Assessing Hidden Hearing Loss After Impulse Noise in a Mouse Model

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Abstract

Introduction: There are several key differences between impulse and continuous noise: the nature of the noise itself, the cochlear and neuronal structures affected, the severity to which they damage the auditory system, and the period of time in which damage occurs. Notably, no work on hidden hearing loss after impulse noise exposure has been done to this point, though it has been extensively studied after continuous noise. Hidden hearing loss manifests physiologically with reductions in suprathreshold amplitudes of the first wave of the auditory brainstem response, while auditory thresholds can remain relatively normal. Objective: This study aimed to assess the extent to which, if at all, hidden hearing loss is present after exposure to impulse noise in C57BL6/J mice. Methods: Thirty-one C57BL6/J mice were used in the experiment, in accordance with IACUC protocols. Auditory brainstem responses were recorded before and after noise exposures. The noise exposures consisted of 500 impulses at 137 dB peSPL. Results: Suprathreshold amplitude reductions in the P1 wave of the mouse auditory brainstem response were seen, but only at frequencies with significant threshold shift. Conclusion: These amplitude changes were consistent with hidden hearing loss, and we conclude that impulse noise can cause hidden hearing loss, but future studies are required to determine the specific mechanisms involved and if they parallel those of hidden hearing loss after continuous noise.

Keywords: Auditory brainstem response, cochlea, hidden hearing loss, impulse noise, noise

INTRODUCTION

Hidden hearing loss is the physiologic and behavioral manifestation of synaptopathy of the inner hair cells (IHCs) and spiral ganglion neuron (SGN) synapses and has been documented in several animal models and noise conditions. The causes of SGN synaptopathy are numerous, including presbycusis, disease and noise exposure. The initial terminal retraction by the SGN occurs during the noise exposure, and within hours of the noise exposure, roughly half of the pre and postsynaptic ribbons have disappeared as a result of glutamate excitotoxicity following the noise exposure. Once a neuron has been disconnected from the associated IHC, it no longer has access to various neurotrophic factors that support the cell. Subsequently, over the course of weeks and months, the neuron can die if it does not re-attach to an IHC. Clinically, this phenomenon is difficult to detect because it can occur without significant threshold shift, which has been the gold standard measure that defines noise-induced injury for decades. This has led to SGN synaptopathy and neuropathy being labeled “hidden hearing loss”. Even after a continuous noise exposure that creates only a temporary threshold shift (TTS), hidden hearing loss. The observations of hidden hearing loss after noise have heretofore come from continuous noise exposures. These experiments used an octave band (8–16 kHz) noise exposure at 100 dB SPL for 2 hours and found suprathreshold reductions in amplitudes of the first wave of the auditory brainstem response (ABR), which correlated with SGN synaptopathy. Though hidden hearing loss is common following continuous noise, it has not been observed after impulse noise exposure up to this point.
Most noise exposures fall into the category of continuous or transient, with many exposures being a combination of the two. Transient exposures include impulse and impact noises. Continuous noise exposures carry key differences compared to impulse and impact noises. The basic difference between the physical characteristics of impulse and continuous noise is that impulse noise results from explosions that occur once or intermittently, punctuated by periods of silence. Typically, because of the brief duration of the signal, most of the exposure interval is silent gap in between impulses. In contrast, continuous noise is a largely uninterrupted noise, and the bulk of the duration of the exposure is noise rather than silent gaps. The specific characteristics of impulse noises depend on the characteristics of the source of the exposure. However, the ideal impulse noise has a waveform with zero rise time, such that it reaches maximum pressure instantaneously. This is immediately followed by a brief fall time, making the total impulse noise short in duration.[7] Impulse and continuous noise injure many of the same cell populations in the organ of Corti, but in potentially different ways and to different degrees. These cochlear injuries include separation of supporting cells in certain regions, damaged outer hair cells (OHCs), holes in the reticular lamina, and disrupted stereocilia. [8] OHCs are frequently damaged, and the subsequent lesion spreads primarily through apoptosis with some necrosis.[9-10] The damage to the reticular lamina created by these noises causes endolymph to surround the OHCs, mixing with the cortilymph, and causing further damage.[11-12] The lesion caused by initial damage to the cochlea and the mixing of cochlear fluids spreads away from the original location, primarily toward the base of the cochlea, first through apoptosis, and subsequently through necrosis.[13] Depending on the degree of damage, the lesion may continue to expand for over a month after the exposure and affects OHCs, IHCs, and populations of supporting cells as it expands.[13-15] After impulse noise, these injuries have a rapid onset, which allows little time for repair before causing a necrotic lesion. In comparison, the cell death caused by initial damage to the cochlea and the mixing of cochlear fluids spreads away from the original location, primarily toward the base of the cochlea, first through apoptosis, and subsequently through necrosis.[13] However, in the case of extremely intense continuous noise, OHCs may die quickly enough that some endolymph is able to mix with cortilymph and cause further damage.[17] The difference here is largely due to the typically higher peak pressures of impulse noises compared to continuous.[7] Finally, the cell death caused by impulse noise largely occurs after the noise has stopped because impulse noises are, by definition, transient. In contrast, much of the damage caused by continuous noise occurs during the noise exposure itself.

While impulse noise exposures have been strongly linked to loss of the cochlear amplifier, which results in permanent threshold shift (PTS), it is unclear whether impulse noises can cause audiometric patterns consistent with hidden hearing loss. Given that impulse noise is common in a variety of industries, including the military, forestry, and shipbuilding, [18] if impulse noise can cause hidden hearing loss as seen in continuous noise, it would be a serious public health concern and worthy of investigation. To best diagnose hidden hearing loss in animals models, various histological methods for synaptopathy are used. However, of perhaps greater clinical relevance to the human are the behavioral or physiologic changes in the auditory system that are indicative of hidden hearing loss. Suprathreshold changes in the amplitudes of the first wave of the ABR are currently the most effective means of diagnosing hidden hearing loss in both human clinical patients[19] and animal models.[2] The current study used suprathreshold amplitude changes in the P1 wave of the ABR in a mouse model to determine if there was evidence of impulse noise-induced hidden hearing loss.

**MATERIALS AND METHODS**

**Animals**

Thirty-one mice were bred in a vivarium at The Ohio State University from parents with the C57BL6/J background. All original mice were obtained from Jackson Labs. There were no significant differences in audiometry or susceptibility to NIHL between the breeder parents. The mice were housed in a university-run vivarium. All animal procedures were approved by The Ohio State University’s Institutional Animal Care and Use Committee.

**ABR threshold and amplitude testing**

Prior to noise exposure, all subjects underwent baseline ABR testing. For pre- and post-exposure testing, mice were anesthetized with a mixture of gaseous isoflurane (2.5% for induction, 1.2% for maintenance) and oxygen (1 L/min flow rate) from an oxygen concentrator (Pureline OC4000, Supera, Clackamas, OR). Testing was performed in a sound-attenuating booth. Three 6 mm platinum electrodes (Rochester Electro-Medical, Lutz, FL) were inserted subdermally. The inverting, non-inverting, and ground electrodes were inserted behind the right pinna, behind the left pinna, and near the right rear leg, respectively. The stimuli were generated using Tucker Davis Technologies (TDT, Gainesville, FL) SigGen software. Each tone burst was 1 ms in duration and had a 0.5 ms rise/fall time with no plateau. Stimuli were presented at a rate of 19/s. Signals were routed to a speaker (TDT Model MF1) positioned at 90 degrees azimuth, 3 cm from the vertex of each mouse’s head. The evoked responses of the mice were amplified with a gain of 50,000 using a TDT RA4LI headstage connected to an RA4PA preamplifier and bandpass filtered from 100 to 3000 Hz. Six frequencies were tested: 4, 8, 12, 16, 24, and 32 kHz at various levels from 90 to 20 dB SPL in 5 dB decrements. The levels were calibrated with a SoundTrack LxT1 sound level meter ( Larson Davis, Depew, NY) with a 1/2 inch condenser microphone (PCB Piezotronics 377B02,
Depew, NY), placed at the level of the animal’s head. Three hundred sweeps were averaged at each stimulus level using TDT BioSigRz software. Thresholds were defined as the lowest sound pressure level at which a repeatable response could be detected. In addition, P1–P2 amplitudes were obtained by placing cursors at the positive P1 peak and the subsequent negative trough. The amplitudes at each stimulus level were assembled into input-output (I-O) functions for analysis. During all ABR analysis, the evaluator was blind to the experimental condition for each animal. Following noise exposure, the ABR testing was repeated at 3, 7, and 21 days to measure threshold shift, with the day 21 measure used as PTS.

Noise exposures

Mice received a single exposure of impulse noise at 137 dB peSPL for 8 minutes and 20 seconds (500 clicks at 1 click/second). The impulses were created on TDT SigGen software, generated using a TDT RZ6 signal processor, amplified by a Marathon DJ-5000 power amplifier (New York, NY), and delivered to a speaker driver (model 2446H, JBL, Inc., Northridge, CA) and acoustic horn (JBL model 2380A) hanging 12 inches above the level of the animals’ heads. The cage lids were removed, but the wire grid remained in place to both allow the full noise dose to reach the animals and prevent escape. The peak equivalent noise level was calibrated at the level of the animals’ heads utilizing an oscilloscope (model TDS 1012B, Tektronix, Inc., Beaverton, OR), preamp (model PS9200, ACO Pacific, Inc., Belmont, CA) and a 1/4 in condenser microphone (model 7016 and model 4016, ACO Pacific, Inc.). Each animal was exposed to noise in a separate individual cage to prevent any shielding from the noise and to ensure as uniform of a noise dose across animals as possible.

Statistical analyses

ABR thresholds were analyzed with a two-factor (day x frequency) analysis of variance (ANOVA). Both factors were treated as within-subjects variables. ABR amplitudes were analyzed with a two-factor ANOVA (day x stimulus level) for each frequency tested. Both factors were treated as within-subjects variables. In cases where frequencies or levels are different, paired $t$-tests were performed to determine these differences. All statistical analyses were performed using IBM SPSS version 23 (IBM, Armonk, NY) and all associated figures were created using SigmaPlot version 11.0 (Systat Software Inc., London, UK).

RESULTS

ABR thresholds

Mouse ABR thresholds [Figure 1] were analyzed at 8, 16, and 24 kHz to ensure that at least TTS was induced with the noise exposure. We found a significant interaction of day x frequency ($F(6.120) = 5.372, P < 0.001$). In order to determine for which frequencies differences in thresholds occurred, we performed a series of one-way ANOVAs, with paired samples $t$-tests for pairwise comparisons over different days. For 8 kHz, there was a main effect of day ($F(3.84) = 20.925, P < 0.001$) and significant differences between pre-exposure thresholds and those on Day 3 ($t$-test).
In addition, there was a significant difference between thresholds at Day 3 and Day 21 for this frequency ($t(28) = -2.112, P = 0.044$). For 16 kHz, there was a main effect of day ($F(3.81) = 32.466, P < 0.001$) and significant differences between pre-exposure thresholds and those on Day 3 ($t(30) = -8.570, P < 0.001$), Day 7 ($t(29) = -9.696, P < 0.001$), and Day 21 ($t(29) = -9.523, P < 0.001$). We therefore concluded that significant PTS was induced by the noise exposure and that thresholds at 8 kHz worsened between Day 3 and Day 21.

**ABR amplitudes**

ABR amplitudes were analyzed at 8, 16, and 24 kHz [Figure 2]. A two-way repeated measures ANOVA (day x level) was performed at each frequency and, in each case, found significant interactions between the day on which the ABR was taken and the presentation level ($F(42.1176) = 1.731, P = 0.003$; $F(42.1176) = 1.633, P = 0.007$; $F(42.1176) = 7.392, P < 0.001$ for 8, 16, and 24 kHz respectively). Following this, one-way repeated measures ANOVAs (day) with paired samples $t$-test for pairwise comparisons were performed to determine at which levels there were differences between days. There were main effects of day at a subset of stimulus levels all three frequencies measured. At 8 kHz, main effects of day were found at all levels except 60, 35, and 20 dB SPL; at 16 kHz, all levels from 65 to 20 dB SPL showed main effects of day; and at 24 kHz, all levels from 30 to 90 showed main effects of day. The most relevant pairwise comparisons were those comparing Pre-exposure to Day 21. The paired samples $t$-tests for Pre-exposure vs. Day 21 revealed that at 8 kHz, differences between amplitudes occurred at all levels, except 35 and 20 dB SPL. At 16 kHz, only levels 65 dB SPL and lower (except 60 dB SPL) were significantly different. Finally, at 24 kHz, all levels except 20–30 dB SPL were significantly different. Not all animals experienced amplitude changes to the same degree. At 8 kHz, the maximum change was 4.465 nV and the minimum was $-1.431$ nV. At 16 kHz, the maximum change was 7.576 nV and the minimum was $-2.177$. Finally, at 24 kHz the maximum and minimum changes were 4.271 and $-2.589$ nV, respectively.

**DISCUSSION**

The degree to which impulse noise would induce hidden hearing loss was difficult to predict because of the differences between impulse and continuous noise and the variability of cochlear injury within a population exposed to the same impulse noises. The highly kurtotic nature of impulse noise makes it drastically different from flatter continuous noise.[20] Continuous noise does not vary as greatly in intensity as impulse noise, which has levels of high intensity followed by periods of silence. Because it is a high-level sound that will depolarize a large number of IHCs, it is conceivable that impulse noise could affect the afferent synapses in a manner similar to that of continuous noise. However, because of the periods of silence between impulses, however brief, there may be time to remove enough...
excess glutamate from the OHC-SGN synapse to prevent excitotoxicity. The current study was undertaken to examine suprathreshold ABR P1 wave amplitudes to determine if they indicate a pattern after noise exposure that is consistent with hidden hearing loss.

The noise caused a PTS by Day 21 of ∼15–35 dB, depending on frequency. Low-intensity amplitudes, 50 dB SPL and below, were steeply decreased at all three frequencies tested, consistent with cochlear amplifier injury and the threshold shifts that were measured. At 16 kHz, there were no significant differences between pre-exposure thresholds and Day 21 for levels above 65 dB SPL. However, 16 kHz did show a significant difference at 90 dB SPL on Day 7, which implies that there was a difference between ABR amplitudes between Day 7 and pre-exposure thresholds, but the amplitude reduction was temporary and recovered by Day 21. At 8 and 24 kHz, the amplitude reductions in high level (55–90 dB SPL) ABR amplitudes that appeared immediately after the noise exposure did not recover and were still significantly lower at Day 21. At 8 and 24 kHz, these differences were present from high levels (90 dB SPL) to low (25–35 dB SPL). Suprathreshold impairment was a good predictor of minimum long-term damage. All levels that showed significant ABR threshold differences at Day 3 showed at least those differences at Day 21. At 16 and 24 kHz all levels that showed impairment at Day 3 showed the same impairment at Day 21. However, at 8 kHz, there were more significant changes at Day 21 than there were at Day 3. This does not hold for prediction of long-term PTS. At Day 21 all three frequencies showed significant PTS, whereas only 24 kHz showed consistent significant Day 3 amplitude changes at levels above 65 dB SPL. However, amplitude reductions for responses to stimuli below 60 dB SPL declined at all frequencies, and was a good predictor of PTS. Therefore, response amplitudes from low-SPL stimuli appear to be a more effective predictor of PTS than suprathreshold amplitudes.

Qualitatively, the amplitude pattern at 8 kHz shows a pattern of amplitude recruitment similar to what would be predicted with cochlear amplifier injury, in which the differences in Pre versus Day 21 amplitude I-O functions narrowed as the intensity level increased. At 24 kHz, this recruitment effect is not evident, though the PTS is much greater (∼35 dB compared to ∼15 at 8 kHz) and may account for the differences between the Pre and Day 21 I-O functions. The pattern of significant ABR amplitude changes for high intensities across frequencies is interesting. The reductions at 24 kHz are consistent with findings of suprathreshold amplitude reductions at the higher frequencies in other noise exposure models.[2] However, the lack of reductions at 16 kHz combined with the statistically significant reductions at 8 kHz are surprising. One possible explanation is that the differences at 8 kHz are statistically anomalous. This idea is supported by the relatively small magnitude of reductions at the highest stimulus levels. There is an apparent recruitment effect in which the biggest differences at 8 kHz are in response to the lowest stimulus levels, and the differences get smaller and smaller as the stimulus level increases. At 16 kHz, by the highest levels, the differences are gone, but at 8 kHz the differences are still statistically significant even though the mean differences are small. Therefore, we conclude that the data after impulse noise are consistent with that the notion that hidden hearing loss is more evident at higher frequencies (i.e. 24 kHz) before low-to-mid frequencies. Overall, we conclude that the impulse noise that created the PTS also created a decrease in suprathreshold amplitudes. For comparison, Kujawa and Liberman[3] reported significant ABR amplitude changes only in the high frequencies (30 kHz and greater), though a different mouse strain was used. The permanent changes to the ABR amplitudes shown in the mouse imply the ability of impulse noise to cause suprathreshold changes to the amplitude of the ABR that are consistent with previous examples of hidden hearing loss. Whether this is a direct result of impulse noise or secondary to permanent damage to the cochlea is still unknown, and further research is indicated to determine if the suprathreshold P1 wave amplitude decreases can occur without significant PTS. The pattern of hearing loss at 24 kHz, which is consistent with changes present in hidden hearing loss, suggests that hidden hearing loss may be a side effect of impulse noise exposure, and is not unique to the continuous noise exposure conditions in which it has been detected previously.[2] As stated above, if impulse noise were shown to cause cochlear synaptopathy, it would be a serious public health concern for the workers in industries with a high rate of exposure to impulse noise.[18] Though no behavioral changes were apparent in this study, human cochlear synaptopathy is associated with behavioral changes and perceptual impairments like impaired speech recognition and discrimination, particularly in conditions of competing background noise. Given the nature of the auditory deficit, it is much harder to detect with typical audiometric evaluations, and because of this concern, the results of this study warrant future investigation.

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Conflicts of interest
There are no conflicts of interest.

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