Evidence of "hidden hearing loss" following noise exposures that produce robust TTS and ABR wave-I amplitude reductions

Edward Lobarinas [a, *], Christopher Spankovich [b], Colleen G. Le Prell [a]

[a] The University of Texas at Dallas, School of Behavioral and Brain Sciences, USA
[b] University of Mississippi Medical Center, Department of Otolaryngology and Communicative Sciences, USA

ARTICLE INFO

ABSTRACT

In animals, noise exposures that produce robust temporary threshold shifts (TTS) can produce immediate damage to afferent synapses and long-term degeneration of low spontaneous rate auditory nerve fibers. This synaptopathic damage has been shown to correlate with reduced auditory brainstem response (ABR) wave-I amplitudes at suprathreshold levels. The perceptual consequences of this "synaptopathy" remain unknown but have been suggested to include compromised hearing performance in competing background noise. Here, we used a modified startle inhibition paradigm to evaluate whether noise exposures that produce robust TTS and ABR wave-I reduction but not permanent threshold shift (PTS) reduced hearing-in-noise performance. Animals exposed to 109 dB SPL octave band noise showed TTS >30 dB 24-h post noise and modest but persistent ABR wave-I reduction 2 weeks post noise despite full recovery of ABR thresholds. Hearing-in-noise performance was negatively affected by the noise exposure. However, the effect was observed only at the poorest signal to noise ratio and was frequency specific. Although TTS >30 dB 24-h post noise was a predictor of functional deficits, there was no relationship between the degree of ABR wave-I reduction and degree of functional impairment.

1. Introduction

Noise-induced hearing loss (NIHL) is a significant public health problem in industrialized nations across the world. In the United States, the Occupational Safety and Health Administration (OSHA) provides workplace guidelines for noise exposure limits whereas the National Institute for Occupational Safety and Health (NIOSH) provides more conservative recommendations on exposure limits (OSHA, 1983; NIOSH, 1998). Although there are differences between these organizations with respect to recommended exposure limits, both OSHA and NIOSH monitor noise-induced damage to the inner ear using the metric of permanent changes in hearing thresholds. In the military, noise exposure is monitored by the Department of Defense and NIHL is a major safety and healthcare concern that can affect mission readiness and long-term quality of life for service members. The majority of reports on noise and hearing loss in military personnel, workers exposed to occupational noise, and adolescents and young adults exposed to recreational noise are cross-sectional in nature. Like civilian and military occupational noise, unregulated recreational noise exposures can also influence hearing health. The World Health Organization (WHO), for example, recently issued a press release stating, "Some 1.1 billion teenagers and young adults are at risk of hearing loss due to the unsafe use of personal audio devices, including smartphones, and exposure to damaging levels of sound at noisy entertainment venues such as nightclubs, bars and sporting events," (WHO, 2015).

Until recently, noise exposures that produced temporary threshold shift (TTS) but no evidence of permanent threshold shift (PTS) in the audiogram were not typically considered hazardous (for discussion of pathological TTS see Dobie and Humes, 2016). Increased thresholds are generally well correlated with outer hair cell (OHC) damage in the inner ear (Dallos and Harris, 1978; McGill and Schuknecht, 1976; Ohlms et al., 1991; Patuzzi et al., 1989; Stebbins et al., 1979). However, recent data from animal models suggest that a permanent form of noise-induced inner ear neural damage can occur in the absence of OHC loss and accompanying PTS. This trauma has been termed "synaptopathy" because the site of lesion appears to involve inner hair cell (IHC) synaptic ribbons and corresponding afferent type-I auditory nerve fibers (for review see, Kujawa and Liberman, 2015).

---

* Corresponding author. The University of Texas at Dallas, Callier Center, 1966 Inwood Road, Dallas, TX, 75235, USA.
   E-mail address: Edward.Lobarinas@utdallas.edu (E. Lobarinas).

Abbreviations		WHO	World Health Organization
		AALAC	American Association for Laboratory Animal Care
ABR	auditory brainstem response	IHC	inner hair cell
BBN	broadband noise	OHC	outer hair cell
DPOAE	distortion product otoacoustic emissions	IACUC	Institutional Animal Care and Use Committee
NIHL	noise-induced hearing loss	dB	decibel
NIOSH	National Institute for Occupational Health	dB SPL	decibel sound pressure level
NBPIAPS	noise-burst prepulse inhibition of the airpuff startle	kHz	kilohertz
OSHA	Occupational Health and Safety Administration	FFT	Fast Fourier Transform
PTS	permanent threshold shift	ANOVA	analysis of variance
SNR	signal-to-noise ratio	SP	summating potential
TTS	temporary threshold shift	AP	action potential

Evidence for noise-induced synaptopathy has been found in both mice (Fernandez et al., 2015; Hickox and Liberman, 2014; Jensen et al., 2015; Kujawa and Liberman, 2009) and guinea pigs (Furman et al., 2013; Lin et al., 2011) following acute noise exposures that result in robust TTS. Based on the initial rodent studies in which decreased ABR amplitudes and synaptopathic damage were limited to those frequencies with the largest measured TTS, our team suggested that the "critical boundary" at which synaptopathic change begins might be on the order of approximately 30-dB TTS measured 24-h following an acute noise exposure (Le Prell et al., 2012; Spankovich et al., 2014). Indeed, more recent data have confirmed that whereas decreased auditory brainstem response (ABR) amplitudes are observed following larger TTS changes (>30 dB 24 h post-noise), smaller TTS (<30 dB 24 h post noise) changes are generally not accompanied by long-term ABR amplitude decreases (Fernandez et al., 2015; Hickox et al., 2014; Jensen et al., 2015). However, recent data from Fernandez et al. (2015) clearly show that the relationship will be more complicated than a simple TTS criterion change at each frequency. A TTS of approximately 30-dB 24-h post noise at the 22.6 kHz test frequency was synaptopathic (in mice) when there was a more robust TTS at the higher frequencies, but the same 30-dB TTS 24-h post-noise at the 22.6 kHz test frequency was not synaptopathic (in mice) when there was not a more robust TTS at the higher frequencies. In addition, longer duration (168 h) exposures at lower levels (84 dB SPL) that produce much lower levels of TTS (<15 dB less than 1 h post-exposure) appear to reduce wave-I suprathreshold amplitudes as a consequence of synaptopathic damage (Maison et al., 2013), although a recent comparison of ribbon counts across studies revealed that ribbon counts in the noise-exposed ears were generally equivalent to ribbon counts published in other studies with larger samples of mice (Le Prell and Brungart, 2016).

When there is a significant and permanent reduction in the ABR wave-I amplitude to suprathreshold stimuli, it is possible that there may be corresponding supra-threshold functional deficits despite the complete recovery of threshold measures. In chinchillas with significant IHC loss, thresholds are largely unaffected, but listening in noise is compromised, particularly at frequencies more than one critical bandwidth from the target stimulus (Lobarinas et al., 2016). For human patients, it is well known that some patients have disproportionate difficulty understanding speech in noisy backgrounds, and it has been suggested that synaptopathic damage in human ears might perhaps explain these deficits (Kujawa and Liberman, 2015; Liberman et al., 2015). This suggestion is supported by the evidence that synaptopathic damage appears to primarily affect lower and medium spontaneous rate fibers that are activated at higher intensity sound levels (Furman et al., 2013) as these lower spontaneous rate fibers are resistant to masking due to their higher thresholds and wider dynamic range (Kujawa and Liberman, 2015). Another suprathreshold deficit that has been suggested as a possible functional correlate of synaptopathic damage is hyperacusis (Hickox et al., 2014). To date, however, there is no direct evidence that synaptopathy produces (or fails to produce) measurable hearing deficits. Interestingly, in contrast to the changes observed after noise exposure, when low spontaneous rate fibers were damaged in gerbils using oubain, the amplitude of the compound action potential and by extension the amplitude of ABR wave-I were unchanged (Bourien et al., 2014). Taken together, it appears that significant damage to the hair cell/auditory nerve fiber interface can occur before changes in threshold or evoked potential amplitudes become evident. Given the potential for undetected cochlear pathology, particularly for noise exposures that generate robust TTS but no evidence of PTS, it is imperative to determine if there are any corresponding functional deficits.

To address this gap in the evidence, we developed a rat model to determine if noise exposures that produce robust TTS and permanent reductions in suprathreshold ABR wave-I amplitude, a correlate of significant synaptopathic damage, also negatively impact hearing-in-noise performance. If permanent reductions in suprathreshold ABR wave-I amplitude are accompanied by evidence of hearing-in-noise deficits in animals, the data would provide significant support for the suggestion that noise exposures once considered benign need to be reevaluated with respect to assumed noise hazard and potential functional deficits in the absence of overt threshold shift (Kujawa and Liberman, 2009, 2015; Maison et al., 2013).

2. Materials and methods

2.1. Subjects

Ten adult male Sprague Dawley rats (9–12 months) were used. Animals were housed in an AALAC-approved animal facility at the University of Florida. Rats were housed in Plexiglass cages with free access to food and water and were maintained on a normal 12-h light/dark cycle in a temperature-controlled room. All of the experimental procedures used were approved by the University of Florida Institutional Animal Care and Use Committee (IACUC).

2.2. Anesthesia

All physiological experiments and noise exposures were performed under ketamine and xylazine anesthesia (ketamine 100 mg/kg i.p.; xylazine, 10 mg/kg, i.p).

2.3. Physiological assessment equipment

Subjects were tested in a dedicated walk-in double-walled sound-attenuating chamber. ABR threshold and amplitude were assessed using Tucker-Davis Technology (TDT, Alachua, FL, USA) software (SigGen, BioSig) and System III hardware. ABR responses were recorded via subdermal needle electrodes (vertex-ventrolateral to pinna). The response from the electrodes was amplified (10,000×), filtered (0.3–3 kHz), digitized, and averaged (1052 stimuli; BioSig). Distortion Product Otoacoustic Emissions (DPOAE) testing was performed using the same TDT system with two calibrated speakers connected via tubing to an ER10B + low noise microphone probe assembly (Eytmotic Research, Elk Grove Village, IL). DPOAEs were recorded using two primary tones, f1 and f2, using an f2/f1 ratio of 1.2., and levels based on the scissor paradigm, L12 = 0.4L2 + 39, where L2 = 70, 60, 50, 40, and 30 dB. During DPOAE testing, the probe assembly was placed in the animal's external ear canal.

2.4. DPOAE and ABR

DPOAE amplitude data provided an indirect measure of OHC integrity at frequency specific regions of the inner ear. DPOAE suprathreshold amplitudes were recorded at 2f1-f2. Input/output functions were obtained by increasing the L1 (and corresponding L2) in 10-dB steps from 30 to 70 dB SPL at f2 frequencies of 8, 16, 24 and 32 kHz. ABR threshold and suprathreshold amplitude were measured, and the amount of threshold shift and suprathreshold amplitude shift induced by noise exposure were calculated relative to pre-noise baseline. ABR testing was performed at 8, 16, 24, and 32 kHz. Acoustic stimuli were initially presented at 90-dB SPL and decremented in 10-dB steps to approximately 10 dB below threshold. Threshold was defined as the lowest intensity of stimulation that yielded a repeatable waveform based on an identifiable ABR wave-I. The wave-I peak-to-peak amplitude was computed by off-line analysis of stored waveforms. ABR and DPOAE testing was performed 24 h before noise exposure and at 24-h and 2-weeks post noise exposure.

2.5. Noise exposure

Rats were exposed bilaterally under ketamine/xylazine anesthesia to bandpass noise (8–16 kHz) of 106 or 109 dB SPL for 2 h in a calibrated sound field.

2.6. Noise exposure equipment

Noise exposure stimuli were generated digitally (Tucker-Davis Technology, RP2.1), filtered (RPVDS software), amplified (AudioSource AMP One/A) and delivered inside a sound-attenuating chamber via a single speaker (Fostex FT17) in a calibrated sound field. The level was calibrated using a ½ inch microphone (Brüel & Kjær instruments, 4134) and a sound level meter with a fast Fourier transform (FFT) analyzer (Larson Davis 831).

2.7. Behavioral assessment equipment

Hearing-in-noise was assessed in cages (20 cm L, 7 cm W, 6 cm H) constructed of acoustically transparent wire-mesh (0.5 cm × 0.5 cm). Cages were mounted on Plexiglas bases (20 cm × 10 cm) resting on pressure-sensitive 35-mm piezoelectric transducers (MCM 28–745) that generated a voltage proportional to the magnitude of the animal's startle response. The output of the startle platform was calibrated using an oscilloscope and various weights (10–40 g) dropped from a fixed distance (3 cm). The startle platform was housed in a commercial, sound-attenuating cubicle (Med-Associates ENV-022 V, 25.0″ W x 16.5″ H x 15.5″ D) lined with acoustic foam (noise floor less than 20 dB SPL at frequencies greater than 4000 Hz). Sound stimuli were generated in a calibrated sound field (TDT RP2.1 ~100 kHz sampling rate), amplified, and delivered via a free-field speaker (Fostex FT17H) placed above the startle platform (25 cm). A startling airpuff stimuli (20 ms) was generated using a pressure valve (19 PSI) to regulate house air and was controlled by triggering a solenoid air valve (Med Associates ESUB-PHM-276) placed 3 cm above the animal in the startle chamber. The output of the startle platform was amplified, digitized and low-pass filtered by an A/D converter (TDT RP2.1, ~6 kHz sampling rate), and stored on a computer for offline analysis.

2.8. Behavioral assessment of hearing in noise

Hearing-in-noise was assessed using a narrowband noise burst cue presented prior to a tactile startle elicitor (airpuff) in the presence of an ongoing noise background, a method described in detail in our previous publication (Lobarinas et al., 2013). The main advantage of using a tactile startle elicitor is that noise exposure has the potential of reducing an acoustically evoked startle response, independent of the audibility of the preceding cue. In contrast, a tactile startle elicitor retains its efficacy even in the presence of hearing loss or changes in loudness perception. Because the dependent variable is the inhibition of the startle response as a function of a preceding acoustic cue, maintaining a robust pre- and post-noise startle response was essential to the experimental design.

Briefly, animals were conditioned (2 acclimatizing sessions, 5 baseline runs) to associate the acoustic cue with a subsequent airpuff such that the acoustic cue served as a warning signal and attenuated the startle response. The noise background and the acoustic target were then independently manipulated to assess the efficacy of the target in the noise background in suppressing the startle response. Under the noise burst pre-pulse inhibition of the airpuff startle (NBPIAPS) paradigm, acoustic targets were presented prior to the onset of a 12–15 PSI airpuff. Audible stimuli presented before the airpuff reliably produced a robust suppression of the startling response to the tactile stimulus. In contrast, inaudible cues produced startle responses indistinguishable from control trials containing no pre-airpuff acoustic stimuli.

Narrowband target stimuli were presented in competing background noise in experimental sessions containing 216 trials. Of these trials, 36 served as control trials with only a continuous broadband background noise (BBN) carrier presented at 30, 50, 55, or 60 dB SPL. The remaining 180 trials contained 70 dB SPL narrowband noisebursts (50 ms, 100 Hz bandwidth, centered at 8, 12, 16, 20, and 24 kHz, ~36 dB/octave roll-off) generated with a digital filter function (TDT RPVDS) and presented 100 ms before an airpuff startle stimulus in the presence of the continuous BBN masker. The signal-to-noise ratio (SNR) of the 50 ms noiseburst relative to the BBN was therefore 40, 20, 15 or 10 dB SNR, with the quietest BBN (30 dB SPL) condition having the best SNR (40 dB). Audibility of the target stimuli at each SNR was determined by statistical comparison of the average startle response amplitude for cued versus uncued trials within a given session. During the experiments, animals were run once per day, three days a week.

2.9. Study design and analysis

A repeated measures design was used. ABR and DPOAE were evaluated 24 h prior to noise exposure (baseline), 24 h post-exposure, and 2 weeks post-exposure. Baseline NBPIAPS was assessed prior to the noise exposure and 2 weeks post exposure.

Data were analyzed using 2-way repeated measures analysis of variance (ANOVA) to determine the statistical reliability of the observed effects of noise exposure on ABR threshold, ABR wave-I amplitude, DPOAE amplitude, and NBPIAPS in varying SNR. All statistical comparisons used an alpha level of 0.05 and post-hoc analysis was performed using the Student-Newman-Keuls method to avoid type-I errors associated with multiple comparisons. Sigma Stat 12.5 was used for these statistical analyses. All results are presented as mean ± standard deviation (SD). Additional analyses to determine covariate relationships were performed using Spearman rho correlations. ABR wave-I amplitude at 90 dB SPL, ABR wave-I amplitude shift at 90 dB SPL (baseline - 2 weeks post noise), NBPIAPS at 20 dB SNR, NBPIAPS at 20 dB SNR shift (baseline − 2 weeks post), post noise ABR threshold, and TTS were compared. SPSS software was used with an alpha level offset to 0.05 for all comparisons.

3. Results

3.1. ABR threshold shift

ABR thresholds were determined 24 h pre, 24 h post, and 2 weeks post noise exposure. Initially, two rats were exposed to 106-dB SPL noise. The 106-dB SPL noise exposure produced TTS of 20−25 dB at 24 hours with full recovery of threshold (Fig. 1a) at the 2 week post noise assessment. Given the working hypothesis that there is a critical synaptopathic boundary associated with approximately 30-dB TTS 24-h post-noise, the exposure level was increased to 109-dB SPL for eight additional rats. After 109-dB SPL exposure, TTS was on average ~35 dB 24-h post noise (Fig. 1a) with full recovery by 2 weeks post-noise. The pattern of the TTS was consistent with the 8−16 kHz octave band noise exposure. A repeated measures ANOVA revealed a significant main effect of noise exposure [$F(2,42) = 146.457$, $p < 0.001$] and an interaction effect between frequency and noise exposure [$F(6,42) = 14.238$, $p < 0.001$] on ABR thresholds. Post-hoc analysis showed a significant difference between baseline and 24 h post noise ($p < 0.001$) and 24 h post noise and 2 weeks post noise ($p < 0.001$) The small differences between thresholds at baseline and 2 weeks post-noise were not statistically significant ($p = 0.236$). These results suggested that ABR thresholds showed generally complete recovery 2 weeks post noise.

3.2. DPOAE amplitudes

DPOAE amplitudes were measured 24 h pre (baseline), 24 h post, and 2 weeks post-exposure. Both 106 and 109 dB SPL noise exposures reduced DPOAE amplitude 24 h post exposure, results that were consistent with the ABR TTS. A 2-way repeated measures ANOVA showed a statistically significant effect of noise exposure on DPOAE amplitudes ($p < 0.001$). A post-hoc analysis showed a significant difference at 24 h post noise ($P < 0.001$) but no significant difference between baseline and 2 weeks post-noise DPOAE amplitude ($p = 0.283$). Fig. 2 shows DPOAE ($L2 = 70$ dB SPL) amplitudes at baseline, 24 h, and 2 weeks post 106 and 109 dB SPL noise exposure. Amplitudes returned to baseline levels 2 weeks post noise at all frequencies tested, suggesting no significant damage of OHC function. Similarly, complete recovery was found at L2 levels of 30, 40, 50, and 60 dB SPL ($p = 0.137$) (data not shown).

3.3. ABR amplitudes

The most commonly reported biomarker of synaptopathic noise-induced trauma is a marked reduction in ABR wave-I amplitude at suprathreshold stimulus levels in the absence of a
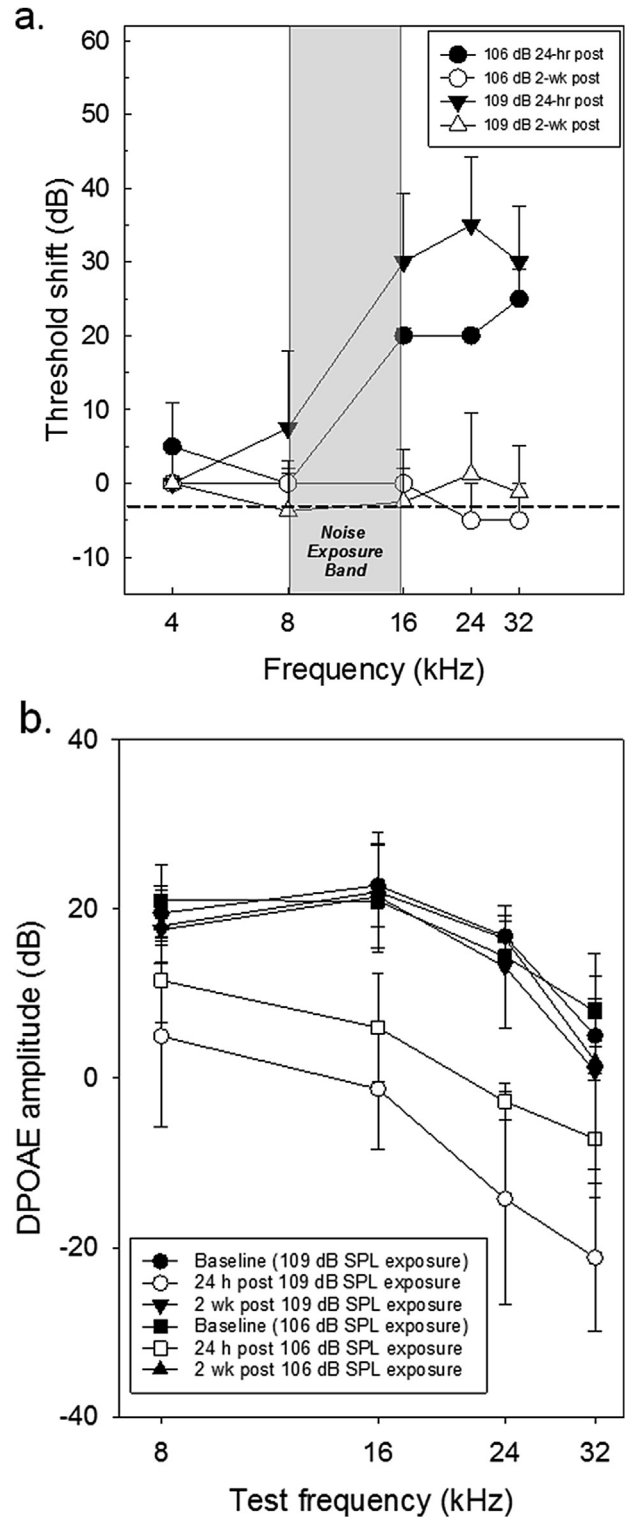

Fig. 1. (a.) Average ABR temporary threshold shift as a function of test frequency 24 h and 2w post 106 or 109 dB SPL, 2 h exposure (8−16 kHz octave band noise, shaded area). TTS of 20−25 dB was observed for the 106 dB SPL exposure and >30 dB for the 109 dB SPL exposure. Thresholds completely returned to baseline levels 2w post-exposure. (b.) Average DPOAE amplitudes at 24 h and 2wk post-106 and 109 dB SPL exposure as a function of test frequency. DPOAE amplitudes were reduced at 24 h and returned to baseline levels by 2w; effects consistent with a temporary threshold shift.

PTS and with no reduction in DPOAE amplitude. Following the 106-dB SPL noise exposure, there were no permanent changes in DPOAE
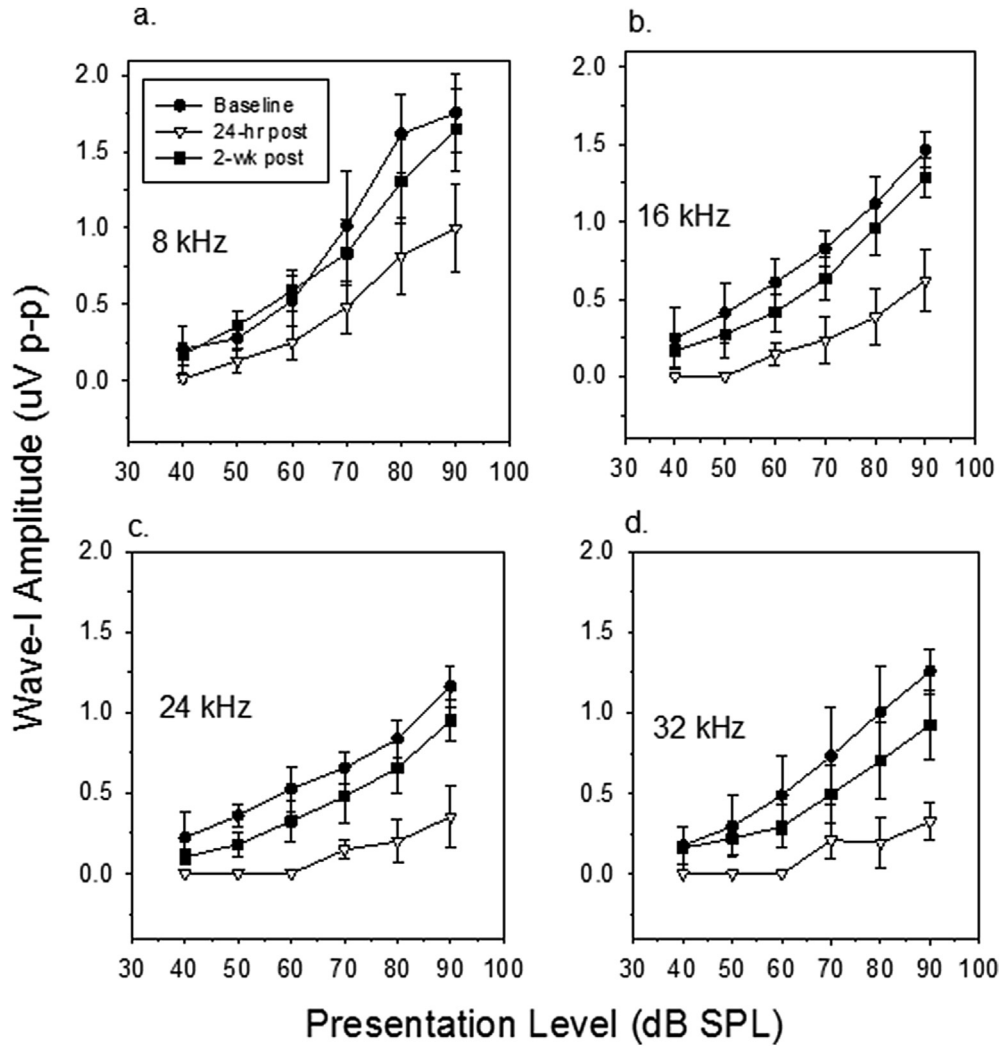
Fig. 2. Average ABR wave-I amplitudes 24 h and 2w post 109 dB SPL exposure as a function of presentation level. Panels a–d show the input-output function of ABR wave-I amplitudes for frequencies 8–32 kHz. Although thresholds returned to baseline levels 2w post-exposure, amplitudes at 16, 24, and 32 kHz failed to return to baseline levels, results interpreted as evidence of hidden hearing loss.

amplitudes or ABR wave-I amplitude 2 weeks post exposure; all of the observed changes were temporary and reversible, at least for these two preliminary test subjects. In contrast, as shown in Fig. 3, there was a reduction in ABR wave-I amplitude 24 hours after the 109-dB SPL noise exposure at all four test frequencies (8, 16, 24, and 32 kHz) that failed to recover at 16, 24, and 32 kHz 2-weeks post exposure test. A 2-way repeated ANOVA revealed a significant main effect of noise exposure on ABR wave-I amplitude for 8 kHz [$F_{(2,70)} = 2.320$, $p < 0.001$], 16 kHz [$F_{(2,70)} = 3.334$, $p < 0.001$], 24 kHz [$F_{(2,70)} = 2.739$, $p < 0.001$], and 32 kHz [$F_{(2,70)} = 3.230$, $p < 0.001$]. There were also interaction effects between ABR presentation level and condition (i.e. baseline, 24 hours, and 2 wk post noise) for 8 kHz [$F_{(10,70)} = 5.740$, $p < 0.001$], 16 kHz [$F_{(10,70)} = 2.133$, $p = 0.033$], 24 kHz [$F_{(10,70)} = 5.710$, $p < 0.001$], and 32 kHz [$F_{(10,70)} = 11.634$, $p < 0.001$]. Post-hoc analysis showed a significant difference in ABR wave-I amplitude between baseline and 24 hours post noise ($p < 0.001$) and between baseline and 2 weeks post noise ($p < 0.05$) indicating that ABR amplitudes did not return to baseline levels 2 week post exposure. ABR amplitude differences were found at 16, 24, and 32 kHz between baseline and 2 weeks post exposure for presentation levels of 60, 70, 80 and 90 dB SPL ($p < 0.05$) but no amplitude differences were found at
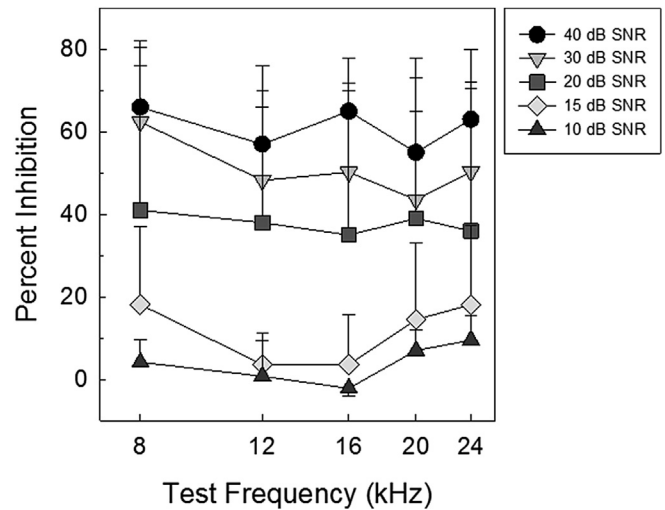

Fig. 3. Average percent inhibition as a function of frequency and SNR. Inhibition was robust at high SNR (30–40) and 20 dB SNR was the lowest condition with reliable and statistically significant startle suppression. The lowest SNR conditions (10–15 dB SNR) were not statistically different from uncued trials.

stimulus levels of 40 and 50 dB SPL. The effects of the 109-dB SPL noise exposure on ABR wave-I amplitudes observed here were consistent with changes reported previously in mice and guinea pigs with confirmed synaptopathic damage in that there was a selective decrease in ABR wave-I amplitude despite recovery of ABR threshold and DPOAE amplitude.

3.4. Startle amplitudes

In a previous study we showed that whereas acoustically evoked startle response amplitudes were susceptible to the effects of noise exposure, tactile evoked startle such as the airpuff used in this study were relatively resistant to the effects of noise exposure and hearing loss (Lobarinas et al., 2013). In order to rule out the potential confounding effects of noise exposure on startle amplitude, we evaluated the mean startle amplitude of uncued trials before and 2 weeks post noise exposure. A one-way repeated measures ANOVA showed that there was no statistically significant difference between startle amplitudes before the noise exposure and 2 weeks post exposure [$F(1,7) = 0.440$, $p = 0.528$].

3.5. Functional deficits

Prior to noise exposure, baseline hearing-in-noise performance was assessed with the SNR manipulated from 40 dB (easiest) to 10 dB (hardest). As shown in Fig. 4, in the 40 dB SNR condition, there was robust attenuation of the startle response indicating the subjects easily detected the NBN in the competing BBN background. Lowering the SNR to 20 dB reduced NBPIAPS but performance was still statistically significantly different ($p < 0.05$) from uncued control trials. In contrast, NBPIAPS at 10–15 dB was not statistically significant from uncued trials, indicating that the rats could not readily detect the 70-dB SPL NBN cue in the higher level noise backgrounds (55–60 dB SPL BBN) during baseline testing prior to the experimental noise exposure. The 40 and 20 dB SNR conditions were therefore the only conditions re-evaluated after noise exposure to determine the effects of noise exposure on hearing-in-noise. The two animals exposed to 106 dB SPL noise, that showed no reduction in ABR wave-I 2-weeks post-noise, did not show any changes in NBPIAPS at either the 20 or 40 dB SNR (not shown). In the eight animals exposed to 109-dB SPL noise, NBPIAPS at 40 dB SNR remained unchanged 2 weeks post exposure (Fig. 4a). NBPIAPS performance at 20 dB SNR, 2 weeks post noise, remained similarly unchanged at 8 and 12 kHz but was reduced at 16, 20, and 24 kHz (Fig. 4b). A 2-way repeated measures ANOVA showed no significant change in NBPIAPS at 40 dB SNR, 2 weeks post noise at any test frequency [$F1,28) = 0.0375$, $p = 0.852$]. In contrast, when we evaluated NBPIAPS at 20 dB SNR we found a statistically significant effect of noise exposure [$F(1,28) = 38.652$, $p < 0.001$]. A post-hoc analysis showed a significant effect of noise exposure for 16 kHz ($p = 0.002$), 20 kHz ($p = 0.009$) and 24 kHz ($p = 0.013$). The noise exposure had no reliable effect on 8 kHz ($p = 0.056$) and 12 kHz ($p = 0.221$) hearing-in-noise performance.

3.6. Relationship among TTS, ABR wave-I amplitude, and NBPIAPS

To determine whether subjects with the poorest post-noise performance also showed greater TTS and ABR wave-I amplitude changes, subjects were divided into two groups (Group 1: the four animals with the least post exposure signal-in-noise performance change; Group 2: the four animals with the most post exposure signal-in-noise performance change). There were no statistically significant baseline or post noise differences between Group 1 and Group 2 at 40 dB SNR ($p = 0.151$, $p = 0.223$) or 20 dB SNR ($p = 0.101$, $p = 0.327$) or interaction effect as a function of frequency for 40 dB
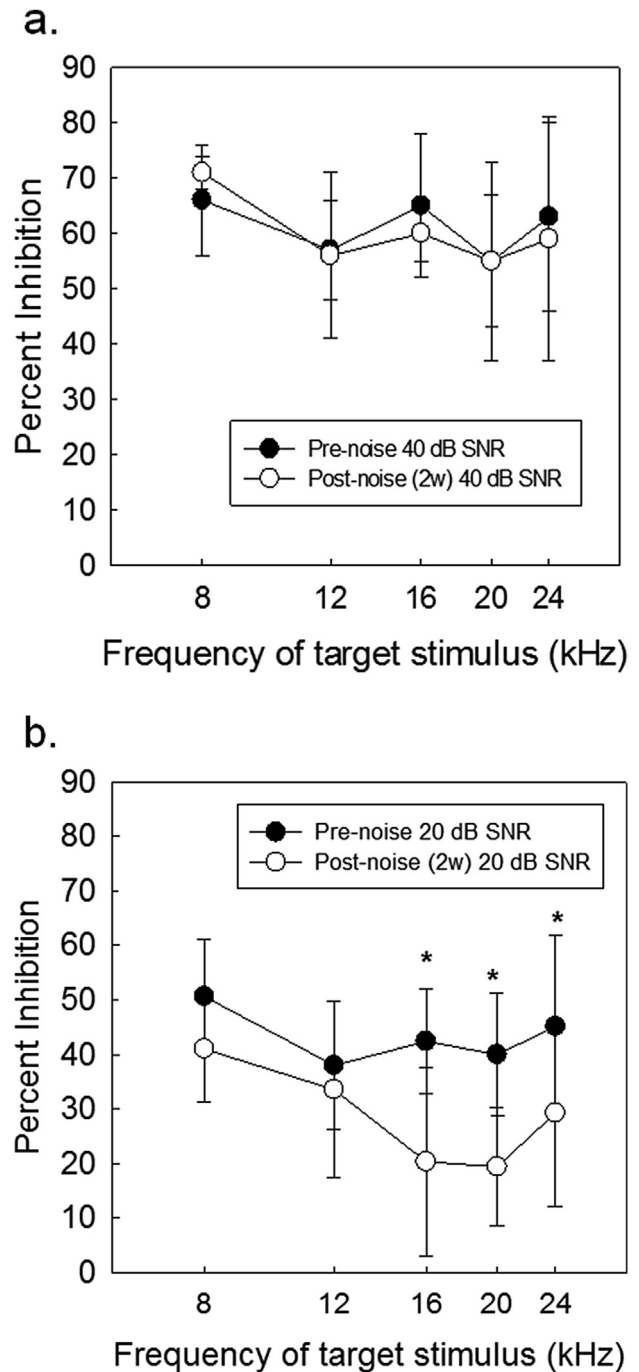

Fig. 4. (a.) Average NBPIAPS at 40 dB SNR as a function of test frequency before and 2w post exposure. 109 dB SPL noise exposure had no effect on NBPIAPS at any test frequency 2w post-exposure. (b.) Average NBPIAPS at 20 dB SNR as a function of test frequency before and 2w post exposure. 109 dB SPL noise exposure significantly reduced NBPIAPS at 16–24 kHz 2w post-exposure, results suggesting functional deficits despite recovery of ABR thresholds.

SNR ($p = 0.368$) or 20 dB SNR ($p = 0.187$). There were also no overall differences between the groups for baseline ABR wave I amplitude nor differences at 90 dB SPL ($p = 0.494$) or 80 dB SPL ($p = 0.788$). Fig. 5 shows a composite of NBPIAPS, TTS, and ABR wave-I amplitude at 16 kHz for both groups. A 2-way repeated measures ANOVA showed a main effect for noise on NBPIAPS for Group 1 [$F(1,12) = 11.798$, $p = 0.041$] and Group 2 [$F(1,12) = 29.428$, $p = 0.012$]. However, Group 1 (5a) showed significant post noise
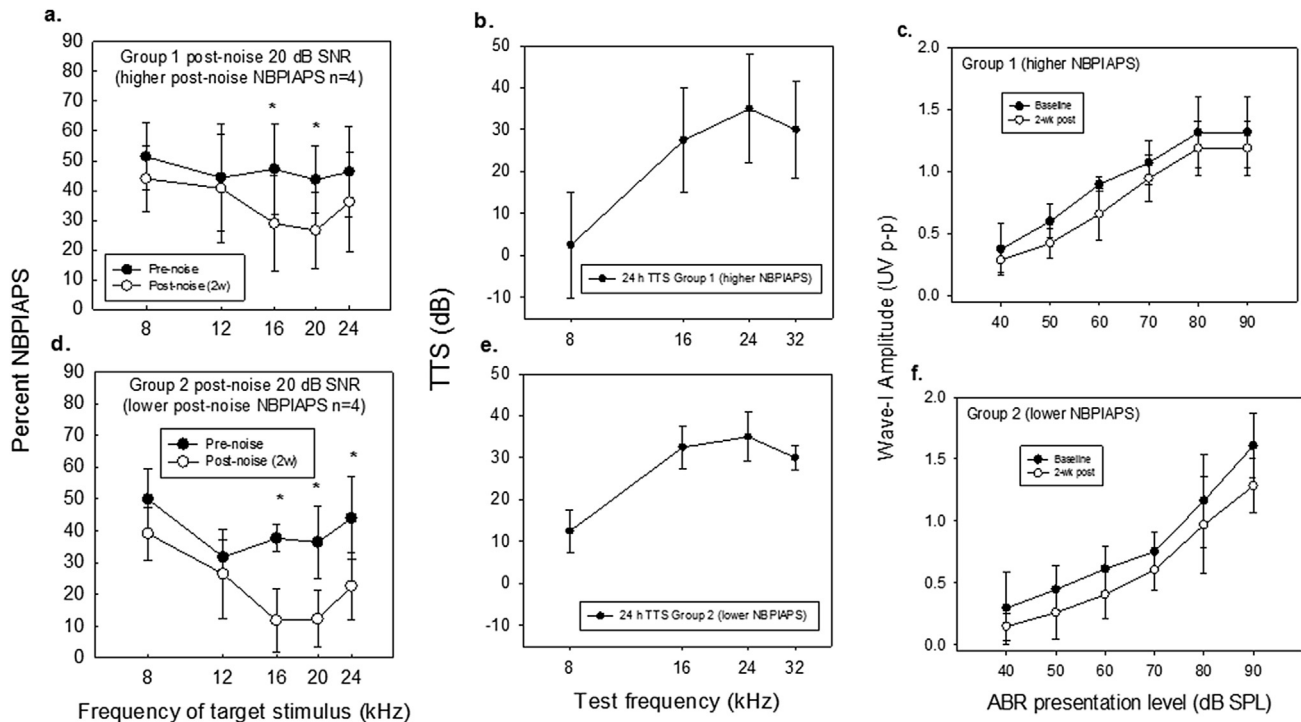
Fig. 5. Mean NBPIAPS, TTS, and ABR wave-I for animals with the highest NBPIAPS post-noise performance at 2w (Group 1, a-c) and for the animals with the lowest 2w post-noise NBPIAPS (Group 2, d-f). Both groups showed similar TTS and reduced ABR wave-I amplitudes 2w post-noise suggesting that neither TTS nor ABR wave-I reduction were reliable predictors of hearing in noise deficits.

deficits at 20 dB SNR for only 16 kHz and 20 kHz whereas Group 2 showed deficits at 16 kHz, 20 kHz, and 24 kHz. Fig. 5b and 5e show the TTS for each group. There was no significant overall difference between groups regarding post noise TTS (p = 0.768). Fig. 5c and 5f show the respective ABR wave-I, 2 week, post noise change at 16 kHz, the frequency showing the greatest degree of post noise NBPIAPS change. There was no significant difference in 2 week, post noise, ABR wave-I reduction between Group 1 and Group 2 at 16 kHz or any other frequency (8 kHz [F(1,29) = 0.006, p = 0.941], 16 kHz [F(1,29) = 1.324, p = 0.294], 24 kHz [F(1,29) = 0.019, p = 0.895], or 32 kHz [F(1,29) = 0.143, p = 0.718]). Thus, in the present experiment, although 109 dB SPL noise exposure produced both ABR wave-I reduction and NBPIAPS deficits, the degree of NBPIAPS impairment did not seem to vary as a function of TTS or wave-I amplitude reduction.

3.7. Covariate relationships

Next, we considered correlations between wave-I amplitude and NBPIAPS at frequencies with the greatest functional effects of noise (16 and 24 kHz). First, Spearman rank correlations were performed to determine relationships between baseline measures. Baseline thresholds, baseline wave-I amplitude, and baseline NBPIAPS at 40 and 20 dB SNR were compared at each frequency separately. No significant correlations were observed (p > 0.05). Spearman rank correlations were also performed to determine the relationship between TTS and post noise outcomes (post-noise threshold, wave-I amplitude, wave-I amplitude shift, 20 dB SNR NBPIAPS, and 20 dB SNR NBPIAPS shift) at 16 and 24 kHz at 2 weeks post noise exposure. No correlations were found between TTS or wave-I amplitude, wave-I amplitude shift, NBPIAPS post noise, or NBPIAPS shift. Wave-I amplitude and wave-I amplitude shift were not correlated with post noise ABR threshold or NBPIAPS performance at 20 dB SNR for either 16 or 24 kHz. No clear correlations

were seen between size of TTS, wave-I amplitude, or NBPIAPS outcomes.

4. Discussion

Noise exposures that produce TTS but no evidence of PTS have not historically been considered hazardous if thresholds recover and DPOAE remain intact, indicating there is no evidence of hair cell loss. However, a series of recent animal experiments have shown that noise exposures that produce robust TTS (>30 dB 24 hours post noise) can result in long term synaptic damage without hair cell loss, a form of hidden hearing loss. A hallmark of this hidden hearing loss is loss of low and medium spontaneous rate fibers and a permanent reduction in suprathreshold ABR wave-I amplitude. These changes have been suggested to underlie suprathreshold deficits such as poorer hearing-in-noise. It is worth noting, however, that selective loss of low spontaneous rate fibers in ouabain treated gerbils has been found to have little effect on the amplitude of the CAP (Bourien et al., 2014). Reductions in ABR wave-I amplitude after oubain were obtained with loss of medium and high spontaneous rate fibers. If these findings extend to noise-induced synaptopathy then substantial neural damage to both the low and medium spontaneous rate neurons may occur before ABR wave-I is affected and before there are any measurable threshold changes. There is now evidence suggesting that even if synaptic damage undergoes repair and evidence of reconnection, neural deficits may still persist after thresholds have recovered. In Guinea pigs, exposed to synaptopathic noise, deficits in single unit temporal and intensity coding, presumed correlates of functional hearing, continued to persist long after thresholds recovered (Song et al., 2016). It appears that regardless of which specific population is affected (selective low spontaneous rate fiber disruption, or disruption of both low and medium spontaneous rate fibers) or whether partial synaptic repair occurs post-noise, it is essential to

develop techniques to identify early markers of hearing loss and to determine when subclinical damage begins to impair functional measures of hearing.

In the experiments presented here, we explored the relationship among noise exposures that produced large TTS, significant ABR wave-I reduction, and hearing-in-noise deficits. Our results show that noise exposures producing TTS greater than 30 dB at 24 h reduced both ABR wave-I amplitudes and hearing-in-noise performance at a low SNR. However, the degree of functional impairment (poorer hearing in noise) was not correlated with the degree of ABR wave-I post-noise amplitude reduction or magnitude of the TTS suggesting a more complex relationship between noise exposure and functional impairment. Recent experiments suggest that perhaps other measures such as ABR wave-V latency, more easily measured in humans (Mehraei et al., 2016), or the summating potential (SP) to action potential (AP) ratio (Liberman et al., 2016), could provide more sensitivity to underlying synaptic damage. The latter could potentially be promising as differences in the SP/AP ratio were found to be correlated with poorer word recognition in noise and abnormal loudness sensitivity in humans (which were largely driven by SP changes).

The data presented here documented changes in performance on a signal-in-noise listening task based on the decreased effectiveness of a "warning" pre-pulse suprathreshold acoustic signal that served to attenuate an air-puff induced startle response. Decreased effectiveness of the pre-pulse signal was not explained by decreases in audibility per se as thresholds were not changed. Our results provide the first direct confirmation of a change in performance on a signal-in-noise task after a robust TTS that resolves to a long-term decrease in ABR amplitude. Although we did not measure synaptopathy directly, the data are consistent with an underlying synaptopathy given the significant ABR wave-I amplitude reductions and a functional deficit in noise at 20 dB SNR. However, caution in interpreting these results is warranted because the observed functional deficits were limited to only those frequencies with the most robust TTS, and the deficits were observed only in the most difficult listening condition. Deficits on the signal-in-noise task were not related to the size of the TTS; i.e., animals with the poorest listening in noise performance did not have larger TTS than those with the best listening in noise performance. In addition, deficits on the signal-in-noise task were not related to the relative decrease in ABR amplitude; i.e., animals with the poorest listening in noise performance did not have greater ABR amplitude reductions than those with the best listening in noise performance. The lack of direct predictive relationships reduces confidence that the observed deficits are directly related to either the magnitude of the TTS or the reduction in ABR amplitude, but the data do support the potential for noise-induced deficits in the detection of the signals in noisy backgrounds after a single acute noise exposure that induces TTS, but not PTS. It is possible, however, that a larger sample size could reveal a relationship between the magnitude of the ABR reduction and the degree of functional deficit observed in our results.

Additional basic research is clearly warranted to determine the relationships among synaptopathic damage, TTS, evoked potentials, and functional hearing deficits. Indeed, the accumulating data on the development of synaptopathic loss after TTS and the assumption that this neural loss drives a corresponding suprathreshold functional impairment, labeled hidden hearing loss, has led to an NIH-sponsored workshop titled, "Synaptopathy and Noise-Induced Hearing Loss: Animal Studies and Implications for Human Hearing" (May 4–5, 2015, Rockville, MD). Basic scientific data identifying mechanisms of damage and the potential for protection were identified as urgent needs, as were new diagnostic tools and prevalence data in humans (NIH, 2015), and specific

funding announcements have since been issued (PAR-16-170, RFA-DC-17-002).

The current human data show a paucity of evidence regarding noise-induced synaptopathy, including the inability to distinguish preventable noise-induced synaptopathy from expected age-related synaptopathy as both manifest as decreased ABR amplitude (Makary et al., 2011; Sergeyenko et al., 2013; Viana et al., 2015). There is a lack of diagnostic tools for humans and although several metrics have been proposed, there are little data documenting differences as a function of noise exposure (Bharadwaj and Shinn-Cunningham, 2014; Le Prell and Brungart, 2016; Mehraei et al., 2016; Shaheen et al., 2015). Further, there is a lack of evidence to guide any potential new human damage-risk criteria. There are no human data establishing risk for a single acute injury (see Dobie and Humes, 2016), and there are no data in humans or even in animals addressing the relative risk of repeated exposures, for less intense exposures that result in small, repeated TTS insults. Nonetheless, there have been calls for changes in noise exposure guidelines in an effort to protect the public from potential exposures to noise once considered safe, and these recommendations have been suggested to potentially extend even to recreational noise, given questions and media reports about a potential "epidemic" of hearing loss related to music player use (Portnuff, 2016). The data reported here directly support deficits on a signal-in-noise detection task after a robust TTS, but the lack of reliable relationships in which neither the degree of TTS above 30 dB nor post-noise ABR wave-I amplitude change were reliably associated with changes in NBPIAPS suggest that caution is warranted with respect to inferring causal relationships between TTS and NBPIAPS deficits, or ABR wave I amplitude and NBPIAPS. It is important to note that the levels of TTS necessary to begin to impair performance in noise with this model far exceeded levels observed in humans after recreational noise, civilian workplace noise, or routine noise among active military members during non-combat operations, and the noise exposures used here and in other animal studies have higher frequency content relative to human exposures that tend to have a long-term spectrum that falls off with 1/f. It remains unknown if smaller but repeated TTS will produce similar functional outcomes, and the extent to which decreased ABR amplitude is a precursor to these functional deficits is not known. The relationship among the size of the TTS, duration of exposure (noise dose) and change in wave-I amplitude does not appear to be straightforward. This observation is supported by human data demonstrating limited relationships between recreational noise history and wave-I amplitude. Stamper and Johnson (2015a) reported noise exposure was significantly correlated with wave-I amplitude in humans. However, when the data were reanalyzed to account for known sex differences in both ABR amplitude and noise exposure, the finding was only reproduced in female participants (2015b). In addition, when Bramhall et al. assessed the relationship between ABR amplitude and speech-in-noise performance, they determined that ABR amplitude decreases were related to speech-in-noise deficits only in the presence of overt hearing loss — a condition that does not fit the definition of hidden hearing loss. More recently, speech in noise deficits in young adults with tinnitus but normal hearing, presumably afflicted with hidden hearing loss, did not show any differences in ABR thresholds or amplitudes relative to non-tinnitus controls (Gilles et al., 2016). Collectively, these emerging data suggest that the use of ABR wave-I amplitude alone is unlikely to provide a reliable or adequate predictor of hearing-in-noise deficits and cannot provide a causal explanation for such deficits in normal hearing individuals. As these findings are extended to humans, even greater heterogeneity is to be expected due to variance in noise exposures across the life span and differences in individual risk factors for noise damage.

Nevertheless, noise exposures that produce large TTS may indeed produce cumulative long term damage that could ultimately lead to functional impairment. Our current standard of care, based primarily on audiometric thresholds, is unlikely to capture early markers of potential hearing loss. Suprathreshold functional measures such as hearing-in-noise testing, as our data suggest, could reveal otherwise hidden deficits and thus new data are urgently needed to establish where risk begins, and how risk grows as a function of increasing level and duration or with repetition of the insult throughout a working career. At this time, it is recommended that hearing-in-noise testing be considered as part of hearing healthcare and monitoring programs for civilians and military personnel who may be exposed to occupational noise in order to assess safe exposure boundaries and the growth of risk with increases in level or duration.

Conflict of interest

The authors declare no conflict of interest.

Author contributions

EL, CS, and CL designed the experiments, performed data collection and analysis and wrote the manuscript.

Acknowledgements

References

Bharadwaj, H.M., Shinn-Cunningham, B.G., 2014. Rapid acquisition of auditory subcortical steady state responses using multichannel recordings. Clin. Neurophysiol. 125, 1878–1888.

Bourien, J., Tang, Y., Batrel, C., Huet, A., Lenoir, M., Ladrech, S., Desmadryl, G., Nouvian, R., Puel, J.L., Wang, J., 2014. Contribution of auditory nerve fibers to compound action potential of the auditory nerve. J. Neurophysiol. 112, 1025–1039.

Dallos, P., Harris, D., 1978. Properties of auditory nerve responses in absence of outer hair cells. J. Neurophysiology 41, 365–383.

Dobie, R.A., Humes, L.E., 2016. Commentary on the regulatory implications of noise-induced cochlear neuropathy. Int. J. Audiol. 16, 1–5.

Fernandez, K.A., Jeffers, P.W., Lall, K., Liberman, M.C., Kujawa, S.G., 2015. Aging after noise exposure: acceleration of cochlear synaptopathy in "recovered" ears. J. Neurosci. 35, 7509–7520.

Furman, A.C., Kujawa, S.G., Liberman, M.C., 2013. Noise-induced cochlear neuropathy is selective for fibers with low spontaneous rates. J. Neurophysiol. 110, 577–586.

Gilles, A., Schlee, W., Rabau, S., Wouters, K., Fransen, E., Van de Heyning, P., 2016. Decreased speech-in-noise understanding in young adults with tinnitus. Front. Neurosci. 10, 288.

Hickox, A.E., Liberman, M.C., 2014. Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus? J. Neurophysiol. 111, 552–564.

Jensen, J.B., Lysaght, A.C., Liberman, M.C., Qvortrup, K., Stankovic, K.M., 2015. Immediate and delayed cochlear neuropathy after noise exposure in pubescent mice. PLoS One 10, e0125160.

Kujawa, S.G., Liberman, M.C., 2009. Adding insult to injury: cochlear nerve degeneration after "temporary" noise-induced hearing loss. J. Neurosci. 29, 14077–14085.

Kujawa, S.G., Liberman, M.C., 2015. Synaptopathy in the noise-exposed and aging cochlea: primary neural degeneration in acquired sensorineural hearing loss. Hear Res. 330, 191–199.

Le Prell, C.G., Brungart, D.S., 2016. Speech-in-Noise tests and supra-threshold auditory evoked potentials as metrics for noise damage and clinical trial outcome measures. Otology & neurotology : official publication of the American otological society. Am. Neurotol. Soc. Eur. Acad. Otology Neurotol. 37, e295–302.

Le Prell, C.G., Dell, S., Hensley, B., Hall 3rd, J.W., Campbell, K.C., Antonelli, P.J., Green, G.E., Miller, J.M., Guire, K., 2012. Digital music exposure reliably induces temporary threshold shift in normal-hearing human subjects. Ear Hear. 33, e44–58.

Liberman, L.D., Suzuki, J., Liberman, M.C., 2015. Dynamics of cochlear synaptopathy after acoustic overexposure. J. Assoc. Res. Otolaryngol. 16, 205–219.

Liberman, M.C., Epstein, M.J., Cleveland, S.S., Wang, H., Maison, S.F., 2016. Toward a differential diagnosis of hidden hearing loss in humans. PLoS One 11, e0162726.

Lin, H.W., Furman, A.C., Kujawa, S.G., Liberman, M.C., 2011. Primary neural degeneration in the Guinea pig cochlea after reversible noise-induced threshold shift. J. Assoc. Res. Otolaryngol. 12, 605–616.

Lobarinas, E., Hayes, S.H., Allman, B.L., 2013. The gap-startle paradigm for tinnitus screening in animal models: limitations and optimization. Hear Res. 295, 150–160.

Lobarinas, E., Salvi, R., Ding, D., 2016. Selective inner hair cell dysfunction in chinchillas impairs hearing-in-noise in the absence of outer hair cell loss. J. Assoc. Res. Otolaryngol. 17, 89–101.

Maison, S.F., Usubuchi, H., Liberman, M.C., 2013. Efferent feedback minimizes cochlear neuropathy from moderate noise exposure. J. Neurosci. 33, 5542–5552.

Makary, C.A., Shin, J., Kujawa, S.G., Liberman, M.C., Merchant, S.N., 2011. Age-related primary cochlear neuronal degeneration in human temporal bones. J. Assoc. Res. Otolaryngol. 12, 711–717.

McGill, T.J., Schuknecht, H.F., 1976. Human cochlear changes in noise induced hearing loss. Laryngoscope 86, 1293–1302.

Mehraei, G., Hickox, A.E., Bharadwaj, H.M., Goldberg, H., Verhulst, S., Liberman, M.C., Shinn-Cunningham, B.G., 2016. Auditory brainstem response latency in noise as a marker of cochlear synaptopathy. J. Neurosci. 36, 3755–3764.

NIH, 2015. Synaptopathy and Noise-induced Hearing Loss: Animal Studies and Implications for Human Hearing [Online]. https://www.nidcd.nih.gov/workshops/synaptopathy-and-noise-induced-hearing-loss-animal-studies-and-implications/2015.

NIOSH, 1998. Criteria for a Recommended Standard [Online]. https://www.cdc.gov/niosh/docs/98-126/pdfs/98-126.pdf.

Ohlms, L.A., Lonsbury-Martin, B.L., Martin, G.K., 1991. Acoustic-distortion products: separation of sensory from neural dysfunction in sensorineural hearing loss in human beings and rabbits. Otolaryngology head neck Surg. Official J. Am. Acad. Otolaryngology Head Neck Surg. 104, 159–174.

OSHA, 1983. Regulation Standards 29 CFR [Online]. https://www.osha.gov/pls/oshaweb/owadisp.show_document?p_table=STANDARDS&p_id=9735.

Patuzzi, R.B., Yates, G.K., Johnstone, B.M., 1989. Outer hair cell receptor current and sensorineural hearing loss. Hear. Res. 42, 47–72.

Portnuff, C.D., 2016. Reducing the risk of music-induced hearing loss from overuse of portable listening devices: understanding the problems and establishing strategies for improving awareness in adolescents. Adolesc. Health Med. Ther. 7, 27–35.

Sergeyenko, Y., Lall, K., Liberman, M.C., Kujawa, S.G., 2013. Age-related cochlear synaptopathy: an early-onset contributor to auditory functional decline. J. Neurosci. 33, 13686–13694.

Shaheen, L.A., Valero, M.D., Liberman, M.C., 2015. Towards a diagnosis of cochlear neuropathy with envelope following responses. J. Assoc. Res. Otolaryngol. 16, 727–745.

Song, Q., Shen, P., Li, X., Shi, L., Liu, L., Wang, J., Yu, Z., Stephen, K., Aiken, S., Yin, S., Wang, J., 2016. Coding deficits in hidden hearing loss induced by noise: the nature and impacts. Sci. Rep. 6, 25200.

Spankovich, C., Griffiths, S.K., Lobarinas, E., Morgenstein, K.E., de la Calle, S., Ledon, V., Guercio, D., Le Prell, C.G., 2014. Temporary threshold shift after impulse-noise during video game play: laboratory data. Int. J. Audiol. 53 (Suppl. 2), S53–S65.

Stamper, G.C., Johnson, T.A., 2015a. Auditory function in normal-hearing, noise-exposed human ears. Ear Hear. 36, 172–184.

Stamper, G.C., Johnson, T.A., 2015b. Letter to the editor: examination of potential sex influences in. Auditory function in normal-hearing, noise-exposed human ears. Ear Hear 36, 172–184. Ear and hearing 36, 738-40.

Stebbins, W.C., Hawkins Jr., J.E., Johnson, L.G., Moody, D.B., 1979. Hearing thresholds with outer and inner hair cell loss. Am. J. Otolaryngology 1, 15–27.

Viana, L.M., O'Malley, J.T., Burgess, B.J., Jones, D.D., Oliveira, C.A., Santos, F., Merchant, S.N., Liberman, L.D., Liberman, M.C., 2015. Cochlear neuropathy in human presbycusis: confocal analysis of hidden hearing loss in post-mortem tissue. Hear Res. 327, 78–88.

WHO, 2015. 1.1 Billion People at Risk of Hearing Loss [Online]. http://www.who.int/mediacentre/news/releases/2015/ear-care/en/.