Noise Trauma Induced Plastic Changes in Brain Regions outside the Classical Auditory Pathway

Guang-Di Chen*, Adam Sheppard, Richard Salvi

Center for Hearing and Deafness, SUNY at Buffalo, Buffalo, NY 14214, USA

Send correspondence to:

Guang-Di Chen, Ph.D.

Center for Hearing and Deafness, State University of New York at Buffalo, 137 Cary Hall, 3435 Main Street, Buffalo, NY 14214, USA

Tel: +1-716-829-5298

Fax: +1-716-829-2980

E-mail: gchen7@buffalo.edu

Abstract:

The effects of intense noise exposure on the classical auditory pathway have been extensively investigated; however, little is known about the effects of noise-induced hearing loss on non-classical auditory areas in the brain such as the lateral amygdala (LA) and striatum (Str). To address this issue, we compared the noise-induced changes in spontaneous and tone-evoked responses from multiunit clusters (MUC) in the LA and Str with those seen in auditory cortex (AC). High-frequency octave band noise (10-20 kHz) and narrow band noise (16-20 kHz) induced permanent threshold shifts (PTS) at high-frequencies within and above the noise band but not at low frequencies. While the noise trauma significantly elevated spontaneous discharge rate (SR) in the AC, SRs in the LA and Str were only slightly increased across all frequencies. The high-frequency noise trauma affected tone-evoked firing rates in frequency and time dependent manner and the changes appeared to be related to severity of noise trauma. In the LA, tone-evoked firing rates were reduced at the high-frequencies (trauma area) whereas firing rates were enhanced at the low-frequencies or at the edge-frequency dependent on severity of hearing loss at the high frequencies. The firing rate temporal profile changed from a broad plateau to one sharp, delayed peak. In the AC, tone-evoked firing rates were depressed at high frequencies and enhanced at the low frequencies while the firing rate temporal profiles became substantially broader. In contrast, firing rates in the Str were generally decreased and firing rate temporal profiles become more phasic and less prolonged. The altered firing rate and pattern at low frequencies induced by high frequency hearing loss could have perceptual consequences. The tone-evoked hyperactivity in low-frequency MUC could manifest as hyperacusis whereas the discharge pattern changes could affect temporal resolution and integration.

Introduction:

Intense noise exposures have long been known to induce temporary or permanent noise-induced hearing loss (NIHL) and cochlear damage that reduces neural output of the cochlea. Paradoxically, NIHL often results in enhanced sound-evoked responses in the central auditory pathway indicative of enhanced central gain, a form of homeostatic plasticity that partially compensates or overcompensates for peripheral hearing loss (Salvi et al., 1990, Salvi et al., 2000, Syka and Rybalko, 2000, Popelar et al., 2008, Sun et al., 2008). The enhanced sound-evoked activity observed in central auditory structures is often greatest at frequencies below the region of NIHL possibly due to a reduction in lateral inhibition (Salvi et al., 2000). In addition to changes in central gain, NIHL typically results in an increase in spontaneous rates (SR) in the cochlear nucleus (CN) which in some cases continues rostral up to the auditory cortex (AC) (Eggermont and Komiya, 2000, Kaltenbach and Afman, 2000, Zhang et al., 2006, Dong et al., 2010, Mulders and Robertson, 2013, Luo et al., 2014). Enhanced sound-evoked responses and increased central gain have been linked to hyperacusis or loudness intolerance (Sun et al., 2012) whereas elevated SR in tonotopic regions associated with hearing loss is considered by some as the neural correlate of tinnitus (Chen and Jastreboff, 1995, Kaltenbach and Afman, 2000). NIHL also induces frequency map reorganization in the AC and enhanced inter-neuronal synchrony; these neurophysiological changes have also been linked to tinnitus (Muhlnickel et al., 1998, Seki and Eggermont, 2002, Eggermont, 2006, Norena and Eggermont, 2006).

NIHL also exerts effects beyond the classical auditory pathway. NIHL suppresses neurogenesis in the hippocampus and alters the spatially tuning of hippocampal neurons as animals navigate through a maze (Goble et al., 2009, Kraus et al., 2010, Newman et al., 2015). The amygdala, which attaches emotional significance to sounds, plays a central role in auditory fear conditioning and modulates the acoustic startle reflex (LeDoux, 2000, Kraus and Canlon, 2012). In this context, it is interesting to note

that the amplitude of acoustic startle reflex is greatly depressed by unilateral NIHL. Since unilateral ear plugging also suppresses startle reflex amplitude, it seems likely that the sensory or motor circuits that regulate the startle reflex requires information from both ears ([Kraus et al., 2010](#), [Lobarinas et al., 2013](#)). The Str, which is involved in motor planning and movements, receives auditory inputs and can modulate the startle reflex ([Bordi and LeDoux, 1992](#), [Kodsi and Swerdlow, 1995](#)). Sound-evoked neural activity in the Str is also greatly enhanced by sodium salicylate, an ototoxic drug that induces tinnitus and hyperacusis ([Chen et al., 2014b](#)). Although the neural correlates of tinnitus and hyperacusis have been extensively studied in the classical auditory pathway, there is growing awareness that non-classical auditory areas such as the amygdala and Str may contribute directly or indirectly to these aberrant perceptions ([Jastreboff, 2007](#), [Rauschecker et al., 2010](#), [Chen et al., 2012](#)). Some clinical reports suggest that the amygdala and Str are involved in tinnitus and hyperacusis ([De Ridder et al., 2006](#), [Cheung and Larson, 2010](#)). The lateral amygdala (LA) and striatum (Str) have extensive connections with the classical auditory pathway ([Romanski et al., 1993](#), [Budinger et al., 2008](#)) and respond robustly to acoustic stimulation ([Bordi et al., 1993](#), [Romanski et al., 1993](#), [Quirk et al., 1995](#), [Cromwell et al., 2007](#), [Chen et al., 2012](#)). Therefore the response properties of neurons in the LA and Str are likely to be altered by NIHL. Although the effects of acoustic trauma have been explored at many different sites in the classical auditory pathways, the effects of NIHL on non-classical auditory centers are poorly understood. To address this issue, we studied the effects of high-frequency NIHL on the sound-evoked responses and SR in the LA and Str of the rat and compared the results to similar data obtained from primary and secondary AC.

Materials and Methods:

Subjects: Sprague–Dawley rats (2 months of age) were acquired from Charles River Laboratories Inc. (Wilmington, MA) and housed in the Laboratory Animal Facility (LAF) of the University at Buffalo and given free access to food and water. The colony room was maintained at 22 °C with a 12-h light-dark cycle. All procedures regarding the use and handling of animals were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the University at Buffalo.

Noise exposure: NIHL was induced with either an octave band noise (OBN, 10-20 kHz) or a narrowband noise (NBN, 16-20 kHz). At three months of age, the rats were exposed to the NBN (24 h/day for 8 days, 7 rats) or to the OBN (24 h/day for 35 days, 4 rats) at an overall intensity level of 104 dB SPL. The rationale for using these two exposures is that they are identical to those being used in ongoing behavioral studies designed to obtain behavioral measures of hearing loss, hyperacusis and tinnitus. The advantage of using long-duration exposures is that all animals develop the similar asymptotic threshold shift after they have been in the noise for >24 h ([Carder and Miller, 1972](#), [Salvi et al., 1978](#), [Chen et al., 2014a](#)). These long-duration exposures reduce between subject variability in the amount of hearing loss and cochlear pathology compared to intense, short duration (e.g., 1-3 h) exposures. Control rats (n=9) were housed in the colony room without intense noise exposure. The two exposures also allowed us to evaluate the effect the bandwidth of the hearing loss on the electrophysiological results.

The OBN and NBN stimuli were generated using a TDT RP2 Real time signal processor (TDT, Gainesville, FL), amplified by AMP 300 power amplifier (AudioSource Inc., Portland, OR) and delivered to a speaker (Vifa D25AG35 1" Dome Tweeter, Madisound Speaker Components, Inc., Middleton, WI). Each rat was housed in a single cage (28 x 25.4 x 20.3 cm) equipped with a speaker

located on the top of the cage. The rats were awake during the exposure and both ears were exposed to the OBN or NBN. The rats had free access to food and water, moved freely about in the cage during the binaural exposure. The noise intensity level in the cage was calibrated with a sound level meter (Larson Davis System 824) with a free-field ½" microphone (model 2540, Larson Davis). The overall intensity and intensity in one-third octave bands were measured. Figure 1 presents spectra of the OBN (blue open bars) and the NBN (black filled bars).

Measurement of noise-induced hearing loss (NIHL): The auditory brainstem response (ABR) was used to estimate the magnitude of the NIHL. The ABRs were recorded pre- and one month post-exposure and the pre-post threshold differences were used to calculate the NIHL or permanent threshold shift (PTS). Rats were anesthetized initially with 4% isoflurane in O₂ at a flow rate of 0.5 L/minute and then maintained at 1.5% isoflurane. Needle electrodes (Grass Technologies) were placed at the vertex (active), posterior bulla (reference) and behind the shoulder blade (ground) for ABR recording. The biological signals were amplified 5020 times by a TDT Headstage-4 bio-amplifier. Tone bursts (5 ms duration with 1 ms rise/fall time, cosine² gated) at 4, 8, 12, 16, 20, 24 and 32 kHz were generated by using a TDT RP2.1 Real time signal processor (TDT, Gainesville, FL) using TDT SigGen software. The stimuli were presented at a rate of 21/s and delivered binaurally through a speaker (FT28D, Fostex). The speaker was calibrated using a sound level meter (Larson Davis System 824) with a ½" microphone (model 2540, Larson Davis). The tone bursts were presented at 100 dB SPL and then decreased in 10 dB steps to 0 dB SPL. The tone-evoked ABR was band-pass filtered (10-3000 Hz with a notch filter at 60 Hz) and averaged 200 times using TDT BiosigRP software. The ABR threshold was defined as the lowest intensity that produces a just noticeable ABR.

Noise-induced hair cell loss: After completing the neural recording in the brain, the cochleae were harvested for histological analysis. Immediately after the cochlea was removed, the round

window, oval window, and the cochlear apex were opened to facilitate the cochlear perfusion with the incubation solution which contained 0.05% nitroterazolium blue chloride (cat#N6876, Sigma), 50 mM sodium succinate and 50 mM phosphate buffer. Afterwards the cochlea was then incubated in the solution for 1 h (at 37 °C). The stained cochleae were fixed in 10% buffered formalin for 2 days and then decalcified in 7% EDTA (ethylenediaminetetraacetic acid) solution for 3-5 days. The entire length of the organ of Corti was then dissected out as a flat surface preparation and examined over its entire length using a light microscope (DMBA300 Digital Microscope, Microscope World) to count loss of both inner and outer hair cells (IHC and OHC) and produce a cochleogram.

Spontaneous and tone-evoked activities in AC, LA, and Str: Spontaneous and tone-evoked neural activity were recorded from the entire AC (primary and secondary areas), the Str and LA in noise-exposed rats 3-5 months post-exposure using a 16-channel linear silicon microelectrode array (A-1x16-10mm 100-177, NeuroNexus Technologies); similar recordings were also obtained from control rats of approximately the same age. Rats were anesthetized with ketamine and xylazine (50 mg/kg and 6 mg/kg i.m.) and then placed in a stereotaxic apparatus with two blunted ear bars. The dorsal surface of the skull was exposed, a small stainless steel screw was screwed into the right parietal bone and a head bar holder was firmly attached to the head using dental cement. The head bar was used to hold the rat's head in the stereotaxic frame after the right ear bar was removed to allow for acoustic stimulation using a free-field loudspeaker. An opening was made on the skull at the appropriate location to gain access to the left (contralateral) AC, Str, and LA. The dura was removed from the surface of the cortex and the electrode was inserted into the brain and advanced into the primary/secondary AC or Str-LA by using the stereotaxic coordinates ([Paxinos and Watson, 2004](#)). Recordings were made from both the primary and secondary AC as defined by electrophysiological response properties and reversal of tonotopy in mapping of the AC ([Di and Barth, 1992](#), [Polley et al.,](#)

2007, Stolzberg et al., 2011, Chen et al., 2013). As described in our recent paper, our recordings in the LA were confined to the dorsal portion of the LA, the primary auditory receiving area. The distinct discharge patterns in this region were generally prolonged with one of two distinct profiles (see Fig. 3C1-C2)(Chen et al., 2014b). As described in our recent paper, our recording from the Str were restricted to the acoustically responsive area just above the dorsal boundary of the LA; the discharge patterns in this region were characterized by a single, sharp onset peak of relatively short latency (Fig. 3B)(Chen et al., 2014b). The firing patterns in the Str and LA were clearly distinguishable from one another.

Tone bursts (50 ms of duration, 1 ms rise/fall time, cosine² gated) were generated by a TDT X6-2 (~100 kHz sampling rate) and presented at a rate of 2/s. The stimuli were delivered through a loudspeaker (FT28D, Fostex), which was located in front of the right ear and calibrated by using a microphone preamplifier (Larson Davis, model 2221) with a ¼" microphone (Larson Davis, model 2520). The biological signals picked by the electrode were sampled with a resolution of 40.96 µs using a RA16PA preamplifier and RX5 base station (Tucker-Davis Technologies System-3, Alachua, FL) using custom-written data acquisition and analysis software (MATLAB R2007b, MathWorks) as previously described (Stolzberg et al., 2011, Chen et al., 2012). Multiunit clusters (MUC) in response to tone bursts presented at different frequencies (1.0, 1.5, 2.3, 3.5, 5.3, 8.0, 12.1, 18.3, 27.7, 42.0 kHz) and intensities (0, 20, 40, 60, 80, 100 dB SPL) were averaged 50 stimulus presentations. A peristimulus time histogram (PSTH) of responses to each frequency-intensity combination was constructed (see Fig. 3). PSTHs of a MUC were plotted as a function of frequency (x-axis) and stimulation level (y-axis) to generate a PSTH frequency-intensity matrix, demonstrating the frequency receptive field (FRF) of each MUC (see Fig. 4). PSTHs of all MUC recorded in an auditory center were averaged to obtain an estimate of the population response and used to make between group

comparisons. Spontaneous discharge rates of the MUC were also obtained and compared between the noise-exposed and the control groups.

Statistical analysis: GraphPad Prism (version 5) was for the statistical analyses as detailed below.

Results:

ABR threshold shift and hair cell loss: Figure 2A shows the magnitudes of ABR threshold shifts of four rats as a function of frequency caused by the 10-20 kHz OBN (104 dB SPL for 35 days). The exposure caused a mean PTS of 20-40 dB between 12 and 32 kHz, but had little or no effect at 4 and 8 kHz. The mean PTS in the noise band (12-20 kHz) was ~33 dB. Figure 2B shows percent OHC and IHC loss of the 4 rats as a function of percent distance from the apex of the cochlea; cochlear location is related to frequency on the upper x-axis ([Muller, 1991](#)). Many OHCs and IHCs were missing in the basal half of the cochlea (50-100% distance from apex) after the OBN exposure. OHC loss occurred in the region of the noise band (red horizontal bar) and reached a peak loss of 67% at the 65% location which corresponds to the high frequency edge of the OBN (arrow). After the peak, OHC loss declined to 13% at the 77% location and then increased to 100% OHC loss in the 90-100% location. Profound IHC loss was only observed in the 85-100% location, a tonotopic location above the noise band. The 16-20 kHz NBN exposure (104 dB SPL, 8 days) caused a mean threshold shifts of 20-30 dB from 16 to 32 kHz, but little shift at lower frequencies (Fig. 2C). The mean PTS in the noise band (16-20 kHz) was ~23 dB. The NBN exposure induced only minor (21%) OHC loss in the 4 rats in the region of the NBN (red horizontal bar, Fig. 2D) but caused nearly 100% OHC loss in the 80-100% location and 100% IHC loss at the 90-100% location.

Firing patterns and frequency selectivity in AC, LA and Str: Figures 3A-C show representative PSTHs of MUC recorded in the primary/secondary AC, Str, and LA respectively in response to tone bursts presented at 100 dB SPL. Figure 3D is a schematic showing a coronal section of the brain with the approximate stereotaxic recording positions of the four MUC shown in A-C. Consistent with our previous results (Wang et al., 2002a, Chen et al., 2014b), the response of the primary/secondary AC MUC had a short latency of ~10 ms (Fig. 3A). The mean response latency across 30 MUC recorded in the primary/secondary AC was 10.6 ms (± 0.63 ms). Similar to the AC, the tone-evoked MUC in the Str also had a short latency onset peak (Fig. 3B) (Chen et al., 2014b). Nearly all Str MUC that responded to sound had a sharp onset, phasic response with a short latency similar to the AC. The mean latency of 52 MUC in the Str was 10.5 ms (± 0.45 ms). The firing patterns of acoustically responsive LA MUC were distinctly different from those in the Str and AC (Chen et al., 2012). The PSTHs of many MUC in the LA had two peaks, an early onset peak followed by a late peak. Approximately 35% of our LA MUC (66/186) had an early response component with or without a late response component (see Fig. 3C1); the mean latency of the early onset response was 15.3 ms (± 0.6 ms), slightly longer than the latency observed in the primary AC and the Str. The remaining 65% of LA MUC had only a late response component like that shown in Figure 3C2 in which the peak latency was long, in this case ~40-75 ms. Most of the LA MUC had a peak latency of ~50-100 ms and the longest latency observed was ~450 ms.

Auditory neurons in the AC, Str and LA responded to tone bursts with varying sensitivity across frequency. Figure 4A shows a frequency-intensity matrix of PSTHs from a representative MUC in the primary AC. The PSTH within each rectangle in the matrix shows the firing pattern obtained with a particular frequency-intensity combination within the 1-42 kHz frequency range and intensities from 0 to 100 dB SPL in 20 dB steps. The PSTHs in blue show the frequency-intensity combinations where

the stimulus-evoked spike rate clearly exceeded the ongoing SR. In this example, the frequency at which a response could be obtained at the lowest intensity occurred at 27.7 kHz at 20 dB SPL; this was defined as the characteristic frequency (CF) of the MUC. Figure 4B presents the PSTH frequency-intensity matrix of a representative MUC in the Str; the CF of this MUC was 12.1 kHz and the threshold was 20 dB SPL. Figure 4C shows the PSTH frequency-intensity matrix of a representative MUC in the LA; in this example the CF was 18.3 kHz at 20 dB SPL.

Spontaneous activity in the AC, LA and Str: Spontaneous discharge rates (SR) were measured from MUC in the primary/secondary AC, LA and Str. Figure 5A shows the mean SRs of all MUC in the AC, LA, and Str regardless of CF. In the AC, the mean SR in the normal control rats was 27.5 ± 1.5 spikes/s ($\pm$ SE, $n=233$ MUC), significantly higher than the mean SR in the LA (13.3 ± 1.5 spikes/s, $n=103$ MUC) and the mean SR in the Str (17.3 ± 1.4 spikes/s, $n=98$). A one way ANOVA showed a significant difference in SR in normal rats between AC, LA and Str ($F=21.68$, 2 and 431 df, $p<0.0001$); a Newman-Keuls Multiple Comparison Test showed significant difference between AC-LA ($p<0.001$) and AC-Str ($p<0.001$), but no difference between LA-Str. After the noise exposure, the mean SR of MUC in the AC increased to approximate 39 spikes/s in the OBN group and ~47 spikes/s in the NBN group; these values were significantly greater than in the normal control group (27.5 spikes/s). A one way ANOVA revealed a significant difference between the control and the noise-exposed groups ($F=29.74$, 2, 690 df, $p<0.0001$); a Newman-Keuls Multiple Comparison Test revealed significant difference between the Control group vs. the OBN group and the Control group vs. the NBN group ($p<0.001$). The mean SRs of MUC in the LA and Str were slightly increased after the noise exposure. In the LA, a one way ANOVA showed a significant difference between the three groups ($F=10.12$, 2, 423 df, $p<0.0001$); a Newman-Keuls Multiple Comparison Test only revealed a significant difference between the Control group and OBN ($p<0.001$). In the Str, a one way ANOVA

revealed a significant difference between the three groups ($F=3.727$, 2, 582 df, $p=0.0246$); a Newman-Keuls Multiple Comparison Test revealed a significant differences between the Control group and the NBN group ($p<0.05$) and the Control group and the OBN group ($p<0.05$). Since there was a significant high-frequency hearing loss in OBN and NBN groups, the SR data were separated into a low-CF group (≤ 8 kHz, normal hearing region) and a high-CF group (≥ 12 kHz, hearing loss region) and reanalyzed. In the low-CF region of primary/secondary AC the mean SR in the control group was ~ 30 spikes/s compared to ~ 38 and ~ 44 spikes/s for the OBN and NBN groups respectively (Fig. 5B). In the low-frequency normal threshold region a one way ANOVA revealed a significant differences between the three groups ($F=4.462$, 2, 321 df, $p=0.0123$); a Newman Keuls Multiple Comparison Test only revealed a significant difference between the Control group and the NBN group ($p<0.05$). In the high-CF hearing loss region, the mean SR in the control group was ~ 27 spikes/s compared to ~ 41 and ~ 50 spikes/s for the OBN and NBN groups respectively (Fig. 5B). A one way ANOVA showed a significant difference between the three groups ($F=29.85$, 2, 366 df, $p<0.0001$); a Newman-Keuls Multiple Comparison Test revealed significant difference between the Control group and OBN group and the Control group and the NBN group ($p<0.001$). In the LA in the low-frequency normal threshold region the mean SR of MUC was ~ 10 spike/s in the Control group, ~ 22 spikes/s in the OBN group and ~ 14 spikes/s in the NBN group (Fig.5C). A one way ANOVA revealed a significant difference between the three groups ($F=13.54$, 2, 220 df, $p<0.0001$); a Newman Keuls Multiple Comparison Test only revealed a significant difference between the Control group and the OBN group ($p<0.001$). In the high frequency hearing loss region, the mean SR of MUC in the Str was ~ 15 spike/s in the Control group, ~ 18 spikes/s in the OBN group, and ~ 15 spikes/s in the NBN group. There were no significant differences between the three groups.

Population PSTHs in LA: The 10-20 kHz OBN and 16-20 kHz NBN exposures tended to reduce or eliminate sound evoked discharges in high-CF MUC whereas they enhanced activity in low-CF MUC. In order to summarize the changes from hundreds of MUC, we first obtained a grand mean PSTH for each frequency-intensity combination in the matrix (10 frequencies x 6 intensities, 50 ms tone bursts); this was accomplished by averaging the individual PSTHs from every MUC. In order to show the sound evoked activity minus SR, the responses obtained at 0 dB SPL were subtracted from responses measured at 20, 40, 60, 80 and 100 dB SPL at each frequency. Figure 6A presents the population PSTHs in the LA for each frequency-intensity combination in the matrix (x-axis = 500 ms, y-axis = 60 spikes/s shown at the upper right and the lower right respectively). Mean data are shown for 103 MUC in the control group (blue) and 140 MUC in the OBN group (red) as a function of frequency (x-axis) and intensity (y-axis). The OBN exposure enhanced the population responses to low frequencies (see the red arrowhead), where there was no hearing loss (Fig. 2). In contrast, responses to high frequencies were reduced or eliminated (see the black arrowhead); this reduction is likely due to the high frequency hearing loss. Figure 6B-D elaborates on the functional changes seen at a low-, high-, and the edge-frequency. Figure 6B presents sound evoked responses at 1.0 kHz obtained at 100, 80, and 60 dB SPL. Sound evoked activity at 1 kHz was substantially greater in the OBN group (red) than the Control group (blue). At 100 dB SPL (Fig. 6B, top), a two way ANOVA was performed on the first 200 ms of mean PSTH. The results revealed a significant difference between the Control group and the OBN group at 100 dB SPL ($F=25.97$, 1, 80 df, $p<0.0001$). Responses at 1 kHz were also significantly greater in the OBN group than the Control group at 80 dB SPL ($F=7.61$, 1, 80 df, $p=0.0072$) and at 60 dB SPL ($F=25.13$, 1, 80 df, $p<0.0001$). The temporal profile of the driven response was also substantially different; the peak response in the OBN PSTH was delayed to 70-80 ms compared to ~10 ms in the control group. Figure 6C presents mean sound-

evoked responses to a frequency in the noise band (12.1 kHz), the ABR threshold shift at this frequency was 31 dB. There was a large reduction in sound evoked activity in the OBN-group (red) compared to the control group (blue); however, the peak in the response occurred at roughly the same time in both groups. At 100 dB SPL (Fig. 6C, top), a two way ANOVA was performed on the first 200 ms of mean PSTH. The results revealed a significant difference between the Control group and the OBN group at 100 dB SPL ($F=10.45$, 1, 80 df, $p=0.0018$). Responses at 12.1 kHz were also significantly greater in the OBN group than the Control group at 80 dB SPL ($F=13.94$, 1, 80 df, $p=0.0004$) and at 60 dB SPL ($F=8.95$, 1, 80 df, $p=0.0037$). At 8 kHz, an edge-frequency in the intact area where the ABR threshold was normal (Fig. 2A), sound evoked responses were significantly reduced at 100 and 80 dB SPL but not at 60 dB SPL or lower intensities (Fig. 6D). At 100 dB SPL (Fig. 6D, top), a two way ANOVA was performed on the first 200 ms of mean PSTH; the results revealed a significant difference between the Control group and the OBN group ($F=10.18$, 1, 80 df, $p=0.002$). Responses at 8 kHz were also significantly greater in the OBN group than the Control group at 80 dB SPL ($F=15.99$, 1, 80 df, $p=0.0001$), but not at 60 dB SPL.

Figure 7A presents population PSTHs in the LA reflecting the mean responses of 103 MUC in the control group (blue) and 183 MUC in the NBN group (red) as a function of frequency (x-axis) and intensity (y-axis). Similar to the OBN, the NBN exposure enhanced responses to the low frequencies (see the red arrowhead) while it reduced responses to the high frequencies (see the black arrowhead). The precise nature of the sound-evoked changes at low-, high-, and the edge-frequency are elaborated upon in Figure 7B-D. The responses to 1.0 kHz at 100, 80, and 60 dB SPL showed a significant enhancement of sound evoked activity after the NBN exposure (Fig. 7B). At 100 dB SPL (Fig. 7B, top), a two way ANOVA was performed on the first 150 ms of the mean PSTH. The results revealed a significant difference between the Control group and the OBN group ($F=13.57$, 1, 75 df, $p=0.0004$).

The responses were also significantly greater in the OBN group than the Control group at 80 dB SPL ($F=13.24$, 1, 75, df, $p=0.0005$) and at 60 dB SPL ($F=16.38$, 1, 75 df, $p=0.0001$). In addition, the onset response shifted from ~10 ms in the Control group to approximately 80-90 ms in the NBN group. In response to 18.3 kHz stimulus, within the region of hearing loss, sound evoked responses were reduced slightly due to less threshold shift (~23 dB) at this frequency (Fig. 7C). The magnitudes of the PSTHs were not significantly different between the Control group and the NBN group. Figure 7D presents responses to 12.1 kHz, located at the edge-frequency of the NBN exposure. Unlike PSTH in the OBN group, the responses to the edge-frequency were largely unaffected by the NBN exposure at 100 and 80 dB SPL, but was significantly enhanced at 60 dB SPL (Two way ANOVA, 150 ms analysis window, $F=9.17$, 1, 75 df, $p=0.0034$).

Figure 8A presents tone-evoked responses of LA-MUC at 80 dB SPL from two NBN-exposed rats in this group with a mean PTS of ~30 dB. In this subgroup, responses were enhanced at 1.0 kHz (normal threshold region), but reduced at 18.3 kHz (region of hearing loss); however, no change was seen at the edge-frequency (12.1 kHz). A two way ANOVA was performed on the first 200 ms of the mean PSTH at 1.0 kHz and the results revealed a significant increase in the response from the Control group vs. the NBN group ($F=85.58$, 1, 60 df, $p<0.0001$). At 18.3 kHz (Fig. 8A, right), the response in the NBN group was significantly reduced compared to the Control group ($F=12.14$, 1, 60, $p=0.0009$). No significant difference was seen at the edge frequency of 12.1 kHz. Figure 8B presents responses of LA-MUC from the other two NBN-exposed rats with less hearing loss (16 dB). In these cases, a slight reduction was observed at the high frequency (18.3 kHz) and there was no enhancement of responses at the low frequency (1.0 kHz) but an enhancement at the edge-frequency (12.1 kHz). At 18.3 kHz (Fig. 8B, right), a two way ANOVA was performed on the first 200 ms of the mean PSTH and revealed a significant decrease in the response from the NBN group compared to the Control group

($F=6.08$, 1, 60 df, $p=0.0166$). The enhancement at the edge-frequency (12.1 kHz) did not reach a statistical significance when analysis was performed in a 200-ms time window. No significant difference was observed at 1.0 kHz.

Population PSTHs in AC: Figure 9A presents the population PSTHs of 548 AC-MUC in the control group (blue) and 224 MUC in the OBN group (red) as a function of frequency (x-axis) and stimulation level (y-axis). Similar to the LA, the OBN enhanced responses to low-frequency stimuli at intensities from 20-100 dB SPL (see the red arrowhead), but reduced responses to high frequency stimuli (see the black arrowhead). The responses to low- (1.0 kHz), high- (12.1 kHz), and edge-frequencies (8.0 kHz) are illustrated in more detail in Figure 9B-D. Mean responses to the low frequency stimulus (1.0 kHz) in the OBN exposed group (red) was generally much greater than the control group (blue, Fig. 9B). At 100 dB SPL (Fig. 9B, top), a two way ANOVA was performed on the first 100 ms of mean PSTH. The results revealed significantly greater responses in OBN group compared to the Control group ($F=422.2$, 1, 700 df, $p<0.0001$). Responses at 1 kHz were also significantly greater in the OBN group than the Control group at 80 dB SPL ($F=372.4$, 1, 700 df, $p<0.0001$) and at 60 dB SPL ($F=820.8$, 1, 700 df, $p<0.0001$). In addition, the duration of the responses increased to nearly twice the duration of the stimulus.

Figure 9C presents the mean PSTH to a 12.1 kHz tone located within the noise band. During the stimulus, the mean PSTH in the OBN group was greatly reduced compared to the control, consistent with the large threshold shift at this frequency (black down arrow). At 100 dB SPL (Fig. 9C, top), a two way ANOVA was performed on the first 50 ms of mean PSTH. The results revealed significantly smaller responses in OBN group compared to the Control group ($F=226$, 1, 350, $p<0.0001$). Responses at 12.1 kHz were also significantly smaller in the OBN group than the Control group at 80 dB SPL ($F=183.9$, 1, 350, df, $p<0.0001$) and at 60 dB SPL ($F=140.8$, 1, 350, df, $p<0.0001$).

In addition, at 100 dB SPL, there was a delayed rebound response in the PSTH in the OBN group compared to the control group; this delayed response peaked around 90 ms and was as large as the onset response (see red up arrows).

Figure 9D presents the mean PSTHs at the edge-frequency (8 kHz). Compared to controls, the onset response in the mean PSTH of the OBN group was reduced (Fig. 9D, black down arrow) despite the fact that there was no threshold shift at 8 kHz. At 100 dB SPL (Fig. 9D, top), a two way ANOVA was performed on the first 50 ms of mean PSTH. The results revealed significantly smaller responses in OBN group compared to the Control group ($F=120.5$, 1, 350 df, $p<0.0001$). Responses at 8.0 kHz were also significantly smaller in the OBN group than the Control group at 80 dB SPL ($F=121.4$, 1, 350, df, $p<0.0001$) and at 60 dB SPL ($F=16.59$, 1, 350, df, $p<0.0001$). The onset response in the OBN group was followed by a delayed response which peaked around 150 ms (Fig. 9D, red up arrows).

Figure 10A presents population PSTHs of 548 AC-MUC in the control group (blue) and 236 MUC in the NBN group (red) as a function of frequency (x-axis) and stimulation level (y-axis). Similar to the OBN, responses to low frequency stimuli (red up arrow) were generally enhanced in the NBN group compared to controls; these differences were most pronounced at 40 and 60 dB SPL. In contrast, responses to high frequencies were generally reduced in the NBN group compared to controls (black down arrow). The changes in the PSTH profiles induced by the NBN are illustrated in more detail for a low- (1.0 kHz), high- (18.3 kHz), and edge-frequency (12.1 kHz). At 100 dB SPL and 1 kHz (Fig. 10B, top), a two way ANOVA was performed on the first 100 ms of mean PSTH. The results revealed a significantly larger response in NBN group compared to the Control group ($F=153.1$, 1, 700 df, $p<0.0001$). At 1 kHz, responses in the NBN group were also significantly greater than the Control group at 80 dB SPL ($F=63.32$, 1, 700 df, $p<0.0001$) and 60 dB SPL ($F=119.9$, 1, 700 df,

$p < 0.0001$). Interestingly, at 1 kHz, the duration of the PSTH response was prolonged and extended well beyond the duration of the stimulus (Fig. 10B, red, up arrow).

At 18.3 kHz, within the noise band, responses during the stimulus were significantly reduced (Fig. 10C, black down arrow). At 100 dB SPL (Fig. 10C, top), a two way ANOVA was performed on the first 50 ms of mean PSTH. The results revealed a significantly smaller response in NBN group compared to the Control group ($F=44.45$, 1, 350 df, $p < 0.0001$). At 18.3 kHz, responses in the NBN group were also significantly smaller than the Control group at 80 dB SPL ($F=27.52$, 1, 350 df, $p < 0.0001$) and 60 dB SPL ($F=95.01$, 1, 350 df, $p < 0.0001$). At the edge frequency of 12.1 kHz, the responses during the stimulus were much greater in the NBN group than the Control group (Fig. 10D). At 100 dB SPL (Fig. 10D, top), a two way ANOVA was performed on the first 100 ms of mean PSTH. The results revealed a significantly larger response in NBN group compared to the Control group ($F=86.76$, 1, 700 df, $p < 0.0001$). At 12.1 kHz, responses in the NBN group were also significantly greater than the Control group at 80 dB SPL ($F=79.63$, 1, 700 df, $p < 0.0001$) and 60 dB SPL ($F=216.9$, 1, 700 df, $p < 0.0001$). Interestingly there was a slight upsurge of post-stimulus activity extending from 100 ms (red up arrows). In addition, at the 12.1 kHz edge-frequency, the PSTH profiles in the NBN group were substantially broader than in the control group (Fig. 10D). The PSTH profiles in the control group consisted of a single peak whose duration was shorter than the stimulus whereas the PSTH profiles in the NBN group often consisted of a double-peak followed by a slow decay that extended out to 100-150 ms. Thus, the NBN exposure tended shift the PSTH profiles from a predominantly phasic-onset response in the control group to broader onset response followed by slow decay resulting in a broader response duration.

Population PSTHs in Str: Figure 11A presents the population PSTHs of 95 MUC in the Str in the control group (blue) and 261 MUC in the OBN group (red) as a function of frequency (x-axis) and

level (y-axis). At the two highest intensities, 100 and 80 dB SPL, the PSTH profiles in the OBN group were below the control group; this trend was most evident in the region of high-frequency hearing loss (≥ 8 kHz). In general, suprathreshold sound-evoked activity was reduced in the Str in contrast to the hyperactivity observed in the AC. At moderate to high intensities, the PSTH profiles in the OBN and control group consisted of a sharp onset peak followed by a rapid decay. While the OBN altered the shape of the AC PSTH profiles by increasing response duration, the shape of the PSTH profiles in the Str were either largely unaffected by the OBN or slightly shorter (e.g., Fig. 11, 5.3 kHz, 80-100 dB SPL). Figure 11B presents population PSTHs of 226 Str MUC (red) in the NBN group and 95 MUC in the control group (blue). Similar to the OBN, the amplitudes of the PSTH profiles in the NBN group were clearly below those of controls in the region of high-frequency hearing loss (≥ 12.1 kHz), but PSTH amplitudes were sometimes less than controls in regions of normal hearing (≤ 8 kHz) (Fig. 11B, 80 dB SPL). The shapes of the PSTH profiles in the NBN group were either similar to the controls or more phasic and of shorter duration (e.g., Fig. 11B, 3.5 kHz, 80-100 dB SPL).

Discussion:

Noise-induced hearing loss reduces the neural output of the cochlea leading to unexpected functional changes in the central auditory pathway such as tonotopic map reorganization in AC and medial geniculate body (Willott et al., 1993, Eggermont and Komiya, 2000, Kamke et al., 2003), loss of surround inhibition in AC, (Rajan, 1998), suprathreshold sound-evoked hyperactivity in the inferior colliculus (IC) and higher auditory centers (Salvi et al., 1990, Syka, 2002) and increased SR in the dorsal cochlear nucleus (DCN), IC and AC (Eggermont and Komiya, 2000, Kaltenbach and Afman, 2000, Mulders et al., 2011). While past research on noise-induced hearing loss has been auditory-centric, many areas outside the classical auditory pathway receiving acoustic information have not

been studied. These non-classical auditory areas combine acoustic information with other endogenous and exogenous cues from concerned with emotional, cognitive, motor and other sensory systems in order to respond to events in the environment. The loss of relevant auditory cues can lead to maladaptive changes such as impaired speech production (Waldstein, 1990, Weatherley et al., 1997, Higgins et al., 2005) or emotional responses to sound (Husain et al., 2014). To address this issue, we exposed rats to a high-frequency OBN (10-20 kHz, 104 dB SPL, 35 days) or narrowband noise (16-20 kHz, 104 dB SPL, 8 days) and compared the responses of the LA and Str, which lie outside the classical auditory pathway, with those seen in the primary/secondary AC. While the responses of the MUC in the Str were reduced, the responses of the MUC in the LA were frequency-dependently altered similar to that observed in the AC.

PTS and Hair Cell Loss: The OBN induced 25-40 dB of PTS from 12 to 32 kHz; this was associated with a bimodal hair cell lesion consisting of a mild to moderate OHC loss in the 12-32 kHz tonotopic region plus a severe loss of IHC and OHC above 32 kHz (Fig. 2A-B). The PTS from the NBN was approximately 20-30 dB, extended over a narrow frequency range, 16-32 kHz, and was associated with OHC and IHC lesions that increased from a mild loss around 20-24 kHz region to a severe loss above 32 kHz (Fig. 2C-D). Regardless of the spectrum of the noise exposure, there was nearly total loss of IHC and OHC in the 80-100% region of the cochlea. The location of this large lesion was roughly an octave above the high frequency edge of the noise similar to the basalward shifts seen in many studies of noise-induced hearing loss (Salvi et al., 1983, Mulders et al., 2011). This shift towards the base has been linked to neural and/or mechanical cochlear nonlinearities (Cody and Johnstone, 1981). While cochlear mechanics may be an important factor another possibility is that antioxidant enzymes that protects cells from oxidative stress are lower in the base than the apex (Sha et al., 2001). In addition, several discrepancies were observed between hearing loss and OHC loss (Chen

and Fechter, 2003, McGowan et al., 2008). With the OBN, 40 dB PTS at 32 kHz was associated with a minor 20% OHC loss. With the NBN, 20-30 dB of PTS from 16-24 kHz was correlated with a small OHC loss of 20% or less. These discrepancies suggest that other subtle pathologies such as damage to the stereocilia or the synapse between the IHC and type I afferent fibers may be contributing to PTS (Liberman and Beil, 1979, Lin et al., 2011, Furman et al., 2013).

Spontaneous Activity and Tinnitus: Noise and drug-induced cochlear damage reduces the neural output from the cochlea leading to a variety of time and frequency dependent changes in SR in the central auditory pathways. In the DCN, ventral cochlear nucleus (VCN) and IC SRs begin to increase approximately one week post-exposure and continue to rise for a few more weeks before stabilizing. In the brainstem, this SR-increase occurs predominantly in high-threshold, high-CF neurons associated with hearing loss (Zhang and Kaltenbach, 1998, Kaltenbach et al., 2000, Mulders et al., 2011, Manzoor et al., 2012, Robertson et al., 2013). Since the CF-SR profile matches the tinnitus pitch, spontaneous hyperactivity in the brainstem has been considered a neural correlate of tinnitus. However, the neural and perceptual measures are generally mismatched temporally; spontaneous hyperactivity builds up slowly over several weeks whereas noise-induced tinnitus normally begins immediately post-exposure (Loeb and Smith, 1967, Atherley et al., 1968, Norena et al., 2002, Seki and Eggermont, 2003, Roberts et al., 2008).

Spontaneous hyperactivity combined with enhanced neural synchrony has also been observed in the primary AC in animals with noise-induced hearing loss (Seki and Eggermont, 2002, Eggermont, 2006, Norena and Eggermont, 2006). However, the SR increases were not correlated with threshold and occurred at CFs both below and above the traumatizing tone exposure (Seki and Eggermont, 2003). This generalized increase in SR across CF was attributed to an overall reduction in inhibition (Wang et al., 2002b, Seki and Eggermont, 2003, Scholl and Wehr, 2008). Consistent with these studies, we

observed increased SR in both low CF, low-threshold neurons and high CF, high-threshold in primary/secondary AC neurons (Fig. 5B). Since SR increased over the entire CF range, the increases we observed are not simply inherited from the auditory brainstem where SR only increased in the region of hearing loss ([Kaltenbach, 2006](#), [Mulders et al., 2011](#)).

Acoustically responsive neurons in the Str play an important role in motor control of vocalizations ([Hu et al., 2014](#), [Grant et al., 2015](#)). SR in the Str only slightly increased after the NBN or OBN exposures (Fig. 5A). Thus, our results suggest that SR in the Str are unaffected by the SR increases in the DCN, VCN, IC and AC. SR in the Str is mostly likely determined by endogenous factors or non-auditory inputs to the Str.

The amygdala, which assigns emotional significance to sensory stimuli ([Davis, 1992](#), [Muramoto et al., 1993](#), [LeDoux, 2000](#)), has been implicated in the affective aspects of tinnitus ([Wallhausser-Franke et al., 2003](#), [De Ridder et al., 2006](#), [Knipper et al., 2013](#), [Singer et al., 2013](#), [Chen et al., 2015](#)). Therefore, we expected that SR might increase in high-CF MUC in the LA following the OBN or NBN exposures. Although SR did not increase after the NBN exposure, an overall increase occurred after the OBN exposure (Fig. 5A). It is unclear why SR increased after the OBN, but not the NBN. One possibility is that the magnitude and bandwidth of the hearing loss was much greater with the OBN than the NBN. This suggests that LA SR only increase when a critical amount of damage occurs. This overall increase in LA SR was largely due to higher rates among low-CF, low-threshold MUC (Fig. 5C). One possibility for the increase in low-CF LA neurons is that high-frequency hearing loss reduces lateral inhibition to the low-frequency regions. Finally, the SR increase among low-CF LA neurons is at odds with the SR increases seen in high-CF, high-threshold neurons in the VCN, DCN and IC ([Kaltenbach, 2006](#), [Mulders et al., 2011](#)). Taken together, these results indicate that SR

in the LA is largely independent of the SR changes seen in more peripheral parts of the classical auditory pathway.

Low-Frequency Hyperactivity: Noise induced hearing loss depresses the neural output from damaged regions of the cochlea and lower regions of the auditory brainstem (Salvi et al., 1979, Salvi et al., 2000, Popelar et al., 2008). Paradoxically, sound-evoked responses from more rostral auditory centers become hyperactive (Popelar et al., 1987, Syka and Rybalko, 2000, Popelar et al., 2008). We previously reported that activity in the IC was enhanced after acoustic trauma and that the most robust increases occurred at frequencies below the high-frequency hearing loss. In contrast, suprathreshold responses in regions of moderate hearing loss were normal or slightly depressed (Salvi et al., 1990, Salvi et al., 2000). Sound-evoked hyperactivity due to noise trauma or other forms of cochlear pathology tends to progressively increase along the caudal-rostral axis (Popelar et al., 1987, Qiu et al., 2000, Salvi et al., 2000, Syka and Rybalko, 2000, Syka, 2002, Popelar et al., 2008, Auerbach et al., 2014, Chen et al., 2015). Consistent with our previous observations, we observed a pronounced increase in suprathreshold sound-evoked firing in the AC with maximal hyperactivity occurring at frequencies below the hear loss (Fig. 9-10). Sound-evoked hyperactivity as reflected in the population PSTH was most pronounced at moderate intensities (40-80 dB) (Fig. 9-10). One interpretation of these results is that low-frequency hyperactivity arises from the loss of inhibition emanating from regions with high frequency hearing loss (Fig. 9-10) (Salvi et al., 1990, Wang et al., 1996, Milbrandt et al., 2000) consistent with results seen in AC immediately after acute acoustic trauma (Scholl and Wehr, 2008).

The complex inhibitory circuitry in the AC provides some clues that can explain how noise-induced high-frequency hearing loss can lead to low-frequency hyperactivity (Wehr and Zador, 2003, Scholl and Wehr, 2008, Oviedo et al., 2010). Many neurons in the AC are sharply tuned and respond

weakly to moderate intensity sounds; however, when treated with GABAergic antagonist, they respond robustly to a broad range of frequencies (Wang et al., 1996, Suga et al., 1997). More detailed *in vitro* and *in vivo* measurements have shown that neurons in layer 2 of AC receive inputs from layers 6 neurons tuned to the same frequency; layer 2 neurons respond robustly to tone bursts (Oviedo et al., 2010). In contrast, neurons in layer 3 receive inputs from off-CF neurons in layer 6 tuned to frequencies 1-3 octaves higher than those in layer 3; these neurons seem to play an important role in spectral integration of complex sounds. Layer 3 neurons respond weakly to tone burst suggesting they receive strong inhibitory inputs. Immediately after acute, high-frequency noise-induced hearing loss, *in vivo* whole cell recordings showed that inhibition was greatly reduced and excitatory responses greatly enhanced and prolonged in low-frequency neurons located far below the trauma tone frequency (Scholl and Wehr, 2008). Taken together, these results suggest that the low-frequency hyperactivity and prolonged responses we observed in AC of animals with permanent high-frequency hearing loss arise from the loss of inhibitory inputs originating from high-threshold, high-frequency neurons located several octaves above the region of hyperactivity. While it is tempting to assume that the hyperactivity and prolonged responses seen in our low-frequency neurons are due to disruptions in layer 6-layer 2 circuitry in AC, similar changes may occur elsewhere in the brain. For example, low-frequency hyperactivity has been observed in the IC after both chronic and acute high-frequency acoustic trauma (Wang et al., 1996, Wang et al., 2002a, Wang et al., 2002b). Most likely, the changes we observed in the AC reflect the combined effects at multiple stages of auditory processing.

Response Diversity at the edge-frequency: There was no threshold shift at the edge-frequencies (8 kHz for OBN exposure; 12 kHz for NBN exposure, see Fig. 2). Nevertheless, suprathreshold responses at the edge of the hearing loss varied in the AC and LA. In some cases, suprathreshold responses at the 8 kHz edge frequency were reduced after the OBN exposure (Figs. 6D

and 9D). In other cases, responses at the 12.1 kHz edge frequency were enhanced slightly at low to moderate intensities after the NBN exposure (Figs. 7D, 8B, and 10D) while in some cases suprathreshold responses at the edge-frequency were unchanged (Fig. 7D at 100 and 80 dB SPL and Fig. 8A). The change of activity at the edge-frequency appeared to be related to severity of hearing loss as schematized in Figure 12. In cases of severe high frequency hearing loss (Figure 12A), the neural output from the high frequencies is greatly reduced (hypoactive); in cases such as this suprathreshold responses in the low frequency region is extremely hyperactive whereas responses at the edge frequency are hypoactive. Hyperactivity in the low-frequency region may result from a loss of lateral inhibition from the high-frequency region. If the high frequency hearing loss is moderate (Figure 12 B), the neural output from the high frequency region is moderately hypoactive and little change is seen in suprathreshold responses at the edge frequency. Finally, in cases of minor high frequency hearing loss, the neural output at the high frequencies shows a modest degree of hypoactivity (Figure 12C); in cases such as this suprathreshold response in the low frequency region show little change whereas mild hyperactivity is seen at the edge frequency possibly reflecting a shift of frequency receptive field (FRF) of high-frequency neurons to the edge-frequency (Stolzberg et al., 2011, Chen et al., 2012, Chen et al., 2013). The lack of hyperactivity in the low-frequency region suggests that lateral inhibition in the low-frequency regions is maintained in cases of mild high-frequency hearing loss. Hyperactivity in the low-frequency regions may occur only when high frequency hearing loss exceeds some critical level.

Response Duration and Temporal Processing: The two most prominent features of suprathreshold hyperactivity were: (1) an increase in the peak firing rate of the population PSTHs and (2) prolongation of PSTH response duration by as much as 30-40 ms. The noise-induced increase in PSTH duration occurred even in cases where the peak firing rates was comparable to controls (Fig. 9B,

100 dB; Fig. 10B, 100 dB). Consistent with our results, *in vivo*, whole cell patch clamp recordings from AC revealed an increase in the duration (up to 30 ms) of sound-evoked depolarization immediately post-exposure; the increase in response depolarization duration was accompanied by a reduction in hyperpolarization indicative of diminished inhibition (Scholl and Wehr, 2008). The noise-induced increase in response duration has implications for hyperactivity and temporal integration of loudness. If spike rate is integrated over the duration of the PSTH response (Fig 9B, ~50 ms duration in controls; ~90 ms duration in exposed), the total number of spikes would be substantially higher in noise-exposed animals versus controls even when there is little change in peak firing rate (Fig. 9B, 100 dB SPL; Fig. 10B, 100 dB SPL). In other words, hyperactivity is reflected by the integration of firing rate across both the time and amplitude domain. The increase in response duration also has implications for loudness perception. It is well known that the loudness of a sound increases with stimulus duration (Algom et al., 1989, Anweiler and Verhey, 2006). Therefore, if loudness is determined by the summation of firing rate over the duration of the neural response, then a high-intensity, low-frequency sound of fixed duration would generate more spikes and be perceived as louder than normal in noise-exposed animals with high-frequency hearing loss as discussed below.

Response Duration and Temporal Resolution: The increase in response duration has implication for psychophysical measures of temporal resolution such as gap detection and forward masking. High-frequency noise-induced hearing loss disrupts temporal resolution and these impairments have generally been attributed to abnormal cochlear function (Giraudi-Perry et al., 1982, Henderson et al., 1984, Moore, 2002). The noise-induced prolongation of tone-evoked responses in the AC would also negatively impact temporal resolution because neural activity continues beyond the offset of the first stimulus thereby reducing the onset response to the second stimulus. Our results suggest that impairments in auditory temporal resolution may have a central component, namely the

loss of inhibition leads to an increase in response duration due to the failure of inhibitory circuits to quickly shut off the response to prior to the onset of the next stimulus. This interpretation is consistent with the improved gap detection performance seen in aged gerbils after drug treatment with vigabatrin, a drug that boosts GABA levels in the central nervous system ([Gleich et al., 2003](#)).

Noise-induced high-frequency hearing loss also induced sound-evoked hyperactivity in the LA. The maximum population PSTH firing rates in the LA were higher than normal at low-frequencies and below normal in regions of high-frequency hearing loss (Fig. 6-7). In addition, the temporal profile of the population PSTH was dramatically altered at low-frequencies; the peak firing rate shifted from approximately 15 ms in controls to nearly 90 ms in the noise-exposed animals (Fig. 6B & 7B). In addition, the PSTH profile was transformed from a broad, flat profile in controls to a wide, triangular PSTH shape in noise-exposed animals. The low-frequency triangular PSTH profiles in the noise-exposed animals resemble the high-frequency PSTH profiles seen in controls (Fig. 6C-D). Therefore, the low-frequency triangular PSTHs may reflect the combined response of low-threshold, low-frequency neurons plus high-frequency neurons with hypersensitive and hyperactive low-frequency tails. Alternatively, the high-frequency hearing loss could cause tonotopic map reorganization in the LA similar to that seen in the AC and medial geniculate body ([Dietrich et al., 2001](#), [Kamke et al., 2003](#), [Norena and Eggermont, 2005](#), [Eggermont, 2006](#)). Tonotopic map reorganization could occur within the LA itself or be inherited from the AC or medial geniculate.

Acoustically responsive neurons in the Str modulate the motor circuits involved in vocalizations. This audio-vocal negative feedback system influences the intensity, frequency and temporal features of phonation ([Lee et al., 2013](#)) as illustrated by the increased vocal output (Lombard effect) and altered speech qualities that occur in background noise or in severely hearing impaired subjects ([Weatherley et al., 1997](#), [McGowan et al., 2008](#)). In contrast to the LA and AC, the peak

firing rates of low-frequency neurons were equal to or less than controls while the output of high-frequency neurons was greatly reduced. In addition, the duration of low-frequency PSTHs were shorter than normal (i.e., more phasic) at high-intensities (Fig. 11A-B) in contrast to the lengthening seen in the AC. The decreased responsiveness of Str neurons, particular at the high frequencies, would alter the temporal features and amount of negative feedback that regulate phonation; this could conceivably be responsible for the changes in vocal intensity, fundamental frequency and temporal structure of speech in hearing impaired subjects ([Weatherley et al., 1997](#), [Lee et al., 2013](#)).

Hyperacusis: Enhanced sound-evoked responses and increased central gain have been linked to hyperacusis, a condition in which every day sounds are perceived as intolerably loud ([Formby et al., 2003](#), [Sun et al., 2012](#), [Auerbach et al., 2014](#), [Chen et al., 2015](#)). Both noise exposures used in this study increased sound-evoked hyperactivity in the AC and LA; these results are consistent with previous reports in which sound-evoked hyperactivity was observed in the AC and LA of rats with behavioral evidence of salicylate-induced hyperacusis ([Chen et al., 2012](#), [Chen et al., 2014b](#), [Hayes et al., 2014](#), [Chen et al., 2015](#)). The noise-induced hyperactivity in AC and LA occurred in the low-frequency region of animals with high-frequency hearing loss; this would be expected to increase the loudness of low frequency sounds (low-frequency hyperacusis). In preliminary experiments, we found behavioral evidence of low-frequency hyperacusis in rats with noise-induced, high-frequency hearing loss. Hyperactivity in the AC would by itself seem sufficient to account for low-frequency hyperacusis. However, additional hyperactivity in the LA could add emotional tone to loud sounds. Ethologically, intense sounds act as warning signals that increase arousal, vigilance and fear ([Liddell et al., 2005](#), [Williams et al., 2005](#)). In subjects who have lost valuable high-frequency acoustic cues, enhancing the gain of low-frequency LA neurons would increase the biological significance of residual

low-frequency hearing. Indeed, high-frequency hearing loss has been found to enhance behavioral responses to low frequency sounds (Carlson and Willott, 1998).

In summary, our results show for the first time that the neurophysiological changes occurring outside the classical auditory pathway as a consequence of noise-induced, high-frequency hearing differ in a number of fundamental respects from those seen in the primary/secondary AC. While the AC, LA and Str all show reduced tone-evoked responses at the high frequencies, responses to low-frequency stimuli are greatly enhanced in the AC and LA while those in the Str are generally depressed despite the absence of low-frequency hearing loss. Low-frequency hyperactivity in the AC and LA, likely due to the loss of long-range inhibition emanating from damaged high frequency regions, would be expected to affect loudness perception (e.g., hyperacusis) whereas hypoactivity in the Str could disrupt vocalizations. High-frequency hearing loss also disrupts the temporal firing patterns evoked by low-frequency tone bursts. Response durations in the AC are substantially prolonged; those in the Str are shortened and become more phasic while LA firing patterns switch from a flattened profile to one with a delayed, triangular peak. The alterations in temporal coding likely impact a listener's ability to integrate and segregate acoustic information needed to process complex stimuli such as speech. The effect of high-frequency, noise-induced hearing loss on SR was quite heterogeneous. SR in the Str was largely unchanged whereas SR in the AC increased in both low and high-CF neurons; in the LA, SR only increased in low-CF neurons after the OBN exposure. These results suggest that SR outside the classically defined auditory pathway are largely unaffected by cochlear hearing loss and that endogenous factors or non-auditory inputs to the LA and Str regulate activity in these areas.

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Figure Legends

Figure 1: Blue open bars and black filled bars show the dB SPL measured in one-third octave bands for the 10-20 kHz OBN and 16-20 kHz NBN. The dashed line and hatched area show the background noise in the colony.

Figure 2: Mean ($\pm$ SEM) ABR threshold shifts as a function of frequency induced by (A) the OBN and (C) the NBN. Mean ($\pm$ SEM) OHC and IHC loss in the group exposed to the OBN noise (B) and the NBN (D). Red horizontal bars show location of the noise bands. The arrows show the high-frequency edge of the noise band.

Figure 3: Representative responses from MUC in the primary AC, Str and LA. (A): Peristimulus time histograms (PSTH) of a MUC recorded in the AC in response to 18.3 kHz tone bursts presented at 100 dB SPL; note short-latency, sharp onset response. (B): PSTH of a MUC in the Str to 18.3 kHz tone bursts presented at 100 dB SPL; note short-latency, sharp onset response. (C1): PSTH of a MUC in the LA to 3.5 kHz tone bursts presented at 100 dB SPL; note early and late response components. (C2) PSTH of a MUC in the LA in response to 18.3 kHz tone bursts presented at 100 dB SPL; note broad, late response. Horizontal bars show location of 50 ms tone burst stimulation. The approximate locations of the three recording sites are shown in the coronal schematic (D).

Figure 4: Examples of frequency receptive field of MUC in the AC (A), Str (B), and LA (C). Each panel shows the frequency (x axis, 10 frequencies, 1-42 kHz) vs intensity (y axis, 0-100 dB, 20 dB steps) matrix of MUC PSTHs for the AC (A), Str (B), and LA (C). The line in each plot outlines the tuning curve of the MUC with approximate CF shown below. X-axis of PSTH is 200 ms; y-axis is 500 spikes/s for A and B and 150 spikes/s for C; Stimulus duration is 50 ms.

Figure 5: (A) Overall group mean ($\pm$ SEM) SR in the AC, LA and Str of control rats and rats exposed to the OBN or NBN (see inset). In the AC, the mean SR of all neurons in the OBN group and

the NBN group were significantly greater than the control group. In the LA, the mean SR of all MUC in the OBN group was significantly greater than the control group. In the Str, mean SR in the OBN and NBN groups were significantly greater than the control groups. (B) The AC-MUC were separated into low-CF (≤ 8 kHz) and high-CF (≥ 12 kHz) groups. The mean SR of high-CF MUC were significantly greater than the control group in both the OBN and NBN groups, but for the low-CF MUC the significance was only observed in the NBN group. (C) The LA-MUC were separated into low-CF (≤ 8 kHz) and high-CF (≥ 12 kHz) groups. The mean SR of low-CF MUC in the OBN group was significantly greater than the control group, but all other noise-exposed LA-MUC did not show significant difference from the control. * $p < 0.05$; *** $p < 0.001$, see text for details.

Figure 6: (A): Population PSTH frequency-intensity matrix for LA MUC in the control group ($n = 103$, blue line) and the OBN group ($n = 140$, red line). Tone burst (50 ms) frequencies and intensities indicated on abscissa and ordinate. Red-up arrowhead and black-down arrowhead point to the approximate frequency-intensity regions where there were large increase (hyperactivity) and decrease (hypoactivity) in firing rate in the OBN group compared to the control group. (B): Population PSTH responses in the control group (blue line) and OBN group (red line) to 1 kHz tone bursts (50 ms) presented at 100, 80, and 60 dB SPL. Red-up arrowheads show the region of the PSTH where there was a large increase (hyperactivity) in firing rate in the OBN group compared to the control group. (C) Population PSTH responses in the control group (blue line) and OBN group (red line) to 12.1 kHz tone bursts (50 ms) presented at 100, 80, and 60 dB SPL. Black-down arrowheads show regions of PSTH where there was a large decrease in spike rate in the OBN-group. (D): Population PSTH responses in the control group (blue line) and OBN group (red line) in response to an 8 kHz (edge frequency) tone burst (50 ms) presented at 100, 80, and 60 dB SPL. Black-down arrowheads show regions where there

was a large decrease in spike rate. PSTH: 500 ms x-axis; 60 spikes/s y-axis. Significant changes in PSTH profiles: ** $p < 0.01$; *** $p < 0.001$; see text for details.

Figure 7: (A) PSTH frequency-intensity matrix for LA MUC in the control group ($n = 103$, blue line) and the NBN group ($n = 183$, red line). Tone burst (50 ms) frequencies and intensities indicated on abscissa and ordinate. Red-up arrowhead and black-down arrowhead point to the approximate frequency-intensity regions where there were large increases (hyperactivity) and decreases (hypoactivity) in firing rate in the NBN group compared to the control group. (B): Population PSTH responses in the control group (blue line) and NBN group (red line) to 1 kHz tone bursts (50 ms) presented at 100, 80, and 60 dB SPL. Red-up arrowheads show the region of the PSTH where there was a large increase (hyperactivity) in firing rate in the NBN group compared to the control group. (C) Population PSTH responses in the control group (blue line) and NBN group (red line) to 18.3 kHz tone bursts (50 ms) presented at 100, 80, and 60 dB SPL. Black-down arrowheads show regions of PSTH where there was a large decrease in spike rate in the NBN group. (D) Population PSTH responses in the control group (blue line) and NBN group (red line) in response to a 12.1 kHz (edge-frequency) tone burst (50 ms) presented at 100, 80, and 60 dB SPL. Red arrowhead shows region of PSTH where there was an increase in spike rate in the NBN group. PSTH: 500 ms x-axis; 60 spikes/s y-axis. Significant changes in PSTH profiles: ** $p < 0.01$; *** $p < 0.001$; see text for details.

Figure 8: (A): Population PSTH (500 ms) of LA MUC in control group (blue line, $n=103$) and NBN exposed rats (red line, $n=88$) with 30 dB of PTS in the noise band (16-20 kHz); data shown for 80 dB SPL tone bursts (50 ms) presented at 1.0, 12.1 and 18.3 kHz. Note large increase in firing rate at 1 kHz (red-up arrowhead) and large reduction at 18.3 kHz (black-down arrowhead), but no change at 12.1 kHz (edge frequency). (B) Population PSTH (500 ms) of LA MUC in the control group (blue line, $n=103$) and NBN exposed rats (red line, $n=95$) with 16 dB of PTS in the noise band (16-20 kHz); data

shown for tone bursts (50 ms) presented at 1.0, 12.1 and 18.3 kHz at 80 dB SPL. No change in PSTH at 1.0 kHz, slight enhancement at 12.1 kHz (edge frequency), and slight reduction at 18.3 kHz.

Significant changes in PSTH profiles: * $p < 0.05$; *** $p < 0.001$; see text for details.

Figure 9: (A): Population PSTH frequency-intensity matrix for AC MUC in the control group ($n = 548$, blue line) and the OBN group ($n = 224$, red line) Tone burst (50 ms) frequencies and intensities indicated on abscissa and ordinate. Red-up arrowhead and black-down arrowhead point to the approximate frequency-intensity regions where there were large increase (hyperactivity) and decrease (hypoactivity) in firing rate in the OBN group compared to the control group. (B): Population PSTH responses in the control group (blue line) and OBN group (red line) to 1 kHz tone bursts (50 ms) presented at 100, 80, and 60 dB SPL. Red-up arrowheads show the region of the PSTH where there was a large increase (hyperactivity) in firing rate in the OBN group compared to the control group. (C) Population PSTH responses in the control group (blue line) and OBN group (red line) to 12.1 kHz tone bursts (50 ms) presented at 100, 80, and 60 dB SPL. Black-down arrowheads show regions of PSTH where there was a large decrease in spike rate in the OBN-group. (D): Population PSTH responses in the control group (blue line) and OBN group (red line) in response to an 8 kHz (edge frequency) tone burst (50 ms) presented at 100, 80, and 60 dB SPL. Black-down arrowheads show regions where there was a large decrease in spike rate. PSTH: 200 ms x-axis; 250 spikes/s y-axis. Significant changes in PSTH profiles: *** $p < 0.001$; see text for details.

Figure 10: (A): Frequency-intensity matrix showing population PSTHs (200 ms duration, ordinate; 250 spikes/s) of AC MUC in the control group (blue line, $n=548$) and NBN group (red line, $n=236$); data obtained with 50 ms tone bursts (1 to 42. kHz, 0-100 dB SPL, 20-dB steps). Red-up arrowhead shows low-frequency regions where firing rates and response duration increased; black-down arrowhead shows high-frequency region where spike rate decreased. (B) Population PSTHs to

1.0 kHz tone bursts (50 ms) presented at 100, 80, and 60 dB SPL; note significant increase in response duration and mean discharge rates in 100 ms duration (red-up arrowheads); (C): Population PSTHs (200 ms, 250 spikes/s) to 18.3 kHz tone bursts presented at 100, 80 and 60 dB SPL showing reduction in spike rate during the stimulus (black-down arrowheads) followed by late response (red up arrows). (D) Population PSTHs (200 ms; 250 spikes/s) to 12.1 kHz tone bursts (edge frequency) at 100, 80, and 60 dB SPL. Note significant increase in response duration and mean discharge rate (red up arrowheads) and increased late component (red up arrows). Significant changes in PSTH profiles: *** $p < 0.001$; see text for details.

Figure 11: (A): Frequency-intensity matrix of population PSTHs (100 ms duration, ordinate 200 spikes/s) of Str MUC in the control group (blue line, $n=95$) and the OBN group (red line, $n=261$). Note reduction firing rate and response duration in high-frequency, high-intensity PSTH in the OBN group. (B) Frequency-intensity matrix of population PSTHs (100 ms duration; ordinate 200 spikes/s) in Str MUC of the control group (blue line, $n=95$) and the NBN group (red line, $n=226$). Note reduction in spike rate and decrease in response duration in the NBN group compared to the control group.

Figure 12: Schematic describing the relation between the degree of high-frequency hearing loss (severe, moderate and minor) and changes in suprathreshold population response profiles in the low-frequency, edge-frequency and high-frequency regions. (A) In cases of severe high-frequency hearing loss, low-frequency regions become extremely hyperactive, the edge-frequency becomes mildly hypoactive and the high-frequency region become very hypoactive. (B) In cases of moderate high-frequency hearing loss, low-frequency regions become mildly hyperactive, little change occurs at the edge-frequency region and the high-frequency region becomes moderately hypoactive. (C) In cases of minor high-frequency hearing loss, the low-frequency region shows no change at suprathreshold

intensities; the edge-frequency becomes mildly hyperactive and the high-frequency region is mildly hypoactive.

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Graphical Abstract