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Research paper

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.

1. 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; 1972; Mills and Talo, 1972; Johnson et al., 1976; Mills et 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; 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; 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

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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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).

2. Methods

2.1. 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.

2.2. 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. Fig. 1 presents the spectrum of the NBN at 104 dB SPL. The sound level in each $^1/_3$ octave band was measured using a sound level meter (Larson Davis System824) and a free-field ½″ microphone (model 2540, Larson Davis). The sound levels in $^1/_3$ 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 to 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).

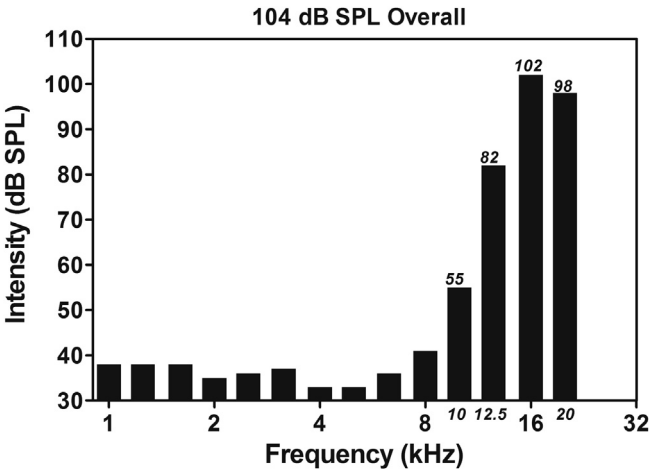

Fig. 1. One-third octave band analysis of the 16–20 kHz NBN measured with the overall level set at 104 dB SPL.

2.3. 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 O_2 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, cosine^2-gated) at 4, 8, 12, 16, 20, 24, and 32 kHz and clicks (50 μs 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 ½″ 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 (band-pass 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. Fig. 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. Fig. 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.

2.4. 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