Tinnitus. International Journal of Environmental Research and Public Health, 14(10), 1139. https://doi.org/10.3390/ijerph14101139

Solis-Angeles, S., Juárez-Pérez, C. A., Jiménez-Ramírez, C., Cabello-López, A., Aguilar-Madrid, G., & Del Razo, L. M. (2021). Prestin and otolin-1 proteins in the hearing loss of adults chronically exposed to lead. Toxicology and Applied Pharmacology, 426, 115651. https://doi.org/10.1016/j.taap.2021.115651

Sutton, L. A., Lonsbury-Martin, B. L., Martin, G. K., & Whitehead, M. L. (1994). Sensitivity of distortion-product otoacoustic emissions in humans to tonal over-exposure: Time course of recovery and effects of lowering L2. Hearing Research, 75(1), 161–174. https://doi.org/10.1016/0378-5955(94)90067-1

Themann, C. L., Byrne, D. C., Davis, R. R., Morata, T. C., Murphy, W. J., & Stephenson, M. R. (2015). Early prognosis of noise-induced hearing loss: Prioritizing prevention over prediction. Occupational and Environmental Medicine, 72(2), 83–84. https://doi.org/10.1136/oemed-2014-102453

Tronstad, T. V., & Gelderblom, F. B. (2016). Sound Exposure During Outdoor Music Festivals. Noise & Health, 18(83), 220–228. https://doi.org/10.4103/1463-1741.189245

Verbeek, J. H. A. M. (2015). Comments on TTS as a predictor of noise-induced hearing loss. Occupational and Environmental Medicine, 72(2), 160–160. https://doi.org/10.1136/oemed-2014-102555

Verbeke, M. (2000). Linear Mixed Models for Longitudinal Data. Springer. https://doi.org/10.1007/b98969

Welch, D., & Fremaux, G. (2017). Why Do People Like Loud Sound? A Qualitative Study. International Journal of Environmental Research and Public Health, 14(8). https://doi.org/10.3390/ijerph14080908

WHO. (2022). WHO global standard for safe listening venues and events. https://www.who.int/publications-detail-redirect/9789240043114

Yang, Zhang, W., Liu, X.-L., Liang, Y., Yuan, Y.-W., Ren, C., & Peng, J.-H. (2014). Localization of prestin and expression in the early period after radiation in mice. European Archives of Oto-Rhino-Laryngology, 271(12), 3333–3340. https://doi.org/10.1007/s00405-014-3180-6

Zhang, X.-D., Thai, P. N., Ren, L., Perez Flores, M. C., Ledford, H. A., Park, S., Lee, J. H., Sihn, C.-R., Chang, C.-W., Chen, W. C., Timofeyev, V., Zuo, J., Chan, J. W., Yamoah, E. N., & Chiamvimonvat, N. (2021). Prestin amplifies cardiac motor functions. Cell Reports, 35(5), 109097. https://doi.org/10.1016/j.celrep.2021.109097

All statistical analyses were performed using R (R Core Team (2023). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.)

Figure legends

Figure 1. An illustration of the experimental procedure. According to our original protocol, PTA and DPOAEs would be conducted at five times points after music exposure. During the study, we decided to stop measurements at the point where PTA thresholds and OAE amplitude returned to baseline.

Figure 2: Mean prestin level before and at specified intervals following music exposure. Error bars show 1 standard error and shadow area the 95% confidence intervals.

Figure 3: Spaghetti plots of serum prestin level in different participants across the different time points.

Figure 4: Mean pure tone thresholds before and at specified intervals following music exposure. Error bars show 1 standard error and grey-shaded

area the 95% confidence intervals. All participants returned to baseline at 4-8 hours after exposure, so no pure tone audiometric thresholds are reported after that.

Figure 5. Mean PTA threshold shifts immediately at 4 minutes after exposure to intense music. Error bars show 1 standard error and grey-shaded area the 95% confidence intervals.

Figure 6: Mean shift of distortion product amplitude 2 minutes after music exposure. Error bars show 1 standard error and grey-shaded area the 95% confidence intervals.

Figure 7: Scatter plot of prestin concentration at baseline and TTS at 6 kHz with a fitted regression line (solid blue) representing the best linear fit. The grey-shaded area around the regression line indicates the 95% confidence interval ($p = 0.031$, $R2 = 0.401$)

Figure 1

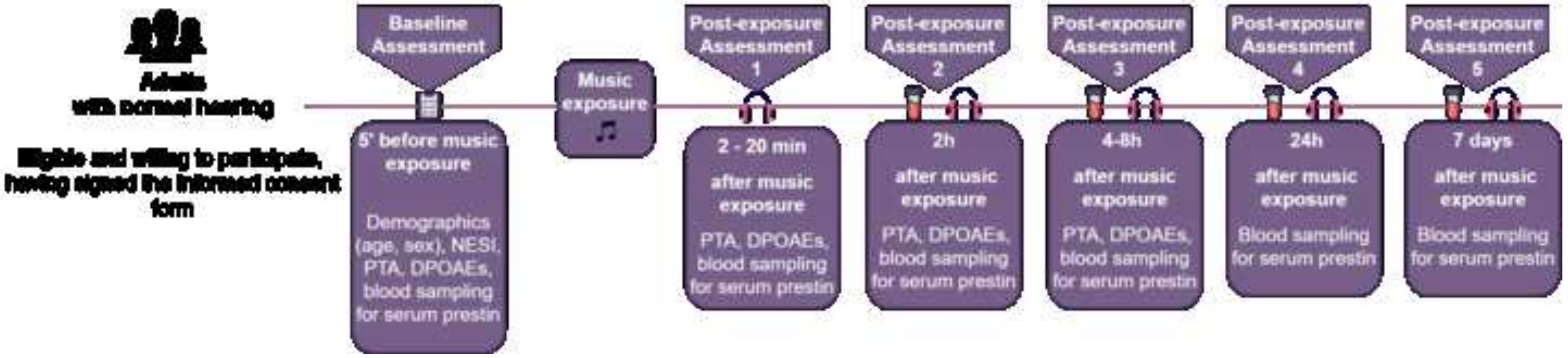

Adults
with normal hearing
Eligible and willing to participate,
having signed the informed consent form
Baseline Assessment
5' before music exposure
Demographics (age, sex), NESI, PTA, DPOAEs, blood sampling for serum prestin
Music exposure
Post-exposure Assessment 1
2 - 20 min
after music exposure
PTA, DPOAEs, blood sampling for serum prestin
Post-exposure Assessment 2
2h
after music exposure
PTA, DPOAEs, blood sampling for serum prestin
Post-exposure Assessment 3
4-8h
after music exposure
PTA, DPOAEs, blood sampling for serum prestin
Post-exposure Assessment 4
24h
after music exposure
Blood sampling for serum prestin
Post-exposure Assessment 5
7 days
after music exposure
Blood sampling for serum prestin

Figure 2

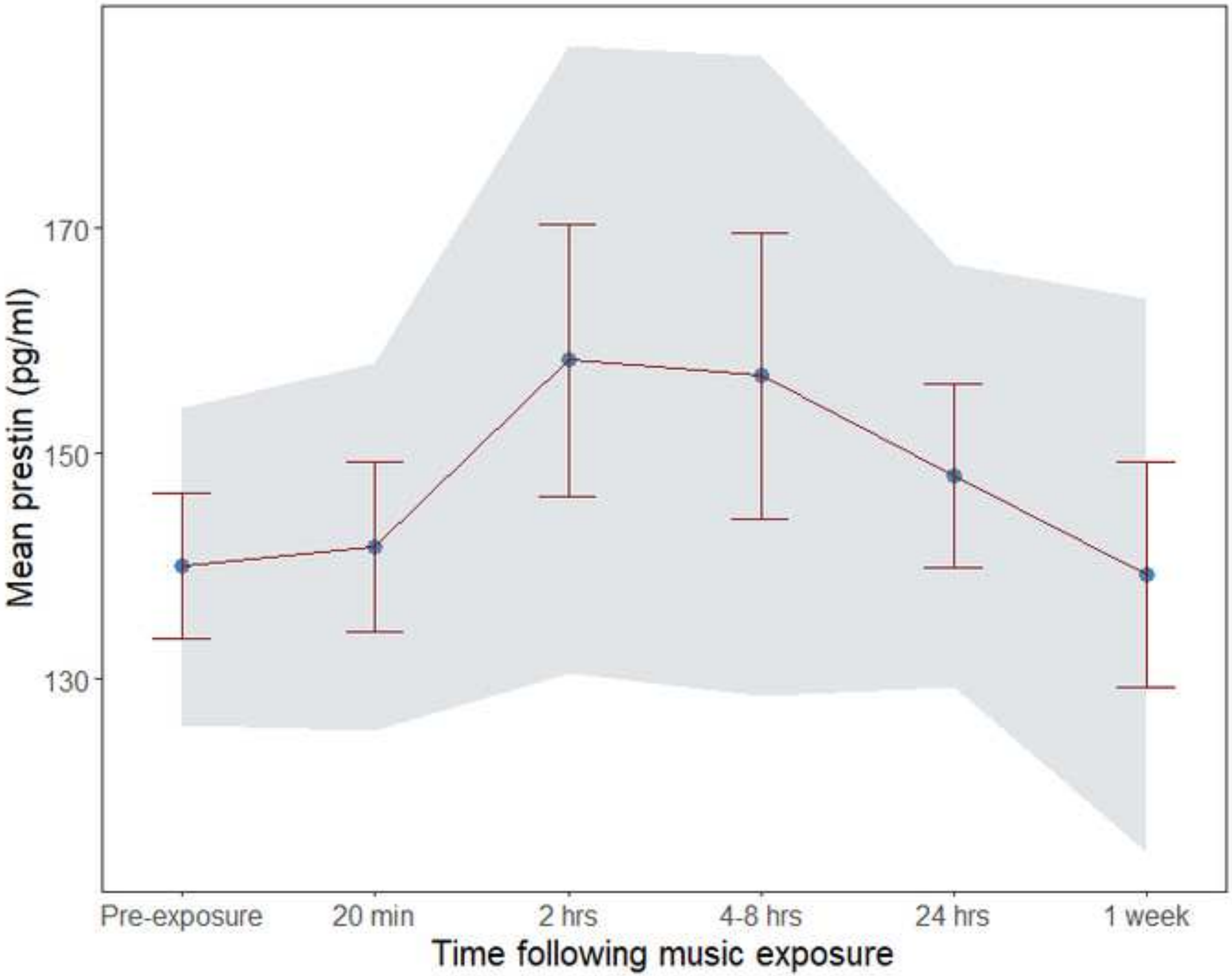

170
150
130
Mean prestin (pg/ml)
Pre-exposure
20 min
2 hrs
4-8 hrs
24 hrs
1 week
Time following music exposure

Figure 3

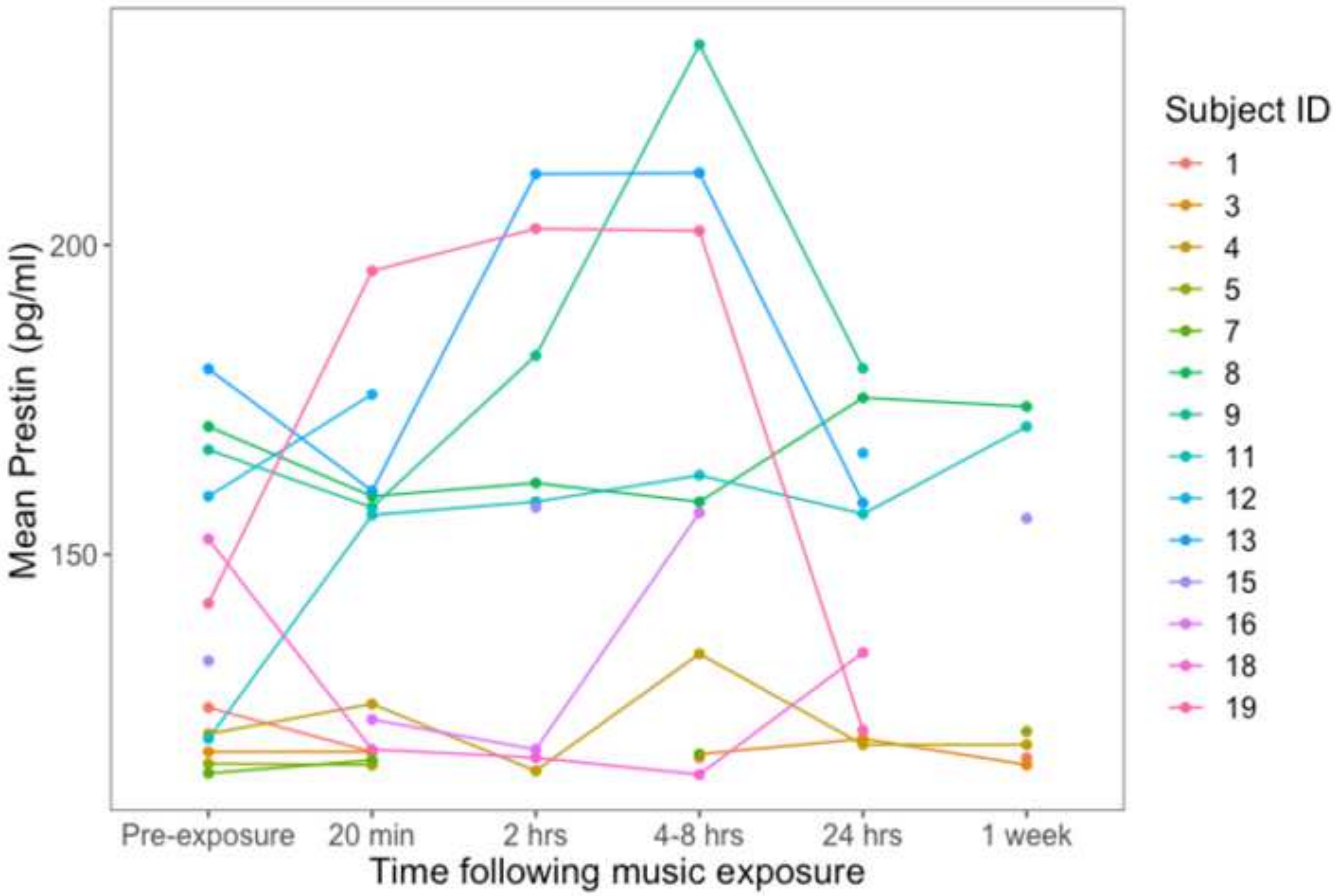

Mean Prestin (pg/ml)
200
150
Pre-exposure
20 min
2 hrs
4-8 hrs
24 hrs
1 week
Time following music exposure
Subject ID
1
3
4
5
7
8
9
11
12
13
15
16
18
19

Figure 4

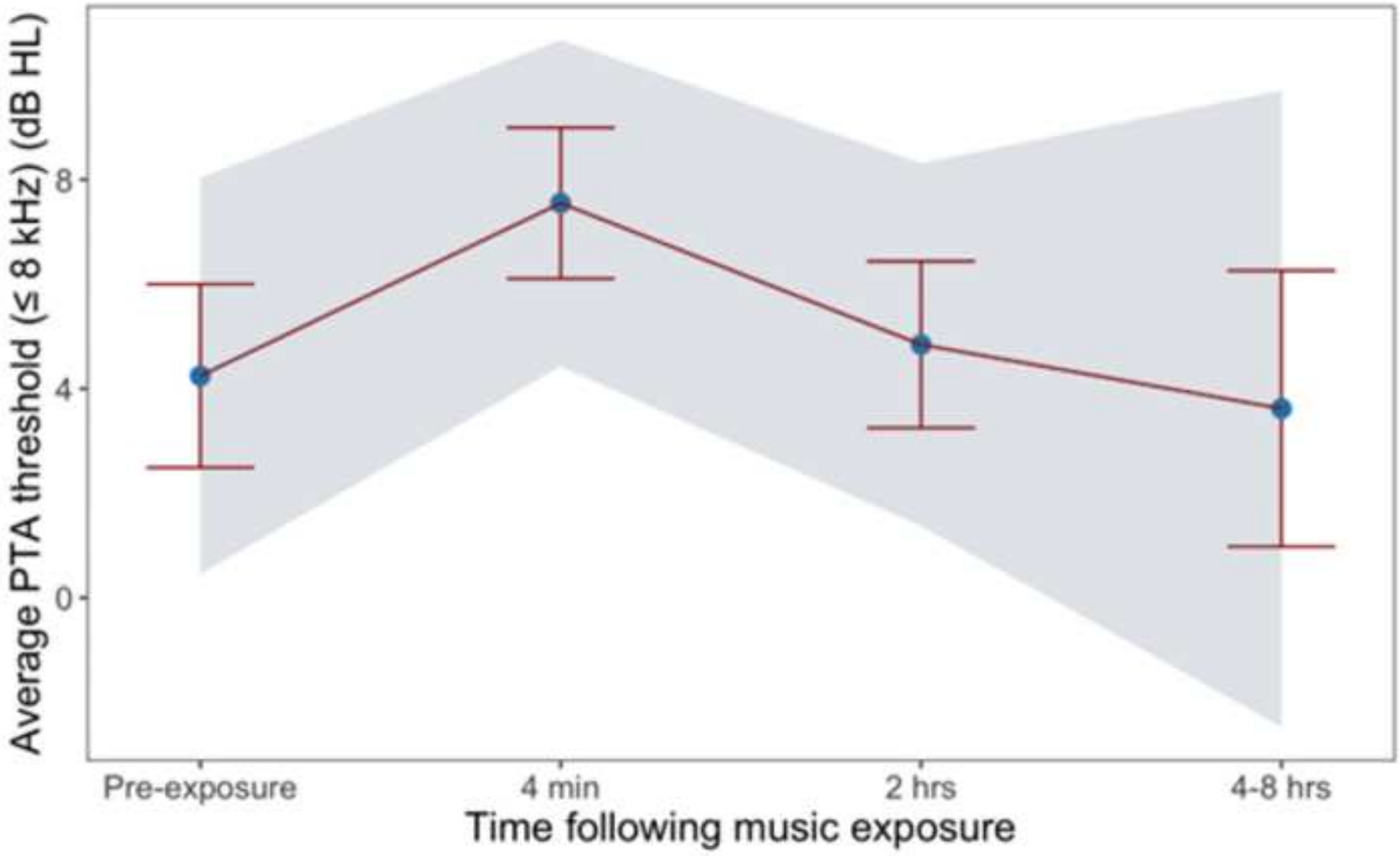

Average PTA threshold (≤ 8 kHz) (dB HL)
8
4
0
Pre-exposure
4 min
2 hrs
4-8 hrs
Time following music exposure

Figure 5

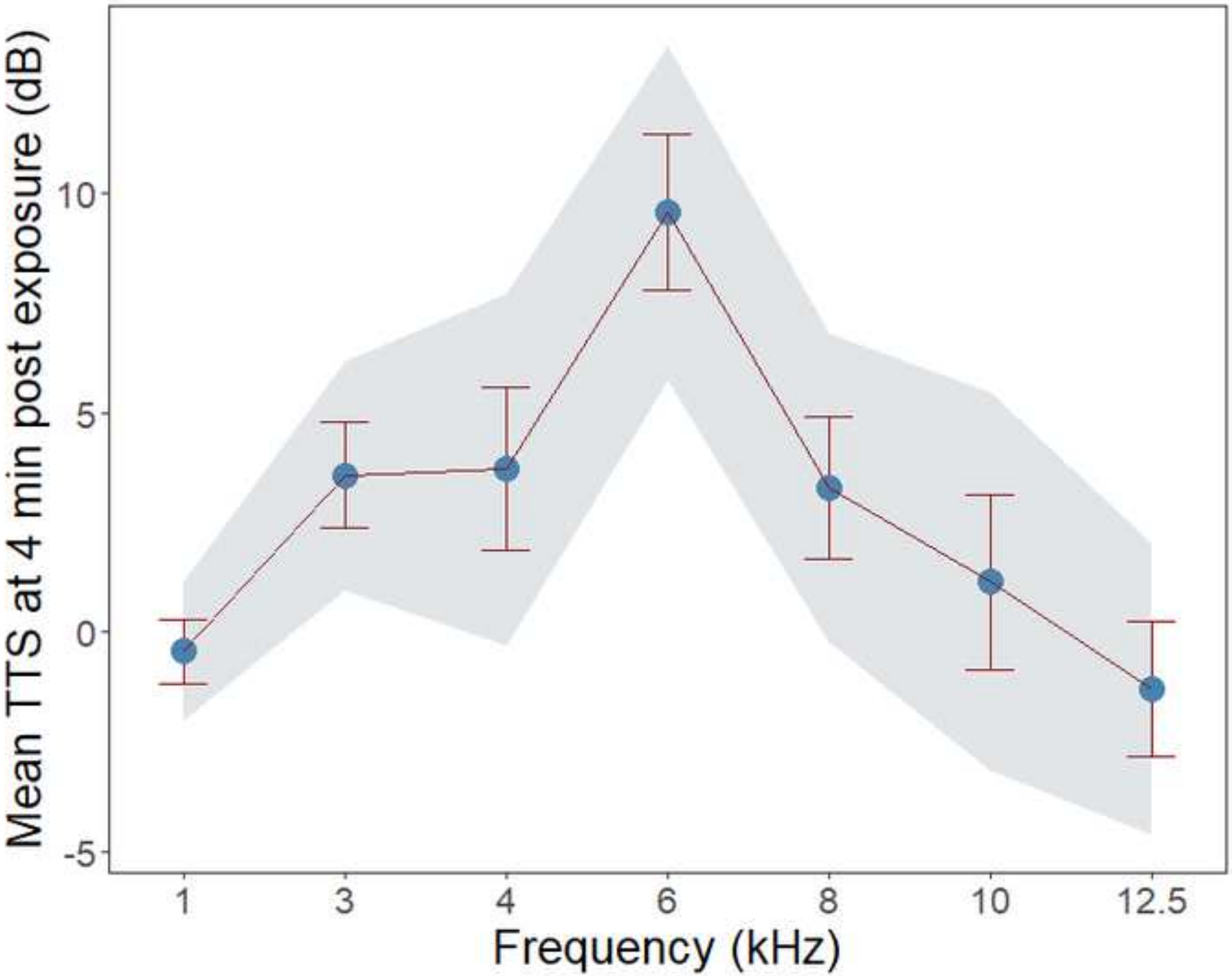

Mean TTS at 4 min post exposure (dB)
10
5
0
-5
1
3
4
6
8
10
12.5
Frequency (kHz)

Figure 6

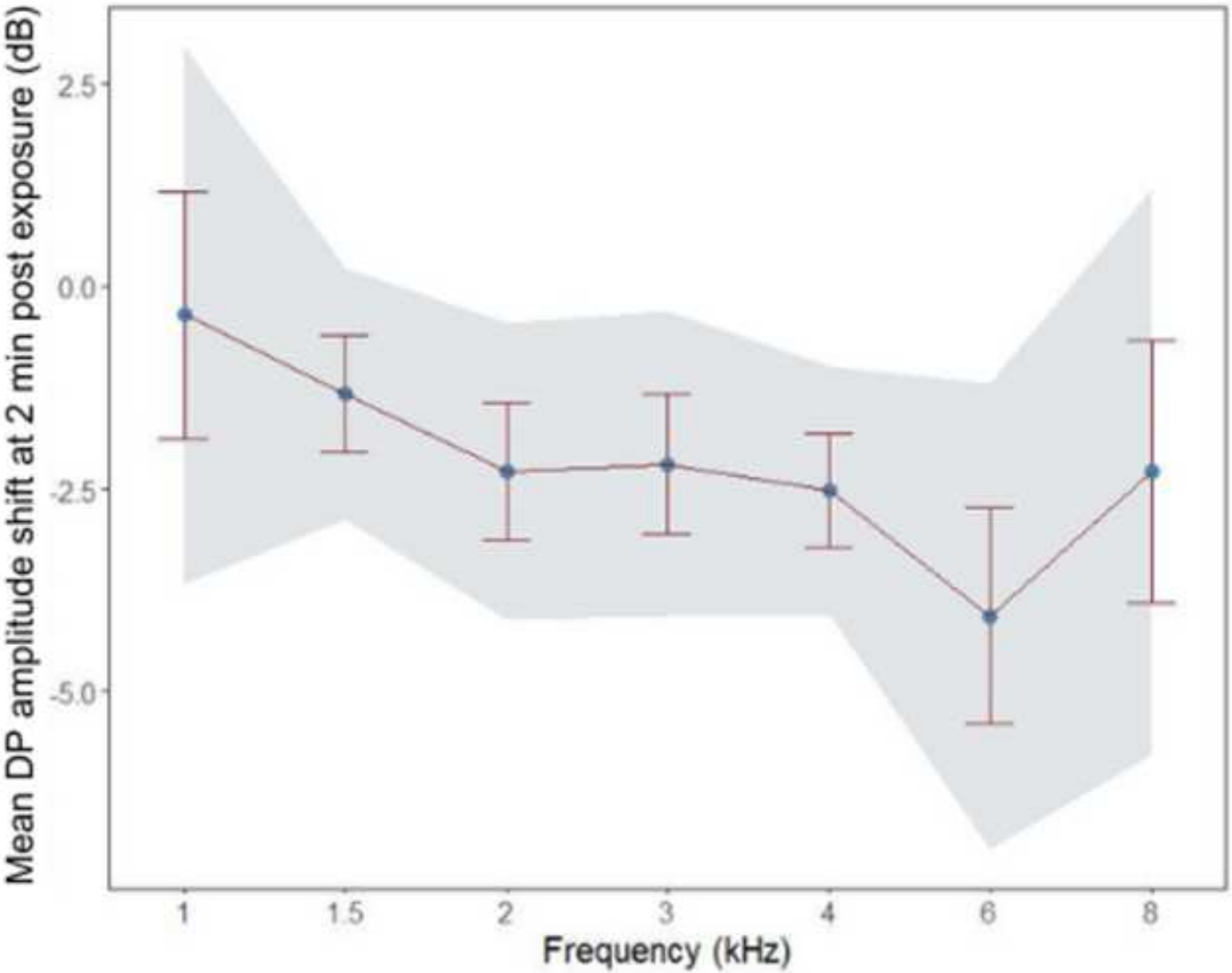
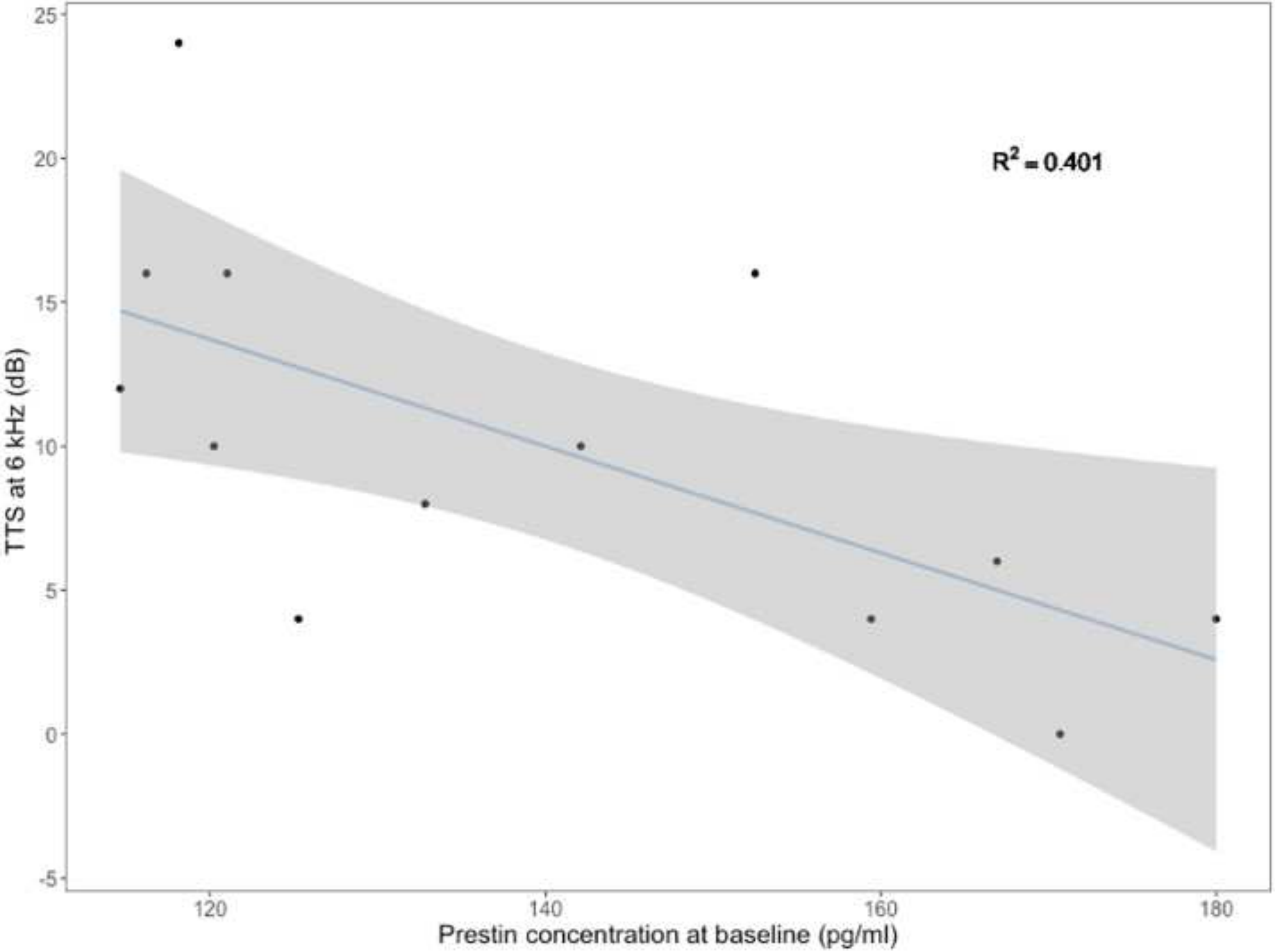

Mean DP amplitude shift at 2 min post exposure (dB)
2.5
0.0
-2.5
-5.0
1
1.5
2
3
4
6
8
Frequency (kHz)

Figure 7

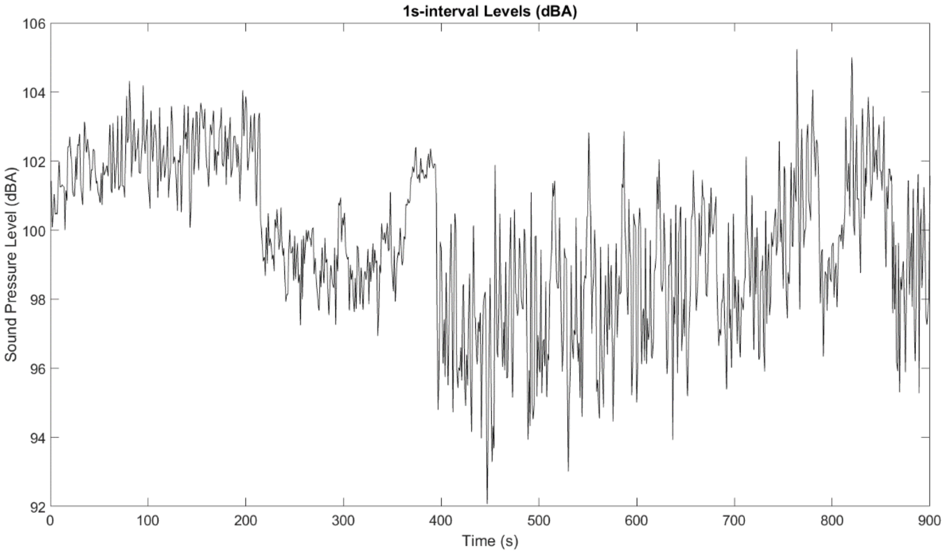

$R^2 = 0.401$
TTS at 6 kHz (dB)
25
20
15
10
5
0
-5
120
140
160
180
Prestin concentration at baseline (pg/ml)

Supplemental Data File (.doc, .tif, pdf, etc.)

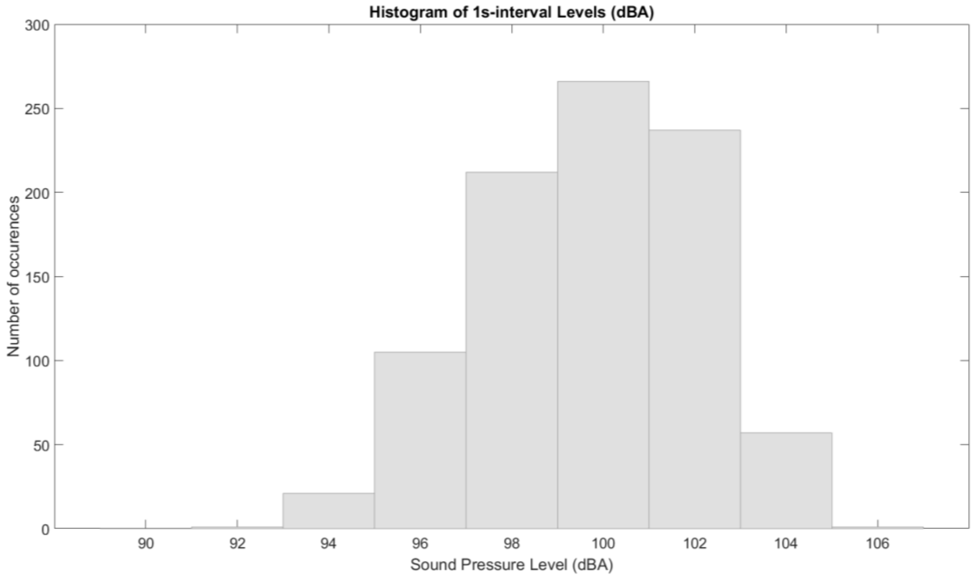
Supplementary material 1: Leq,1s SPL (dBA) statistics of the 30’ audio material

Mean	Median	SD	IQR
99.68	99.73	2.29	3.38 (98.12-101.5)

Supplementary material 2. Sound Pressure Levels (dB A) of 15’ audio material, measured on a BK4128 HATS with TDH-39 headphones

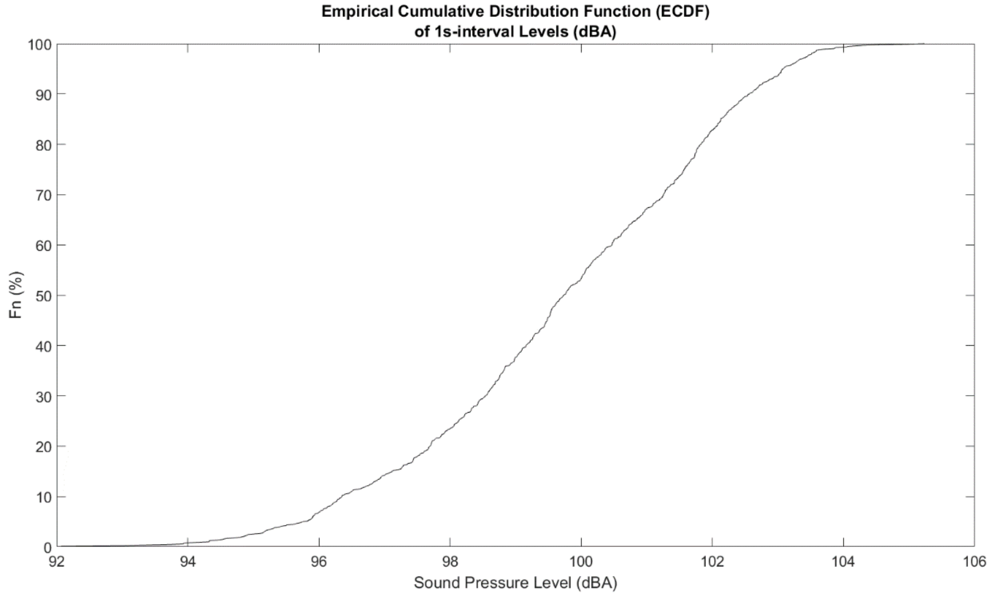


Supplemental Data File (.doc, .tif, pdf, etc.)



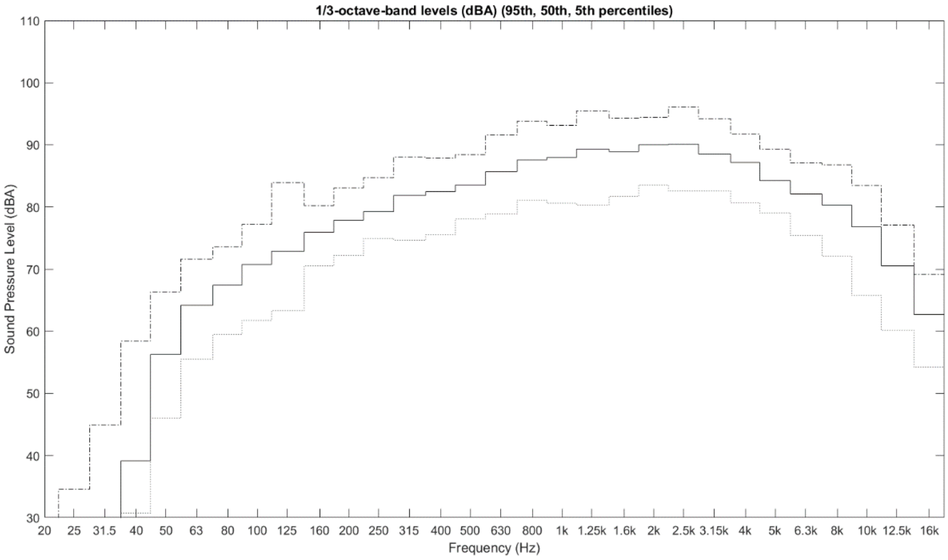
Supplementary Material 3: Histogram of SPL (dB A) of the 15’ audio material

Supplemental Data File (.doc, .tif, pdf, etc.)



Supplementary Material 4: Empirical cumulative distribution of SPL (dB A) of the 15’ audio material

Supplemental Data File (.doc, .tif, pdf, etc.)



Supplementary Material 5: 1/3-octave LTAS of the 15' audio material

Supplemental Data File (.doc, .tif, pdf, etc.)

Supplementary material 6: Differences in DPOAE levels between pre- and post-exposure measurements (at 2 minutes). Highlighted cells correspond to those values that lie outside the 95% CIs.

	1k Hz	1.5 kHz	2 kHz	3 kHz	4 kHz	6 kHz	8 kHz
	2.5	1.1	1.3	1.9	-3.7	-15.6	-10.3
	-1	-1.5	-3.5	-2.8	-1.7	-4	-2.8
	-1.7	-0.2	-1	-1.4	-5.5	-7.4	-2.8
	-0.4	-1.5	-0.2	0.7	-0.4	-3.9	-5.5
	2.1	1.8	-1.2	-1.6	-1.7	-3.5	-4.7
	2.3	-2.3	-2.9	-2.9	-2.4	-2.4	-3.8
	-1.8	-2.6	-1.5	-0.1	-1.6	-2.3	-3.2
	2.2	-1.5	-0.7	-1.2	-1.6	-1.6	-0.1
	0.8	0.5	0	-0.1	-0.3	-2.1	-3.2
	-2.4	-1.3	-0.2	-2.5	-1.9	-2.9	-1.4
	13.9	1.5	-5.8	-9.7	-10.2	-10.4	-5
	-7.1	-4.1	-7.9	-3.1	-1.5	-6.2	-4.6
	-2.3	0.2	0.5	0.4	0.4	-0.9	-1.5
	-12.1	-8.7	-8.9	-8.3	-3.2	6.2	17
SE of measurement	**1.20**	**0.80**	**0.72**	**0.84**	**0.91**	**1.90**	**1.24**
95% CI of the difference	**-3.71**	**-2.47**	**-2.21**	**-2.58**	**-2.82**	**-5.85**	**-3.82**