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Prolonged noise exposure-induced auditory threshold shifts in rats

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Abstract

Noise-induced hearing loss (NIHL) initially increases with exposure duration, but eventually reaches an asymptotic threshold shift (ATS) once the exposure duration exceeds 18-24 h. Equations for predicting the ATS have been developed for several species, but not for rats, even though this species is extensively used in noise exposure research. To fill this void, we exposed rats to narrowband noise (NBN, 16-20 kHz) for 5 weeks starting at 80 dB SPL in the first week and then increasing the level by 6 dB per week to a final level of 104 dB SPL. Auditory brainstem responses (ABR) were recorded before, during, and following the exposure to determine the amount of hearing loss. The noise induced threshold shift to continuous long-term exposure, defined as compound threshold shift (CTS), within and above 16-20 kHz increased with noise level at the rate of 1.82 dB threshold shift per dB of noise level (NL) above a critical level (C) of 77.2 dB SPL i.e. $CTS = 1.82(NL - 77.2)$. The normalized amplitude of the largest ABR peak measured at 100 dB SPL decreased at the rate of 3.1% per dB of NL above the critical level of 76.9 dB SPL, i.e., $\%ABR\ Reduction = 3.1\%(NL - 76.9)$. ABR thresholds measured >30 days post-exposure only partially recovered resulting in a permanent threshold shift of 30-40 dB along with severe hair cell loss in the basal, high-frequency region of the cochlea. In the rat, CTS increases with noise level with a slope similar to humans and chinchillas. The critical level (C) in the rat is similar to that of humans, but higher than that of chinchillas.

Introduction

Numerous studies have shown that noise-induced hearing loss initially increases with exposure duration above a critical level. However, once the exposure duration exceeds 18-24 h, the hearing loss reaches a plateau referred to as asymptotic threshold shift (ATS) (Carder and Miller, 1971, Carder, 1972, Mills and Talo, 1972, Johnson et al., 1976, Mills et

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al., 1979, Clark, 1991). Theoretically, the ATS is believed to represent the upper limit of a permanent threshold shift (PTS) that can result from an exposure of infinite duration. However, hearing loss from prolonged noise exposures can completely recover if the exposure duration lasts only a few days and the level of the exposure is low to moderate; higher level and prolonged noise exposures can result in PTS (Carder and Miller, 1971, Carder, 1972, Mills and Talo, 1972, Johnson et al., 1976, Mills et al., 1979, Clark, 1991). The ATS in humans, chinchillas, monkeys and guinea pigs increases with noise level (NL, in dB SPL) above a critical level (C, in dB SPL) with a slope (R) of 1.5-2.0 dB thresholds shift for each dB greater than C, i.e., $ATS = R (NL - C)$ (Carder and Miller, 1971, Carder, 1972, Mills and Talo, 1972, Mills et al., 1979, Syka and Popelar, 1980, Clark, 1991, Eddins et al., 1999, Coomber et al., 2014). Noise exposures leading to ATS would benefit studies investigating the mechanisms underlying noise-induced hearing loss (NIHL) and noise-induced auditory perceptual disorders such as tinnitus and hyperacusis since the degree of threshold shift is predictable and highly consistent across animals (Atherley et al., 1968, Blakeslee et al., 1978).

Intense noise causes hearing loss which is accompanied in some cases with auditory perceptual disorders, such as tinnitus and hyperacusis (Axelsson and Sandh, 1985, Axelsson and Hamernik, 1987, Phoon et al., 1993, Konig et al., 2006, Moon et al., 2011, Chen et al., 2013). Auditory perceptual disorders such as tinnitus are often related to characteristics of the NIHL including its severity, frequency characteristics and the slope of the audiogram (Axelsson and Sandh, 1985, Konig et al., 2006). Nearly identical NIHL in a group of animals would be beneficial in characterizing the relationship between NIHL and the noise-induced neural alterations in the brain (e.g., spontaneous activity) that underlie auditory perceptual disorders (e.g., tinnitus). However, the hearing loss induced by intense, short duration exposures are often highly variable (Henderson et al., 2001, Luebke and Foster, 2002).

Rats have been used extensively in the study of NIHL (Cappaert et al., 2000, Chen, 2002), noise-induced tinnitus and hyperacusis (Zhang and Kaltenbach, 1998, Kaltenbach et al., 2000, Heffner, 2011, Sun et al., 2012, Pace and Zhang, 2013, Chen et al., 2014). However, hearing loss from prolonged noise exposure has not yet been assessed in rats. Therefore, the current study was designed to measure the degree of noise exposure-induced threshold shift to continuous long-term exposure, defined as CTS (compound threshold shift), in a group of rats exposed to a weekly high-frequency narrowband noise (16-20 kHz) exposure that increased from 80 to 104 dB SPL in 6 dB steps. Since prior sound exposure can affect the hearing loss resulting from subsequent exposures, a phenomenon sometimes referred to as “toughening” (Subramaniam et al., 1992, Canlon, 1997, Pukkila et al., 1997, Canlon and Fransson, 1998, Niu and Canlon, 2002, Hamernik et al., 2003, Niu et al., 2004, Tahera et al., 2007), we intentionally use CTS to describe the hearing loss resulting from our sequential weekly noise exposures to distinguish it from ATS studies without prior noise exposure. The data showed that beyond a critical noise level of ~77 dB SPL, the degree of CTS increased by ~1.8 dB per dB increase in exposure level, consistent with results observed in human subjects and chinchillas. The 55 dB of CTS resulting from the final 104 dB SPL exposure only partially recovered when the rats were removed from the noise resulting in a significant

PTS at the high frequencies similar to that observed in guinea pigs (Syka and Popelar, 1980).

Methods

Subjects

Eleven male Sprague Dawley rats (2 months of age) were acquired from Charles River Laboratories Inc. (Wilmington, MA). Six were exposed to noise and five were used as controls for hair cell counting. The animals were housed in the Laboratory Animal Facility at the University at Buffalo and given free access to food and water. The colony room was maintained at 22°C with a 12-hour light-dark cycle. All procedures used in this project were approved by the Institutional Animal Care and Use Committee (HER05080Y) at the University at Buffalo and carried out in accordance with NIH guidelines.

Noise exposure

Each of the six noise-exposed rats was housed in an individual noise exposure cage (L=19", W=10", H=8") with a speaker (Vifa D25AG35 100 Dome Tweeter, Madisound Speaker Components, Inc., Middleton, WI) mounted above the center of the cage (3.5" above the cage). After 10 days of environmental adaptation and acquiring baseline auditory brainstem response (ABR) measurements, the animals were exposed to a narrowband noise (NBN, 16-20 kHz). Sound levels at the height of animal's ear (3" from the bottom of the cage) were measured directly below the speaker; those measured near the edge of the cage at this height could be 1-2 dB lower. Figure 1 presents the spectrum of the NBN at 104 dB SPL. The sound level in each $\frac{1}{3}$ octave band was measured using a sound level meter (Larson Davis System824) and a free-field $\frac{1}{2}$ " microphone (model 2540, Larson Davis). The sound levels in $\frac{1}{3}$ octave bands centered at 20, 16, 12.5 and 10 kHz were 98, 102, 82 and 55 dB SPL respectively; at lower frequencies the levels ranged from 30-40 dB SPL. The animals were exposed to the NBN at 80 dB SPL for the first week and the noise level was subsequently increased by 6 dB in each following week (i.e., 86 dB SPL in the second week, 92 dB SPL in the third week, 98 dB SPL in the fourth week, and 104 dB SPL in the last week).

Auditory brainstem response

The ABR was recorded pre-exposure, during the exposure (at the 25th h and the 121st h after the onset of each weekly exposure), and following the cessation of the noise exposure until one month post-exposure. During the noise exposure, each animal was removed from the noise-exposure cage for one hour for ABR recording. Rats were anesthetized with 4% isoflurane in O₂ at a flow rate of 0.5 L/minute and subsequently maintained at 1.5% isoflurane. Body temperature was maintained at 37°C using a homeothermic blanket (Harvard Apparatus). Alternating phase tone bursts (5-ms duration with 1-ms rise/fall time, cosine²-gated) at 4, 8, 12, 16, 20, 24, and 32 kHz and clicks (50 μ s pulse) were generated using TDT SigGen software and presented at a rate of 21/sec. The stimuli were delivered binaurally through a speaker (FT28D, Fostex) calibrated using a sound level meter (Larson Davis System 824) and a $\frac{1}{2}$ " microphone (model 2540, Larson Davis). Needle electrodes (Grass Technologies) were placed at the vertex (active), posterior bulla (reference) and behind the shoulder blade (ground). The responses were amplified 5020 times by a TDT

Headstage-4 bio-amplifier (bandpass filter: 10-3000 Hz with a notch filter at 60 Hz) and averaged 200 times. At each frequency, the sound level was decreased in 10-dB steps from 100 dB SPL to 0 dB SPL. The animals usually woke within 2-5 min after removal of isoflurane and were immediately returned to the noise-exposure cage. ABR threshold was defined as the lowest level that produced a noticeable ABR response. Figure 2A presents mean ABR thresholds of the animals as a function of frequency. The difference between the pre-exposure threshold and the ABR thresholds obtained during or after the exposure was defined as ABR threshold shift. ABR amplitude was also measured. Figure 2B presents the average (n=6) ABR waveform from all animals in response to clicks of 100 dB pSPL; the amplitude of the largest positive peak occurred at ~2.2 ms and the largest negative peak was located at ~3.3 ms. ABR amplitude was the peak to peak distance.

Hair Cell Loss

At the conclusion of the experiment, the cochleae were removed and the round window, oval window, and the cochlear apex were opened to facilitate perfusion of staining solutions and fixative as described in detail previously (Chen, 2002, Atik, 2014). Briefly, the cochleae were perfused with an incubation solution containing 0.05% nitroterazolium blue chloride (cat#N6876, Sigma), 0.05M sodium succinate, and 0.05 M phosphate buffer and then incubated in the solution for 1 h (37 °C). The stained cochleae were fixed in 10% buffered formalin for 2 days and then decalcified in 7% EDTA (ethylenediamine tetraacetic 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 the number of missing inner hair cells (IHC) and outer hair cells (OHC). Cell counts were converted to percent OHC and IHC loss using laboratory norms and plotted as function of percent distance from the apex; cochlear position was related to frequency using a rat cochlear-frequency place map (Bohne and Clark, 1982).

Results

CTS

Figure 3 presents mean (n=6) ABR threshold shifts at 4, 8, 12, 16, 20, 24 and 32 kHz measured during the five NBN exposures and after the noise exposure when thresholds were recovering. In the first exposure week at 80 dB SPL, CTSs within and above the exposure frequency (16-32 kHz) increased 6-9 dB whereas there was little change in threshold at lower frequencies (< 12 kHz). During the second week of exposure at 86 dB SPL, CTSs within and above the exposure frequency (16-32 kHz) were 15-20 dB whereas the low frequency (4-12 kHz) threshold shifts were roughly 2-9 dB. During the third week of exposure at 92 dB SPL, CTSs at the high frequencies increased to approximately 19-29 dB, while threshold shifts at the low frequencies ranged from 5 to 13 dB. In the fourth week exposure at 98 dB SPL, CTSs within and above the exposure frequency were between 25 and 46 dB while thresholds shifts at 8-12 kHz were 8-12 dB and those at 4 kHz were 4-8 dB. Finally, during the last 104 dB SPL exposure which lasted 8 days, thresholds were measured three times on days 1, 5 and 8 of the exposure. CTSs were 45-58 dB within and above the exposure frequency while threshold shifts at 8-12 kHz were 22-28 dB and those at 4 kHz

were approximately 15 dB. During the 4-week recovery period, thresholds essentially fully recovered at 4, 8 and 12 kHz whereas thresholds only partially recovered at frequencies within and above the exposure resulting in PTSs of 25, 35, 35 and 38 dB at 16, 20, 24, and 32 kHz respectively. The largest, threshold shift at 32 kHz occurred approximately one octave above the NBN exposure. The threshold shifts of the click-evoked ABR (not shown) were similar to those observed at 8 kHz.

Each panel in figure 4 (A-G) presents the mean (n=6) CTSs (2 measurements/week) as a function of noise level for 4, 8, 12, 16, 20, 24 and 32 kHz respectively. The data were fitted by linear regression to the function, $CTS = R(NL - C)$, and the slope (R) and critical level (C) and goodness of fit (r^2) for each stimulus are shown in each panel. At high frequencies within and above the NBN frequency the slopes (R), and critical intensities (C) were similar across frequency. At 16, 20, 24 and 32 kHz, the slopes were 1.86, 1.85, 1.84 and 1.73 dB/dB respectively and the critical intensities were 75.5, 77, 76.3 and 77.1 dB SPL respectively. At the low frequencies below the NBN, the slopes were shallower than at the high frequencies. For 4, 8, and 12 kHz, the slopes were 0.46, 0.75 and 0.87 dB/dB respectively and the critical intensities were 78.8, 75.3 and 77.0 dB SPL. The data indicate that the growth of CTS at low frequencies below the NBN was much smaller than that at the high frequencies within and above the NBN despite similar critical intensities. Since the CTS growth functions at frequencies within and above the NBN were quite similar, the average CTSs were computed for 16, 20, 24, and 32 kHz as a means of summarizing the high frequency data. As shown in figure 5, the relationship between CTS and noise level can be expressed as: $CTS = 1.82 * (NL - 77.2)$ where the critical level, C, equals 77.2 dB SPL and the slope, R, equals 1.82 dB/dB increase in CTS for each dB increase above C, the critical level.

ABR Amplitude

Figure 6 presents ABR amplitudes in response to tones at different frequencies at 100 dB SPL. Consistent with the threshold shift, the amplitude of the suprathreshold ABR decreased as the level of the NBN increased. The largest amplitude reductions occurred at frequencies within or above the NBN (16-32 kHz). The smallest amplitude reductions occurred at the lowest two frequencies (4-8 kHz) with moderate reductions occurring at 12 kHz. The ABR amplitudes were completely recovered at the low frequencies (4-12 kHz), but only partially recovered at the high frequencies (16-32 kHz).

The ABR amplitudes measured at 100 dB SPL during the five NBN noise exposure levels were normalized to their baseline amplitude and expressed as percent reduction in ABR amplitude. Figure 7 presents ABR amplitude reductions (%) measured at high frequencies within and above the NBN. Figure 7(A-D) shows the results at 16, 20, 24 and 32 kHz respectively. ABR amplitude decreased approximately linearly with increase in NBN level. The data in each panel were fit to the equation, $ABR \text{ Amplitude Reduction (\%)} = R(NL - C)$, where R is the slope in % reduction per dB SPL of the NBN, NL is the level of the NBN and C is the critical level above which the amplitude begins to decrease. Each panel shows the value for R, C, and goodness of fit (r^2) to the data. The critical levels, C, for 16, 20, 24 and 32 kHz were 75.6, 78.9, 73.8 and 76.9 dB SPL respectively and the slopes for 16, 20, 24 and 32 kHz were 2.8, 3.3, 2.8 and 3.5 % reduction per dB NL. Figure 7E shows the mean %

amplitude reduction vs. sound level function for 16, 20, 24 and 32 kHz. The mean critical level for these 4 frequencies was 76.9 dB SPL and the mean slope was 3.1% amplitude reduction per dB increase in the noise level. Based on the equation, the 100 dB tone-evoked ABR at frequencies within and above the noise band would disappear (100% of reduction) when the NBN increased to 109 dB SPL.

Permanent Threshold Shift and Hair Cell Loss

Because the series of five exposures lasted five weeks and reached a high level that caused significant CTS, thresholds did not fully recover at the high frequencies after four weeks of recovery. As shown in figure 8A, the largest mean PTS, ~38 dB, occurred at 32 kHz. PTSs at 20-24 kHz were approximately 34 dB and roughly 25 dB at 16 kHz. Thresholds from 4 to 12 kHz were nearly normal with 3 dB or less threshold shift. Thus, the greatest hearing loss occurred at 32 kHz, nearly an octave above the center frequency of the exposure consistent with many previous studies (Cappaert et al., 2000, Cave et al., 2007, Cacace et al., 2014).

Figure 8B shows the mean ($\pm$ SEM) loss of OHC and IHC as a function of percent of total distance from the cochlear apex; the upper x-axis shows the frequency-place map for the rat (Bohne and Clark, 1982). OHC loss from the five-week exposure increased from 20% to 100% in the cochlear locations that extend from 20 kHz to above 48 kHz. IHC losses increased from approximately 20% to 100% at frequencies extending from approximately 32 kHz to above 48 kHz. Although there was a 20-30 dB threshold shift from 16-20 kHz, there was less than 25% OHC loss and no IHC loss at the cochlear locations that map to these impaired frequencies consistent with our previous finding (Chen and Fechter, 2003). In general, the hearing loss extends to lower frequencies than would be expected by the location of the cochlear OHC and IHC lesion. The control rats (n=5) did not show hair cell loss.

Discussion

In this study, we used the term CTS to describe the hearing loss resulting from weekly noise exposures that increased in intensity from 80 to 104 dB in 6 dB steps to distinguish it from the ATS that occurs without prior noise exposure. This distinction was made because prior noise exposure could make the ear more or less vulnerable to subsequent exposure. Thus, in the following discussion the reader should keep in mind that the results from our CTS exposures may be different from ATS.

CTS

In humans, hearing loss initially increases with noise exposure duration and then reaches an asymptote or plateau after 12-18 h (Johnson et al., 1976, Viall and Melnick, 1977, Mills et al., 1979, Laroche et al., 1989). The time required for chinchillas to reach its asymptote value appears to be slightly longer than that observed in humans (Carder and Miller, 1971, Carder, 1972, Clark, 1991). In guinea pigs exposed to an one-third octave band noise centered at 2 kHz, asymptote of hearing loss was observed at 4 kHz around 6 h of exposure; however, at 2 kHz, a quasi- plateau was observed when thresholds increased slowly between 6 h and 5 days of exposure (Syka and Popelar, 1980). An asymptote of threshold shift has

also been observed in monkeys (Coomber et al., 2014), but one study suggested that the asymptote may require more than 4 days of exposure to reach (Nielsen et al., 1984). Close inspection of our results (i.e., 16, 20 and 24 kHz during the exposure week 4, Figure 3), revealed a few cases where the threshold shift measured on day 5 of the exposure was slightly greater than that measured after day 1 of the exposure implying that the hearing loss had not fully stabilized between day 1 and day 5 of the exposure. Other data suggest that thresholds are stable between day 1 and day 5 of exposure, for example, threshold shifts at 16, 20 and 24 kHz were nearly identical after 1 day and 5 days of exposure. Because thresholds were only measured on day 1 and day 5 of the exposure, we do not know precisely when the asymptote is reached in rat, but this could be established in future studies with a more detailed temporal analysis of hearing loss growth. Nevertheless, given that the plateau of hearing loss occurs in many other species with exposures lasting a day or two, it seems highly likely that the plateau of hearing loss would be well established in rats by day 5 of each of our weekly exposures.

ATS vs. sound level

It has been demonstrated that ATS in chinchillas and humans increases linearly with noise intensity level after a critical level has been exceeded. The critical level and the slope of the ATS function vary with species and with the spectrum of the noise. In the chinchilla, the critical level for an octave band of noise centered at 0.5 kHz is 65 dB SPL whereas for an octave band of noise centered at 4.0 kHz, the critical level is quite low, only 46.8 dB SPL. For both octave band frequencies, the growth of ATS with sound level is 1.6 dB hearing loss per dB noise level increase (Carder and Miller, 1971, Carder, 1972). In humans exposed to broad band noise, the critical level was approximately 78 dBA and the growth of ATS was 1.7 dB per dB noise level increase (Mills et al., 1981). Other studies report ATS slopes ranging from 1.5-2.0 dB per dB of noise level increase (Carder and Miller, 1971, Carder, 1972, Mills and Talo, 1972, Ward, 1975, Melnick, 1976, Saunders et al., 1977, Mills et al., 1979, Stephenson et al., 1980, Mills et al., 1981, Clark, 1991, Melnick, 1991). The CTS slopes in the rat obtained at frequencies within and above the 16-20 kHz noise band ranged from 1.73-1.86, in good agreement with previous findings of ATS in humans, chinchillas and other species. For our 16-20 kHz noise band, which lies in the most sensitive region of the rat's audiogram, the critical level for frequencies from 16-32 kHz was 77.2 dB SPL. Thus, the critical level for the rat is comparable to that measured in humans (76.3-78.5 dB). However, the critical level in the rat is much higher than that observed in chinchillas at both low frequencies and high frequencies (Carder and Miller, 1971, Carder, 1972). In the present study, rats were continuously exposed to noise for one week at each noise level. Such paradigms have been shown to cause resistance and toughening resulting in reduced damage to the later exposure (Subramaniam et al., 1992, Canlon, 1997, Pukkila et al., 1997, Canlon and Fransson, 1998, Niu and Canlon, 2002, Hamernik et al., 2003, Niu et al., 2004, Tahera et al., 2007). In this study, the ABR was measured after 1 day and 5 days of noise exposure. A reduction of ABR threshold shift at the second measurement (day-5) compared to the first one (day-1) was not observed, indicating that the cochlea is not toughened. Instead, ABR threshold shift measured at the second time in each exposure week was often greater than that measured at the first time, indicating accumulative disruption. Second, in previous prolonged noise exposure studies (Carder and Miller, 1971, Carder, 1972, Clark, 1991),

animals were exposed to a new-level of noise after a rest-period of 7 days from a previous exposure, at which time the hearing thresholds had recovered. It has been found that every dB of noise level increase above a critical level produces ~1.7 dB of extra threshold shift. We obtained a similar result; every dB of noise level increase produced ~1.8 dB of extra threshold shift. The similar growth of hearing loss with increasing intensity indicates that the exposure to the lower-level noise in our study did not slow or prevent the growth of hearing loss caused by subsequent higher level noise. Accordingly, toughening was unlikely a major factor influencing the growth of hearing loss or amount of hearing loss, though we concede that this has not been directly shown here. We also measured CTS growth functions at frequencies located below the frequency of the noise exposure; at these frequencies the CTS slopes were much smaller (0.46-0.87 dB/dB) than those observed at the high frequencies; but the critical intensities were unexpectedly similar (75.3-78.8; Figure 4). Why the critical intensities are similar for both low and high frequencies is unclear, but one possibility is that the onset response of the ABR is biased towards the 16-24 kHz region where the rat's hearing is most sensitive. Consequently, the ABR elicited by low frequency stimuli may be composed of neural responses from both high and low frequency regions. When the high frequency noise begins to damage high frequency region of the cochlea, the number of neurons contributing to the ABR elicited by a low frequency tone burst may be reduced due to diminished contributions from high frequency neurons. This would explain why the critical levels are similar at low and high frequencies and why the CTS slopes are shallower at low versus high frequencies. Another factor that contributes to the spread of hearing loss to the low frequencies is the apical expansion of the traveling wave towards the low-frequency region of the cochlea. At extremely high sound levels, significant hearing loss can occur at frequencies far below the frequency of the noise exposure resulting in considerable damage in the apical region of the cochlea (Davis et al., 1950).

ABR Amplitude vs. CTS

Consistent with the threshold shifts, the ABR amplitude evoked by high frequency tone bursts presented at 100 dB decreased nearly linearly as the level of the noise increased. The reduction of ABR amplitude (%) was linearly related to the noise intensity level with a slope of 2.5% to 3.5% (i.e. each dB increase of noise level reduced the ABR amplitude 2.5-3.5% from its initial amplitude). These results imply that as the level of CTS increases, there is progressively less neural activity or neural synchronization occurring within the auditory brainstem, that is, neural hypoactivity. An important theoretical and clinically relevant question posed by these results is do subjects in a state of CTS experience loudness decruitment (abnormally slow loudness growth) similar to patients with acoustic neuromas or brain damage (Hebert et al., 2013, Dawes et al., 2014) or do they experience loudness recruitment characteristic of patients with sensorineural hearing loss or hyperacusis that is often associated with tinnitus (Loeb and Smith, 1967, Martins et al., 2013, Mahmood et al., 2014)?

PTS and Cochlear Pathology

In humans and chinchillas, behavioral measures of ATS completely or almost completely recovered from ATS exceeding 60 dB as long as the exposure duration lasted only 1 week; however, for very long exposure durations lasting as long as 161 weeks, hearing thresholds

from a 50 dB ATS only recovered 10-15 dB resulting in PTS (Carder and Miller, 1971, Carder, 1972, Mills and Talo, 1972, Mills et al., 1979, Cody and Johnstone, 1981, Clark, 1991, Melnick, 1991). Likewise, hearing thresholds from a prolonged noise exposure only partially recovered when guinea pigs were continuously noise exposed for 120 days (Syka and Popelar, 1980). For 40-45 dB of ATS, there was only 15-20 dB of hearing recovery leaving a significant permanent hearing loss of 20-35 dB at frequencies within and above the noise band. In the present study in rats, a 55 dB CTS was reached. After the five week escalating noise exposure, there was only ~20 dB of recovery resulting in a permanent threshold shift of 30-40 dB and significant OHC and IHC loss in the high frequency region of the cochlea.

ATS and Tinnitus

Exposure to intense noise has long been known to induce tinnitus. Most humans exposed to high level tones or narrow band noise for minutes or hours experience the phantom sound of tinnitus immediately after the traumatizing stimulus is turned off (Mencher et al., 1973, Moody et al., 1976, Muller, 1991, Ortmann et al., 2011). If the traumatizing exposure is not too severe, the tinnitus may last minutes or hours and eventually disappear as hearing thresholds recover. Since rats are frequently used to evaluate the neural correlates of tinnitus, the use of moderate level prolonged noise exposures lasting one to five days might be especially useful for studying temporary noise-induced tinnitus since the magnitude and spectral location of the hearing loss is consistent and predictable across animals. Studies are underway to exploit the CTS paradigm for studying transient noise induced tinnitus using an operant behavioral paradigm (Stolzberg et al., 2013) and preliminary results indicate that rats exposed to our NBN for 1-5 days develop behavioral evidence of tinnitus immediately after they are removed from the prolonged noise and that the tinnitus-like behavior dissipates with recovery time.

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Abbreviations

ABR	auditory brainstem response
ATS	asymptotic threshold shift
IHC	inner hair cell
PNITS	prolonged noise exposure-induced threshold shift
NBN	narrowband noise
NIHL	noise-induced hearing loss
NL	noise level
OHC	outer hair cell

PTS permanent threshold shift

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- We measured prolonged noise exposure-induced threshold shifts in rats
- The threshold shift increases with noise level at a slope of 1.82 dB per dB
- The critical noise level that induces threshold shift is 77.2 dB SPL
- Consistently, ABR amplitude decreases with noise level at a slope of 3% per dB
- The critical noise level that induces ABR reduction is 76.9 dB SPL

Figure 1.

One-third octave band analysis of the 16-20 kHz NBN measured with the overall level set at 104 dB SPL.

Figure 2.

(A): Normal ABR thresholds as a function of frequency. (B) Assessment of ABR amplitude; the amplitude was measured between the highest positive peak (~2.2 ms) and the greatest negative peak (~3.3 ms).

Figure 3.

Mean ABR threshold shifts measured during and after the 16-20 kHz NBN exposure. The rats ($n=6$) were exposed for seven consecutive days at 80 dB SPL in the 1st week, 86 dB SPL in the 2nd week, 92 dB SPL in the 3rd week, 98 dB SPL in the 4th week, and for 8 consecutive days at 104 dB SPL in the 5th week. ABR threshold shifts were measured at the 25th hour and the 121st hour in each exposure-week and at 2, 4, 8, 16, and 30 post-exposure days. For clarity, error bars not shown.

Figure 4.

Mean ($n=6$ animals, SEM error bar) ABR threshold shifts for (A) 4 kHz, (B) 8 kHz, (C) 12 kHz, (D) 16 kHz, (E) 20 kHz, (F) 24 kHz and (G) 32 kHz versus intensity level of the NBN. Threshold shifts, measured at the 25th and 121st h after the start of each NBN level were used to compute the CTS for each NBN level increase. Data in each panel fit by linear regression (solid line) to the function $CTS = R (NL - C)$ where NL is noise intensity level in dB SPL, C is the critical level in dB SPL and R is the slope expressed as dB CTS per dB intensity level increase of the NBN. Each panel shows the goodness of fit (r^2), the slope, R, and the critical level, C.

Figure 5.

Mean (SEM error bar) threshold shifts as a function of NBN intensity level for frequencies within and above the NBN (16, 20, 24 and 32 kHz). Data fit by linear regression (solid line) to the function $CTS = R (NL - C)$ where NL is noise level in dB, C is the critical level in dB SPL and R is the slope expressed as dB CTS per dB level increase of the NBN; goodness of fit (r^2), slope, R, and critical level, C, shown in inset.

Figure 6.

ABR amplitude (see Figure 2) at 4, 8, 12, 16, 20, 24 and 32 kHz before (0 d), during the 1st through 5th week of the NBN exposure at the intensity level indicated and during the recovery period. For clarity, SEM and click data are not shown.

Figure 7.

Mean ($n=6$, SEM) percent reductions of ABR amplitude relative to baseline amplitude as a function of NBN intensity level. ABR amplitudes declined approximately linearly with increase in NBN level. Data in each panel fitted to function: ABR Amplitude Reduction (%) = $R(NL-C)$; R , C and goodness of fit (r^2) shown in each panel. Percent reduction of ABR amplitude for test frequencies: (A) 16 kHz, (B) 20 kHz, (C) 24 kHz, (D) 32 kHz and (E) the average of 16, 20, 24 and 32 kHz.

Figure 8.

(A) Mean ($n=6$, SEM) permanent ABR threshold shifts as a function of frequency. (B) Mean ($n=6$, SEM) OHC and IHC loss as a function of percent distance from the apex of the cochlea; percent distance from the apex related to frequency using the cochlear frequency-place map for the rat. Solid and dashed lines are OHC and IHC counting respectively in control rats ($n=5$).