Can long-term exposure to non-damaging noise lead to hyperacusis or tinnitus?

MARTIN PIENKOWSKI[*]

Osborne College of Audiology, Salus University, Philadelphia, PA, USA

Hearing loss triggers changes in the central auditory system, some maladaptive. A region of primary auditory cortex (A1) deprived of input responds more strongly to cochlear lesion-edge frequencies, and its spontaneous firing rate (SFR) increases. This spontaneous and sound-evoked hyperactivity has been associated with tinnitus and hyperacusis, respectively. Regional increases in A1 spontaneous and sound-evoked activity are also observed after long-term exposure to non-damaging levels of noise. Adult cats exposed to such noise bands had suppressed SFR and evoked activity in the A1 region mapped to the noise band, but had increased SFR and evoked activity in A1 regions above and below the band. We hypothesized that, post-exposure, frequencies within the noise band should for some time be perceived as softer than before (hypoacusis), whereas frequencies outside of the noise band might be perceived as louder than before (hyperacusis), and might even be internalized as tinnitus. To investigate this possibility, adult CBA/Ca mice were exposed for >2 months to 8–16 kHz bandpass noise at 70 dB SPL, and tested for hypo/hyperacusis and tinnitus using prepulse inhibition (PPI) of the acoustic startle reflex (ASR), and gap-PPI of the ASR (GPIAS), respectively. ABRs and DPOAEs showed that the 70 dB SPL exposure was indeed non-damaging, whereas the same noise band at 75 dB SPL appeared to cause cochlear synaptopathy. Contrary to hypothesis, long-term exposure to non-damaging noise had no significant effect on PPI ASR and GPIAS testing. These negative findings nevertheless have important implications for PPI and GPIAS testing, and for the mechanisms of tinnitus and hyperacusis.

INTRODUCTION

Loud noise exposure can destroy cochlear outer and inner hair cells (OHCs and IHCs) and nerve fibers (ANFs), resulting in permanent hearing loss, typically at higher sound frequencies (Kujawa and Liberman, 2015). This can trigger a number of changes, some maladaptive, at various levels of the central auditory system. For example, the high-frequency area of primary auditory cortex (A1), when deprived of cochlear input, becomes more active spontaneously, and responds more strongly to input from the better-preserved mid-frequency turn of the cochlea, which becomes over-represented in A1 (Eggermont, 2017). Although this can enhance aspects of hearing at the over-represented frequencies, it could also lead to tinnitus – phantom ringing or hissing perceived as originating in the ears or head (Eggermont, 2012), and to hyperacusis – a reduced tolerance of moderate to loud sounds (Tyler *et al.*, 2014; Pienkowski *et al.*, 2014).

*Corresponding author: mpienkowski@salus.edu

Spontaneous central hyperactivity has been linked with behavioral evidence of tinnitus in animals with permanent noise-induced hearing loss (Kaltenbach *et al.*, 2004; Engineer *et al.*, 2011; Li *et al.*, 2013; Ropp *et al.*, 2014; Coomber *et al.*, 2014; Basura *et al.*, 2015; Longenecker and Galazyuk, 2016; Wu *et al.*, 2016; Sturm *et al.*, 2017), and with temporary hearing loss induced by salicylate (Eggermont and Kenmochi, 1998). Likewise, sound-evoked central hyperactivity has been linked with animal models of hyperacusis after noise trauma (Sun *et al.*, 2012; Chen *et al.*, 2013; Hickox and Liberman, 2014), salicylate injection (Turner and Parish, 2008; Sun *et al.*, 2009), and hereditary progressive hearing loss (Carlson and Willott, 1996; Ison *et al.*, 2007; Xiong *et al.*, 2017). Still, aspects of these animal data are puzzling (see Discussion), and evidence from human brain imaging studies linking spontaneous central hyperactivity with tinnitus (Elgoyhen *et al.*, 2015), and sound-evoked hyperactivity with hyperacusis (Gu *et al.*, 2010), remains more preliminary.

Regional increases in A1 spontaneous and sound-evoked activity are also observed after long-term exposure to non-damaging levels of noise (Pienkowski and Eggermont, 2011). In a series of studies on adult cats exposed to various tone pip ensembles and noise bands at ~70 dB SPL for weeks to months at a time, it was shown that A1 responses were strongly suppressed at frequencies within the exposure band (particularly at its edges), but were generally enhanced at frequencies above and/or below the exposure band (Pienkowski and Eggermont, 2009; 2010a; 2010b; Pienkowski *et al.*, 2011; 2013; note: the seminal study by Noreña *et al.*, 2006, used an exposure of ~80 dB SPL). We attributed the suppression to a homeostatic reduction in central gains in response to the persistent sound stimulus, and the enhancement to decreased lateral inhibition from the suppressed region (Pienkowski and Eggermont, 2012). These changes slowly reversed (also over weeks or months) after the end of the exposure (Pienkowski and Eggermont, 2009; 2010a; 2010b). Interestingly, the spontaneous hyperactivity was generally seen in the enhanced regions of A1 (Noreña *et al.*, 2006; Pienkowski and Eggermont, 2009; 2010b; Munguia *et al.*, 2013), not in the deprived region as is the case with permanent hearing loss (Eggermont, 2017). Given these data, we wondered whether long-term exposure to non-damaging noise could lead to hyperacusis or tinnitus. Specifically, we hypothesized that, post-exposure, frequencies within the noise band should for some time be perceived as softer than before (hypoacusis), whereas frequencies outside of the noise band might be perceived as louder than before (hyperacusis), and might even be internalized as tinnitus.

To investigate this possibility, adult CBA/Ca mice were exposed for >2 months to 8–16 kHz bandpass noise at ~70 dB SPL, and tested for hypo/hyperacusis and tinnitus using prepulse inhibition (PPI) of the acoustic startle reflex (ASR), and gap-PPI of the ASR (GPIAS), respectively. Auditory brainstem responses (ABRs) and distortion product otoacoustic emissions (DPOAEs) were used to show that the 70 dB SPL exposure was indeed non-damaging, whereas the same stimulus at 75 dB SPL appeared to cause cochlear synaptopathy. Contrary to hypothesis, long-term exposure to non-damaging noise had no significant effect on PPI ASR and GPIAS testing. As will be discussed, these negative findings nevertheless have important implications for PPI and GPIAS testing, and for the mechanisms of tinnitus and hyperacusis.

METHODS

Animals and noise exposure

This work was approved by the Institutional Animal Care and Use Committee of Salus University. Nine normal-hearing male CBA/Ca mice (Jackson Laboratories) served as subjects in the main experiment, and were exposed bilaterally for 2 months continuously to sharply-filtered 8–16 kHz noise at ~70 dB SPL, beginning at about 3 months of age. Six mice served as unexposed controls, and another six were exposed to the same 8–16 kHz noise at ~75 dB SPL. The noise was synthesized in Adobe Audition, and played out by a free-field loudspeaker (Tucker Davis Technologies [TDT], Model MF1), which was mounted just above the cages housing the mice. All mice were kept on a 12-h light/dark schedule (light 8 am–8 pm) and were given free access to food and water. There were no signs of long-term distress in any of the noise-exposed mice.

Assessment of loudness perception and tinnitus using the acoustic startle reflex

The ASR is a protective reflex elicited by an intense sound (Koch, 1999). In mice, it involves a whole-body flinch and jump, the force of which was measured using a motion-sensitive platform in an anechoic foam-lined, sound-attenuating startle chamber (San Diego Instruments, SR-LAB). ASR amplitudes can be reduced by preceding the intense, startling sound with a less-intense, non-startling "prepulse", known as prepulse inhibition (PPI) of the ASR. The degree of ASR reduction, termed the magnitude of the PPI, is related to the behavioral salience of the prepulse. For example, the greater the perceived loudness of the prepulse, the greater the magnitude of the PPI (e.g., Carlson and Willott, 1996). Thus, an estimate of the rodent's loudness function (i.e., perceived loudness vs. sound intensity) can be obtained by measuring the magnitude of the PPI as a function of the prepulse intensity, at a given prepulse frequency. The GPIAS variant of PPI (Galazyuk and Hébert, 2015) substitutes a silent gap in a narrowband noise (NBN) background for the tone prepulse. It is believed that a ringing tinnitus with a similar pitch to the NBN background reduces the salience of the gap, decreasing the magnitude of the PPI. Figure 1 illustrates these ideas, including the stimulus parameters used in the present study. For PPI and GPIAS testing, startle stimuli were 20-ms bursts of broadband noise (BBN) at 105 dB SPL. Tone prepulses were also 20 ms long (including 1 ms on/off $\cos^2$ ramps), preceded the startle noise by 100 ms (onset-to-onset), and were presented at 50 or 70 dB SPL. For GPIAS testing, silent gaps 20 or 50 ms long were embedded in 1/3-octave NBN at 65 dB SPL, and also preceded the startle burst by 100 ms. These sound stimuli were synthesized using Adobe Audition, and played out by a HiVi Isodynamic Tweeter (Model RT2C-A). Stimulus levels were calibrated with a 1/4 inch ACO Pacific microphone (Model 7017) placed inside the startle chamber.

Figure 2A shows the experiment timeline, and Fig. 2B a block diagram of a single startle session. Each session consisted of 362 startle trials with an average inter-startle interval of 5 s (range 3–7 s), for a total session time of ~30 min. GPIAS testing was conducted at NBN frequencies of 6, 8, 11, 16, 23 and 32 kHz, and in BBN. PPI testing was conducted using tone prepulses at frequencies of 4, 6, 8, 11, 16, 23 and 32 kHz. Each gap-in-noise or prepulse frequency was presented in a block of 21 trials in pseudorandom order, with 7 startle-only trials, and 7 trials each for 20 and 50-ms gaps, or for 50 and 70 dB SPL prepulses. The ratio of the ASR amplitude with a gap or a prepulse to that without a gap
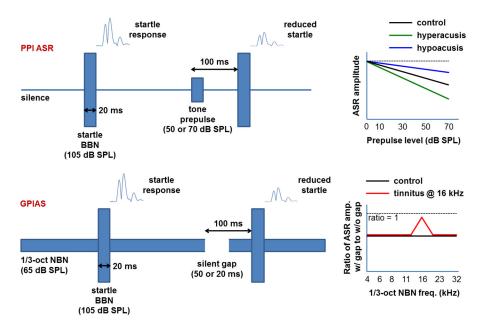
Fig. 1: Schematic diagrams of PPI ASR (top) and GPIAS (bottom), including the stimulus parameters used in the present study. Also shown are hypothetical PPI functions in animals with hypo- and hyperacusis (top-right), and hypothetical GPIAS results that are positive for tinnitus at 16 kHz (bottom-right).

or prepulse was calculated for each block, and constituted the "raw data" for the session. In addition, 3 blocks of "I/O functions" were run, in which startle-only amplitudes were measured in response to 20 ms-long tones (including 1 ms on/off ramps) at 4, 6, 8, 11, 16 and 23 kHz, and to BBN, at both 85 and 105 dB SPL. Finally, one block of startle-only trials to 105 dB SPL BBN was measured at the beginning and end of the session to track within-session adaptation of the startle response. Each mouse completed many such sessions (see below), with the order of the GPIAS and PPI blocks interchanged and randomized between sessions to offset adaptation effects. All sessions were conducted during the day, but in darkness, with the lights off inside the startle box.

Prior to the first ASR test session, the 9 mice were gradually acclimated to the startle chamber and test stimuli over a period of 2 weeks. Each mouse was then tested during 12 sessions, as described above, and the final results were averaged across sessions. Each mouse was limited to one session per day, and completed the 12 sessions over a period of 3–4 weeks. This was followed by the 2 month noise exposure, and then another 3–4 weeks of ASR testing after a short startle re-acclimation period. During this post-exposure ASR testing, the noise stimulus was left on for 12 h each night (8 pm–8 am). The mice were tested in random order during the day, but no earlier than 10am, 2 h after noise offset for the day. Maintaining the noise exposure at night eliminated the potential confound on post-exposure testing of the gradual reversal of noise-induced changes after the cessation of exposure (Pienkowski and Eggermont, 2009; 2010a; 2010b).

Startle response analysis was automated using custom software written in Mathematica. Reliable responses were identified using a template-matching algorithm similar to that

Non-damaging noise and hyperacusis or tinnitus?

Fig. 2: A. Experiment timeline. **B.** Block diagram of a single startle session. See Methods for a detailed description.

described by Grimsley *et al.* (2015). Thousands of startle trials were checked by eye and the performance of the algorithm was found to be excellent. Startle amplitude was taken as the largest peak in each trace. Individual mouse and group-averaged ASR results were compared pre- and post-exposure using two-way ANOVAs with post-hoc Bonferroni tests.

DPOAE and ABR recording

DPOAEs and ABRs were measured from the left ears and mastoid areas 2 weeks following the completion of post-exposure (or control) ASR sessions. Mice were anesthetized with a mixture of 50 mg/kg ketamine and 10 mg/kg xylazine, injected intraperitoneally, and were topped up with half doses of this mixture as needed to maintain a state of areflexia. They were placed on a homeothermic blanket which kept their body temperature at 36.5°C, inside a single-walled sound-attenuating chamber (ETS-Lindgren). Following the completion of DPOAE and ABR testing, extracellular recordings were attempted from A1, after which the mice were sacrificed. However, the cortical data are too preliminary to report here.

ABRs were always recorded first. Stimuli were tone bursts at 4, 6, 8, 11, 16, 23 and 32 kHz, and were 3 ms in duration including 1-ms $\cos^2$ on and off ramps. Stimuli were synthesized using TDT software (SigGen), and played out by a TDT MF1 speaker coupled to the animal's left ear canal with a 10 cm tube and sealed probe. Sound levels were calibrated with the probe coupled to a 1/4 inch microphone (ACO Pacific, 7017) with an additional 7 mm-long plastic tube, intended to approximate the length of the mouse ear canal. Stimuli were presented at 10–90 dB SPL at each frequency, in 10 dB steps, with 512 repetitions per level and a presentation rate of 21.1 /s. The ABR was recorded differentially between the left mastoid area and vertex (ground electrode in the nape of the neck) using subdermal needle electrodes (Rochester Electro-Medical, Inc., Model S83018-R9). Potentials were amplified, digitized, and filtered between 100 and 3,000 Hz under the control of TDT software (BioSig). At low stimulus levels, measurements were repeated twice, and ABR

threshold was defined as the lowest SPL that yielded a reproducible ABR, minus 5 dB (half step size). Peak-to-trough amplitudes were then determined for mouse ABR waves 1–4 at all supra-threshold SPLs, if the wave was distinct.

Following ABR recording, DPOAEs were measured from the left ear using an OAE probe coupled to a pair of TDT MF1 speakers and to an Etymotic Research microphone (ER-10B+). Stimuli were synthesized using TDT software (SigGen). The frequency of the higher primary tone (f_2) was again set to 4, 6, 8, 11, 16, 23 or 32 kHz, and the frequency of the lower primary tone (f_1) was given by $f_1 = f_2/1.2$. Levels of f_1 (L_1) ranged from 20 to 80 dB SPL in 10 dB steps, with $L_2 = L_1 - 10$ dB. DPOAEs at frequency $2f_1 - f_2$ were amplified and digitized under the control of TDT software (BioSig). DPOAE amplitudes are reported in units of dB V, and DPOAE threshold was defined as the lowest level of L_1 (again minus the step size of 5 dB) at which the DPOAE amplitude was above the 99% confidence interval for the microphone noise floor, averaged across the six frequency bins adjacent to $2f_1 - f_2$.

RESULTS

ABRs and DPOAEs

Nine normal-hearing adult male CBA/Ca mice were exposed 24 h/day for 2 months and then 12 h/day for 1 month to sharply-filtered 8–16 kHz noise at 70 dB SPL. ABRs and DPOAEs were measured 2 weeks after the end of the 3 month exposure. They were compared to measurements made at the same age in six unexposed control mice, and in another six mice which were exposed to the same 8–16 kHz noise at 75 dB SPL. Group-averaged ABR results are shown in Fig. 3A ($\pm$ 1 SE or standard error). There were no significant differences in ABR thresholds between the three groups ($p=0.56$ for the main effect of group across frequency; two-way ANOVA). ABR wave 1 input-output functions were not affected after exposure to 70 dB SPL noise, but were significantly reduced after exposure to 75 dB SPL at frequencies of 8 ($p=0.031$), 11 ($p=0.004$), and 16 kHz ($p=0.007$) (i.e., only at frequencies within the noise band; all other frequencies were $p>0.05$ as indicated). These p-values reflect the main effect of group across ABR stimulus level (two-way ANOVA), and were not corrected for multiple comparisons at the various stimulus SPLs. Note that none of the differences at individual stimulus SPLs were significant at the $p=0.05$ level after post-hoc Bonferroni correction, only the main effects. Importantly, no significant differences were found between groups in the amplitudes of ABR waves 2–4 (data not shown). Also, there were no significant differences between groups in DPOAE thresholds and DPOAE input-output functions at any primary tone frequency (Fig. 3B). As will be discussed, these results are consistent with mild noise-induced "hidden hearing loss" or cochlear synaptopathy (Kujawa and Liberman, 2015) following exposure at 75 dB SPL, but no noise-induced cochlear damage following exposure at 70 dB SPL.

PPI ASR

ASR results are compared pre- and post-exposure for the 9 mice exposed to 8–16 kHz noise at the non-damaging level of 70 dB SPL. It was hypothesized that this exposure would cause sound frequencies within the noise band to be perceived as softer than before (hypoacusis), whereas frequencies above and/or below the noise band would be perceived as louder than before (hyperacusis).
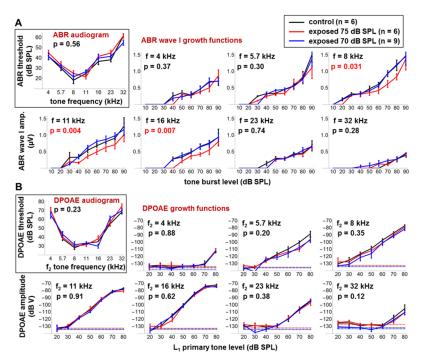
Fig. 3: A. ABR audiograms, and wave 1 growth functions at different stimulus frequencies, for unexposed control mice, and for mice exposed for 3 months to 8–16 kHz noise at 70 dB SPL and 75 dB SPL. Error bars show ±1 SE. **B.** As 3A but showing DPOAE audiograms and growth functions at different stimulus frequencies, for unexposed and exposed mice.

Figure 4A shows group-averaged ASR amplitudes (± 1 SE) in response to BBN and tonal startle stimuli presented at 85 dB SPL (black traces) and 105 dB SPL (grey traces). There were no significant differences pre- vs. post-exposure at any startle frequency or level ($p>0.05$, as indicated, for the main effect of group across frequency; separate two-way ANOVAs were run at each SPL). Group-averaged PPI results (± 1 SE) are shown in Fig. 4B, for prepulse levels of 50 dB SPL (black traces) and 70 dB SPL (grey traces). Note that the PPI effect was highly significant ($p<<0.05$) at the group level for all prepulse frequencies and SPLs (i.e., ASR amplitude ratios with vs. without prepulse are all $<<1$). However, again there were no significant differences post-exposure ($p>0.05$, as indicated). Individual animal results (not shown) also do not support the hypothesis that at least some of the mice may have developed frequency-specific hypo- or hyperacusis post-exposure.

GPIAS

GPIAS results are also reported pre- and post-exposure for the 9 mice exposed to 8–16 kHz noise at the non-damaging level of 70 dB SPL. Figure 5 shows group-averaged ASR amplitude ratios (± 1 SE) with and without 20-ms (black traces) and 50-ms (grey traces) silent gaps embedded in 1/3-octave NBN at a range of frequencies, and in BBN. As will be discussed further, GPIAS testing was performed using both 50 ms and 20 ms gaps because previous auditory cortical ablation studies have suggested that cortex is not essential for GPIAS with gaps of 50 ms or longer, but is required at gap durations of <30 ms (Ison *et al.*, 1991; Bowen *et al.*, 2003; Weible *et al.*, 2014). However, there
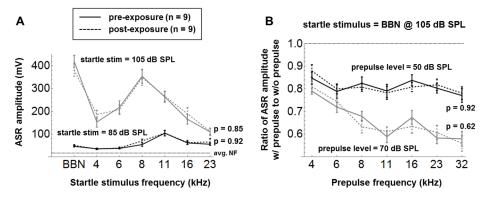
Fig. 4: A. Group-averaged ASR amplitudes (± 1 SE) in response to BBN and tonal startle stimuli presented at 85 dB SPL (black traces) and 105 dB SPL (grey traces), pre- and post-exposure. The average startle system noise floor is drawn in. **B.** Group-averaged PPI results (± 1 SE) for prepulse levels of 50 dB SPL (black traces) and 70 dB SPL (grey traces).
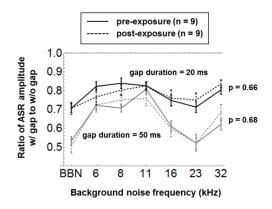

Fig. 5: Group-averaged GPIAS results (± 1 SE) for gap durations of 20 ms (black traces) and 50 ms (grey traces).

were no significant differences post-exposure with either 50 or 20-ms gaps, despite the fact that the GPIAS effect itself was highly significant ($p<<0.05$) at the group level for both gap durations in all NBN backgrounds (i.e., ASR amplitude ratios with vs. without gap are all $<<1$). Again, individual animal results (not shown) do not support the idea that at least some of the mice may have developed tinnitus post-exposure.

DISCUSSION

Effects of exposure to moderately loud noise on the auditory periphery

There was evidence of cochlear synaptopathy in CBA/Ca mice following a 3-month exposure in adulthood to 8–16 kHz noise at 75 dB SPL, but not at 70 dB SPL. ABR wave 1 amplitudes at suprathreshold SPLs were significantly reduced 2 weeks after the end of the 75 dB SPL exposure, and this was specific to stimuli at 8, 11 and 16 kHz (i.e., frequencies within the exposure band; Fig. 5A), while DPOAEs were not affected at any stimulus frequency (Fig. 5B), nor were ABR wave 2–4 amplitudes (data not shown). This pattern of damage differs to some extent from that observed following shorter exposures to louder noise. A study by Fernandez *et al.* (2015) compared the effects on CBA/Ca mice of 2 hour exposures to 8–16 kHz noise at 100 and 91 dB SPL. By 2 weeks post-exposure, DPOAEs had returned to pre-exposure baselines in both cases, while ABR wave 1 amplitudes were

reduced (indicative of synaptopathy) only after the louder, 100 dB SPL exposure. However, the pattern of DPOAE and ABR temporary threshold shifts (TTS), measured at 1 day post-exposure, was instructive: After the 100 dB exposure, maximum TTS was observed at the highest frequencies tested, >30 kHz, but after the 91 dB exposure, maximum TTS developed at around 20 kHz, only slightly above the 8–16 kHz exposure band (Fernandez *et al.*, 2015). These results can likely be explained by the greater "spread of excitation" to more basal regions of the cochlea at higher exposure levels. At the more moderate level of 75 dB SPL (present study), this spread of excitation is small, and the damage appears to be limited to the cochlear region mapped to the exposure band. Maison *et al.* (2013) exposed CBA/Ca mice to 8–16 kHz noise at 84 dB SPL for 1 week, and found reduced ABR wave 1 amplitudes and IHC synapse counts 1 week post-exposure; as in the present study, the reduction was greatest at 8–16 kHz, although some reduction was also seen above the exposure band.

The present study appears to be the first to suggest that cochlear synaptopathy can occur after prolonged exposure to noise at levels as low as 75 dB SPL. At least in CBA/Ca mice, this is close to the threshold for a damaging exposure, as mice exposed to 70 dB SPL did not show any ABR-based evidence of synaptopathy. A recent opinion piece argued that humans are less susceptible than rodents to TTS and by extension to cochlear synaptopathy, and that the bandpass exposures used in the animal studies are not representative of real-world noise (Dobie and Humes, 2017). While these are fair points, the present data suggest that long-term exposure at currently permissible occupational levels (e.g., 85 dB A for 8 h/day; NIOSH, 1998; OSHA, 2002) may not in fact be safe for the ear.

Effects of exposure to moderately loud noise on the central auditory system

Preliminary data, not presented here, suggest similar noise-induced changes in mouse A1 to those reported previously in cats. These changes are also likely exhibited at the level of the thalamic medial geniculate body (MGB), as inferred from cortically-recorded local field potentials (Pienkowski and Eggermont, 2011). Lau *et al.* (2015) performed whole brain functional magnetic resonance imaging following long-term BBN exposure of adult rats at 65 dB SPL, and found reduced noise-evoked activation of A1 and MGB, but no change in the inferior colliculus (IC) and lower brainstem. Thus, it seems that the effects of non-damaging noise are mostly limited to the thalamocortex, while the effects of damaging noise are already prominent at the level of the IC and lower brainstem (Eggermont, 2017).

Negative PPI ASR and GPIAS findings after exposure to non-damaging noise

There were no significant exposure-induced changes in ASR amplitudes measured in response to tones and BBN at 85 and 105 dB SPL (Fig. 4A), and no changes in ASR amplitude ratios with and without tone prepulses at 50 and 70 dB SPL (Fig. 4B), and with and without 20 and 50-ms silent gaps embedded in NBN and BBN backgrounds (Fig. 5). Thus, there was no evidence of hypo/hyperacusis or tinnitus, as assessed by the ASR, in mice exposed to non-damaging noise.

A possible reason for why hypo/hyperacusis was not detected in the present study is that exposure to moderately loud noise causes changes mainly at the thalamocortical level (see above), whereas PPI of the ASR appears sensitive to changes mainly at the (pre-attentive) brainstem level, as suggested by previous studies on decerebrate rats (Davis and Gendelman, 1977; Fox, 1979; Li and Frost, 2000). Thus, PPI of the ASR should not be used to study the behavioral correlates of neural changes that are observed mainly at the thalamocortical but not at the brainstem level.

This caveat does not necessarily apply to the GPIAS form of PPI, which at present is widely adopted for tinnitus screening in rodents. In the present study, GPIAS testing was performed using both 50 and 20 ms gaps, as previous work has suggested that auditory cortex is not essential for GPIAS with gap durations of 50 ms or longer, but is required for gaps less than ~30 ms (Ison *et al.*, 1991; Bowen *et al.*, 2003; Weible *et al.*, 2014). Nevertheless, results here were negative for both gap durations, implying that moderately noise-exposed mice did not develop tinnitus, at least as assessed by GPIAS, in spite of our previous work showing that adult cats exhibited noise-induced regional increases in A1 spontaneous activity. If GPIAS screening for tinnitus can indeed be trusted (see below), the negative findings suggest that auditory cortical hyperactivity is not sufficient to cause tinnitus.

Recent studies on rats (Ropp *et al.*, 2014), guinea pigs (Coomber *et al.*, 2014), and mice (Longenecker and Galazyuk, 2016) found increased spontaneous firing rates (SFRs) in the IC of all noise-exposed animals, *including those testing negative for tinnitus using GPIAS*. Interestingly, Hickox and Liberman (2014) also failed to find GPIAS-based evidence of tinnitus in CBA/Ca mice after a damaging noise dose (8–16 kHz at 100 dB SPL for 2 h). This is especially surprising in light of more recent data showing that the 100 dB SPL noise dose increased IC SFRs by an even greater margin than the same noise at 105 dB SPL, which of course caused more damage to the cochlea (Hesse *et al.*, 2016).

Several studies have reported evidence of cochlear damage in people with tinnitus and clinically normal audiograms (Weisz *et al.*, 2006; Schaette and McAlpine, 2011; Gu *et al.*, 2012; Paul *et al.*, 2017), who comprise about 10% of all tinnitus cases. A large study of Danish workers reported that the prevalence of tinnitus increased with occupational noise exposure level and duration in workers with hearing loss, but was not associated with the noise dose in workers with clinically normal hearing (Rubak *et al.*, 2008). On the other hand, Guest *et al.* (2017) found a link between tinnitus and noise exposure history in young adults with normal audiograms, but no evidence of synaptopathy or other cochlear damage. Thus, it remains possible that despite the negative GPIAS results reported here, tinnitus could be induced by prolonged exposure to non-damaging noise even in the absence of hearing loss.

REFERENCES

Basura, G.J., Koehler, S.D., and Shore, S.E. (**2015**). "Bimodal stimulus timing-dependent plasticity in primary auditory cortex is altered after noise exposure with and without tinnitus," J. Neurophysiol., **114**, 3064-3075.

Bowen, G.P., Lin, D., Taylor, M.K., *et al.* (**2003**). "Auditory cortex lesions in the rat impair both temporal acuity and noise increment thresholds, revealing a common neural substrate," Cereb. Cortex., **13**, 815-822.

Carlson, S., and Willott, J.F. (**1996**). "he behavioral salience of tones as indicated by prepulse inhibition of the startle response: relationship to hearing loss and central neural plasticity in C57BL/6J mice," Hear. Res., **99**, 168-175.

Chen, G., Lee, C., Sandridge, S.A., *et al.* (**2013**). "Behavioral evidence for possible simultaneous induction of hyperacusis and tinnitus following intense sound exposure," J. Assoc. Res. Otolaryngol., **14**, 413-424.

Coomber, B., Berger, J.I., Kowalkowski, V.L., *et al.* (**2014**). "Neural changes accompanying tinnitus following unilateral acoustic trauma in the guinea pig," Eur. J. Neurosci., **40**, 2427-2441.

Davis, M., and Gendelman, P.M. (**1977**). "Plasticity of the acoustic startle response in the acutely decerebrate rat," J. Comp. Physiol. Psychol., **91**, 549-563.

Dobie, R.A., and Humes, L.E. (**2017**). "Commentary on the regulatory implications of noise-induced cochlear neuropathy," Int. J. Audiol., **56**, 74-78.

Eggermont, J.J., and Kenmochi, M. (**1998**). "Salicylate and quinine selectively increase spontaneous firing rates in secondary auditory cortex," Hear. Res., **117**, 149-160.

Eggermont, J.J. (**2012**). *The Neuroscience of Tinnitus*. Oxford University Press.

Eggermont, J.J. (**2017**). "Acquired hearing loss and brain plasticity," Hear. Res., **343**, 176-190.

Elgoyhen, A.B., Langguth, B., De Ridder, D., *et al.* (**2015**). "Tinnitus: perspectives from human neuroimaging," Nat. Rev. Neurosci., **16**, 632-642.

Engineer, N.D., Riley, J.R., Seale, J.D., *et al.* (**2011**). "Reversing pathological neural activity using targeted plasticity," Nature, **470**, 101-104.

Fernandez, K.A., Jeffers, P.W., Lall, K., *et al.* (**2015**). "Aging after noise exposure: Acce-leration of cochlear synaptopathy in "recovered" ears," J. Neurosci., **35**, 7509-7520.

Fox, J.E. (**1979**). "Habituation and prestimulus inhibition of the auditory startle reflex in decerebrate rats," Physiol. Behav., **23**, 291-297.

Galazyuk, A., and Hébert, S. (**2015**). "Gap-prepulse inhibition of the acoustic startle reflex (GPIAS) for tinnitus assessment: Current status and future directions," Front. Neurol., **6**, 88.

Grimsley, C.A., Longenecker, R.J., Rosen, *et al.* (**2015**). "An improved approach to separating startle data from noise," J. Neurosci. Meth., **253**, 206-217.

Gu, J.W., Halpin, C.F., Nam, E.C., *et al.* (**2010**). "Tinnitus, diminished sound-level tolerance, and eleva-ted auditory activity in humans with normal hearing sensitivity," J. Neurophysiol., **104**, 3361-3370.

Gu, J.W., Herrmann, B.S., Levine, R.A., *et al.* (**2012**). "Brainstem auditory evoked potentials suggest a role for the ventral cochlear nucleus in tinnitus," J. Assoc. Res. Otolaryngol., **13**, 819-833.

Guest, H., Munro, K.J., Prendergast, G., *et al.* (**2017**). "Tinnitus with a normal audiogram: Relation to noise exposure but no evidence for cochlear synaptopathy," Hear. Res., **344**, 265-274.

Hesse, L.L., Bakay, W., Ong, H.C., *et al.* (**2016**). "Non-monotonic relation between noise expo-sure severity and neuronal hyperactivity in the auditory midbrain," Front. Neurol., **7**, 133.

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

Ison, J.R., O'Connor K., Bowen, G.P., *et al.* (**1991**). "Temporal resolution of gaps in noise by the rat is lost with functional decortication," Behav. Neurosci., **105**, 33-40.

Ison, J.R., Allen, P.D., and O'Neill, W.E. (**2007**). "Age-related hearing loss in C57BL/6J mice has both frequency-specific and non-frequency-specific components that produce a hyperacusis-like exaggeration of the acoustic startle reflex," J. Assoc. Res. Otolaryngol., **8**, 539-550.

Kaltenbach, J.A., Zacharek, M.A., Zhang, J., *et al.* (**2004**). "Activity in the dorsal cochlear nucleus of ham-sters previously tested for tinnitus following intense tone exposure," Neurosci. Lett., **355**, 121-125.

Koch, M. (**1999**). "The neurobiology of startle," Prog. Neurobiol., **59**, 107-128.

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

Lau, C., Zhang, J.W., McPherson, B., *et al.* (**2015**). "Functional magnetic resonance imaging of the adult rat central auditory system following long-term, passive exposure to non-traumatic acoustic noise," Neuroimage, **107**, 1-9.

Li, L., and Frost, B.J. (**2000**). "Azimuthal directional sensitivity of prepulse inhibition of the pinna startle reflex in decerebrate rats," Brain Res. Bull., **51**, 95-100.

Li, S., Choi, V., and Tzounopoulos, T. (**2013**). "Pathogenic plasticity of Kv7.2/3 channel acti-vity is essential for the induction of tinnitus," Proc. Natl. Acad. Sci. USA., **110**, 9980-9985.

Longenecker, R.J., and Galazyuk, A.V. (**2016**). "Variable effects of acoustic trauma on behavioral and neural correlates of tinnitus in individual animals," Front. Behav. Neurosci., **10**, 207.

Maison, S.F., Usubuchi, H., and Liberman, M.C. (**2013**). "Efferent feedback minimizes cochlear neuropathy from moderate noise exposure," J. Neurosci., **27**, 5542-5552.

Munguia, R., Pienkowski, M., and Eggermont, J.J. (**2013**). "Spontaneous firing rate changes in cat primary auditory cortex following long-term exposure to non-traumatic noise: Tinnitus without hearing loss?" Neurosci. Lett., **546**, 46-50.

NIOSH (**1998**). "Criteria for a recommended standard: Occupational noise exposure," National Institute for Occupational Safety and Health Publication No: 98-126.

Noreña, A.J., Gourévitch, B., Aizawa, N., *et al.* (**2006**). "Spectrally enhanced acoustic environ-ment disrupts frequency representation in cat auditory cortex," Nat. Neurosci., **9**, 932-939.

OSHA (**2002**). *Hearing Conservation.* Occupational Safety and Health Administration, U.S. Department of Labor, Publication No: OSHA 3074.

Paul, B.T., Bruce, I.C., and Roberts, L.E. (**2017**). "Evidence that hidden hearing loss underlies amplitude modulation encoding deficits in individuals with and without tinnitus," Hear. Res., **344**, 170-182.

Pienkowski, M., and Eggermont, J.J. (**2009**). "Long-term, partially-reversible reorganization of frequency tuning in mature cat primary auditory cortex can be induced by passive exposure to moderate-level sounds," Hear. Res., **257**, 24-40.

Pienkowski, M., and Eggermont, J.J. (**2010a**). "Intermittent exposure with moderate-level sound impairs central auditory function of mature animals without concomitant hearing loss," Hear. Res., **261**, 30-35.

Pienkowski, M., and Eggermont, J.J. (**2010b**). "Passive exposure of adult cats to moderate-level tone pip ensembles differentially decreases AI and AII responsiveness in the exposure frequency range," Hear. Res., **268**, 151-162.

Pienkowski, M., and Eggermont, J.J. (**2011**). "Cortical tonotopic map plasticity and behaviour," Neurosci. Biobehav. Rev., **35**, 2117-2128.

Pienkowski, M., Munguia, R., and Eggermont, J.J. (**2011**). "Passive exposure of adult cats to bandlimited tone ensembles or noise leads to long-term response suppression in auditory cortex," Hear. Res., **277**, 117-126.

Pienkowski, M., and Eggermont, J.J. (**2012**). "Reversible long-term changes in auditory processing in mature auditory cortex in the absence of hearing loss induced by passive, moderate-level sound exposure," Ear. Hearing, **33**, 305-314.

Pienkowski, M., Munguia, R., and Eggermont, J.J. (**2013**). "Effects of passive, moderate-level sound exposure on the mature auditory cortex: Spectral edges, spectrotemporal density, and real-world noise," Hear. Res., **296**, 121-130.

Pienkowski, M., Tyler, R.S., Roncancio, E.R., *et al.* (**2014**). "A review of hyperacusis and future directions: Part II. Measurement, mechanisms, and treatment," Am. J. Audiol., **23**, 420-436.

Ropp, T.J., Tiedemann, K.L., Young, E.D., *et al.* (**2014**). "Effects of unilateral acoustic trauma on tinnitus-related spontaneous activity in the inferior colliculus," J. Assoc. Res. Otolaryngol., **15**, 1007-1022.

Rubak, T., Kock, S., Koefoed-Nielsen, B., *et al.* (**2008**). "The risk of tinnitus following occupational noise exposure in workers with hearing loss or normal hearing," Int. J. Audiol., **47**, 109-114.

Schaette, R., and McAlpine, D. (**2011**). "Tinnitus with a normal audiogram: physiological evi-dence for hidden hearing loss and computational model," J. Neurosci., **31**, 13452-13457.

Sturm, J.J., Zhang-Hooks, Y.X., Roos, H., *et al.* (**2017**). "Noise trauma induced behavioral gap detection deficits correlate with reorganization of excitatory and inhibitory local circuits in the inferior colliculus and are prevented by acoustic enrichment," J. Neurosci., **37**, 6314-6330.

Sun, W., Lu, J., Stolzberg, D., *et al.* (**2009**). "Salicylate increases the gain of the central auditory system," Neuroscience, **159**, 325-334.

Sun, W., Deng, A., Jayaram, A., *et al.* (**2012**). "Noise exposure enhances auditory cortex responses related to hyperacusis behaviour," Brain Res., **1485**, 108-116.

Turner, J.G., and Parish, J. (**2008**). "Gap detection methods for assessing salicylate-induced tinnitus and hyperacusis in rats," Am. J. Audiol., **17**, S185-S192.

Tyler, R.S., Pienkowski, M., Roncancio, E.R., *et al.* (**2014**). "A review of hyperacusis and future directions. Part I. Definitions and manifestations," Am. J. Audiol., **23**, 402-419.

Weible, A.P., Moore, A.K., Liu, C., *et al.* (**2014**). "Perceptual gap detection is mediated by gap termination responses in auditory cortex," Curr. Biol., **24**, 1447-1455.

Weisz, N., Hartmann, T., Dohrmann, K., *et al.* (**2006**). "High-frequency tinnitus without hearing loss does not mean absence of deafferentation," Hear. Res., **222**, 108-114.

Wu, C., Martel, D.T., and Shore, S.E. (**2016**). "Increased synchrony and bursting of dorsal cochlear nucleus fusiform cells correlate with tinnitus," J. Neurosci., **36**, 2068-2073.

Xiong, B., Alkharabsheh, A., Manohar, S., *et al.* (**2017**). "Hyperexcitability of inferior colli-culus and acoustic startle reflex with age-related hearing loss," Hear. Res., **350**, 32-42.