ELSEVIER

Hearing Research 145 (2000) 111–122

HEARING RESEARCH

www.elsevier.com/locate/heares

Noise damage in the C57BL/CBA mouse cochlea☆

Henry C. Ou [1], Barbara A. Bohne *, Gary W. Harding

Washington University School of Medicine, Department of Otolaryngology, St. Louis, MO 63110, USA

Received 24 January 2000; accepted 24 April 2000

Abstract

The present study was designed to determine the response to noise of the auditory system of a genetically well-defined laboratory mouse in preparation for examining the effect of noise on mice with specific genetic mutations. The mice were C57BL/CBA F1 hybrids. Eight mice served as non-noise-exposed controls and 39 mice were exposed for 1–24 h to an octave band of noise with a center frequency of 2, 4 or 8 kHz and a sound pressure level of 100–120 dB. Auditory brainstem response thresholds were measured pre-exposure and several times post-exposure (i.e., 0–27 days) to determine the magnitude of the temporary threshold shift (TTS) and permanent threshold shift (PTS). After fixation by cardiac perfusion, the cochleas from each mouse were embedded in plastic, dissected into quarter turns of the cochlear duct and analyzed quantitatively. Immediately post-exposure, all mice had sizable TTSs at the tested frequencies (i.e., 3–50 kHz). At this time, two mice were killed. Thresholds of the other 37 mice recovered somewhat in the first 4 days post-exposure. One mouse fully recovered from its TTS; 10 mice were left with PTSs at all frequencies; 26 mice recovered at some frequencies but not others. Most mice with PTSs for 30–50 kHz had focal losses of inner and outer hair cells in the basal 20% of the organ of Corti, often with degeneration of adjacent myelinated nerve fibers in the osseous spiral lamina. On the other hand, mice with PTSs for the lower frequencies (i.e., 3–20 kHz) had stereocilia disarray without significant hair cell losses in the second and first turns. Considerable variability was found in the magnitude of hair cell losses in those mice that received identical noise exposures, despite their genetic homogeneity.

Key words: Mouse; Noise exposure; Temporary threshold shift; Permanent threshold shift; Auditory brainstem response; Quantitative histopathology

1. Introduction

Over the 100+ year history of noise research, humans (e.g., Davis et al., 1950) and a number of animal species (e.g., guinea pig, chinchilla, cat, rabbit, mouse) have been used to examine the effects of noise on auditory function and structure, and to determine how noise parameters (i.e., frequency, intensity, duration and scheduling) are related to the damage potential of a particular exposure. Human studies have provided valuable information on the relation between noise exposure and the loss of auditory function (e.g., Taylor et al., 1965; Mills et al., 1970; Sulkowski, 1973; Melnick, 1976). However, research with humans has not been able to provide much information on the amount or pattern(s) of cochlear damage induced by excessive exposure to noise. This latter point is important in view of the fact that hair cell injury and loss generally precede any measurable permanent shift in pure tone thresholds (e.g., Bredberg, 1968; Clark and Bohne, 1978).

Despite the large number of histopathological studies involving the effects of noise on the cochlea, issues remain unanswered (e.g., how excessive noise first injures then destroys cells in the organ of Corti; why degeneration in the cochlea continues a long time after the exposure has terminated; why exposure at one frequency often damages two or more regions in the cochlea). These issues appear to have arisen because some researchers have attempted to elucidate general princi-

* Corresponding author. Tel./Fax: +1 (314) 362-7497; E-mail: bohneb@msnotes.wustl.edu

☆ This work was completed as part of a thesis by H.C. Ou for the MA/MD program in Biological Sciences at Washington University School of Medicine.

[1] Present address: Department of Otolaryngology, VMB Hearing Research Center, Box 357923, University of Washington Medical School, Seattle, WA 98195-7923, USA.

0378-5955/00/$ – see front matter
PII: S0378-5955(00)00081-2

ples of noise damage but used only intense, short-duration exposures and/or evaluated only a portion of the organ of Corti.

It appears that there are several ways in which noise may damage the cochlea. Intense noises, such as explosions (i.e., >140 dB SPL), damage the cochlea instantaneously, probably by purely mechanical means. Explosions produce high-amplitude vibrations of the cochlear partition that exceed its elastic limits. The organ of Corti is torn from its attachment to the basilar membrane. With time, the bare basilar membrane is covered by undifferentiated squamous epithelium (e.g., Lurie, 1942; Hawkins et al., 1943). Explosions result in an abrupt severe hearing loss from which there is little recovery of function post-exposure (Ward and Glorig, 1961). On the other hand, noises, such as those associated with the workplace and recreational activities, probably damage the cochlea by non-mechanical mechanisms. In these instances, structural damage begins as degeneration of scattered sensory cells and continues to increase with longer or repeated exposures (e.g., Bohne, 1976; Clark and Bohne, 1978). The associated hearing loss gradually develops over time (Taylor et al., 1965).

The term 'temporary threshold shift' (TTS) has been used to indicate a transient impairment of auditory function due to trauma such as noise exposure. With increasing post-exposure time, a TTS completely disappears and hearing thresholds return to pre-exposure levels. The term 'permanent threshold shift' (PTS) has been used in instances when, post-exposure, hearing thresholds have stabilized, but are poorer than pre-exposure values. The term 'compound threshold shift' (CTS) was coined by Miller et al. (1963) to describe threshold shifts that have both temporary and permanent components. A CTS is at a maximum immediately post-exposure, then recovers somewhat as its temporary component resolves. It has been suggested that when a CTS is present, the TTS component masks the PTS component (e.g., Miller et al., 1963) and that the partial recovery of thresholds with increasing post-exposure time parallels the disappearance of the TTS. This suggestion implies that the structural correlates of TTS and PTS are both present immediately post-exposure. However, it was recently shown that when an animal is exposed to a moderate-level, short-duration noise, it manifests a moderate TTS immediately post-exposure and the histopathological changes are almost exclusively confined to the supporting cells. If the animal subsequently develops a PTS, the structural correlates of the PTS are not present immediately after exposure, but develop in the post-exposure period (Nordmann et al., 2000). These latter data suggest that the biochemical and cellular processes underlying TTS are distinct from those which eventually result in PTS. For this reason, the term 'TTS' is used here to refer to thresholds measured 0–7 days post-exposure, regardless of whether or not the animal's thresholds eventually recovered (i.e., pure TTS) or stabilized at a reduced level (i.e., pure PTS).

It is well known that a human or animal exposed to noise of predominantly one frequency does not develop its maximum hearing loss at that frequency. Instead, the maximum threshold shift may occur one-half to two octaves above the frequency of the exposure. Humans sustain one-half to one-octave shifts after exposures to noise resulting in TTSs (Davis et al., 1950; Mills et al., 1970; Melnick, 1976). Mice have been found to sustain their maximum threshold shifts up to two octaves higher than the frequency of the exposure (Henry, 1984). The actual basis for this shift is not known, especially why the shifts range from one-half to more than two octaves, depending on species. However, it has been shown that increasing the intensity of a stimulus causes a shift in the maximum effect of the exposure toward higher frequencies (e.g., McFadden and Plattsmier, 1982). Regardless of the mechanism for the octave shift phenomenon, it is necessary to measure auditory thresholds over a range of frequencies at multiple intervals post-exposure, and to examine the organ of Corti in its entirety from apex to base to characterize fully the effects of noise on the function and structure of the cochlea.

In recent years, mice have become very desirable subjects for medical research because of the ready availability of genetically defined strains and the ability to generate knockout mice lacking specific genes. Knockout mice may be valuable for testing some of the hypothesized mechanisms of noise damage. However, in order to interpret data from knockouts and mice with other experimental perturbations, it is important to characterize the functional and structural changes in a typical mouse's auditory system to different noises and to develop an anatomically based frequency–place map for the mouse cochlea (see companion paper).

2. Materials and methods

2.1. Animals

The mice, purchased from The Jackson Laboratory (Bar Harbor, ME), were the first generation of a cross between homozygous C57BL mice that carry the *ahl* gene for age-related hearing loss and are susceptible to noise-induced hearing loss, and homozygous CBA mice that do not carry the *ahl* gene and are relatively resistant to age-related and noise-induced hearing loss (Erway et al., 1993). The mice were 1.75–3 months old when pre-exposure auditory brainstem response (ABR) thresholds were first determined and were 3–4 months

old at the time of death. The animals' pinnae were tagged to identify the mice individually.

The protocol for the care and utilization of the animals in this study was reviewed and approved by the Animal Studies Committee at Washington University (#96315; Development of a frequency–place map for the mouse organ of Corti, G.W. Harding, PI).

2.2. ABR technique

The mice were anesthetized with an intraperitoneal injection of a mixture of ketamine (80 mg/kg body weight) and xylazine (16 mg/kg body weight). Ophthalmic ointment (bacitracin–neomycin–polymyxin) was applied to the eyes after anesthesia. Stainless steel needle electrodes were placed subcutaneously at the vertex of the head and inferior to the left bulla; a ground electrode was placed in the left flank. Test sounds were presented binaurally with the tip of the auditory signal delivery tube approximately 1 mm from the entrance to the external auditory canal. Thus, the recorded ABRs reflect the input from both cochleas.

The ABR threshold testing facility and technique are described in detail in Nordmann et al. (2000). Briefly, the equipment consisted of a personal computer, a National Instruments ATMIO16X, 16 bit, 100 kHz, analog I/O board; Tucker-Davis XB-1, PA-4 and HB-6 computer-controlled audiometric modules; a Crown D-150 power amplifier; two Radio Shack 40-1377, high-frequency sound transducers; a BK 2203 sound level meter with a 1/4-inch microphone (flat frequency response to 100 kHz); a Grass P-511, AC-coupled electrophysiological amplifier; a Tektronix 475A oscilloscope; and an Industrial Acoustics AC-3 sound-shielded booth (>60 dB attenuation).

Auditory signals (e.g., 0.1-ms clicks and 10.0-ms tone pips, ramped on and off over 1 ms) were synthesized, D-A converted, passed to the attenuator and both channels of the headphone buffer. This output was then passed to the power amplifier which drove the sound transducers. The output of the sound level meter (at range setting 70 ± 10 dB) was connected directly to channel 1 of the A-D converter. The computer used internal equations to calculate the calibrated signal level in dB.

The ABR threshold testing virtual instrument (National Instruments; LabWindows, CVI) produced auditory test signals with alternating signal polarity and synchronously summed the resulting electrophysiological responses. The instrument delivered the clicks and tone pips at 3, 4, 6, 8, 10, 12, 16, 20, 25, 30, 40 and 50 kHz. Stimuli were presented at 20 per second; 400 responses were averaged at each test intensity. An automated threshold tracking algorithm allowed determination of the threshold with decreasingly small decibel steps (minimum of 2 dB). With tone pips, the testing began at 3 kHz and continued sequentially until the threshold at 50 kHz had been determined. This frequency range was tested because our equipment did not generate reproducible signals below 3 kHz or above 50 kHz.

After completion of the ABR threshold testing, the mice recovered in a holding cage under a warming light. Mice that had not recovered from anesthesia by 30 min post-ABR testing were given an intraperitoneal injection of Atipamezole (0.2 mg/ml) at 0.0025 ml/g body weight. Atipamezole is a specific antagonist of central and peripheral $\alpha 2$-adrenoceptors. This drug reverses the effects of xylazine.

2.3. ABR interpretation

ABR response waveforms were plotted on paper and analyzed at each test frequency. A response was considered positive when there was a recognizable pattern with an amplitude of at least 2.5 μV over 1.3 ms. Click response waveforms measured at the beginning of each mouse's testing session were compared to those at the end of the same testing session. This procedure was done to confirm the stability of the ABR pattern during tone pip testing and to ensure that the animal's level of responsiveness did not change (± 5 dB) during the 30-min testing period.

2.4. ABR thresholds in control animals

Eight mice which ranged in age from 1.75 to 4 months of age were used as controls. Two controls had ABR thresholds determined four or five times over 1 month to assess the repeatability of the ABR thresholds and waveforms. Three controls had ABR thresholds measured once. In order to determine if repeated auditory testing results in hair cell loss, three controls were not ABR-tested.

2.5. ABR thresholds in noise-exposed animals

In the noise-exposed mice, ABR thresholds were determined immediately post-exposure (0.5–1.5 h), and at 2, 4, 6, 14, 21 and 27 days of recovery. Threshold shifts were calculated by subtracting each animal's shifted threshold from its pre-exposure threshold. Threshold shifts were deemed permanent if they stabilized at greater than 10 dB after 14 days of recovery.

2.6. Noise exposure

The noise facility consisted of a wire mesh cage that was suspended from the ceiling of an IAC booth that had been made reverberant by the addition of a 1.9 cm

thick Masonite lining. The mice were exposed to an octave-wide band of noise (OBN) with a center frequency of either 2, 4, or 8 kHz. The sound pressure level (SPL) of a particular exposure ranged from 100 to 120 dB (±0.5 dB) and the duration ranged from 1 to 24 h. Two to four mice were exposed simultaneously in the awake state in individual compartments of the exposure cage. In a few instances, the mice were found occupying the same compartment at the end of the exposure. Thus, they may have partly shielded one another's ears and slightly attenuated their exposure.

2.7. Cochlear preparation

At the time of death, each mouse was anesthetized with an intraperitoneal injection of the ketamine/xylazine cocktail. For local anesthesia, 0.1 ml of 0.25% Marcaine was injected into the chest wall. The animal's chest was opened to expose the heart. A 30-gauge needle, covered with an 8-mm piece of PE-20 tubing, was inserted into the left ventricle. Blood was washed from the vascular system by perfusing 5 ml of lactated Ringer's solution through the heart at a rate of 1–2 ml/min. This flush out was followed by perfusion of 5 ml of fixative (i.e., 1% OsO_4 in Dalton's buffer with 1.65% $CaCl_2$, pH 7.3–7.5). After completion of the perfusion, the mouse was decapitated, its temporal bones separated and the middle ears opened. The stapes was removed from the oval window, and small holes were made in the cochlear apex and in the middle of the superior semicircular canal with a fine pick. Using these holes alternately, cold fixative was gently infused into the perilymphatic spaces for 2–3 min with a fine-tip glass pipette. The left and right cochleas were immersed in 3 ml of cold fixative for 2 h, washed three times (15 min each) in Hanks' balanced salt solution, placed in 70% ethanol and left overnight at 4°C.

The specimens were dehydrated in a graded series of ethanol and propylene oxide for 3 h, infiltrated with Durcupan (epoxy resin) for 5.5 h, then embedded in a 5 mm thick layer of fresh plastic which was polymerized at 60°C for 48 h. After polymerization, the cochlear duct was dissected into quarter-turn segments each of which was trimmed close and parallel to the basilar membrane and re-embedded in thin layers of plastic as described by Bohne and Harding (1997).

2.8. Cochlear evaluation

The trimmed segments were initially examined at 125× or 250× by phase contrast microscopy (Wild M-20) to assess the general pattern of damage and to determine the length of the organ of Corti (OC). Length was measured along the union of the inner and outer pillar heads. Each OC segment was then examined at a high power (625× or 1250×) to determine: (a) the number of present inner (IHC) and outer hair cells (OHC); (b) the number of missing IHCs and OHCs (i.e., those replaced by phalangeal scars); (c) the number of degenerated inner and outer pillar cells; and (d) abnormalities of the hair cell stereocilia (see below and Fig. 1). The approximate amount of degenerated myelinated nerve fibers (MNFs) was determined by comparing the staining intensity of the fibers in a non-dam-

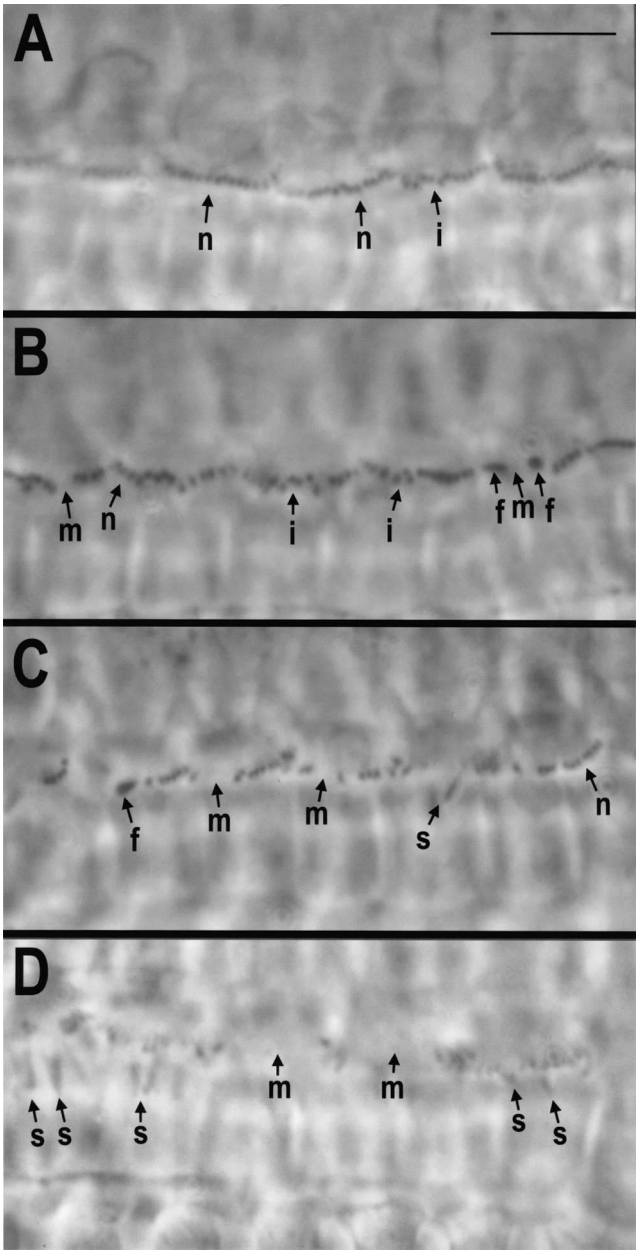

Fig. 1. High-power phase contrast photomicrographs of IHC stereocilia (f, fused; i, irregular; m, missing; n, normal; s, splayed) in control (A) and noise-exposed mice 4 weeks post-exposure (B–D): (A) grade 0 (HO30R, second turn); (B) grade 1 (HO33R, second turn); (C) grade 2 (HO24L, second turn); (D) grade 3 (HO31R, second turn). Bar equals 10 μm.

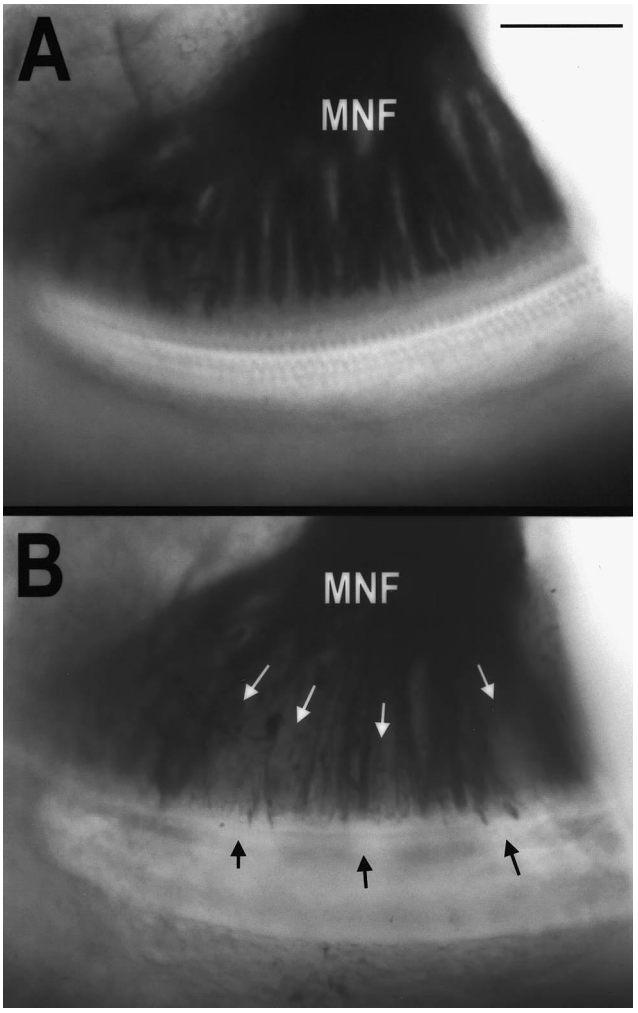

Fig. 2. Low-power bright-field photomicrographs of osseous spiral lamina, organ of Corti and basilar membrane at basal tip from: (A) non-noise-exposed control (HO43L) showing typical density of myelinated nerve fibers (MNF); (B) noise-exposed mouse (HO36R) showing patchy loss of MNFs (white arrows) and OC (black arrows). Bar equals 100 μm.

aged area of the osseous spiral lamina with that of the region in question (Fig. 2). This method provides an estimate of the magnitude of MNF degeneration, but cannot detect a low-level, widespread loss of fibers (Bohne et al., 1985).

One cochlea each from 39 noise-exposed mice was analyzed quantitatively by phase contrast microscopy. This strategy was used because it has been shown that there is a high correlation in the damage between the left and right cochleas of chinchillas exposed to noise binaurally (Bohne et al., 1986). Thus, using the cell loss data from both cochleas of these mice would have inflated the sample size without changing the between-animal variance. In eight mice, however, both cochleas were analyzed quantitatively to determine whether or not noise-exposed mice sustain bilaterally symmetrical damage.

2.9. Cytocochleograms

The percentages of missing IHCs and OHCs were determined by dividing the number of missing hair cells by the total number of hair cells (i.e., present plus absent) in that OC segment. For the hair cells that remained in a particular region, stereocilia abnormalities were classified as follows: grade 0, normal to $<20\%$ splayed or fused; grade 1, $>20\%$ to $<50\%$ splayed or fused; grade 2, $>50\%$ splayed or fused with $<50\%$ missing or severely splayed; and grade 3, $>50\%$ missing or severely splayed (Fig. 1). This classification scheme is based on the hypothesis that splayed and/or fused stereocilia have a reduced number of tip links and therefore, hair cells with these abnormalities will have elevated thresholds.

Stereocilia abnormalities were easier to grade for the IHCs than the OHCs. The tallest IHC stereocilia could be seen individually at $1250\times$ magnification in flat preparations (Fig. 1A). Because OHC stereocilia are smaller in diameter than IHC stereocilia, they could not be resolved individually in flat preparations. In non-damaged regions of the cochlea, the OHC stereocilia formed a smooth 'W' pattern. Damage to the OHC stereocilia appeared as a disruption of the 'W' pattern, usually in the form of irregularities in the limbs of the 'W'.

For each cochlea, the millimeter length of all dissected segments of the cochlear duct was converted to a percentage of the total cochlear length. The percentages of missing IHCs and OHCs, stereocilia grade and amount of MNF loss were plotted on the y-axis as a function of percentage distance from the cochlear apex on the x-axis.

3. Results

3.1. Cochlear morphometrics

Because of the paucity of data on OC parameters in the mouse cochlea, the total number of hair cells had to be counted in order to be able to calculate the percentage of missing IHCs and OHCs. Cell totals were counted in all OC segments in which hair cell losses were minimal or moderate. OC length ranged from 5.66 to 6.16 mm and averaged 5.90 ± 0.12 mm ($n = 47$). IHC and OHC densities per millimeter averaged, respectively, 120 and 418 in the second turn (i.e., 0–42.6% distance from apex), 128 and 425 in the first turn (i.e., 42.7–76% distance) and 107 and 371 at the round window hook (i.e., 76.1–100% distance).

To determine the percentages of missing hair cells in severely damaged segments, the total number of hair cells which should have been present was estimated by

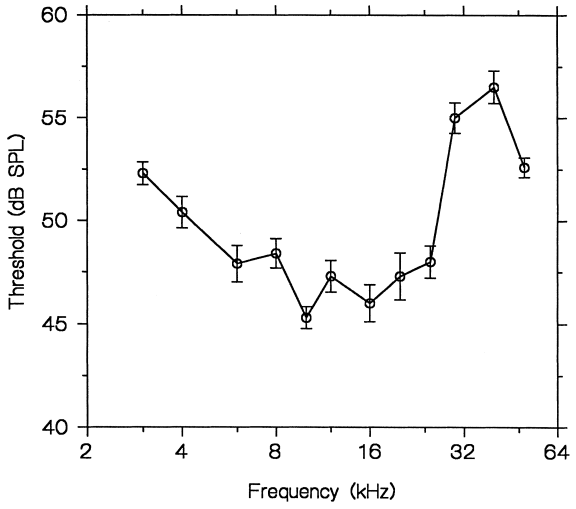

Fig. 3. Mean control thresholds and S.E.M. as a function of frequency for 44 mice (five controls and 39 subsequently noise-exposed).

multiplying segment length by hair cell densities in an adjacent segment. The percentage missing was then estimated by dividing the number missing by the calculated total.

3.2. ABR thresholds

Mean thresholds prior to experimental manipulation for the 44 functionally tested mice (five controls plus 39 noise-exposed) are shown in Fig. 3. Thresholds ranged from 52 dB SPL at 3 kHz, to 45 dB at 10 kHz, 58 dB at 40 kHz and 53 dB at 50 kHz. Standard error of the means ranged from 0.5 to 1.1 dB.

3.3. Controls

IHC and OHC losses in the eight controls were variable, averaging $0.5 \pm 1.3\%$ and $2.4 \pm 4.5\%$, respectively, in the entire cochlea (i.e., 0–100% distance from apex). One of the eight controls (3.5 months old; ABR-tested four times) had 19.8% IHC loss and 73.2% OHC loss in the basal 20% of the OC. At the same location, there was slight loss of MNFs and scattered missing pillar cells. Inspection of the cytocochleograms from the other seven controls revealed that the basal tip damage did not correlate with the number of times the mouse had been ABR-tested, or with its age.

3.4. Noise-exposed mice

Immediately after their noise exposure, all mice had TTSs of varying magnitude at all tested frequencies. Two mice that were exposed to the 4-kHz OBN at 110 dB SPL for 8 h were prepared for histology within 2 h post-exposure. Thirty-seven mice recovered for 2–4 weeks before their cochleas were prepared for histology. For most of these latter mice, maximal recovery of thresholds occurred by 4 days post-exposure. One mouse fully recovered from its TTS, 10 sustained PTSs at all tested frequencies and 26 developed 'notched' audiometric patterns (i.e., PTS at some frequencies and complete recovery at other frequencies).

A range in the magnitude of hair cell damage/loss was found in the cochleas of mice that received identical noise exposures. However, for the eight mice in which both cochleas were evaluated, the patterns and magnitude of hair cell damage and loss were very similar in the two ears. Student's *t*-tests showed no significant difference between hair cell losses in the apical halves, basal halves, or basal 20% of the organ of Corti ($P > 0.3$, 0.9 and 0.2, respectively). On this basis, the contribution of both cochleas of a particular mouse to its ABR thresholds was expected to be symmetrical.

Abnormalities of the IHC stereocilia were more frequent and severe than for the OHCs. Across the three rows of OHCs, stereocilia on the first row tended to be more severely affected than those on the second and third rows. A region was graded as having damaged OHC stereocilia if the stereocilia were abnormal on at least one OHC row.

3.5. 2-kHz OBN group

These mice sustained maximum TTSs of 38–56 dB at 3 or 4 kHz and/or 30–50 kHz. Table 1 summarizes the exposure parameters and resulting PTSs for the eight animals in this group. Two mice (HO31 and 33) that were exposed at 110 dB SPL for 8 h and then at 115 dB SPL for 12 h fully recovered from their threshold shifts. However, these mice plus six others sustained a PTS when they were exposed at 120 dB SPL for 24 h. The

Table 1
Exposure parameters for 2-kHz OBN and resulting ABR threshold shift(s)

Animal #	Exposure parameters	Frequency(ies) of PTS > 10 dB
HO31[a]	110 dB SPL, 8 h	No PTS
HO33[a]	110 dB SPL, 8 h	No PTS
HO31[a]	115 dB SPL, 12 h	No PTS
HO33[a]	115 dB SPL, 12 h	No PTS
HO31[a]	120 dB SPL, 24 h	3–4, 50
HO33[a]	120 dB SPL, 24 h	3–6, 50
HO34	120 dB SPL, 24 h	4–8, 12
HO45	120 dB SPL, 24 h	3–8, 16, 40–50
HO46	120 dB SPL, 24 h	6–10
HO47	120 dB SPL, 24 h	3–6, 16, 40–50
HO48	120 dB SPL, 24 h	3–6
HO49	120 dB SPL, 24 h	3–12

[a]Mice received three exposures because the first two exposures did not result in a PTS.

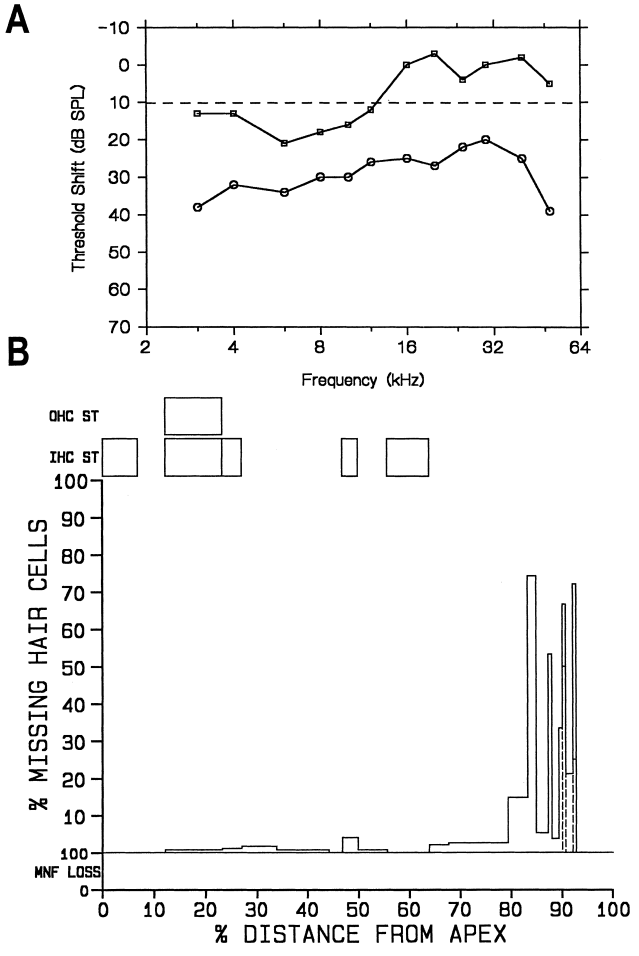

Fig. 4. ABR threshold shifts (dB SPL) and cytocochleogram for HO49 (2-kHz OBN, 120 dB SPL, 24 h). (A) At 1–2 h post-exposure, this animal had a 20–40-dB threshold shift (circles) across all tested frequencies. At 4 weeks post-exposure, the mouse had a 12–20-dB permanent threshold shift (squares) for 3–12 kHz. (B) Histopathological data plotted as the percentage distance from cochlear apex (*x*-axis). The cytocochleogram is divided into three sections: *y*-axis: Top: Stereocilia grade for outer (OHC ST) and inner (IHC ST) hair cells (no box, grade 0; open box, grade 1); Middle: Percentage of missing IHCs (dashed line) and OHCs (solid line); Bottom: Percentage loss of myelinated nerve fibers (MNF LOSS) in the osseous spiral lamina. IHC and OHC losses were minimal in the apical half and averaged 2.5% and 20%, respectively, in the basal 20% of the cochlea. Grade 1 stereocilia damage was present in the apex and middle of the cochlea for IHCs and near the apex for OHCs. There was no loss of nerve fibers in this cochlea.

resulting PTS curves could be divided into two main patterns. The first pattern revealed a 'double notch' with threshold shifts of 11–20 dB at 3–6 kHz, and at 40–50 kHz (see Fig. 1A of our companion paper). Between the two notches, thresholds usually fully recovered. Double-notched patterns were seen in four of eight mice in this group. The second pattern displayed a single low-frequency notch extending from 3–6 to 10–12 kHz (Fig. 4A). There was full recovery from 16 to 50 kHz. The region of full recovery contrasts with the first pattern in which there was a second PTS at the highest test frequencies. Single, low-frequency notched patterns occurred in four of eight mice in this group.

In contrast to the threshold shift curves, the cytocochleograms for this group did not fall into two patterns. Although there was some variation in the extent of the damage, most cochleas had stereocilia damage (primarily grade 1) without a corresponding loss of hair cells in the second turn, and OHC loss in the first turn and round window hook with no associated IHC stereocilia damage (e.g., Fig. 4B). In the basal 20% of the OC, pillar losses were scattered and numbered less than 50. Only one of the eight animals in this group (HO45) had a slight loss of MNFs at the basal tip that coincided with a 9% loss of IHCs (lesions at the basal tip are summarized below; see Table 4).

3.6. 4-kHz OBN group

Six of these mice (HO23, 24, 25, 26, 36 and 37) sustained broad TTSs, which exceeded the output of the equipment (i.e., 110 dB) at 3–6 kHz and/or 30–50 kHz. Table 2 summarizes the exposure parameters and the resulting PTSs for the 11 animals in this group. Two mice (HO19 and 20), exposed at 100 dB SPL for 2 h, and one mouse (HO22), exposed at 105 dB for 4 h, sustained an 11-dB PTS for 30 and/or 40 kHz. Another mouse (HO21), exposed at 105 dB SPL for 4 h, fully recovered from its TTS (Fig. 5A). Seven animals that were exposed at 110 dB SPL for 4 or 8 h developed a double-notched audiometric pattern. In contrast to the double-notched pattern seen in the 2-kHz group, the audiometric pattern in this group tended to have a very limited region of full recovery. Full recovery from the TTS occurred at 10 and/or 12 kHz only. PTSs were greater in magnitude than those in the 2-kHz group, generally 20–40 dB SPL for 3–6 kHz and 40–50 kHz.

Table 2
Exposure parameters for 4-kHz OBN and resulting ABR threshold shift(s)

Animal #	Exposure parameters	Frequency(ies) of PTS > 10 dB
HO19	100 dB SPL, 2 h	30–40
HO20	100 dB SPL, 2 h	30
HO21	105 dB SPL, 4 h	No PTS
HO22	105 dB SPL, 4 h	40
HO25	110 dB SPL, 4 h	3–8, 16–50
HO26	110 dB SPL, 4 h	3–6, 20, 30–50
HO23	110 dB SPL, 8 h	3–8, 12–50
HO24	110 dB SPL, 8 h	3–8, 16–50
HO35	110 dB SPL, 8 h	3–10, 16–50
HO36	110 dB SPL, 8 h	3–6, 40–50
HO37	110 dB SPL, 8 h	3–8, 30–50

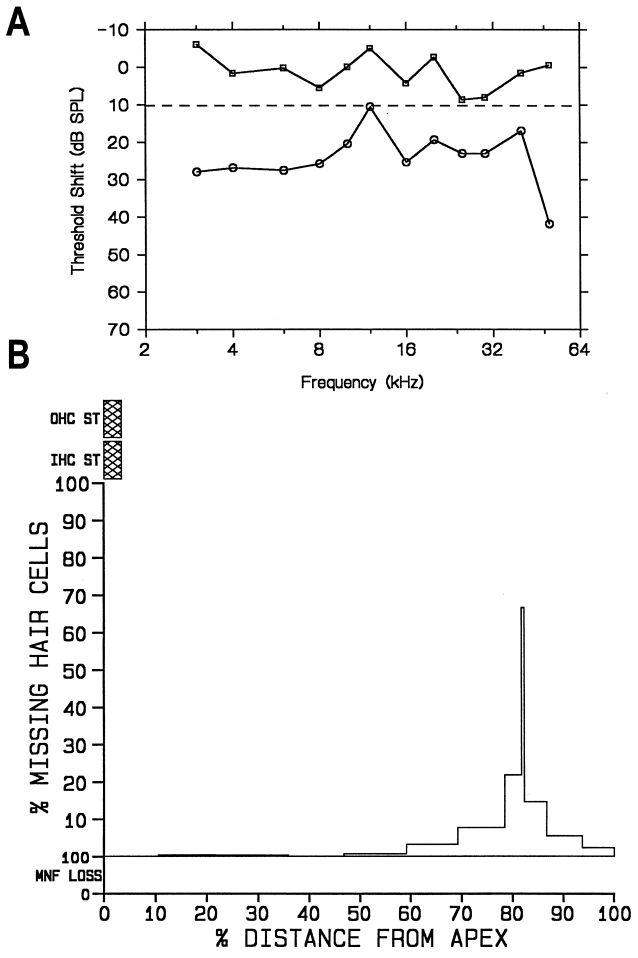

Fig. 5. ABR threshold shifts and cytocochleogram for HO21 (4-kHz OBN at 105 dB SPL for 4 h). (A) TTS (circles) determined 1–2 h post-exposure shows a broad hearing loss ranging from 10 to 40 dB across all tested frequencies. Thresholds at all frequencies returned to pre-exposure values by 4 days post-exposure. Squares indicate threshold shift at 4 weeks. (B) There was a slight loss of outer hair cells (10%) in the basal 20% of the cochlea; all nerve fibers were intact and, except at the extreme apex, the hair cell stereocilia were not damaged. See Fig. 4 legend for description of *x*- and *y*-axes.

The mouse without a PTS and the three mice with an 11-dB PTS at 30 and/or 40 kHz had minimal loss of hair cells and few sensory cells with grade 1 and/or 2 stereocilia damage at the apical tip (e.g., Fig. 5B). The animals with the double-notched PTSs had similar cytocochleograms with short stretches of stereocilia damage (grades 1 and 2) in the second and first turns and focal losses of IHCs and OHCs at the round window hook and the lower first turn (see Fig. 2B of our companion paper). The hair cell lesions had accompanying nerve fiber loss when IHC loss equaled or exceeded 15% (lesions at the basal tip are summarized below; see Table 4). In general, the stereocilia and hair cell lesions in these cochleas were more basally located than those in the 2-kHz group.

3.7. 8-kHz OBN group

Seventeen of 18 mice sustained broad TTSs, which exceeded the output of the equipment at 3–10 kHz and/or 16–50 kHz. Table 3 summarizes the exposure parameters and the resulting PTSs for the mice in this group. Nine mice exposed at 105–115 dB SPL for 2–12 h and one mouse (HO41) exposed at 100 dB for 2 h developed a 20–50-dB PTS, encompassing all tested frequencies (e.g., Fig. 6A).

Eight of nine mice that were exposed at 100 dB SPL for 1 or 2 h recovered somewhat from their TTSs. These mice did not have a consistent pattern of hearing loss. Four mice (HO50, 51, 52 and 53) had triple-notched audiometric patterns with PTSs ranging from 11 to 30 dB SPL. Two mice (HO40 and 54) had double-notched audiometric patterns with PTSs ranging from 11 to 40 dB SPL. Two mice (HO15 and HO16) had 15–25-dB PTSs at 25–30 kHz. In contrast to the audiometric patterns seen in the 2- and 4-kHz groups, it appears that the audiometric patterns in this group are distinct, rather than a simple variation on a single pattern.

The cytocochleograms in this group were also variable. Those animals that did not recover from their TTSs had widespread IHC stereocilia damage (grades 1, 2 and/or 3) in the second and first turns. There was little IHC and OHC loss in the second turn but a variable amount of OHC loss in the first turn, which was contiguous with the sensory cell loss at the round window hook. In the basal 20% of the OC, there was moderate to severe IHC and OHC loss and degeneration of adjacent MNFs (e.g., Fig. 6B). In all but two mice (i.e., HO15 and 16), there was moderate to severe loss of adjacent MNFs when IHC loss equaled or exceeded

Table 3
Exposure parameters for 8-kHz OBN and resulting ABR threshold shift(s)

Animal #	Exposure parameters	Frequency(ies) of PTS > 10 dB
HO15	100 dB SPL, 1 h	25
HO16	100 dB SPL, 1 h	25–30
HO52	100 dB SPL, 2 h	4–6, 12–16, 25, 50
HO51	100 dB SPL, 2 h	3–6, 12–25, 40–50
HO50	100 dB SPL, 2 h	6, 12–16, 30–50
HO53	100 dB SPL, 2 h	12, 20, 50
HO40	100 dB SPL, 2 h	3–10, 16–50
HO54	100 dB SPL, 2 h	3–4, 10–50
HO41	100 dB SPL, 2 h	All tested frequencies
HO13	105 dB SPL, 2 h	All tested frequencies
HO14	105 dB SPL, 2 h	All tested frequencies
HO11	110 dB SPL, 4 h	All tested frequencies
HO12	110 dB SPL, 4 h	All tested frequencies
HO7	110 dB SPL, 8 h	All tested frequencies
HO8	110 dB SPL, 8 h	All tested frequencies
HO9	110 dB SPL, 8 h	All tested frequencies
HO5	115 dB SPL, 12 h	All tested frequencies
HO6	115 dB SPL, 12 h	All tested frequencies

Table 4
Average PTS for high frequencies[a] and damage at basal tip in noise-exposed mice

Animal #	Exposure				Average PTS (dB SPL)	% Missing hair cells[b]		Pillar losses[b,c]	Nerve fiber degeneration[b]
	Freq. (kHz)	SPL (dB)	Dur. (h)	Rec. (wk)		IHC	OHC		
HO33	2	120	24	4	None	0	16	Few	None
HO49	2	120	24	4	None	3	20	Few	None
HO46	2	120	24	4	None	0	25	Some	None
HO31	2	120	24	4	None	1	32	Few	None
HO48	2	120	24	4	None	6	37	Few	None
HO34	2	120	24	4	11	2	30	Some	None
HO45	2	120	24	4	13	9	32	Some	Slight
HO47	2	120	24	4	17	4	28	Few	None
HO19	4	100	2	4	None	0	1	Few	None
HO20	4	100	2	4	None	0	1	None	None
HO21	4	105	4	4	None	0	10	Few	None
HO22	4	105	4	4	None	0	1	Few	None
HO25	4	110	4	4	38	42	85	Many	Moderate
HO26	4	110	4	4	38	46	81	Many	Severe
HO37	4	110	8	2	None	3	38	Few	None
HO36	4	110	8	3	20	15	85	Some	Moderate
HO35	4	110	8	4	38	56	74	Many	Severe
HO24	4	110	8	4	45	55	81	Many	Moderate
HO23	4	110	8	4	47	22	82	Many	Moderate
HO15	8	100	1	4	None	57	82	Some	None
HO16	8	100	1	4	None	40	63	Some	None
HO52	8	100	2	4	None	4	20	Few	None
HO53	8	100	2	4	21	6	28	Few	None
HO50	8	100	2	4	20	4	37	Few	None
HO51	8	100	2	3	21	1	27	Few	None
HO54	8	100	2	4	30	6	51	Some	None
HO40	8	100	2	4	40	69	81	Many	Severe
HO41	8	100	2	3	47	50	80	Many	Severe
HO14	8	105	2	4	42	44	94	Many	Moderate
HO13	8	105	2	4	55	49	99	Many	Severe
HO12	8	110	4	4	52	42	95	Many	Moderate
HO11	8	110	4	4	54	35	97	Many	Moderate
HO7	8	110	8	4	56	39	100	Many	Moderate
HO8	8	110	8	4	57	56	92	Many	Moderate
HO9	8	110	8	4	57	58	97	Many	Severe
HO5	8	115	12	4	37	30	87	Many	Moderate
HO6	8	115	12	4	53	34	91	Many	Moderate

[a]For 30, 40 and 50 kHz.
[b]At 80–100% distance from cochlear apex.
[c]Few: $\leq$10; some: $>$10 and $\leq$50; many: $>$50.

30% at the basal tip (lesions at the basal tip are summarized below; see Table 4). Most of the mice with double and triple audiometric notches had scattered regions of stereocilia damage (usually grades 1 and 2) in the second and first turns and one or more focal hair cell lesions in the basal 20% of the OC (see Fig. 3 of our companion paper).

3.8. Histological appearance of the noise-exposed mouse cochlea fixed acutely

The cochleas of two mice were fixed 2 h post-exposure, when a 25–50-dB TTS was present across all tested frequencies. OHC loss averaged 30.4% in the region 80–100% distance from the apex. At the same location, most IHCs, and all pillars and MNFs were intact. Hair cell stereocilia, except those in the apical 5% of the OC, had a normal arrangement and were scored grade 0. The exposure used for these animals was also used for five 1-month-recovery mice (HO23, 24, 35, 36 and 37) in the 4-kHz OBN group. These latter mice all developed double-notched PTSs and four of them had stereocilia damage in the second turn (see above).

3.9. Basal tip lesions

Table 4 summarizes data from the basal 20% of the

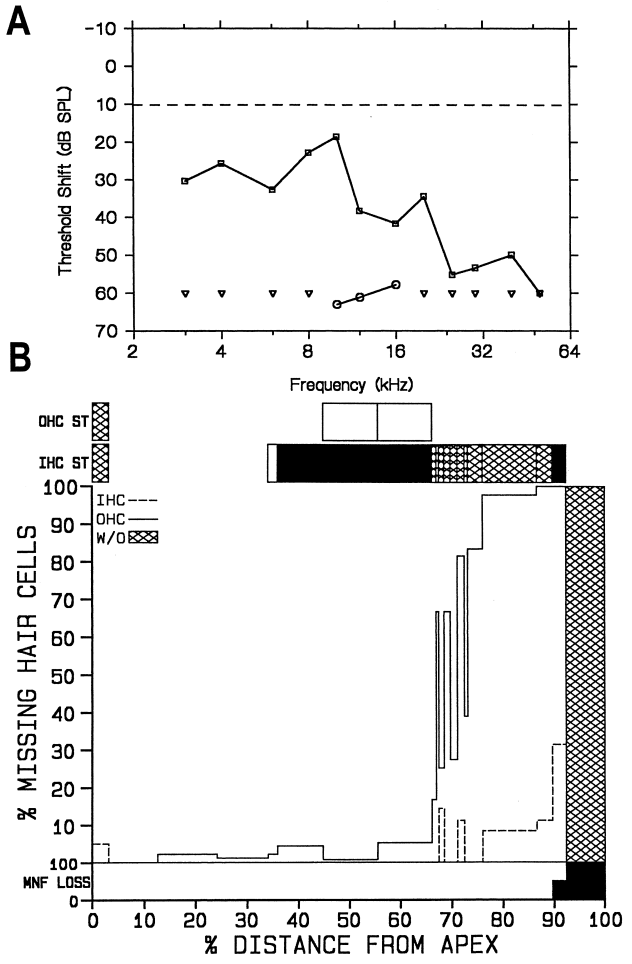

Fig. 6. ABR threshold shifts and cytocochleogram for HO13 (8-kHz OBN at 105 dB SPL for 2 h). (A) At 1–2 h post-exposure, the TTS (circles) was 60 dB for 10–16 kHz. No response (inverted triangles) was elicited by the other tested frequencies (i.e., 3–8 and 20–50 kHz). Four weeks post-exposure, threshold shifts (squares) had improved to 20–40 dB for 3–20 kHz and to 50–60 dB for 25–50 kHz. (B) IHC and OHC losses averaged 49% and 99%, respectively, in the basal 20% of the cochlea. There was a region of total loss of the OC (tall, hatched bar) from 92 to 100% distance from the apex. Many nerve fibers in the basal 10% of the cochlea had degenerated (solid bar, degeneration to modiolus). Grade 2 stereocilia damage (hatched box) was found on both IHCs and OHCs at the apical tip. Stereocilia damage on the IHCs was extensive, extending from 33 to 92% distance from the apex, while that on the OHCs was found from 46 to 68% distance (open box, grade 1; hatched box, grade 2; solid box, grade 3). See Fig. 4 legend for description of *x*- and *y*-axes.

OC in the 37 mice that recovered 2–4 weeks following their exposure to noise. The average threshold shift at 30, 40, and 50 kHz is shown along with the percentage of missing IHCs and OHCs, the extent of pillar losses and the amount of MNF loss. A lack of threshold shift or a shift less than 20 dB tended to be associated with missing OHCs only, without an associated loss of IHCs or MNFs (e.g., HO31, 37 and 52). Larger threshold shifts (>20 dB) were associated with moderate to severe losses of both OHCs and IHCs, as well as moderate to severe MNF degeneration (e.g., HO26 and 40).

4. Discussion

4.1. ABR thresholds of C57BL/CBA F1 mice

The 44 ABR-tested mice in the present study were maximally sensitive to the 10-kHz test tone, having an average pre-exposure threshold of 45 dB SPL. Thresholds increased by 7–13 dB at lower and higher test frequencies. The shape of the curve is similar to that shown by Mikaelian et al. (1974) for behaviorally tested C57 and CBA mice, but the level is about 15 dB higher at all frequencies. This is probably due to the higher sensitivity of behavioral testing. These thresholds are also somewhat greater than those reported by Li (1992) for 1–4-month-old homozygous CBA/Ca and C57BL/6J mice in which a visual detection level was used to identify a positive response. Erway et al. (1996) required two identifiable ABR waves for a positive response and found similar thresholds to those reported in the present study. In our study, a response was called positive only if its magnitude was at least 2.5 μV over 1.3 ms. These variations in criteria for a positive response probably account for much of the difference in absolute thresholds across studies.

4.2. Variations in threshold shifts with the parameters of the noise exposure

Most noise-exposed mice sustained a broad TTS, regardless of the center frequency of the exposure. The TTSs tended to be greater at 3–4 kHz and/or 30–50 kHz than at the middle frequencies. TTSs tended to be greater with the 4- and 8-kHz OBN than the 2-kHz OBN, despite the fact that the 2-kHz noise was presented at the highest SPL (i.e., 120 dB) and for the longest duration (i.e., 24 h). Exposure to the 4- and 8-kHz OBN also resulted in more PTS than the 2-kHz OBN. Thus, C57BL/CBA F1 mice appear to be more sensitive to a 8-kHz OBN than a 2- or 4-kHz OBN.

4.3. Importance of the preparation technique in assessing noise damage in the mouse cochlea

The use of the plastic-embedding technique (Bohne and Harding, 1997) was essential to minimize artifactual injury to the hair cell stereocilia, to preserve the entire cochlear duct for evaluation and to permit accurate determination of the apex-to-base location(s) of damage in the OC. These points were important for

relating a particular mouse's inner ear damage to its PTS and to identify the presence of a dual cochlear lesion.

4.4. Stereocilia damage

Most non-noise-exposed control cochleas had hair cells with grade 1 stereocilia damage extending from 0% to approximately 12% distance from the apex of the OC. This damage may represent a preparation artifact because the affected area was directly beneath the apical perfusion hole. Conversely, the apex of the OC is often found to contain unusually shaped hair cells. Thus, hair cells at the apex having slight stereocilia abnormalities may be a normal variation.

Except at the apical tip, the cochleas that were fixed 1–2 h post-exposure, when they had broad TTSs, had no hair cells with stereocilia damage. In contrast, hair cells with stereocilia damage were scattered throughout the second turn in nearly all 2–4-week-recovery cochleas that had a PTS for one or more frequencies from 3 to 20 kHz. It is hypothesized that the stereocilia damage found in the present study did not occur during the exposure but rather developed belatedly, perhaps because of injury to cellular repair mechanisms in the noise-exposed hair cells. In general, the greater the magnitude of the PTS, the more severe was the stereocilia damage, and the greater was its apex-to-base extent. Also, there was a tendency for more severe stereocilia damage (i.e., grades 2 and 3) to be located more basally when the animal had been exposed to the 8-kHz OBN, compared to the 2-kHz or 4-kHz OBN. IHCs immediately apical to focal losses of IHCs and OHCs at the basal tip often had severe stereocilia damage (e.g., Fig. 6B).

4.5. Patterns of PTS and cochlear damage in the noise-exposed C57BL/CBA mouse

Exposure of this mouse to an OBN with a center frequency of 2, 4 or 8 kHz often resulted in a double-notched PTS and two separate regions of damage in the OC. The more apical lesion, which correlated with PTS for 3–16 kHz, generally consisted of stereocilia damage only. The more basal damage, which correlated with PTS for 30–50 kHz, usually extended to the basal tip and consisted of one or more focal losses of IHCs and OHCs. In most cases, nerve fiber degeneration was evident at the basal tip when IHC loss exceeded 20%. These patterns of noise-induced cell damage and loss in the mouse are different from those described in other species [e.g., chinchilla (Bohne and Harding, 2000); guinea pig (Stockwell et al., 1969); rabbit (Engström and Borg, 1983); human (Bredberg, 1973)] after comparable exposures.

4.6. Intra-species variability

Considerably variability was found among the mice exposed to the same noise (e.g., HO40, 41, 50–54, 8-kHz group; Table 4). This variability was somewhat surprising, given the fact that the mice had genetic homogeneity. In a few cases, mice that were supposed to receive identical noise may have had a slightly attenuated exposure. However, this possibility seems insufficient to account for the wide variation in the magnitude of PTS (e.g., 0–47 dB for 30–50 kHz) and the extent of damage in the basal 20% of the OC (e.g., IHC, 1–69% loss; OHC, 20–81% loss). It was also found that there was sizable variation in magnitude of hair cell loss (e.g., IHC, 0–20% loss; OHC, 0–73% loss) in the basal 20% of the non-noise-exposed controls. The control mouse with extensive cell loss at the basal tip could have had premature aging of its peripheral auditory system, which is again a surprising finding. The bases for variations in the rate of aging and in susceptibility to noise damage in genetically homogeneous mice are currently unknown.

Acknowledgements

This work was supported by the National Organization for Hearing Research, the American Heart Association, and the Department of Otolaryngology at Washington University School of Medicine. We gratefully acknowledge the technical assistance provided by Mr. Thomas J. Watkins, and the helpful input concerning experimental design and data analysis provided by the members of Dr. Ou's thesis committee, Drs. Charles Anderson, J. Gail Neely, and Carl Rovainen. The suggestions for revision provided by the two anonymous reviewers are greatly appreciated.

References

Bohne, B.A., 1976. Safe level for noise exposure? Ann. Otol. Rhinol. Laryngol. 85, 711–724.

Bohne, B.A., Harding, G.W., 1997. Processing and analyzing the mouse temporal bone to identify gross, cellular and subcellular pathology. Hear. Res. 109, 34–45.

Bohne, B.A., Marks, J.E., Glasgow, G.P., 1985. Delayed effects of ionizing radiation on the ear. Laryngoscope 95, 818–828.

Bohne, B.A., Bozzay, D.G., Harding, G.W., 1986. Interaural correlations in normal and traumatized cochleas: Length and sensory cell loss. J. Acoust. Soc. Am. 80, 1729–1736.

Bohne, B.A., Harding, G.W., 2000. Degeneration in the cochlea after noise damage: Primary vs. secondary events. Am. J. Otolaryngol., in press.

Bredberg, G., 1968. Cellular pattern and nerve supply of the human organ of Corti. Acta Otolaryngol. (Stockh.) 236 (Suppl.), 1–135.

Bredberg, G., 1973. Experimental pathology of noise-induced hearing

loss. In: Hawkins, Jr., J.E., Lawrence, M., Work, W.P. (Eds.), Otophysiology, Advances in Oto-Rhino-Laryngology, Vol. 20. S. Karger, Basel, pp. 102–114.
Clark, W.W., Bohne, B.A., 1978. Animal model for the 4-kHz tonal dip. Ann. Otol. Rhinol. Laryngol. 51 (Suppl.), 1–16.
Davis, H., Morgan, C.T., Hawkins, J.E., Jr., Galambos, R., Smith, F.W., 1950. Temporary deafness following exposure to loud tones and noise. Acta Otolaryngol. 88 (Suppl.), 1–57.
Engström, B., Borg, E., 1983. Cochlear morphology in relation to loss of behavioural, electrophysiological and middle ear reflex thresholds after exposure to noise. Acta Otolaryngol. 402 (Suppl.), 5–23.
Erway, L.C., Willott, J.F., Archer, J.R., Harrison, D.E., 1993. Genetics of age-related hearing loss in mice: I. Inbred and F1 hybrid strains. Hear. Res. 65, 125–132.
Erway, L.C., Shiau, Y.-W., Davis, R.R., Krieg, E.F., 1996. Genetics of age-related hearing loss in mice. III. Susceptibility of inbred and F1 hybrid strains to noise-induced hearing loss. Hear. Res. 93, 181–187.
Hawkins, Jr., J.E., Lurie, M.H., Davis, H., 1943. Injury of the inner ear produced by exposure to loud tones. Committee on Medical Research of the Office of Scientific Research and Development, Contract OEMcmr-194.
Henry, K.R., 1984. Cochlear damage resulting from exposure to four different octave bands of noise at three ages. Behav. Neurosci. 98, 107–117.
Li, H.-S., 1992. Influence of genotype and age on acute acoustic trauma and recovery in CBA/Ca and C57BL/6J mice. Acta Otolaryngol. 112, 956–967.
Lurie, M.H., 1942. The degeneration and absorption of the organ of Corti in animals. Ann. Otol. Rhinol. Laryngol. 51, 712–717.
McFadden, D., Plattsmier, H.S., 1982. Exposure-induced loudness shifts and threshold shifts. In: Hamernik, R.P., Henderson, D., Salvi, R. (Eds.), New Perspectives on Noise-Induced Hearing Loss. Raven Press, New York, pp. 363–373.
Melnick, W., 1976. Human asymptotic threshold shift. In: Henderson, D., Hamernik, R.P., Dosanjh, D.S., Mills, J.H. (Eds.), Effects of Noise on Hearing. Raven Press, New York, pp. 277–289.
Mikaelian, D.O., Warfield, D., Norris, O., 1974. Genetic progressive hearing loss in the C57/bl6 mouse. Acta Otolaryngol. 77, 327–334.
Miller, J.D., Watson, C.S., Covell, W.P., 1963. Deafening effect of noise on the cat. Acta Otolaryngol. 176 (Suppl.), 1–91.
Mills, J.H., Gengel, R.W., Watson, C.S., Miller, J.D., 1970. Temporary changes of the auditory system due to exposure to noise for one or two days. J. Acoust. Soc. Am. 48, 524–530.
Nordmann, A.S., Bohne, B.A., Harding, G.W., 2000. Histopathological differences between temporary and permanent threshold shift. Hear. Res. 139, 13–30.
Stockwell, C.W., Ades, H.W., Engström, H., 1969. Patterns of hair cell damage after intense auditory stimulation. Ann. Otol. Rhinol. Laryngol. 78, 1144–1168.
Sulkowski, W., 1973. Some epidemiological data on noise-induced hearing loss in Poland, its prophylaxis and diagnosis. In: Proceedings of the International Congress on Noise as a Public Health Problem, US Environmental Protection Agency, 550-9-73-008, pp. 139–155.
Taylor, W., Pearson, J., Mair, A., Burns, W., 1965. Study of noise and hearing in the jute weaving. J. Acoust. Soc. Am. 38, 113–120.
Ward, W.D., Glorig, A., 1961. A case of firecracker-induced hearing loss. Laryngoscope 71, 1590–1596.