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Hearing Research 204 (2005) 90–100
HEARING RESEARCH
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The effect of an age-related hearing loss gene (*Ahl*) on noise-induced hearing loss and cochlear damage from low-frequency noise

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Received 26 October 2004; accepted 10 January 2005
Available online 13 February 2005

Abstract

Inbred C57BL/6J mice carry two copies of an age-related hearing loss gene (*Ahl*). It has been shown that these mice begin losing high-frequency hearing at two months. Several functional studies have reported that the *Ahl* gene renders mice more susceptible to noise-induced hearing loss (NIHL) than strains which do not carry this gene [e.g., Hear. Res. 93 (1996) 181; Hear. Res. 155 (2001) 82; J. Assoc. Res. Otolaryngol. 2 (2001) 233]. Johnson et al. [Hear. Res. 114 (1997) 83] developed a congenic B6.CAST-+^{Ahl} mouse which carries the wild-type allele from *Mus musculus castaneus* at the *Ahl* locus. Five each of young C57BL/6J males and females, and B6.CAST-+^{Ahl} males were exposed to a 4-kHz octave band of noise at 108 dB SPL for 4 h. Non-noise-exposed mice of the same strains and age served as controls. The noise-exposed mice were functionally tested for ABR thresholds and DPOAE levels pre-exposure and three times post-exposure: 0 days to determine the magnitude of temporary threshold shift (TTS); 6 days to determine rate of recovery; and 20 days to determine the magnitude of permanent threshold shift (PTS). At 20 days post-exposure, the animals underwent cardiac perfusion to fix their cochleae. The isolated cochleae were embedded in plastic and dissected into flat preparations. By phase-contrast microscopy, each cochlea was evaluated from apex to base to quantify the losses of hair cells, nerve fibers and stria vascularis and to localize stereocilia damage. Functional data from each mouse were aligned with the cytocochleogram using the frequency–place map of Ou et al. [Hear. Res. 145 (2000) 111; Hear. Res. 145 (2000) 123]. Sizable variation in the magnitude of TTS, PTS and hair-cell loss was found among mice of the same genetic strain. The congenic B6.CAST-+^{Ahl} male mice had significantly less TTS immediately post-exposure than C57BL/6J males or females but not less PTS or hair-cell losses at 20 days post-exposure. These results indicate that, at one month of age, mice carrying two copies of the *Ahl* gene have an increased susceptibility to TTS from a low-frequency noise before they have any indication of age-related hearing or hair-cell loss. However, this appeared not to be the case for PTS. The *Ahl* gene appears to play a role in susceptibility to NIHL but, other genes as well as systemic and local factors must also be involved.

Keywords: *Ahl* gene; TTS; PTS; ABR; DPOAE; Histopathology; C57BL/6J; B6.CAST

Abbreviations: ABR, auditory brainstem response; *Ahl*, age-related hearing loss gene; DPOAE, distortion product otoacoustic emission; IHC, inner hair cell; MNF, myelinated nerve fibers; NIHL, noise-induced hearing loss; PTS, permanent threshold Shift; OBN, octave band of noise; OC, organ of Corti; OHC, outer hair cell; SPL, sound pressure level; TTS, temporary threshold shift

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doi:10.1016/j.heares.2005.01.004

1. Introduction

Inbred C57BL/6J mice carry two copies of a naturally occurring age-related hearing loss gene (*Ahl*; Erway et al., 1993). At two months, these mice begin to develop a high-frequency hearing loss (>20 kHz, Li and Borg, 1993). Homozygous CBA mice carry the wild-type allele at the *Ahl* locus and maintain good hearing out to two years (Li, 1992). Erway et al. (1996) hypothesized that the *Ahl* gene is responsible for the increased susceptibility to noise-induced hearing loss (NIHL) of the C57BL mouse compared to the CBA mouse. Several studies have reported that the *Ahl* gene renders mice more susceptible to NIHL than strains which do not carry this gene (e.g., Erway et al., 1996; Davis et al., 2001; Jimenez et al., 2001). Two inbred strains that do not carry the *Ahl* gene [129/SvEv (Yoshida et al., 2000; Noben-Trauth et al., 2003) and MOLF/Ei (Candreia et al., 2004)] have recently been shown to be exceptionally resistant to NIHL. However, C57BL mice differ genetically from 129/SvEv and MOLF/Ei mice at many other sites besides the *Ahl* locus. Johnson et al. (1997) developed a B6.CAST-+^{Ahl} (B6.CAST) mouse that carries the wild-type allele (from *Mus musculus castaneus*) at the *Ahl* locus. Thus, the B6.CAST mouse differs from the C57BL mouse only at and near the *Ahl* locus. Keithley et al. (2004) have shown that the B6.CAST strain does not show age-related hearing loss at an early age ($\leqslant$15 mo) but shows a substantial hearing loss and some ganglion cell degeneration in the basal turn by 18 months.

This report describes the functional and histopathological changes in young (i.e., 4–15 weeks) female and male C57BL/6 J (i.e., *Ahl*/*Ahl*) and male B6.CAST-+^{Ahl} mice that were exposed to the same low-frequency noise. Group data are compared to determine if the presence of the *Ahl* gene results in an increased susceptibility to this noise.

2. Methods

2.1. Animals

Non-noise-exposed male (1) and female (3) C57BL/6J, and B6.CAST-+^{Ahl} male (1) mice (4–7 weeks old) served as controls for pre-existing hair-cell losses. Their cochleae were processed for quantitative histopathological examination as described below. Noise-exposed mice included five C57BL females (5–9 weeks), five C57BL males (7–9 weeks) and five B6.CAST males (four 7–8 weeks, and one 15 weeks); one member from each of the three groups being exposed simultaneously. These animals were terminated after 20 days of recovery (20-day groups). An additional two female and two male C57BL mice were functionally tested, noise exposed, and terminated (see below) by 2 h after the end of the exposure (0-day group). No B6.CAST males were used for histopathological examination at 0 or 6 days and no B6.CAST females were available for this study. The protocol for the care and use of the animals in this study was approved by the Animal Studies Committee at Washington University School of Medicine (#20000158, B. A. Bohne, PI).

2.2. Noise exposure

Awake mice were exposed for 4 h to an octave band of noise (OBN) with a center frequency of 4 kHz and a sound pressure level (SPL) of 108 dB. During their exposure, the mice were housed in separate compartments of a wire-mesh exposure cage. The cage was suspended in a double-walled, soundproof booth that had been made reverberant with 3/4 in.-thick Masonite. The noise was calibrated before each exposure session (B&K sound level meter with 1/2 in. microphone) and varied by less than $\pm$2 dB in the individual compartments.

2.3. Functional testing

Two days before their exposure, the mice were anesthetized as described below and ABR thresholds were determined at 12 frequencies from 3 to 50 kHz (see Nordmann et al., 2000 and Ou et al., 2000a for details). In addition, $2f_1$–f_2 DPOAE levels were determined in response to 29 pairs of frequencies ($f_2/f_1 = 1.23$, $L_1 = L_2 = 55$ dB) from $f_1 = 2$ to 40 kHz (methods similar to Jimenez et al., 1999). Acoustic ABR click and tone pip and DP tone burst stimuli were calibrated with the B&K sound level meter with a 1/4 in. microphone (flat frequency response out to 100 kHz) just prior to the beginning of this study and at periodic intervals during the study. Five each of C57BL females, C57BL males and B6.CAST males were anesthetized and functionally tested pre-exposure and at 0, 6 and 20 days post-exposure, after which their cochleae were fixed and harvested. The functional data were converted to ABR threshold shift and DPOAE level shift by subtracting post-exposure values from pre-exposure values.

2.4. Cochlear fixation and processing

The mice were anesthetized with a mixture of ketamine (80 mg/kg) and xylazine (16 mg/kg) given i.p. Our techniques for fixing, processing and analyzing the mouse cochlea have been described in detail elsewhere (Bohne and Harding, 1997; Bohne et al., 2001). Briefly, after clamping the descending aorta, cardiac perfusion with 5 ml of lactated Ringer's solution was performed to wash the blood out of the vascular system. Both cochleae were then fixed by cardiac perfusion with 5 ml of 1% osmium tetroxide in Dalton's buffer with 1.65% $CaCl_2$. The cochleae were removed from the head and immersed

in cold fixative for 2 h. The specimens were dehydrated and embedded in plastic (Durcupan). One cochlea from each mouse was dissected into 10 plastic-embedded segments that ranged in length from 0.28 to 0.95 mm.

2.5. Quantification of histopathological changes

The length of the organ of Corti (OC) was measured in each segment along the junction of the pillar heads using an image analysis system (i.e., Nikon SMZ-U microscope, Javelin camera, Data Translations frame grabber board, and Foster-Findley software). Each segment was then examined by phase contrast microscopy at 1250×. Phalangeal scars that replaced missing inner hair cells (IHC) and outer hair cells (OHC), and missing pillar cells were counted. In cochleae selected for illustration in each group, damage to the stereocilia bundle on the remaining IHCs in each segment was graded on a 0–3 scale as follows: 0 – within normal limits; 1 – slight abnormality with mild disarray, or few missing and/or fused; 2 – moderate abnormality with moderate disarray or splaying, or more than half missing/fused; 3 – severe abnormality with most severely splayed, missing or fused. The number of IHCs having each grade was totaled and converted to a percentage of the IHCs remaining in that segment.

The stria vascularis was examined to locate regions of degeneration (i.e., lack of osmium staining). The osseous spiral lamina was examined to identify areas with decreased osmium staining which signifies degeneration of the peripheral processes (i.e., MNF) of the spiral ganglion cells. The percentage of MNF loss and the dieback distance toward Rosenthal's canal were estimated by comparing staining intensity of the region in question to non-damaged regions.

Cytocochleograms were prepared showing the percentage of IHC and OHC losses, the percentage of remaining IHCs with stereocilia grades 1–3, stria-vascularis degeneration and MNF loss as a function of percentage distance from the OC apex.

Bohne and Clark (1982) defined focal hair-cell lesions (Fig. 1) as regions in which 50% or more of the hair cells are missing over a distance of at least 0.03 mm (i.e., 3 IHCs). Each cochlea was inspected for focal hair-cell lesions. The length, apex-to-base location and percentage loss of hair cells in all focal lesions were determined and were plotted on the cytocochleograms.

2.6. Height of the OC

In mice, the OC is small in cross-section and the pillars are very short. For these reasons, it is difficult to identify distorted regions of the OC in flat preparations of mouse cochleae as can be done in comparable specimens from larger animals [e.g., chinchillas (Nordmann et al., 2000)].

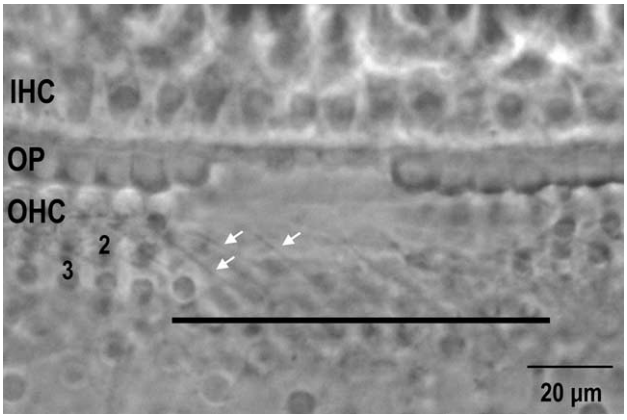

Fig. 1. C57BL/6J Female (#9; 20-day recovery). Photomicrograph of OHC focal lesion (0.08 mm; long horizontal black bar) at 88% distance from apex in cytocochleogram shown in Fig. 4. Inner hair cells (IHC) and inner pillars are intact. Five outer pillars (OP) and most OHC (2, 3) are missing in lesion. Deiters' cell processes (white arrows) are visible at left edge of lesion.

In 10 mice (i.e., three controls; four 0-day and three 20-day), 2.5-μm-thick sections of the cochlear duct were cut at a radial angle at three percentage locations [approximately 22% (4 kHz), 48% (10 kHz) and 71% (24 kHz) distance from the apex]. The sections were mounted on glass slides, stained with a mixture of methylene blue and azure II (Richardson et al., 1960) and examined by bright-field microscopy at 1250×. Evaluation of the radial sections allowed the identification of distorted OC and measurement of OC height. Height from the reticular lamina to the upper surface of the basilar membrane was measured at three sites: the lateral edge of the apical membrane of the inner hair cell (IHC); the medial edge of the first outer hair cell apex (OHC1); and the lateral edge of the third outer hair cell apex (OHC3) (Baggot et al., 1987). Using the same measurement system for determining OC length, height was measured in five sections from each percentage location in each sectioned cochlea. The results were averaged for each location. The sections were also assessed for the condition of the hair cells, supporting cells and nerve fibers.

2.7. Qualitative evaluations of other histopathological changes

The condition of the OHC stereocilia was assessed qualitatively as normal, somewhat, or severely damaged within particular segments. The area below the IHC bases was screened to identify regions where vacuoles were present which are indicative of swollen nerve terminals.

2.8. Correlation of histopathological changes and functional data

The functional data were overlaid upon the cytocochleograms using the OC frequency–place map

for the mouse (Ou et al., 2000b). The DPOAE level shifts and the differential noise floor (i.e., pre-exposure DPOAEs – noise floor) were plotted at f_2. Because the dB scale is nonlinear, the group means were calculated by converting dB to pressure ratio (P/P_0) in pascals, calculating the average, and converting the result back to dB. Arithmetic standard deviations are shown because converting the mean plus and minus standard deviation in pascals is inherently asymmetric on the nonlinear dB scale. An ANOVA was not done because the small sample size precluded its use. Group-summary statistics were compared within the low- (1–8 kHz), middle- (8–25 kHz), and high- (25–96 kHz) frequency regions of the OC to determine if there were significant differences between groups in functional shifts and hair-cell losses. The cutoff frequency for the low and middle groups were slightly below 8 and 25 kHz, respectively, so that data were not averaged in more than one region.

3. Results

3.1. ABR thresholds and DPOAE levels pre-exposure

Pre-exposure ABR thresholds were within the limits shown in Ou et al. (2000a); (Fig. 3). Pre-exposure DPOAE levels were similar to those shown in Jimenez et al. (1999, Fig. 2A; at 2 months of age). The magnitudes of the pre-exposure DPOAEs in the 1-month-old mice were generally smallest in the 3–8 kHz region, largest in the 8–25 kHz region, and variable in the 25–50 kHz region. In the latter region, some animals had robust DPOAEs out to 50 kHz while others were close to the noise floor. There were moderate to substantial left ear versus right ear asymmetries in DPOAEs for 11 of the 16 animals tested (69%) in both the 8–25 and 25–50 kHz regions.

3.2. Controls

Three female C57BL mice, one male C57BL and one B6.CAST mouse ranging in age from 4 to 7 weeks served as non-noise-exposed controls. The length of the OC for this group was 6.09 ± 0.15 mm. IHC losses were minimal throughout the OC. OHC losses were small in the low- and middle-frequency regions but larger in the high-frequency region. One of five control mice had a 0.09 mm OHC focal lesion centered at 96.4 percent distance from the apex. None of the cochleae had stria-vascularis or MNF degeneration. In three of five cochleae, there were vacuoles beneath the IHCs over a variable distance in the OC (i.e., 0–43% & 55–85%; 69–95%; 0–65%). In the apical half of the cochlea, approximately 1/3 of the IHC stereocilia bundles were graded 1 while in the basal half, less than 10% were graded 1. The remainder of the IHC stereocilia bundles were graded 0. OHC stereocilia bundles were within normal limits.

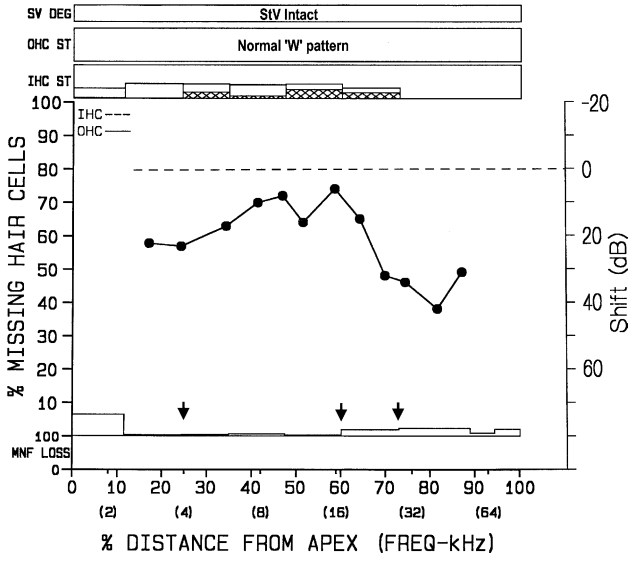

Fig. 2. C57BL/6J Female (#4; 0-day recovery). Histopathological data are plotted as a function of percentage distance (x-axis) from cochlear apex – % OHC loss (solid line) and % IHC loss (dashed line) [left y-axis], stria-vascularis degeneration (top band, SV DEG), OHC stereocilia condition (upper middle band, OHC ST). IHC stereocilia disarray (upper lower band, IHC ST – % of remaining: grade 1, open box; grade 2, hatched box), % nerve-fiber loss (bottom band, MNF LOSS). ABR threshold shifts (filled circles) at 0 days post-exposure are overlaid (right y-axis) as a function of frequency (kHz) (alternate x-axis). (DPOAE level shifts not determined). A substantial threshold shift ($\geqslant 10$ dB) occurred at all frequencies except 8, 10, and 16 kHz. OHC loss was minimal throughout the OC. There was no IHC loss. IHC stereocilia disarray was mild to moderate; OHC stereocilia were normal. The stria vascularis and MNF were intact. Arrows point to percentage locations where radial sections were cut.

In the three sectioned controls, hair-cell shape was within normal limits, pillars were not buckled, the Deiters' bodies paralleled the bodies of the outer pillars and their nuclei were located just inferior to the Deiters' cup region.

In each of the following sections (3.3–3.6), the functional results are presented first, followed by the histopathological findings in the groups of noise-exposed mice.

3.3. Noise-exposed mice, 0-day recovery

At 0 days post-exposure, ABR threshold shifts in the male and female C57BL mice were greatest in the low- (3–8 kHz) and high- (25–50 kHz) frequency regions with less in the middle frequencies (8–25 kHz) (Fig. 2, Table 1). In contrast, the DPOAE level shifts were minimal in the low- and high-frequency regions and maximal in the middle frequencies (Table 2). The length of the OC in this group averaged 6.03 ± 0.15 mm. IHC losses were minimal throughout the OC. OHC losses were greater than IHC losses in all three frequency regions, but did not exceed 3.3% in any region (Fig. 2, Table 3). None of these mice had a focal lesion, degeneration of the stria vascularis, or MNF loss. In three of four cochleae, there

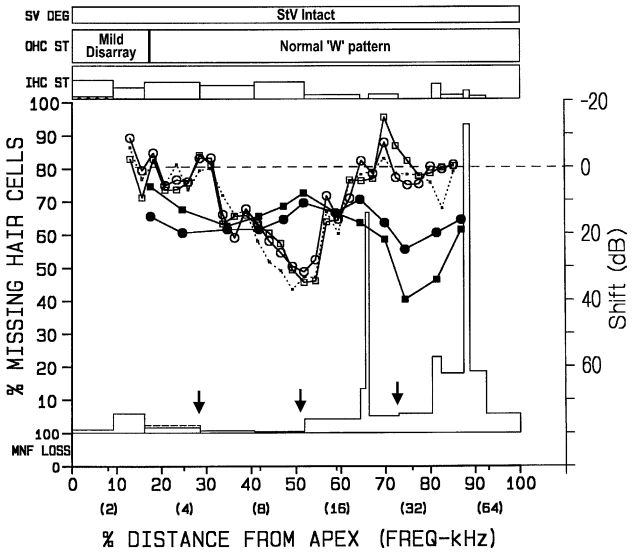

Fig. 3. C57BL/6J Female (#9; 20-day recovery). ABR threshold shifts at 0 days (filled circles) and 20 days (filled squares) post-exposure (see Fig. 2 for other definitions). DPOAE level shifts at 0 days (open circles) and 20 days (open squares) are shown (plotted at f_2; dotted line, differential noise floor). At 0 days, ABR threshold shifts were similar to those seen in the C57BL, 0-day group and DPOAE level shifts were maximal at all frequencies. At 20 days, ABR threshold shifts had recovered a little in the low and middle frequencies, but worsened in the high frequencies. DPOAE levels did not recover. There were two focal OHC focal lesions, a 0.05 mm lesion centered at 66% and a 0.08 mm lesion centered at 88% distance. IHC stereocilia disarray was mild; more cells in the apical half of the OC were affected; OHC stereocilia were normal except mild disarray in the apical tip. The stria vascularis and MNF were intact. Arrows point to percentage locations where radial sections were cut.

were vacuoles beneath the IHCs over a variable distance in the OC (i.e., 38–66%; 11–73%; 14–30%). Approximately half of the IHC stereocilia bundles were graded 1 or 2 in these cochleae. The remainder of the IHC stereocilia bundles were graded 0. OHC stereocilia bundles were within normal limits.

In the radial sections from these cochleae, hair-cell shape was found to be typical. Some IP bodies were slightly bowed in one cochlea. In two cochleae (#3 and 4), the Deiters' bodies were slightly to moderately collapsed towards the tunnel space but the nuclei were in their normal position.

The cytocochleograms in Figs. 3–5 belong to mice that were exposed at the same time and had data which were simultaneously as close as possible to the means shown in Tables 1–3.

3.4. Noise-exposed C57BL females, 20-day recovery

At 0 days post-exposure, ABR threshold shifts (Fig. 3, Table 1) and DPOAE level shifts (Fig. 3, Table 2) showed the same pattern as seen in the C57BL mice terminated on day 0. By 6 days, there was some improvement ($\geqslant$10 dB) in the ABR threshold shifts for the low

Table 1
Logarithmic mean (dB) of ABR threshold shift (±arithmetic SD) at 0, 6 and 20 days post-exposure within three frequency regions (kHz) in noise-exposed groups

Group	Post-exposure (d)	*N*	3–8 kHz	8–25 kHz	25–50 kHz
C57BL (2 F[a] and 2 M[a])	0	4	21 (9)	13 (1)	35 (12)
C57BL (F)	0	5	18 (5)	15 (4)	25 (10)
	6	4	5 (3)	9 (2)	22 (11)
	20	4	8 (5)	10 (4)	24 (10)
C57BL (M)	0	5	21 (4)	15 (3)	25 (6)
	6	5	3 (4)	6 (8)	23 (5)
	20	5	4 (3)	2 (3)	16 (6)
B6.CAST (M)	0	5	15 (6)	5 (4)	2 (5)
	6	5	1 (6)	8 (5)	6 (6)
	20	5	4 (6)	8 (7)	9 (6)

[a] F: female; M: male.

Table 2
Logarithmic mean (dB) of DPOAE level shift (±arithmetic SD) at 0, 6 and 20 days post-exposure within three frequency regions (kHz) for noise-exposed groups

Group	Post-exposure (d)	*N*	3–8 kHz	8–25 kHz	25–50 kHz
C57BL (F[a])	0	1	6 (–)	19 (–)	0 (–)
C57BL (F)	0	5	5 (4)	25 (7)	7 (5)
	6	4	4 (4)	22 (14)	7 (6)
	20	4	5 (4)	19 (13)	2 (4)
C57BL (M[a])	0	5	1 (2)	23 (8)	5 (3)
	6	5	0 (2)	11 (5)	3 (3)
	20	5	1 (2)	11 (7)	3 (3)
B6.CAST (M)	0	5	2 (3)	22 (7)	2 (2)
	6	5	1 (4)	9 (12)	1 (4)
	20	5	2 (4)	12 (5)	2 (4)

[a] F: female; M: male.

but not middle and high frequencies ($n = 4$; 1 female died after functional testing on day 0). There was little change in DPOAE level shifts over those measured at 0 days post-exposure. At 20 days, the ABR threshold shift and DPOAE level shift patterns were similar to that at 6 days.

The length of the OC averaged 5.90 ± 0.10 mm in four C57BL females. IHC losses were minimal throughout the OC (Fig. 3, Table 3). OHC losses were minimal in the low- and middle-frequency regions but larger and more variable in the high-frequency region. One OC (#9) had a 0.05 mm focal lesion in the mid-frequency region centered at 65.9% and a 0.08 mm one in the high-frequency region centered at 88.0%. Another OC (#21) had three focal lesions in the high-frequency region (0.08, 0.05, and 0.07 mm centered at 84.2%, 87.4%, and 92.2%, respectively). The OC in the other two females had no focal lesions. None of the cochleae had stria-vascularis or MNF degeneration. In three of four cochleae, there were vacuoles beneath the IHCs over a variable portion of the

Table 3
Average percentage of missing inner (IHC) and outer (OHC) hair cells (±SD) and number of cochleae with focal lesions within three frequency regions (kHz) for each group

Group	*N*	1–8 kHz	8–25 kHz	25–96 kHz
Controls				
C57BL (3 F[a] and 1 M[a])	4			
IHC		0.7 (1.2)	0 (0)	0.1 (0.3)
OHC		2.7 (0.9)	1.1 (0.5)	3.8 (3.1)
Cochleae w/focal lesions		0	0	1
B6.CAST (M)	1			
IHC		0 (–)	0 (–)	0 (–)
OHC		1.5 (–)	1.2 (–)	1.5 (–)
Cochleae w/focal lesions		0	0	0
Exposed (0-d recovery)				
C57BL (2 F and 2 M)	4			
IHC		0.1 (0.2)	0 (0)	0.1 (0.3)
OHC		1.7 (0.5)	0.6 (0.3)	1.9 (0.4)
Cochleae w/focal lesions		0	0	0
Exposed (20-d recovery)				
C57BL (F)	4			
IHC		0.6 (0.5)	0 (0)	0.1 (0.3)
OHC		3.0 (1.2)	2.2 (1.8)	11.4 (6.2)
Cochleae w/focal lesions		0	1	2
C57BL (M)	5			
IHC		1.4 (1.5)	0.2 (0.2)	1.5 (2.5)
OHC		2.4 (0.6)	1.7 (0.4)	9.2 (12.3)
Cochleae w/focal lesions		0	0	2
B6.CAST (M)	5			
IHC		0.2 (0.3)	0 (0)	0 (0)
OHC		3.4 (1.3)	2.0 (0.7)	3.2 (1.9)
Cochleae w/focal lesions		0	0	2

[a] F: female; M: male.

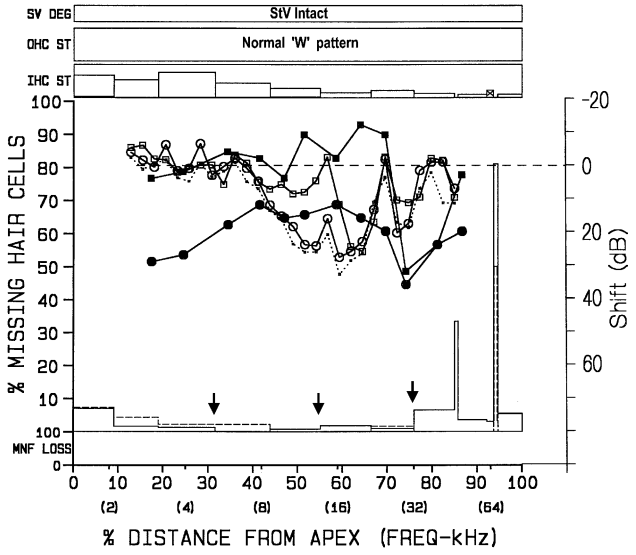

Fig. 4. C57BL/6J Male (#8; 20-day recovery). (See Figs. 2 and 3 for definitions.) At day 0, the ABR threshold shifts (solid circles) and DPOAE level shifts (open circles) were similar to those of the C57BL females. However, by 20 days, ABR threshold shifts returned to pre-exposure values (solid squares) except at 30 and 40 kHz. DPOAE level shifts (open squares) improved somewhat in the middle frequencies. Hair cell losses were minimal throughout the OC, but there was a 0.06 mm combined IHC and OHC focal lesion centered at 94% distance. IHC stereocilia disarray was mild and concentrated in the apical half of the OC; OHC stereocilia were normal. The stria vascularis and MNF were intact. Arrows point to percentage locations where radial sections were cut.

OC (i.e., 9–16%; 41–61%; 24–71%). The condition of the stereocilia on the IHCs was improved over that in the 0-day cochleae. OHC stereocilia were within normal limits, except for slight disarray beneath the apical infiltration hole that was made in the cochlear bone prior to embedding.

In the sectioned cochlea (#9), hair-cell shape was within normal limits. Pillar and Deiters' bodies had typical orientations and their nuclei were in their normal positions.

3.5. Noise-exposed C57BL males, 20-day recovery

Immediately post-exposure, ABR threshold shifts (Fig. 4, Table 1) and DPOAE level shifts (Fig. 4, Table 2) showed a similar pattern to that seen in the mice terminated on day 0. By 6 days, there was some improvement ($\geqslant$10 dB) in the ABR threshold shifts for low frequencies, minor improvement ($\geqslant$5 dB) for middle frequencies, and no change for high frequencies. At 20 days, ABR threshold shifts in the low and middle frequencies were similar to those at 6 days. The ABR threshold shift improved a little in the high frequencies. There was little change in DPOAE level shifts for low and high frequencies at all times tested but a substantial improvement in DPOAE level shifts for middle frequencies at 6 and 20 days.

The length of the OC averaged 5.90 ± 0.08 mm in five C57BL males. Hair-cell losses (Fig. 4, Table 3) were variable in this group and were similar to those in the C57BL females. One OC (#8) had a 0.06 mm focal lesion centered at 94.1% and another (#14) had a 0.54 mm focal lesion centered at 94.6%, that included the basal tip. The other three OC from this group did not have focal lesions. None of the cochleae had stria-vascularis or MNF degeneration. In three of five cochleae, there were vacuoles beneath the IHCs over a variable portion of the OC (i.e., 31–92%; 66–74% & 91–92%; 5–35%). The condition of the stereocilia on the IHCs was improved over that in the 0-day cochleae. OHC stereocilia were within normal limits.

In the sectioned cochlea (#8), hair-cell shape was within normal limits and pillars were not buckled. The bodies of the Deiters cells were slightly to moderately collapsed towards the tunnel, but their nuclei were in their normal position.

3.6. Noise-exposed B6.CAST males, 20-day recovery

At 0 days post-exposure, ABR threshold shifts (Fig. 5, Table 1) were present only in the low-frequency region.

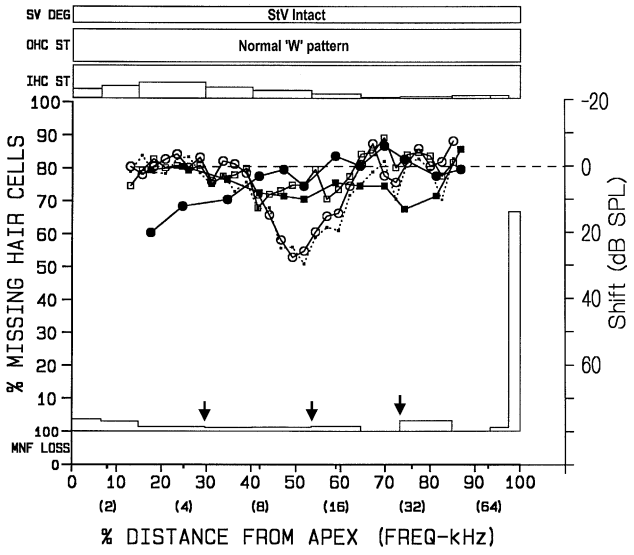

Fig. 5. B6.CAST Male (#10; 20-day recovery). (See Figs. 2 and 3 for definitions.) At 0 days, there were small ABR threshold shifts (solid circles) from 3 to 6 kHz only. DPOAE level shifts (open circles) were similar to those of the C57BL mice. By 20 days, the ABR threshold shifts (solid squares) at low frequencies had recovered but a small shift developed at 30 kHz. DPOAE level shifts (open squares) had nearly recovered at all frequencies. Hair-cell losses were minimal throughout the OC, but there was a 0.15 mm OHC focal lesion centered at 98.5% distance. IHC stereocilia disarray was mild and concentrated in the apical half of the OC; OHC stereocilia were normal. Arrows point to percentage locations where radial sections were cut.

DPOAE level shifts (Fig. 5, Table 2) were minimal in the low- and high-frequency regions and maximal in the middle frequencies. By 6 days, there was some improvement ($\geqslant$10 dB) in the ABR threshold shifts for low frequencies. DPOAE level shifts improved by 10 dB in the middle frequencies, but there was little change in the low and high frequencies. At 20 days, ABR threshold shifts were worse at high frequencies than found at 0 days. DPOAE level shifts at 20 days showed little change over that found at 6 days.

The length of the OC averaged 5.81 ± 0.17 mm in five B6.CAST males. Hair-cell losses in these mice (Fig. 5, Table 3) were less variable and were decreased from those in the C57BL females and males. The hair-cell losses in the 15-week-old mouse were within one standard deviation of the mean for this group. One OC (#10) had a 0.15 mm focal lesion centered at 98.7% that included the basal tip and another (#19) had a 0.04 mm focal lesion centered at 84.9%. The other three OC in this group had no focal lesions. None of the cochleae had stria-vascularis or MNF degeneration. In three of five cochleae, there were vacuoles beneath the IHCs over a variable portion of the OC (i.e., 80–89%; 10–42%; 81–86%). The condition of the stereocilia on the IHCs was improved over that in the 0-day cochleae. OHC stereocilia were within normal limits.

In the sectioned cochlea (#10), hair-cell shape was within normal limits. Pillar and Deiters' bodies had typical orientations and their nuclei were in their normal position.

3.7. Group comparisons of functional data

The group data were compared within each of the three frequency ranges shown in Tables 1–3 using the two-tailed Student t test with functional shifts in dB converted to pressure ratio in pascals.

3.7.1. 0-day recovery mice vs. 0-day data from C57BL females or males sacrificed at 20 days

No significant differences were found in ABR threshold shifts at 0 days. There were insufficient data to test significant differences in DPOAE level shifts.

3.7.2. 20-day C57BL females vs. males

The females had a significantly greater ABR threshold shift for middle frequencies at 20 days ($p = 0.01$). DPOAE level shifts were not significantly different.

3.7.3. 20-day C57BL males vs. B6.CAST males

Significant differences were found in ABR threshold shifts for middle ($p = 0.01$) and high frequencies ($p = 0.03$) at 0 days and for high frequencies ($p = 0.05$) at 6 days. There were no significant differences in ABR threshold shifts at 20 days. Pooling the data from the C57BL males and females did not result in a significant difference in ABR threshold shifts between the C57BL mice and the B6.CAST mice at 20 days. There were no significant differences in DPOAE level shifts.

3.8. Group comparisons of quantitative histopathological data

3.8.1. Control group vs. noise-exposed 0-day group

There were no significant differences in hair-cell losses (Table 3) between these groups. The mean OHC loss in the high-frequency region of the non-noise-exposed mice was a bit higher than in the noise-exposed C57BL mice because one control had a focal OHC lesion near the basal tip.

3.8.2. 0-day group vs. 20-day groups

There were no significant differences in IHC losses between the 0-day group and any of the 20-day groups. Compared to the 0-day group, OHC losses were significantly greater for the 20-day female group in the high-frequency region ($p = 0.03$) and for the 20-day B6.CAST group in the low-frequency region ($p = 0.05$).

3.8.3. 20-day C57BL male group vs. 20-day C57BL female group

There were no significant differences in hair-cell losses between these two groups.

3.8.4. 20-day C57BL male group vs. 20-day B6.CAST male group

IHC and OHC losses were not significantly different. Pooling the data from the C57BL males and females did not result in a significant difference in hair-cell losses between the C57BL mice and the B6.CAST mice at 20 days.

3.9. Qualitative histopathological data

Vacuoles were found below the IHCs over a variable distance in the organ of Corti in three of five controls. Vacuoles were found in three of four 0-day C57BL cochleae. However, the percentage locations of the vacuoles did not correlate with the frequency range of the threshold shifts. At 20 days, vacuoles were found in three of four C57BL females, three of five C57BL males, and three of five B6.CAST males. In the one C57BL male for which both cochleae were examined, vacuoles were present in one OC but not the other. As in the 0-day animals, the regions with vacuoles did not correlate with the location and extent of the permanent threshold shifts.

3.10. Organ of Corti height in control and noise-exposed mice

Organ of Corti height in the sectioned cochleae at three percentage regions is shown in Table 4. In control cochleae, OC height increased from the IHC to OHC3 and decreased from apex to base. Across the three controls, OC height was very uniform.

Mean OC height in 0-day cochleae was nearly identical to that of the controls. However, the variability in height across cochleae was greater. Height was decreased by about 10 μm at all percentage locations in one of four 0-day cochleae (#4; C57 female). In this cochlea, the pillar cell bodies were slightly bowed and the Deiters' cell bodies had partly collapsed toward the tunnel.

Two of the three 20-day cochleae had OC heights within the range of controls. In one 20-day cochlea (#8; C57 male), OC height was about 5–10 μm reduced from controls at all percentage locations. In this cochlea, the Deiters' cell bodies were partly collapsed toward the tunnel.

4. Discussion

The exposure used in the present study (4-kHz OBN, 108 dB SPL, 4 h) was chosen because we had earlier found that this exposure produced maximal hair-cell losses in the first turn, away from the basal tip of the OC (Ou et al., 2000a). Use of this exposure allowed functional testing without the confounding influence of age-related hair-cell loss which begins at the basal tip and progresses apically. The patterns of ABR TTS and PTS in mice are often an "inverted ox-bow" shape regardless of the noise-exposure band (Ou et al., 2000a,b). Noise damage in mice can occur up to three octaves above the frequency of the exposure band (Henry, 1984; Ou et al., 2000a; Wang et al., 2002). However, in the present study the 4-kHz exposure produced a substantial PTS in the low- and high-frequency regions of the OC in C57BL females, one being coincident with the noise exposure band and the largest one at three octaves above the exposure band. For the C57BL males, the PTS was three octaves above the exposure band. In the B6.CAST mice, the PTS region was 2–3 octaves above the exposure band.

Vazquez et al. (2004) compared DPOAE changes from 5.6–48.5 kHz in 2.5-month-old C57BL and

Table 4
Organ of Corti height[a] at three sites for three percentage locations in control, and noise-exposed C57BL and B6.CAST mice

Animal	OC length (mm)	11–33% distance			36–60% distance			63–78% distance		
		IHC (μm)	OHC1 (μm)	OHC3 (μm)	IHC (μm)	OHC1 (μm)	OHC3 (μm)	IHC (μm)	OHC1 (μm)	OHC3 (μm)
Ctrl #1 C57-F[b]	5.87	34	43	49	34	36	46	34	34	40
Ctrl #7 CAST-M[b]	5.93	35	37	46	32	32	39	30	30	37
Ctrl #24 C57-F	6.20	35	40	45	35	37	41	33	35	39
Mean ± SD	6.00 ± 0.18	35 ± 1	40 ± 3	47 ± 2	34 ± 2	35 ± 3	42 ± 4	32 ± 2	33 ± 3	39 ± 2
0-d #3 C57-F[c]	5.84	37	44	52	35	40	47	32	32	37
0-d #4 C57-F[c]	5.98	28	30	36	26	25	28	28	28	33
0-d #5 C57-M	5.87	36	40	49	36	37	42	34	35	37
0-d #6 C57-M	6.18	34	40	46	36	38	42	32	33	35
Mean ± SD	5.97 ± 0.15	34 ± 4	39 ± 6	46 ± 7	33 ± 5	35 ± 7	40 ± 8	32 ± 3	32 ± 3	36 ± 2
20-d #8 C57-M[c]	5.94	29	30	36	30	29	32	28	27	30
20-d #9 C57-F	5.93	37	41	48	34	37	43	31	32	35
20-d #10 CAST-M	5.85	36	40	47	34	34	41	32	32	39

[a] Correction for tissue shrinkage was not made. Because all cochleae were processed in the same fashion, the amount of shrinkage was expected to be similar in all specimens and would not affect comparisons across cochleae.
[b] F: Female; M: male.
[c] Deiters' bodies were partly collapsed toward the tunnel space.

B6.CAST mice that were exposed for 1 hour to a 10 kHz OBN at 105 dB SPL. At 28 days post-exposure, C57BL mice were still 10 dB below baseline while B6.CAST mice had completely recovered. In the present study, initial DPOAE level shifts were in the mid-frequency region and their magnitude, pattern, and location across frequency were similar to that reported for C57BL and B6.CAST mice by Vazquez et al. This result may seem paradoxical, but in other animals it has been shown that exposure to a low-frequency noise produces functional and histopathological changes in both the low- and high-frequency region of the OC (e.g., Bohne and Harding, 2000). By 20 days of recovery in the present study, there were still residual ABR and DPOAE shifts in the C57BL mice. In the B6.CAST mice, ABR threshold shifts recovered at low frequencies and increased slightly at middle and high frequencies while DPOAE level shifts had nearly recovered. No histopathological analysis was performed in the Vazquez et al. study, so hair-cell losses could not be compared.

With the present exposure in C57BL mice, functional shifts were maximal, but hair-cell losses were minimal immediately post-exposure. Some improvement in function occurred over time while hair-cell losses develop belatedly, particularly in the base. Despite genetic homogeneity in C57BL mice, the magnitude of the delayed hair-cell loss following an identical noise exposure was quite variable due to the unpredictable occurrence of focal lesions in the basal turn.

In the present study, the histopathological changes in C57BL mice initially involved IHC stereocilia damage apically but with time, developed into losses of hair cells in the base. Henry (1984) saw similar patterns of functional loss in 6-month-old CBA mice exposed to octave bands of noise. Because CBA mice carry the wild-type allele at the *Ahl* locus and therefore do not develop age-related hearing loss, the losses in Henry's study must have been noise-induced. The patterns of functional and structural losses in mice differ from those found in a number of other species, including humans (e.g., McGill and Schuknecht, 1976), chinchillas (e.g., Bohne and Clark, 1982; Bohne and Harding, 2000) and guinea pigs (e.g., Wang et al., 1994). In the latter three species, a better correlation exists between decrements in auditory function and hair-cell losses, especially in the region of maximum auditory sensitivity.

Swollen nerve endings (vacuoles) below the IHCs have been postulated as one pathological change that underlies TTS (e.g., Puel et al., 1998). In the present study, vacuoles were observed below the IHCs in some control cochleae. Similar vacuoles were also found in some noise-exposed cochleae but not in others that had equivalent TTSs and PTSs. When vacuoles were seen in the 0-day or 20-day cochleae, their location and extent did not correlate with the pattern of TTS or PTS, respectively. Thus, the presence of swollen nerve endings in the IHC region does not correlate with TTS, at least not in noise-exposed mice or chinchillas (Nordmann et al., 2000).

There was little correlation between the functional losses and the histopathological changes in the present study. At 0 days in cochlear regions with sizable TTSs, there was only mild to moderate abnormalities of IHC stereocilia. Whether or not this damage was sufficient to account for the functional losses could not be determined. There was minimal hair-cell loss, minimal supporting cell damage and OC height was normal for three of the four 0-day cochleae (Table 4). The latter finding contrasts with data from chinchillas in which TTS was correlated with buckling of the pillar cells, partial collapse of the Deiters cells, decrease in OC height and uncoupling of the stereocilia from the tectorial membrane (Nordmann et al., 2000). Mouse pillar cells are much shorter and their bodies stouter compared to those in mammals with larger OCs. Perhaps, short, stout pillars do not buckle. Davis et al. (2003) suggested that there is a different mechanism for noise-induced hearing loss in mice homozygous for the *Ahl* gene compared to mice with the wild-type gene. Our data also suggest that the mechanism for noise-induced hearing loss in wild-type mice may be different from that in larger mammals.

The ABR is thought to be dominated by the activity of IHCs (e.g., Nordmann et al., 2000). The DPOAE is thought to be generated by OHCs (e.g., Brownell, 1990). It is known that DPOAE levels in 2-month-old, non-noise-exposed C57BL mice are robust out to 50 kHz (Jimenez et al., 1999). However, the magnitude of pre-exposure DPOAEs reported here for 1-month-old C57BL and B6.CAST mice were variable and not as robust at and above 25 kHz. Because the onset of hearing in mice is at 2 weeks of age (Alford and Ruben, 1963; Saunders et al., 1979), the function of the basal OHCs in our 1-month-old mice may not have been completely developed. Left versus right ear asymmetries were found in 69% of the mice tested pre-exposure. This finding is in agreement with data from Martin et al. (2002) who reported that at 4 months of age, 79% of C57BL mice had left versus right DPOAE-magnitude asymmetries.

Age-related hearing loss in C57BL mice has been reported to begin at two months (Li and Borg, 1993) and younger C57BL mice are more susceptible to noise than older mice (Ohlemiller et al., 2000). These findings suggest that the *Ahl* gene begins to exert its influence on noise susceptibility at two months of age (Li, 1992; Ohlemiller et al., 2000). However, age-related hair-cell loss begins at the basal tip before two months (Bohne et al., 2001) and progresses apically as the mouse ages. Studies that do not test function above 32–40 kHz would not be able to detect this beginning loss. Thus, it is just as likely that the *Ahl* gene is active at or near hearing maturity in C57BL mice (i.e., approx. 1 month; Saunders et al., 1979).

Sato et al. (1991) have shown that there is a significant difference in cochlear length between human males and females, with male cochleae being longer than female. Recently, Bohne et al. (2001) showed a similar gender difference in C57BL/CBA F1 mouse cochleae. Thus, it seemed logical to expect that gender might influence one's susceptibility to noise. For this reason, data from the noise-exposed, 20-day C57BL males and females were analyzed separately. A significant difference between genders was found for permanent ABR threshold shifts for the middle frequencies only, with the female mice sustaining the greater PTS. However, no gender differences were found with respect to cochlear length or hair-cell loss. In contrast, McFadden et al. (1999) found female chinchillas sustained more hair-cell loss than males following impulse noise exposure. On the other hand, Welleschik and Korpert (1980) reported that male and female workers exposed to industrial noise sustained similar amounts of noise-induced hearing loss. Clearly, more studies are needed to determine if gender affects susceptibility to noise.

The sample size in the present study was small and thus, the generality of the findings may be limited. With a larger sample and similar variance, B6.CAST mice might be shown to be less susceptible than C57BL mice to TTS, PTS and hair-cell losses induced by exposure to low-frequency noise. In the present sample, the B6.CAST males were found to be much less susceptible to TTS at middle and high frequencies than C57BL males and females, but they were nearly equally susceptible to TTS in the low frequencies and PTS in the high frequencies. Thus, the *Ahl* gene may play an unexpected role for NIHL in mice, affecting susceptibility to TTS but not PTS. If one hypothesizes that TTS is somewhat protective against the belated development of PTS (Flock et al., 1999; Nordmann et al., 2000), it is difficult to explain why hair cells in C57BL mice eventually die. The lack of a partially protective TTS in the B6.CAST mice at middle and high frequencies could explain why their hair cells ultimately die in nearly equal numbers to those in C57BL mice.

From these results, we conclude that the *Ahl* gene plays a role in susceptibility to noise-induced hearing loss, but that other genes, as well as systemic and local factors must also be involved in determining overall noise susceptibility.

Acknowledgements

This project was supported in part by NIOSH (OH 03973) and by the Department of Otolaryngology, Washington University School of Medicine. The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of NIOSH.

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