Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus?

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Hickox AE, Liberman MC. Is noise-induced cochlear neuropathy key to the generation of hyperacusis or tinnitus? J Neurophysiol 111: 552–564, 2014. First published November 6, 2013; doi:10.1152/jn.00184.2013.—Perceptual abnormalities such as hyperacusis and tinnitus show clinically normal thresholds. Recent work in animals has shown that a “neuropathic” noise exposure can cause immediate, permanent degeneration of the cochlear nerve despite complete threshold recovery and lack of hair cell damage (Kujawa SG, Liberman MC. J Neurosci 29: 14077–14085, 2009; Lin HW, Furman AC, Kujawa SG, Liberman MC. J Assoc Res Otolaryngol 12: 605–616, 2011). Here we ask whether this noise-induced primary neuronal degeneration results in abnormal auditory behavior, based on the acoustic startle response (ASR) and prepulse inhibition (PPI) of startle. Responses were measured in mice exposed either to a “neuropathic” noise or to a lower-intensity, “nonneuropathic” noise and in unexposed control mice. Mice with cochlear neuropathy displayed hyperresponsivity to sound, evidenced by enhanced ASR and PPI, while exposed mice without neuronal loss showed control-like responses. Gap PPI tests, often used to assess tinnitus, revealed limited gap detection deficits in mice with cochlear neuropathy only for certain gap-startle latencies, inconsistent with the presence of tinnitus “filling in the gap.” Despite significantly reduced wave 1 of the auditory brainstem response, representing cochlear nerve activity, later peaks were unchanged or enhanced, suggesting compensatory neural hyperactivity in the auditory brainstem. Considering the rapid postexposure onset of both cochlear neuropathy and exaggerated startle-based behavior, the results suggest a role for cochlear primary neuronal degeneration, per se, in the central neural excitability that could underlie the generation of hyperacusis.

acoustic startle response; prepulse inhibition; auditory brainstem response; noise-induced hearing loss; acoustic trauma

ACOUSTIC OVEREXPOSURE has been linked to numerous hearing problems beyond elevation of audiometric threshold. Tinnitus, the persistent perception of phantom sound in the absence of stimulation, and hyperacusis, the perception of moderate-level sounds as intolerably loud, are common sequelae of acoustic overexposure (e.g., Anari et al. 1999; Schmuziger et al. 2006) and can co-occur (Gu et al. 2010; Hébert et al. 2013; for review, see Baguley 2003), typically with concomitant hair cell damage and elevated cochlear thresholds. Although patients with tinnitus and hyperacusis often report a history of traumatic acoustic overexposure, some have clinically normal thresholds (Anari et al. 1999; Brandy and Lynn 1995; Coelho et al. 2007; Schmuziger et al. 2006), suggesting that these perceptual anomalies can arise through peripheral damage that is undetected by standard audiometry. Recent work in animals has shown that noise exposures causing only transient threshold elevation can nonetheless result in permanent loss of cochlear nerve fibers (Kujawa and Liberman 2009; Lin et al. 2011). Cochlear nerve synapses on hair cells can regenerate after exposure, leaving the surviving cell bodies and central projections unresponsive to sound and lacking spontaneous neural activity, as if the target hair cell were lost (Liberman and Kiang 1978). This neuronal loss may be selective for the subgroup of cochlear neurons with high thresholds and low spontaneous discharge rates (Furman et al. 2013), thus explaining the lack of impact of their loss on cochlear thresholds.

Recent human studies have suggested that tinnitus in patients with normal audiograms may be correlated with a peripheral neuropathy, seen as a reduction in amplitude of the first wave of the suprathreshold auditory brainstem response (Gu et al. 2012; Schaette and McAlpine 2011).

Here, using the acoustic startle response (ASR) and prepulse inhibition (PPI) of startle, we examine how noise-induced primary neuronal degeneration changes startle-based responses to auditory stimuli in mice. The whole body ASR, elicited by an intense tone or noise burst, can be attenuated by the presence of a preceding stimulus, or “prepulse.” The magnitude of tone- or noise-burst PPI can be a useful measure of stimulus detectability (Hoffman and Ison 1980). These startle-based responses are often enhanced after systemic administration of salicylate (Yang et al. 2007) and can be enhanced after noise-induced temporary or permanent threshold shift (Chen et al. 2013; Sun et al. 2012), manipulations known to induce central auditory hyperactivity in animal models (for review, see Kaltenbach 2011 and Eggermont 2013). Consequently,
these overreactions to sound are interpreted as behavioral signs of hyperacusis. The gap-detection variant of PPI, where the prepulse is a silent gap in an otherwise continuous carrier, has been used to assess temporal processing (e.g., Bowen et al. 2003) but recently has gained attention as a potential measure of tinnitus in animals (e.g., Turner et al. 2006). In the latter implementation, a decrease in gap PPI is hypothesized to indicate the presence of a tinnitus percept that obscures, or masks, the silent gap. Gap-PPI deficits in noise-exposed or salicylate-injected animals are often attributed to tinnitus (Turner et al. 2006; Yang et al. 2007), although recent evidence suggests that, in humans, tinnitus does not “fill in the gap” (Fournier and Hébert 2013).

Here we show that noise-exposed mice with primary neuronal degeneration exhibit changes in ASR and PPI compared with both unexposed control mice and noise-exposed mice without neuronal loss. The observed robust enhancements of tone- and noise-burst ASR and PPI suggest hyperacusis-like behavior. However, the dependence of reduced gap-detection PPI on precise stimulus parameters is suggestive of auditory processing difficulties rather than perception of tinnitus. Overall, the results suggest that, in addition to known effects of hair cell damage and associated threshold shifts, widespread primary cochlear deafferentation per se may be a critical factor in the development of some perceptual anomalies after noise exposure.

MATERIALS AND METHODS

Animals. CBA/CaJ male mice were used, because their cochlear thresholds are stable out to 1 yr of age (Ohlemiller et al. 2010) and in order to replicate the primary neuronal degeneration phenotype described previously (Kujawa and Liberman 2009). Mice were obtained from Jackson Laboratories (stock no. 000654) at 10 wk of age and were housed in groups under a 12:12-h light-dark cycle (lights on at 7 AM) and constant temperature (72°F). Groups of mice were exposed to high-level noise at 16–18 wk of age, while cagemates served as unexposed age- and sex-matched controls. All procedures were approved by the Institutional Animal Care and Use Committee of the Massachusetts Eye and Ear Infirmary.

Noise exposure. Mice were exposed to octave-band noise (8–16 kHz) for 2 h at either 100 or 94 dB SPL. This octave-band exposure has been shown to produce extensive cochlear synaptopathy and neural loss in the basal half of the cochlea while sparing the apex, thereby providing a useful internal control (Furman et al. 2013; Kujawa and Liberman 2009). Exposures were performed in a small reverberant chamber with an elevated platform in the center where mice were placed, awake and unrestrained, in an acoustically transparent wire cage. The noise waveform was generated digitally with a fifth-order Butterworth filter, amplified (Crown Power Amplifier D75A), and delivered by a compression driver (JBL model 2446H) coupled to an exponential horn in the roof of the chamber. Sound was delivered to the ear, and Kjaer condenser microphone and, in ear, calibrations were performed before each recording. Custom LabVIEW software controlling National Instruments 16-bit soundcards (6052E) generated all ABR, DPOAE stimuli and recorded all responses.

For DPOAEs, the cubic distortion product 2f1-f2 was measured in response to primaries f1 and f2 (frequency ratio f2/f1 = 1.2, and level ratio L1 = L2 + 10), where f2 varied from 8 to 45.3 kHz in half-octave steps. For each f2 frequency, L2 was swept from 10 to 80 dB SPL in 5-dB steps. Pressure measurements in the ear canal were averaged with spectral and waveform averaging before determination of the amplitude of the 2f1-f2 component in dB SPL. Interpolated 2f1-f2 amplitude functions were used to calculate DPOAE threshold with an isoresponse criterion of 5 dB SPL.

ABRs were recorded differentially between needle electrodes at the vertex and at the ventral edge of the pinna, with a ground at the base of the tail. Waveforms were measured in response to 4-ms tone pips (0.5 ms cos2 rise-fall) with alternating polarity at a rate of 40/s. Tone pips at 11.3 and 32 kHz were swept in level from 15 to 80 dB SPL in 5-dB steps. Average waveforms from 512 presentations were amplified, 10,000×, band-pass filtered from 0.3 to 3 kHz, and stored for off-line analysis. Threshold was determined by visual analysis of stacked waveforms from highest to lowest SPL, where threshold was the lowest level at which a reproducible peak or trough appeared. Average waveforms at 80 dB SPL from control animals were used to guide visual analysis. Wave 1 amplitude was defined as the difference between a 1-ms average of the prestimulus baseline and the wave 1 peak, after additional high-pass filtering to remove low-frequency baseline shifts. For all wave 1 analyses, measurements of suprathreshold ABR represent the average peak 1 amplitude across presentations of 60, 70, and 80 dB SPL. Because wave 5 was difficult to consistently identify across mice, especially for 32 kHz stimuli, wave 5 amplitude was defined as the root-mean-square (rms) amplitude of the filtered waveform (at 80 dB SPL) between 3.5 and 5 ms re peak 1 (see Fig. 1D).

Histology: synaptic ribbon counts. Animals were intracardially perfused with 4% paraformaldehyde while deeply anesthetized, and the left inner ears were extracted and postfixed for 2 h at room temperature. Ears were decalcified in EDTA, and the cochlear spiral was microdissected into six pieces. Cochlear whole mounts were triple-immunostained with primary antibodies overnight at 37°C against the following: 1) CtB2P to visualize synaptic ribbons (mouse anti-CtBP2 at 1:200; BD Transduction Labs), 2) Na+-K+-ATPase to visualize primary afferent terminals (goat anti-NKAα3 at 1:100; Santa Cruz no. sc-16052), and Myosin VIIa to visualize inner hair cells (IHCs) (rabbit anti-Myosin VIIa at 1:200; Proteus Biosciences no. 25-6790). Secondary antibodies were applied for 1 h at 37°C as follows: biotinylated donkey anti-mouse (1:200) followed by streptavidin-conjugated Alexa Fluor 568 (1:1,000); Alexa Fluor 488-coupled chicken anti-goat (1:1,000) followed by Alexa Fluor 488-coupled goat anti-chicken (1:1,000); and Alexa Fluor 647-coupled donkey anti-rabbit (1:200).

Pieces were imaged at low power, and a custom ImageJ plug-in was used to create a frequency map. Confocal microscopy (Leica TCS SP2) was used to image the whole mounts at frequency locations of 11.3 and 32 kHz with an oil-immersion ×100 objective (1.4 NA) at ×2 digital zoom and a z step of 0.25 μm. Each frequency location was imaged in two adjacent regions, with ~10 IHCs per region, for a total of roughly 20 IHCs per location per ear. Z stacks of the IHC base were captured, taking care to include all synaptic ribbons, and then analyzed off-line with Amira (Visage Imaging). Individual ribbons were isolated within Amira with the connected components function and iso-surface tools, counted, and expressed as synaptic ribbons per number of IHCs in the stack.
Reflex modification audiometry: ASR and PPI. ASR and PPI of ASR were measured in noise-exposed mice and in unexposed, age-matched control mice at roughly log-spaced postexposure survivals from 1 day to 10 wk. All ASR and PPI tests were conducted in a table-top sound isolation booth (Mac2, IAC) lined with acoustic foam panels to reduce acoustic reflections. Mice were placed in custom, acoustically transparent cages (7 × 5 × 4 cm) with an elliptical floor designed to restrict explorative behavior while still allowing full expression of the whole body startle response. Each cage was placed on a cantilevered armature designed to couple vertical cage motion to an accelerometer mounted at the base of the armature. An array of three speakers (Fostex FT17H Horn Super Tweeter, flat response from 4 to 50 kHz, ±4 dB) was mounted around the cage to present startle stimuli via one speaker (above the cage) and the prepulse and background stimuli via two speakers (on either side of the cage). All ASR and PPI stimuli and responses were generated and recorded with custom LabVIEW software running on a 24-bit PXI (National Instruments).

ASR and PPI tests were conducted either in quiet or in the presence of continuous broadband noise (BBN) at 60 dB SPL. Startle stimuli were tone or noise bursts, 20 ms in duration with 0.1-ms rise-fall times. For all PPI tests, startle stimuli were BBN bursts at 105 dB SPL. For acoustic PPI tests, the prepulse was 50 ms in duration: tone-burst prepulses had 5-ms rise-fall ramps, whereas gap prepulses had 0.1-ms ramps (Turner et al. 2006). Tone- or noise-burst prepulses were positioned to terminate immediately (0 ms) before startle onset. For gap-PPI tests, the prepulse was a gap in an otherwise continuous carrier at 60 dB SPL. “Near-gap” prepulses were positioned to terminate immediately (0 ms) before startle onset, while “far-gap” prepulses terminated 80 ms before startle onset. The gap carrier was either BBN or 0.5-octave band-pass noise centered at frequencies from 5.6 to 45.3 kHz in 0.5-octave steps. Band-pass filtering of the carrier was performed with cascaded high- and low-pass eighth-order Butterworth filters to achieve a 48 dB/octave roll-off (Turner et al. 2006). For visual PPI tests, the prepulse was a 50-ms light burst (~1,000 lux) from white LEDs on either side of the startle cage, with

![Graphs showing ASR and PPI measurements](http://jn.physiology.org/abstract/554)

![Fig. 1.](http://jn.physiology.org/abstract/554)
a delay of 100 ms between light offset and startle onset. Light PPI tests were conducted in quiet, in continuous BBN, or in continuous 0.5-octave band-pass noise centered at 11.3 kHz.

Each ASR and PPI test consisted of 11 blocks of trials (see, e.g., Ison and Allen 2007), where the first block was excluded from analysis because startle amplitudes in moderate background noise adapt to an asymptotic level during this initial period (Davis 1974). Within each block, each varied parameter was presented once, in randomized order. For ASR and tone- or noise-burst PPI tests, each sound pressure level was presented once per block, and PPI tests included an additional two startle-only (“baseline”) trials in each block. For gap PPI or light PPI, each block consisted of one prepulse trial and two baseline startle trials. For all tests, the interval between trials varied randomly between 15 and 25 s. Each mouse performed, on average, a total of 15 different ASR and/or PPI tests in randomized order. Mice were tested for up to 3 h per day, usually two or three times per week. For each combination of postexposure time and test type, any given mouse performed the test only once. Each session began with a 2-min acclimatization period in the cage before startle testing began, and all tests were conducted in darkness. The experimenter could observe the mouse during testing via a monitor fed by the output of an infrared camera.

For each trial, accelerometer output was digitized from 880 ms before to 120 ms after startle-stimulus onset. Startle amplitude was calculated as the rms of the 100 ms following startle-stimulus onset. In some PPI trials, usually for tone- or noise-burst prepulses at a level approaching startle threshold, the prepulse itself elicited a startle response that obscured any response to the startle elicitor. Therefore an algorithm was developed to exclude trials from analysis if the response waveform during the prepulse window (prepulse onset to prepulse offset) met two criteria: 1) rms amplitude exceeded 2 standard deviations above the lowest activity levels in this window throughout the entire test, and 2) waveform peaks in this window matched stereotyped latencies of startle waveform peaks characteristic of a startle response for our apparatus. The rejection rate of these high-intensity prepulse trials, across all mice within a given exposure group, never exceeded 10%. Within each test, for a given mouse, startle amplitudes were averaged across all trials of the same stimulus conditions. PPI was defined as fractional reduction of startle, i.e., 1 minus the ratio of startle amplitude with vs. without prepulse (see, e.g., Ison and Allen 2007). Thus a value of 0 means no effect of the prepulse, a value of 1 means complete inhibition of startle, and a negative value indicates prepulse facilitation of the startle response.

Statistical analyses. Three-way or two-way ANOVAs were performed, where appropriate, on data from independent mice with SAS software (SAS Institute) with between-subject effect of exposure group and within-subject effects of startle or physiological test parameters. ANOVAs were constructed as mixed models with random effect of subject and a residual correlation structure (either compound symmetry, heterogeneous compound symmetry, or unstructured, based on a likelihood ratio test) to control for repeated measures. Where significant interaction of exposure group with the within-subject variables was found, we examined the simple main effect (slice in SAS) of exposure group at each level, or combination of levels, of the within-subject variable(s). Simple main effects tests were assessed for significance with the Holm-Bonferroni method with α level of 0.05, and, where significant, pairwise comparisons among groups means were performed using Holm-Bonferroni correction, α level 0.05 (ttest2 in MATLAB, MathWorks; P values reported in figure legends).

RESULTS

Neuropathic and nonneuropathic ears. Mice exposed to octave-band noise at 100 dB SPL for 2 h show a large threshold shift 1 day after exposure, followed by complete threshold recovery within 1–2 wk (Kujawa and Liberman 2009). Although cochlear thresholds (and hair cells) recover, there is significant degeneration of cochlear nerve terminals at the bases of IHCs and a commensurate reduction in the amplitude of suprathreshold cochlear neural potentials, such as wave 1 of the ABR (Kujawa and Liberman 2009).

Here we studied the effects of this noise-induced primary neuropathy on auditory behavior, as measured with the ASR and PPI of startle. To control for extra-auditory effects of high-level noise exposure, we sought to define a “nonneuropathic” exposure, which might cause a similar degree of temporary threshold shift (and systemic stress) yet be fully reversible with respect to both hair cell and neuronal function. As shown in Fig. 1A, 2-h exposures at either 100 or 94 dB SPL caused a severe temporary threshold shift (up to 40 dB) in DPOAEs, primarily a metric of outer hair cell function (Lonsbury-Martin and Martin 2003). Similarly, exposure caused temporary shifts of up to 50 dB in ABR thresholds (Fig. 1B). The maximum threshold shifts moved to higher frequencies as exposure level increased, consistent with known level-dependent nonlinearities in cochlear mechanics (Robles and Ruggiero 2001). Both exposures also show nearly complete DPOAE and ABR threshold recovery when measured as soon as 1 wk later.

To assess neuropathy, the amplitude of ABR wave 1 (Fig. 1C), representing the summed activity of cochlear nerve fibers, was measured in response to suprathreshold tone bursts at 11.3 and 32 kHz. On the basis of prior study of the 100-dB exposure (Kujawa and Liberman 2009), we expect a large mean amplitude reduction at 32 kHz and less at 11.3 kHz, as was observed in our 100 dB exposure group (36% vs. 7%, respectively) (Fig. 1C). The later ABR waves, reflecting sound-evoked activity in higher brainstem centers, are not attenuated in the 100 dB group; in fact, wave 5 is enhanced at 32 kHz at 1–2 wk after exposure (Fig. 1D), suggesting possible neuronal hyperactivity in central auditory pathways. Animals exposed at 94 dB (Fig. 1C) showed a smaller reduction in mean wave 1 amplitude at 32 kHz: 15% reduced re control mice vs. 36% in the 100 dB group. Despite the small reduction in group mean amplitude, the majority of the 94 dB-exposed mice showed responses within the range of control values, suggesting that, on an individual basis, many 94 dB mice did not acquire neuropathy. The interaction of exposure group, stimulus frequency, and ABR wave was significant in a three-way ANOVA [F(2,499) = 25.21, P < 0.0001], and the simple main effect of exposure group was significant for wave 1 amplitudes at 32 kHz [F(2,499) = 80.58, P < 0.0001] and for wave 5 amplitudes at 32 kHz [F(2,499) = 15.1, P < 0.0001].

To more directly assess the loss of innervation in the IHC area (Fig. 2A), we selected, for histological analysis, three animals from each exposure group with ABR amplitudes representative of the group mean. Synaptic ribbons, which are easily counted in immunostained cochlear tissue (Fig. 2, B–D), provide a reliable proxy for loss of afferent synapses after acoustic injury (Maison et al. 2013). Frequency and exposure group yielded a significant interaction in an overall ANOVA [F(2,6) = 5.33, P < 0.05], and simple main effect of exposure group on ribbon counts was significant for the 32 kHz region [F(2,6) = 21.07, P < 0.01] but not for the 11.3 kHz region [F(2,6) = 4.41, P = 0.0664]. Ribbon counts (Fig. 2E) showed a large and highly significant reduction in the 100 dB group re control mice (44%) and re the 94 dB group at 32 kHz and only
Coil the spiral, i.e., at cochlear frequencies there was scattered outer hair cell loss in the basalmost 15% of outside of the extreme base. In the ears exposed at 100 dB, light microscopy revealed no hair cell loss in any cochlea, and pathophysiology suggest that the 100-dB and 94-dB exposures achieve the intended contrast between neuropathic and nonneuropathic conditions. Inspection of the organ of Corti by light microscopy revealed no hair cell loss in any cochlea, outside of the extreme base. In the ears exposed at 100 dB, there was scattered outer hair cell loss in the basalmost 15% of the spiral, i.e., at cochlear frequencies > 54 kHz. The neuropathy observed at 11.3 kHz and 32 kHz regions after the 100-dB exposure is thus “primary” in the sense that nerve terminals and synapses disappear from surviving hair cells, in contrast to “secondary” degeneration observed as a consequence of hair cell loss.

Prepulse inhibition and hearing in noise. Central (retrocochlear) processing of threshold and suprathreshold stimuli was assessed with acoustic PPI. Prepulses were tone and BBN bursts, measured both in quiet and in a continuous noise background (see Fig. 3, A and D, insets).

In a quiet background, PPI growth functions in control animals (Fig. 3, A–C) suggest that the prepulse tone bursts at 11.3 (Fig. 3A) or 32 kHz (Fig. 3B) can modulate the startle response at sound pressures as low as 25 dB SPL. The similarity of these PPI “thresholds” to cochlear nerve fiber thresholds (Taberner and Liberman 2005) and to behavioral thresholds (Radziwon et al. 2009) in the same mouse strain (CBA/CaJ) suggests that PPI can be used to estimate the behavioral audiogram. Addition of background noise should elevate behavioral thresholds for tones, and, indeed, PPI thresholds for control mice were elevated by ~20–30 dB by the addition of continuous noise at 60 dB SPL (Fig. 3, D–F), seen as a rightward shift in the PPI-in-noise functions compared with PPI in quiet.

Mice with primary neural degeneration (i.e., the 100-dB exposure group) might be expected to show reduced PPI in the presence of background noise, given that the exposure-induced neural loss is selective for the high-threshold cochlear nerve fibers (Furman et al. 2013) that are normally particularly resistant to masking by continuous background noise (Costalupes et al. 1984). Paradoxically, the neuropathic mice showed significantly greater PPI for higher-level tones in noise (Fig. 3, D and E). This was confirmed by significant main effect of exposure group at several prepulse level × test conditions (conditions in Fig. 3, D and E, with statistical symbols; in each case, P value of simple effect test < 0.001), following a significant three-way interaction by ANOVA ([F(70,1827) = 1.45, P < 0.01]. Subsequent paired comparisons revealed that PPI in noise for the neuropathic (100 dB) group was significantly enhanced compared with both control mice and the 94 dB group for 32-kHz prepulses at 65 and 75 dB SPL and for 11.3 kHz prepulses at 65 dB SPL and was enhanced compared with the 94 dB group for 32-kHz prepulses at 55 dB SPL.

PPI enhancement in the neuropathic ears cannot be due to the noise exposure per se, because responses of the nonneuropathic group to high-level prepulses did not differ significantly from control mice under any stimulus condition (Fig. 3). Nor is PPI enhancement related to the small residual threshold shift seen in some ears exposed at 100 dB. When the neuropathic (100 dB) group is divided into those with full threshold recovery vs. those with residual shift, both subgroups showed PPI enhancement (Fig. 4A). The PPI enhancement does appear to depend on the degree of cochlear neuropathy. When the 94 dB group is divided into subgroups based on ABR amplitude, the cases with smaller wave 1 show greater PPI (Fig. 4B).

To test whether the hyperresponsivity suggested by the enhanced PPI in neuropathic mice was specific to the auditory pathway, we implemented a cross-modal PPI test using a visual prepulse stimulus preceding the acoustic startle (Fig. 5). With appropriate stimulus parameters, a visual stimulus can also suppress startle amplitude (Hoffman and Ison 1980; Tremblay et al. 2012). Using this assay, we found no evidence for generalized sensory hyperresponsiveness in the neuropathic mice compared either with the unexposed control mice or with...
Fig. 3. Prepulse inhibition (PPI) of the acoustic startle is enhanced in mice with noise-induced neuropathy (100-dB exposure). Growth of PPI as a function of prepulse level is shown for 11.3 kHz (A and D), 32 kHz (B and E), or broadband noise (BBN; C and F) prepulses. Means (±SE) are shown (key in F applies to all panels). For tone-burst prepulses (A, B, D, E), n = 11–21 mice/group; for broadband prepulses (C and F), n = 9–13 mice/group. D–F are the same as A–C except that, for the former, PPI is measured in the presence of continuous broadband background noise at 60 dB SPL (compare schematic insets in A and D; “S” indicates startle elicitor). Data were obtained at 1–10 wk after exposure. Statistical significance by t-test: *P < 0.02, 100 dB group different from both control and 94 dB groups; +P < 0.001, 100 dB group different from 94 dB group.

Because PPI measures are expressed as a fractional alteration in startle magnitude, changes in the startle response itself could complicate the interpretation. Indeed, PPI varies with baseline startle threshold and hyperacusis-like behavior. For example, PPI is better predicted by ABR wave 1 amplitude (B) than by residual threshold shift (A). PPI values for 32-kHz prepulses at 75 dB in 60-dB noise, a stimulus condition for which PPI was enhanced in the neuropathic group (Fig. 3E). A: the neuropathic (100 dB) group is divided according to DPOAE threshold: ≤ (red circles) or > (red triangles) 1 SD above the mean of the control group (dotted line). Each small symbol represents a different mouse, and mean PPI is shown for each group/subgroup by the bold symbols. DPOAE threshold for each animal is averaged from 8 to 45.3 kHz and expressed re control mean. B: the nonneuropathic (94 dB) group is divided according to ABR wave 1 amplitude; ≤ (blue triangles) or > (blue circles) 1 SD below the 94 dB group mean (dotted line). Each small symbol represents a different mouse, and mean PPI is shown for each group/subgroup by the bold symbols. ABR wave 1 amplitude is calculated as in Fig. 1C. PPI data are extracted from Fig. 3E and from Fig. 9D, and corresponding DPOAE and ABR data are extracted from Fig. 1A and Fig. 1C, respectively. Color key from previous figures applies here.
magnitude in both humans and animals (Csomor et al. 2008; but see Ison et al. 1997). Thus we measured the thresholds and growth of ASR, without PPI, in the three groups of mice.

Growth of ASR with level was measured for tonal and BBN startle stimuli in quiet and in a continuous-noise background (see Fig. 6, A and D, insets). Startle responses to 105-dB SPL BBN bursts in noise, the startle stimulus used in the PPI-in-noise tests (Fig. 3, D–F), were not different among exposure groups by a test of simple main effect [Fig. 6F; $F(2,3001) = 0.26, P = 0.77$, preceding ANOVA described below]. Moreover, the baseline startle responses extracted from the PPI-in-noise tests where neuropathic mice showed enhanced PPI (Fig. 3, F), were not different among exposure groups.

Fig. 5. Light-evoked PPI of the acoustic startle is not altered for mice with noise-induced neuropathy compared with unexposed or nonneuropathic groups. Mean (± SE) PPI values in response to a 50-ms light burst are shown for quiet (A) and BBN (B) backgrounds and for background noise of a half-octave band centered at 11.3 kHz (narrowband noise, NBN; C) (compare schematic insets across panels; “S” indicates startle elicitor). Data were obtained 6–10 wk after exposure, i.e., at 22–28 wk of age, where photic PPI is still robust in the CBA/CaJ mouse (Tremblay et al. 2012). Data are from $n = 17–21$ samples (from 9–11 mice) per group per condition.

Fig. 6. Acoustic startle response (ASR) is enhanced near startle threshold in mice with noise-induced neuropathy (100-dB exposure). Growth of startle amplitude with level is shown for startle stimuli at 11.3 kHz (A and D), 32 kHz (B and E), or BBN (C and F) startles. Means (± SE) are shown (key in F applies to all panels). For tone-burst startles (A, B, D, E), $n = 13–32$ mice/group; for broadband startles (C and F), $n = 9–17$ mice/group. D–F differ from A–C only by the presence of continuous broadband background noise at 60 dB SPL (compare schematic insets in A and D; “S” indicates startle elicitor). Inset in B shows responses from 75 to 95 dB on an expanded scale. Data were obtained at 1–10 wk after exposure. Statistical significance by $t$-test: $*P < 0.03$, 100 dB group different from both control and 94 dB groups; $#P < 0.001$, 100 dB group different from control group; $^{\dag}P < 0.01$, 100 dB group different from 94 dB group. Nonzero responses at low startle levels (<70 dB SPL) represent background activity, which was not significantly different across groups.
Fig. 7. Regardless of exposure conditions, enhancement of ASR is better predicted by ABR wave 1 amplitude (b) than by residual threshold shift (a). ASR was elicited by 32-kHz tone bursts at 90 dB in 60-dB noise, a stimulus that evoked significantly enhanced ASR in neuropathic mice (Fig. 6E). A: the neuropathic (100 dB) group is divided according to DPOAE threshold: ≤ (red circles) vs. > (red triangles) 1 SD above the control mean (dotted line). B: the nonneuropathic (94 dB) group is split according to ABR wave 1 amplitude: ≤ (blue triangles) or > (blue circles) 1 SD below the mean of the 94 dB group (dotted line). ASRs are extracted from Figs. 6E and 9E, and corresponding DPOAE and ABR data are extracted from Figs. 1, A and C, respectively. Other display conventions are as described for Fig. 4.
making them difficult to visually distinguish.

Note that for BBN carriers (B), pathic group appear within days of exposure and are stable for at least 10 wk. Overall, the longitudinal data considered thus far were averaged over postexposure survivals ranging from 1 to 10 wk. In considering underlying mechanisms, it is informative to examine the onset and offset time courses of the hyperresponsive behavior in the neuropathic exposure group, examined here for 32-kHz stimuli. As shown in Fig. 9A, ABR threshold recovery is complete by 1 wk after exposure, with which time ABR wave 1 amplitude has also reached asymptote (Fig. 9B). ABR wave 5 amplitude is initially reduced during the period of temporary threshold shift but eventually recovers to, and at some time points exceeds, control responses (Fig. 9C). The enhancements of tone-burst PPI (Fig. 9D) and tone-burst ASR (Fig. 9E) are not noticeable 1 day after exposure, when thresholds are still greatly elevated, but appear within 1 wk and stabilize, at least out to 10 wk after exposure. Near-gap PPI is consistently reduced in the neuropathic group for up to 10 wk after exposure (Fig. 9F), while far-gap PPI shows comparable behavior between neuropathic and control mice (Fig. 9G). Overall, the longitudinal data suggest that noise-induced changes in behavior in the neuropathic group appear within days of exposure and are stable for at least 10 wk.

DISCUSSION

Noise exposure and hyperactivity in central auditory pathways. Tinnitus, the perception of phantom sounds, and hyperacusis, the reduced tolerance for moderate sound levels, are often comorbid (for review, see Baguley 2003), and both can be triggered by a single episode of acoustic overexposure (see, e.g., Anari et al. 1999; Schmuziger et al. 2006). Given the nature of these anomalies, it is natural to speculate that they arise from either spontaneous or sound-evoked hyperactivity somewhere in the auditory neural pathways.

In the periphery, acoustic injury typically results in reduced sound-evoked and spontaneous activity in the cochlear nerve (Heinz and Young 2004; Liberman and Dodds 1984a; Liberman and Kiang 1984). This overall reduction in peripheral neural activity occurs both because damage to (or loss of) the sensory cells reduces (or eliminates) synaptic transmission to cochlear nerve terminals (Liberman and Dodds 1984b; Liberman and Kiang 1978) and because overdriving the cochlear neurons during exposure induces glutamate excitotoxicity that destroys their peripheral terminals and leads to widespread cochlear nerve degeneration, even when the hair cells survive (Puel et al. 1998; Wang et al. 2002). In contrast, extent spontaneous and sound-evoked hyperactivity have been observed throughout the central auditory pathway (for review, see Eggermont 2013). Such hyperactivity is measurable within a few hours (Noreña and Eggermont 2003; Salvi et al. 2000) and can persist indefinitely, in the absence of further experimental manipulation (Bauer et al. 2008; Kaltenbach et al. 2000).

Neurophysiological studies in vivo and imaging studies in vitro have suggested that decreased inhibition in an early stage of the ascending pathway, for example in dorsal cochlear nucleus (DCN), leads to the “sign change” that underlies the transformation of reduced cochlear nerve output into enhanced central activity (for review, see Roberts et al. 2010; see also Middleton et al. 2011).

In principle, such central hyperactivity could arise directly from trauma-evoked changes in local neural circuitry during exposure or indirectly as a response to reduced cochlear neural output. Partial reversal of neural hyperactivity in the inferior colliculus (IC) from ablating the cochlea or DCN within the

*Fig. 8. Gap prepulses elicit reduced PPI in neuropathic (100-dB exposure) mice for a narrowband carrier at 32 kHz only when the gap was placed near the startle elicitor. Gap PPI was measured for half-octave band carriers at 7 log-spaced center frequencies and for a BBN carrier, for both “near-gap” (A) and “far-gap” (B) conditions (compare schematic insets in A and B; “S” indicates startle elicitor). Mean (± SE) PPI values are shown (key in B applies to both panels). Gap PPI is significantly reduced in the neuropathic (100 dB) group compared with control mice for near gaps in a 32-kHz narrowband carrier (*P < 0.05). Data were obtained from 1 to 10 wk after exposure, with n = 19–40 samples (from 10–25 mice) per group per condition for near gaps (A) and n = 43–62 samples (from 13–26 mice) per group per condition for far gaps (B). Note that for BBN carriers (A and B), control group means are nearly identical to 94 dB group means, making them difficult to visually distinguish.
Fig. 9. After initial threshold stabilization (~1 wk), performance on all startle-based tests was stable with increasing postexposure time. Threshold (A), wave 1 amplitude (B), and wave 5 amplitude (C) of ABRs at 32 kHz are normalized re control means. Wave 1 amplitudes are calculated as in Fig. 1. Wave 5 is the root-mean-square (rms) amplitude of the 80 dB SPL waveform from 3.5–5 ms re peak 1. Tone-burst PPI for 32-kHz prepulses at 75 dB (D) and tone-burst ASR for 32-kHz startle stimuli at 90 dB (E) were measured in 60-dB background noise. Gap PPI is shown for a half-octave band noise carrier centered at 32 kHz for near-gap (F) and far-gap (G) conditions. Mean (± SE) values are shown. Postexposure times are grouped logarithmically and plotted at the mean time for each time bin.

first few weeks after exposure (Manzoor et al. 2012; Mulders and Robertson 2009) suggests a dependence on altered peripheral activity. At longer survivals, however, central hyperactivity becomes less dependent on input from the periphery (Mulders and Robertson 2011), consistent with clinical reports of longstanding tinnitus that failed to disappear after surgical destruction of the cochlear nerve (House and Brackmann 1981). Alterations in ASR and PPI magnitude observed here in the neuropathic group occurred within the first week after exposure and remained for at least 10 wk (Fig. 9), mirroring this rapid onset and persistence of central hyperactivity after noise exposure. In light of previous studies, it is likely that noise-induced primary cochlear neuropathy initially catalyzes the neural hyperactivity underlying enhanced behavioral response to sound but the persistence of altered behavior weeks later relies on additional plasticity of the central auditory system.

Acoustic startle and prepulse inhibition of startle as measures of hyperacusis. The subcortical circuitry of the acoustic startle in rodents is driven by cochlear nerve input to the cochlear root neurons, which project to the sensorimotor interface in the caudal pontine reticular nucleus that ultimately excites spinal motor neurons responsible for the whole body startle response (Lee et al. 1996; Lingenhöhl and Friauf 1994). Ventral and dorsal cochlear nuclei are also proposed to contribute to the startle response, on the basis of evidence from labeling and lesioning studies (for review, see Yeomans and Frankland 1995; see also Meloni and Davis 1998). Auditory PPI of startle is mediated in large part by the IC, without which auditory, but not visual, PPI is reduced or eliminated (for review, see Fendt et al. 2001). All these ascending auditory nuclei may become hyperactive after noise exposure (for review, see Kaltenbach 2011 and Eggermont 2013), and this hyperactivity may drive the enhancement of ASR and PPI that we observed in the neuropathic mice. Given that the nonneuropathic exposure caused a similar temporary threshold shift (Fig. 1) without enhancing ASR or PPI (Fig. 3, Fig. 6), and that visually elicited PPI was unaffected (Fig. 5), the hyperacusis-like behavior was likely not generated by extra-auditory effects of noise such as stress or fear, which can exaggerate ASR or PPI (Davis 2006; Du et al. 2011). Taken together, the results suggest that cochlear nerve degeneration, per se, is critical to the generation of hyperacusis.

Enhanced ASR amplitude and greater PPI have been reported in animal models of salicylate-induced tinnitus, where they are interpreted as signs of hyperacusis (Sun et al. 2009; Yang et al. 2007). In this systemic salicylate model, increased evoked response amplitude in auditory cortex is thought to underlie the change in behavior, rather than increased “gain” of the subcortical components of startle and startle inhibition, given that systemic salicylate depresses responses in the cochlear nerve and midbrain (see, e.g., Sun et al. 2009). Enhanced cortical, rather than subcortical, activity has also been implicated in hyperacusis-like behavior after acoustic overexposure, where a temporary threshold shift was associated with an acute (1 h after exposure) increase in auditory cortical spiking and in ASR amplitude, with no concurrent enhancement of IC response (Sun et al. 2012). These results were interpreted as a direct effect of exposure on cortical physiology and cortex-influenced behavior. Alternatively, they could arise indirectly from a temporary reduction in spontaneous activity...
in the auditory nerve (Liberman and Dodds 1987), which could lead to temporary changes in central “gain.”

Cochlear neuropathy in the absence of hair cell loss may also be key to the generation of hyperacusis-like behavior in the C57BL/6j mouse model of progressive sensorineural hearing loss. At 7 mo, neuronal counts are reduced throughout the cochlea, whereas hair cells are lost mainly in the basal turn (Hequembourg and Liberman 2001). As these mice age, PPI and startle amplitude are enhanced and startle threshold is reduced for mid- and low-frequency tones, suggestive of hyperacusis-like behavior (Ison et al. 2007; Ison and Allen 2003; Willott et al. 1994). The present results suggest that it could be the loss of apical-turn neurons that drives this low-frequency hyperresponsivity, rather than central reorganization arising from the loss of basilar-turn hair cell loss (for review, see Willott 1996).

Reduction in gap PPI as a measure of tinnitus. Gap-PPI deficits in noise-exposed animals are often attributed to tinnitus, rather than threshold shift, based on the persistence of the deficit after threshold recovery or the presence of a (normal) unexposed ear (for discussion, see Lobarinas et al. 2013). Given that noise-induced cochlear neuropathy correlates with tinnitus-like behavior as assayed by an operant-conditioning paradigm (Bauer et al. 2007), the neuropathic group in the present study might be expected to experience tinnitus. Here, noise-exposed mice with cochlear neuropathy showed gap-PPI deficits only for a narrowband noise carrier at 32 kHz in the near-gap condition (Fig. 8A). This deficit could not be attributed to differences in baseline startle response (Lobarinas et al. 2013) or to skewing of the mean by prepulse facilitation. Comparison with performance in the far-gap condition (Fig. 8B) reveals that the deficit is dependent on the gap-startle interval. At face value, these results are inconsistent with the idea that any perceived tinnitus is simply “filling in the gap.” If central hyperactivity that underlies tinnitus reflects increased “noise” (Zeng 2013), presumably manifested as increased spontaneous firing (for review, see Kaltenbach 2011), the internally generated noise and/or corresponding percept should obscure the gap prepulse at any latency where robust PPI is normally observed.

The discrepancy between near- and far-gap PPI in the neuropathic group could arise for a number of reasons. The absence of noise onset as a cue in the near-gap condition (it occurs simultaneously with the startle elicitor) could be a key difference: the presence of both noise offset and onset cues in the far-gap condition might make the gap more robust to the gap-PPI circuitry. An enhanced, hyperacusis-like response to noise onset at the end of the gap, only present in the far-gap case, could potentially mask reduced gap PPI that would otherwise be observed, as in the near-gap condition. Alternatively, the differences in gap-startle latency could engage different levels of the central auditory pathways. Indeed, it has been reported that lesions of auditory cortex affect far-gap but not near-gap detection (Figs. 2 and 4 of Bowen et al. 2003). Regardless of the underlying reason(s), the results in Fig. 8 suggest caution in the use of gap PPI as an assay for tinnitus.

Cochlear nerve degeneration as an elicitor of hyperacusis and tinnitus. Patients complaining of hyperacusis and tinnitus often have sensorineural hearing loss, while some have normal audiograms (Anari et al. 1999; Brandy and Lynn 1995; Coelho et al. 2007; Schmuziger et al. 2006). Similarly, hyperacusis in central auditory pathways has been seen after traumatic noise exposures that cause severe permanent threshold shifts (e.g., Kaltenbach et al. 2000) but also after moderate exposures that cause only temporary threshold elevations (e.g., Bauer et al. 2008), suggesting that hair cell death is not necessary to evoke compensatory changes in central auditory activity. Although hair cell lesions in the extreme cochlear base (at extra-auditory frequencies) may have a role in perceptual abnormalities, it is possible that noise-induced loss of high-threshold cochlear afferent fibers (Furman et al. 2013), also undetected by clinical audiometry, can trigger neural hyperactivity that underlies hyperacusis and/or tinnitus in patients with normal thresholds.

Here we tested this hypothesis by comparing the sequelae of two types of noise exposure: both caused significant temporary threshold shifts; neither caused significant loss of sensory cells; yet only one caused significant loss of cochlear neurons. Consistent with the hypothesis, only the neuropathic exposure led to central hyperactivity, as evidenced by the enhancement of ABR wave 5 (Fig. 1D), consistent with sound-evoked hyperactivity arising from the IC (Melcher and Kiang 1996). The control-like amplitudes of intermediate waves 2–4, which are generated largely by the bushy cell pathway of the ventral cochlear nucleus and superior olivary complex (Melcher and Kiang 1996), despite the significant wave 1 decrement (Fig. 1D) also reflects a degree of increased “gain” in these central auditory pathways. Recent human studies have reported an enhancement of the wave 5-to-wave 1 ratio in subjects with tinnitus and normal thresholds, compared with audiometrically matched control subjects, suggestive of central neural hyperactivity (Gu et al. 2012; Schaette and McAlpine 2011). The present results provide empirical evidence that the enhancement of wave 5-to-wave 1 ratio after acoustic injury is likely due to the loss of cochlear nerve fibers rather than a direct effect of noise exposure on the central auditory system or damage to the cochlear sensory cells.

Enhanced startle response amplitudes and/or lower startle thresholds, suggestive of hyperacusis, have been reported in animals with hearing loss induced by salicylate (Sun et al. 2009), aging (Ison and Allen 2003), or noise (Chen et al. 2013). In these studies, startle amplitudes were elevated at all levels above startle threshold. Here, in our neuropathic animals with fully recovered thresholds, startle amplitudes were enhanced only at moderate intensities, converging with control responses at the highest stimulus levels (Fig. 6). This difference may arise because the putative increase in central auditory gain grows with increasing peripheral damage. However, it is not clear which type of anomalous startle behavior more closely mirrors the human condition, as the response to high-level sound is difficult to assess in those for whom such stimuli are so profoundly disturbing. Nevertheless, the exaggerated responses for moderate-level sounds is reminiscent of loudness growth functions in hyperacusis individuals (e.g., Brandy and Lynn 1995), suggesting a role for noise-induced cochlear neuropathy in abnormal sound level tolerance.

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