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Susceptibility of DPOAEs to Sound Overexposure in Inbred Mice with AHL

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ABSTRACT

The notion that three inbred strains of mice, i.e., C57BL/6J (C57), BALB/cByJ (BALB), and WB/ReJ (WB), which exhibit differential rates of age-related hearing loss (AHL), may also exhibit differential susceptibility to noise-induced hearing loss was tested by comparing the effects of sound overexposure on these strains. The aftereffects of noise overstimulation on the distortion-product otoacoustic emissions (DPOAEs) of these three strains were compared and contrasted to those for the CBA/CaJ (CBA) strain, which does not show changes in hearing threshold sensitivity up to 15 months of age. Two cohorts of mice, one at 2.5 and the other at 6 months of age, were first exposed to a tonal overstimulation paradigm, were allowed to recover, and then were later re-exposed to an octave band noise (OBN), at 3 or 7 months of age, respectively. The two sound exposure episodes were designed to produce either a temporary (tonal exposure) or permanent (OBN exposure) reduction in the levels of the $2f_1 - f_2$ DPOAE in the WB strain, which exhibited the fastest rate of AHL. Although the tonal paradigm resulted in a temporary decrease in DPOAE levels for all strains at both ages, the 2.5-month BALBs showed the greatest susceptibility to this overexposure, while the 2.5-month WBs exhibited the least effects on DPOAEs. At the older age of 6 months, tonal overexposure produced essentially the same reduction in DPOAE levels for all four strains. In addition, there were no differences noted between CBAs and C57s, at either of the two ages. The OBN paradigm resulted in a permanent decrease in DPOAE levels in all the strains exhibiting early AHL, i.e., the C57, BALB, and WB mice, for frequencies about one-half to an octave higher than the exposure frequency, regardless of age. In contrast, the CBA strain was not significantly affected by the OBN overexposure.

Keywords: Cochlea, age-related hearing loss, sound overexposure, distortion product otoacoustic emissions, inbred mouse strains

INTRODUCTION

It has long been known that humans show differential elevations in hearing thresholds after exposure to excessive sounds (Taylor 1965; Sulkoski 1973; Ward 1973). In fact, in a classic early study, Taylor (1965) noted a large variability in noise-induced hearing loss (NIHL) among Scottish jute weavers, who had been exposed to the same industrial noises for similar lengths of time. It is this variability in response to sound exposure that encouraged the use of laboratory animal models in order to control, or manipulate, a number of the factors that influence susceptibility to NIHL, including the acoustic environment, along with subject-based qualities such as genetic predisposition and age. Although animal models have been used in controlled environments to study the effects of noise exposure on auditory function for many years, the commonly observed between-subject variability has not been significantly reduced (Miller et al. 1963; Clark and Bohne 1978; Cody and Robertson 1983) or the

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basis of this individual susceptibility uncovered. Recently, the laboratory mouse has become a popular experimental model to study noise-induced changes in cochlear function, primarily because of the accumulated knowledge about its genome and the availability of genetically identical inbred strains of mice, which would be expected to reduce between-subject variability.

Studies of the effects of excessive noise on auditory function have focused on two mouse strains in particular, CBA/CaJ (CBA) and C57/BL6J (C57) mice. In general, these investigations have shown that the C57 is more susceptible to NIHL than the CBA strain when age-matched mice were exposed to identical excessive-noise conditions (Shone et al 1991; Li 1992; Li et al. 1993; Miller et al. 1998; Davis et al. 1999). Recently, Davis et al. (1999) showed noise-exposure response curves for 3–4-month-old C57 and CBA mice that demonstrated increased susceptibility of auditory brainstem response (ABR) thresholds for C57s compared with CBAs. However, other research on pre-exposure thresholds, as well as age, both of which might be expected to significantly contribute to noise-exposure susceptibility, resulted in contradictory findings. For example, in an earlier study, Shone et al. (1991) found that age alone, without any age-related dysfunction or presbycusis, was not a factor in increasing NIHL, as tested in either 6- vs. 8-month-old C57s or 8- vs. 21-month-old CBAs. In contrast, a more recent study concluded that advancing age increased susceptibility to NIHL, as demonstrated by shifts in ABR thresholds after acoustic overstimulation, particularly at the higher frequencies (Miller et al. 1998). In addition, detailed studies on the effects of age and acoustic overexposure that regularly compared the C57 with the CBA strain on a monthly basis concluded that age did not increase susceptibility to NIHL, except for very young 1-month-old mice (Li 1992; Li et al. 1993). Moreover, the latter investigators also showed that the CBA strain eventually lost its early susceptibility to NIHL at about 3 months of age.

Over the past few decades, auditory function in several inbred mouse strains, i.e., CBA, C57, BALB/cByJ (BALB), and WB/ReJ (WB), has also been characterized in detail with either measures of ABRs or distortion product otoacoustic emissions (DPOAEs). As a consequence of this resulting information, these strains have been proposed (Henry and Chole 1980; Henry 1983; Li and Borg 1991; Li 1992; Erway et al. 1993; Parham 1997; Willott and Erway 1998; Willott et al. 1998; Jimenez et al. 1999; Parham et al. 1999) as animal models to study the differential rates of age-related hearing loss (AHL). The accelerated rate of AHL in the C57 and BALB mice appears to be the result of the actions of the recessive *Ahl* gene (Erway et al. 1993; Johnson et al. 1997; Willott et al. 1998), which has been mapped to Chromosome 10 of the mouse genome (Johnson et al. 1997). The *Ahl* gene, in addition, has been hypothesized to be responsible for not only the early onset of AHL but also for the increased susceptibility of the C57 mouse to noise overexposure (Davis et al. 1999).

The present study was designed to better understand the effects of differential rates of AHL, likely due to the *Ahl* gene, on temporary and permanent sound-induced alterations to the sensitive outer hair cell (OHC) system of the cochlea. Toward this end, the $2f_1 - f_2$ DPOAEs of four inbred mouse strains i.e., the CBA, C57, BALB, and WB, with unique rates of AHL as previously described by DPOAE testing (Jimenez et al. 1999), were characterized with respect to their response to two distinct noise-exposure paradigms. These overstimulation protocols were designed to produce either a temporary or a permanent reduction in DPOAE levels and were administered at two different ages to determine if advancing AHL significantly affected the response to noise exposure.

MATERIALS AND METHODS

Subjects

Subjects were 40 female mice, with $n = 10$ for each of the four strains. Three strains—CBA, C57, and BALB—were purchased at 6–8 weeks of age from a commercial breeder (The Jackson Laboratory, Bar Harbor, ME). The fourth strain, WB, was bred within the vivarium facilities of the University of Miami School of Medicine from male/female pairs that were purchased from the above supplier. Due to untimely deaths, the final number of mice decreased slightly for each mouse strain and noise-exposure condition (see figure captions for exact numbers). Mice were housed in a temperature- and light-regulated vivarium room in standard polyurethane cages with free access to food and water. Over a representative 2-day work period, the mean noise level of the room was ~60 dBA for 99.3% of the time and ~70 dBA for the remaining (0.7%) time as measured by a noise-logging dosimeter (Quest Technologies, M-27, Oconomowoc, WI). Detailed measures in 1/3-octave bands, from 4 to 80 kHz using a calibrated 1/4-in. microphone (Bruel and Kjaer, Model 4136, Norcross, GA) along with a dynamic-signal analyzer (Hewlett Packard Model 3561A, Palo Alto, CA), showed that the root-mean-square (RMS) average sound level was about 35 dB SPL, with the most intense frequency band centered at 31.5 kHz at a level of about 30 dB SPL.

Mice were divided into two cohorts. One ($n = 5$/strain) was intially exposed at 2.5 months of age to a tonal stimulus and then re-exposed at 3 months to an

octave-band noise (OBN). The second cohort (n = 5/strain) was exposed to the tonal overstimulation at 6 months and subsequently re-exposed to the identical OBN at 7 months. In preliminary experiments on WB mice, the tonal overexposure caused a temporary reduction in DPOAEs which recovered to pre-exposure levels within a few hours, while the OBN exposure produced a permanent decrement in DPOAE levels that lasted for at least 28 days after exposure.

At the beginning of each data-collection session, mice were lightly anesthetized (intramuscularly) with an initial dose of a combination of ketamine hydrochloride (100 mg/kg) and xylazine (4 mg/kg) in equal volumes of bacteriostatic water. Anesthesia was maintained with additional doses (ketamine: 50 mg/kg, xylazine: 2 mg/kg) when twitching of the vibrissae was observed. Ears were examined with an operating microscope for evidence of debris in the external canal or a middle-ear infection (excessive cerumen and/or a reddened or ruptured tympanic membrane, respectively). Core body temperature of the anesthetized mouse was monitored with a feedback-controlled homeothermic blanket (Harvard Apparatus, Holliston, MA) and rigorously maintained at 36.5 ± 0.5°C using a heated table with feedback from a rectal probe.

The acquisition, maintenance, and testing of mice were approved by the University's Institutional Animal Care and Use Committee and closely monitored by the School's Division of Veterinary Resources.

General experimental design

The primary measurement of the present work was the $2f_1 - f_2$ DPOAE. Complete details of the recording procedure used for mice have been described elsewhere (Jimenez et al. 1999). Briefly, the f_1 and f_2 primary tones were generated by a dual-channel synthesizer (Hewlett Packard Model 3326A) and attenuated, under computer control, using customized software. The f_1 and f_2 primaries ($f_2/f_1 = 1.25$) were then presented over two separate earspeakers (Radio Shack, Realistic Dual Radial Horn Tweeters, Tandy Corp., Ft. Worth, TX) and delivered to the outer-ear canal through an acoustic probe, where they were allowed to acoustically mix to avoid artifactual distortion. Ear-canal sound pressure levels, which were measured by an emissions microphone assembly (Etymotic Research, ER-10B$^+$, Elk Grove Village, IL) embedded in the probe, were sampled, synchronously averaged, and Fourier analyzed for geometric mean (GM) frequencies [$(f_1 \times f_2)^{0.5}$] ranging from 5.6 to 19.7 kHz (i.e., f_2 = 6.3–22.5 kHz) by a computer-based DSP board. Corresponding noise floors (NFs) were computed by averaging the levels of the ear-canal sound pressure for five frequency bins above and below the DPOAE frequency bin (±54 Hz).

For test frequencies above 20.1 kHz, a computer-controlled dynamic-signal analyzer (Hewlett Packard Model 3561A) was used. The related NFs were estimated by averaging the levels of the ear-canal sound pressure for the two FFT frequency bins below the DPOAE frequency (i.e., for 3.75 Hz below the DPOAE). No artifactual DPOAEs were ever measured in a hard-walled cavity that approximated the size of the mouse outer-ear canal, which was used to calibrate the tonal stimuli. For both stimulus protocols, DPOAEs were considered to be present when they were at least 3 dB above the NF.

Depending on the exposure paradigm, DPOAEs were measured at a single frequency or as serially obtained DP-grams, i.e. DPOAE levels as a function of GM frequency. That is, for the tonal overexposure experiments, DPOAEs were monitored during both the pre- and the postexposure periods at the test frequency of 13 kHz. This was the frequency expected to be maximally affected by the 10-kHz tonal overstimulation, i.e., the frequency that was ~1/2 octave below the 13-kHz test frequency (see complete details of exposure stimuli below). Two primary-tone paradigms that were used consisted of either $L_1 = L_2 = 55$ dB SPL or $L_1 = 55$ and $L_2 = 45$ dB SPL. These levels were selected on the basis of both their moderate intensities and prior evidence that moderately intense, offset primary-tone levels were more sensitive than equilevel primaries in detecting the effects of reversible overexposure on DPOAEs (Sutton et al. 1994; Whitehead et al. 1995). During an experimental session the two test protocols were systematically alternated at once per second during both the pre- (1 min) and postexposure (10 min) measurement intervals.

For the subsequent OBN overexposure experiments, DPOAEs were measured as DP-grams, referenced to the GM frequency (Martin et al. 1987), during the pre-exposure period and at 2, 7, 14, 21, and 28 days postexposure. Specifically, DP-grams described emission levels in response to primary tones at $L_1 = L_2$ = 55, 65, and 75 dB SPL as a function of the GM frequencies, which ranged from 5.6 to 48.5 kHz (f_2 = 6.3–54.2 kHz), in 0.1-octave increments. Two GM frequencies (17.1 and 18.4 kHz) were not included in the average plots illustrated below because of obvious artifacts related to the 1/4-wave cancellation effect in the mouse ear canal.

Details of noise-exposure paradigms

The tonal overexposure paradigm was designed to produce a reversible decrease in DPOAE levels. Based on past experience, it consisted of a 1-min pure tone at 10 kHz generated by the f_1 channel of the frequency synthesizer at a level of 100 dB SPL as measured in the ear canal with the ER-10B$^+$ microphone assembly.

This exposure tone was delivered through the DPOAE probe in a closed sound field to a randomly selected ear of an anesthetized mouse. The OBN paradigm was designed to produce a permanent decrease in DPOAE levels (still measurable at 28 days postexposure) in the WB strain, which had been shown previously to display the most rapid rate of AHL (Jimenez et al. 1999). Thus, tonal overexposure consisted of a 1-hour OBN centered at 10 kHz at an RMS average level of 105 dB SPL as measured by a 1/2-in. microphone (ACO Pacific 7013, Belmont, CA) in combination with a precision sound-level meter. The OBN was generated by a custom-made, broadband noise generator. This signal was then filtered (Frequency Devices 9002, Haverhill, MA), amplified with a stereo amplifier (NAD Electronics LTD, 325 PE, London, United Kingdom), and transduced by two direct-reflecting loudspeakers (Bose 901, Wrentham, MA) that were controlled by an associated 2-channel active sound equalizer. The resulting spectrum, analyzed with the dynamic-signal analyzer in 1/3-octave frequency bands, ranged from 8 to 15 kHz, with the maximum energy of 100 dB SPL at 10 kHz. During the 1-hour OBN exposure, four mice, with free access to food, were each placed into a small, custom-made, wire-mesh cage. The four-cage unit was centered in a double-walled sound-isolation chamber that was fitted with hard reflecting surfaces that ensured homogenous exposure levels.

Data processing and statistical analysis

The DPOAE and NF levels were measured and converted to ASCII text files using customized software. These data were subsequently imported to a database (Excel 98, v.7.0, Redmond, WA, Microsoft Corp.), plotted, and transposed for statistical analysis by averaging the DPOAE levels from the DP-grams in 1/2-octave steps (except for the highest frequency bin) across the frequency range tested. Thus, seven frequency intervals were examined at: (a) 5.6–7.4, (b) 8–10.6, (c) 11.3–14.9, (d) 15.9–21.2, (e) 22.6–29.9, (f) 31–42, and (g) 45–48 kHz.

Statistical analyses were performed on the database by using commercially available software, including SPSS (SPSS Inc., v.6.1, Chicago, IL) and Excel 7.0, to determine routine descriptive statistics, including means, standard deviations (SDs), and standard errors of the mean (SEMs), as well as parametric statistics based on four-way repeated-measures analyses of variance (ANOVAs). Because the large number of significant interactions made global statements regarding the significance of individual findings problematic, two-tailed Student's *t*-tests were primarily used. Tests of statistical significance used Bonferroni *post hoc* corrections, which adjusted the level of statistical significance based on the number of *t*-tests performed (see Tables 1 and 2).

RESULTS

The overall results can be summarized as follows. First, after the tonal exposure at 2.5 months, the BALB strain, which shows the slowest rate of the AHL strains examined here, exhibited the largest loss in DPOAE levels immediately postexposure and the least recovery to pre-exposure baselines at 5-min postexposure. In contrast, the WB strain, which displays a very rapid progression of AHL, exhibited the least reduction in DPOAE levels and had almost recovered to baseline levels by 5 min following the termination of the exposure. However, for the 6-month exposed mice, the reversible tonal-induced changes in DPOAEs were very similar among the four mouse strains. Second, 28 days after the more severe OBN exposure, the DPOAE levels for the three strains that exhibit very rapid or more gradual rates of AHL, i.e., WB, C57, and BALB, were more adversely affected than CBA, with the BALB displaying the largest amount of DPOAE loss across the measurable frequency range. Finally, for the three strains that were susceptible to the OBN-induced DPOAE decrements, young mice exposed at 3 months of age exhibited losses as severe or more so than the older ones exposed at 7 months of age. These findings are described in greater detail below.

Differences in baseline DPOAE levels

Before the tonal noise exposure at 2.5 months of age, in response to equilevel primaries at $L_1 = L_2 = 55$ dB SPL two of the mouse strains exhibited similar DPOAE levels at the 13-kHz monitoring frequency. That is, CBA showed average DPOAE levels of 27 ± 0.6 dB SPL, whereas C57 had emissions of 25 ± 1 dB SPL. In contrast, compared with CBA, BALB showed statistically significant and larger DPOAE levels at 31 ± 0.8 dB SPL ($p < 0.05$, $t = 3.324$, $df = 8$). This is also true when compared with C57 ($p < 0.01$, $t = 4.426$, $df = 8$) and with WB ($p < 0.001$, $t = 9.604$, $df = 8$), with WB displaying significantly lower levels at 18 ± 4 dB SPL compared with CBA ($p < 0.001$, $t = 6.280$, $df = 8$) and C57 ($p < 0.001$, $t = 5.128$, $df = 8$).

For three of the strains, these baseline levels did not change appreciably by 6 months, of age, with CBAs at 26 ± 1.7, C57s at 25 ± 1.9, and WBs at 22 ± 0.3 dB SPL. However, the 6-month BALBs, at 27 ± 0.9 dB SPL, exhibited levels that were reduced from those measured earlier at 2.5 months, which were now more similar to the other mouse strains. In general, for both

TABLE 1

Average loss from baseline immediately following reversible tonal exposure and loss remaining after 5 minutes of recovery for both primary-tone levels

		55/55 dB SPL		*55/45 dB SPL*	
Strain	*Age (mo)*	*Initial loss (dB)*	*Loss at 5 min (dB)*	*Initial loss (dB)*	*Loss at 5 min (dB)*
CBA	2.5	−40.6 ± 4.5[a]	−6.2 ± 2.3[a]	−37.7 ± 7.4	−9.0 ± 3.0[a]
	6	−37.5 ± 1.1	−2.7 ± 0.7	−36.3 ± 5.1	−5.7 ± 1.8
BALB	2.5	−40.4 ± 1.9[a]	−10.3 ± 5.2[a]	−43.6 ± 8.8[a]	−13.9 ± 4.1[a]
	6	−40.0 ± 4.6	−3.5 ± 0.2	−33.9 ± 2.8	−7.4 ± 0.2
C57	2.5	−34.9 ± 3.9	−7.4 ± 4	−33.9 ± 4.5	−9.7 ± 4.4
	6	−33.3 ± 11.3	−3.2 ± 1.3	−35.32 ± 7.2	−6.6 ± 1.6
WB	2.5	−16.4 ± 11.8	−1.9 ± 1	−19.7 ± 10.39	−2.8 ± 1.3
	6	−30.1 ± 3.9	−6.0 ± 1.9	−30.2 ± 1.8	−8.4 ± 3.1

[a]Significantly different from WB tested at $p < 0.05$ (Bonforroni corrected to $p < 0.008$).

TABLE 2

Statistical significance of the DPOAE level decrements 28 days after a 1-hour OBN exposure[a]

		Frequency bin (kHz)						
Strain	*Age (mo)*	*a (5.6–7.5)*	*b (8–10.6)*	*c (11.3–15)*	*d (16–21)*	*e (23–30)*	*f (32–42)*	*g (45–48)kHz*
$L_1 = L_2 = 55$ dB SPL								
CBA	3	—	—	—	—	—	—	—
	7	—	—	—	—	—	—	—
BALB	3	—	—	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.001$	NF
	7	—	—	$p < 0.01$	$p < 0.01$	—	NF	NF
C57	3	—	—	—	—	$p < 0.001$	—	NF
	7	—	—	—	—	NF	NF	NF
WB	3	—	—	$p < 0.001$	$p < 0.001$	NF	NF	NF
	7	—	—	$p < 0.001$	$p < 0.001$	NF	NF	NF
$L_1 = L_2 = 65$ dB SPL								
CBA	3	—	—	—	—	—	—	—
	7	—	—	—	—	—	—	—
BALB	3	—	—	$p < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$
	7	—	—	$p < 0.01$	—	$p < 0.01$	NF	NF
C57	3	—	—	—	—	$p < 0.001$	—	NF
	7	—	—	—	$p < 0.01$	NF	NF	NF
WB	3	—	—	$p < 0.01$	$p < 0.01$	NF	NF	NF
	7	—	—	$p < 0.01$	$p < 0.001$	NF	NF	NF
$L_1 = L_2 = 75$ dB SPL								
CBA	3	—	—	—	—	—	—	—
	7	—	—	—	—	—	—	—
BALB	3	—	—	$p < 0.01$	$p < 0.001$	$p < 0.001$	$p < 0.001$	$p < 0.01$
	7	—	—	$p < 0.01$	—	$p < 0.01$	—	NF
C57	3	—	—	—	$p < 0.001$	$p < 0.001$	—	NF
	7	—	—	—	—	NF	NF	NF
WB	3	—	$p < 0.01$	$p < 0.05$	$p < 0.01$	NF	NF	NF
	7	—	$p < 0.02$	$p < 0.002$	$p < 0.001$	NF	NF	NF

[a]NF indicates DPOAEs were at the noise floor due to aging. *p* values shown only for differences significantly greater than those resulting from aging.

age groups, DPOAEs elicited with the unequal level primaries of $L_1 = 55$ and $L_2 = 45$ dB SPL were about 1–2 dB lower in magnitude than emissions evoked with equilevel primaries. Because of these baseline DPOAE-level differences, changes following noise exposure were plotted as difference scores from baseline, so that 0 dB represented "no change" for all groups. Because all four strains were shown previously, to have similar DPOAE response/growth or input/output (I/O) functions (Jimenez et al. 1999), pre/post difference scores provided just as accurate a measure of susceptibility as estimates of sensation level or threshold,

assuming that the absolute baseline did not affect the amount that noise shifted the I/O functions to the right, following exposure.

Susceptibility to temporary DPOAE level changes

Following the brief tonal overexposure, a temporary reduction in DPOAE levels was noted for each of the four strains tested with both stimulus-level paradigms at the two ages. As illustrated in Figure 1, the tonal exposure produced strain-specific amounts of initial postexposure decrements in DPOAE levels that were accompanied by different amounts of recovery toward baseline. Specifically, for the initial 5 min of the recovery period, the plots of Figure 1 show the average postexposure DPOAE levels at the 13-kHz GM frequency, normalized to pre-exposure baseline, for the two protocols of $L_1 = L_2 = 55$ (Fig. 1A, C) and $L_1 = 55$, $L_2 = 45$ (Fig. 1B, D) dB SPL and at the two exposure ages of 2.5 (A,B) and 6 (C,D) months. Table 1 provides the average and SDs of initial DPOAE decrements, along with the loss in DPOAE levels at 5 min postexposure for the four strains. It is clear from Figures 1A and B and from Table 1 that at 2.5 months BALBs (small solid circles), CBAs (large solid circles), and C57s (crosses) exhibited the largest tone-induced losses in DPOAE levels, which ranged from about 35 to 40 dB for the $L_1 = L_2 = 55$ dB SPL stimulus condition and from 35 to 43 dB for the $L_1 = 55$, $L_2 = 45$ dB SPL paradigm. In other words, for these strains, DPOAEs were reduced to the NF, or to approximately −10 dB SPL. In contrast, the WBs (open circles) exhibited the least amount of loss, ranging from about 16 (55/55 dB SPL) to 20 dB (55/45 dB SPL). This represents a postexposure level that was about 10 dB above the NF. As indicated in Table 1, these initial losses for the CBA and BALB mice at 55/55 dB SPL and for the BALBs at 55/45 dB SPL were significantly different from those of the WBs.

In contrast to the 2.5-months findings, the functions of Figure 1C and D for the 6-month animals exhibited no statistically significant differences among the four mouse strains for the amount of DPOAE loss initially produced by the tonal exposure (see Table 1). Thus, on average, the trend at 6 months of age was for the tonal exposures to produce a decrement in DPOAE levels of about 30–40 dB. Moreover, at 5 min (300 s) postexposure, as indicated in Figures 1A–D, DPOAE levels had not completely recovered back to baseline, for any of the mouse strains, at either of the two exposure ages or for either of the two primary-tone level paradigms. Thus, as indicated in Table 1, DPOAEs (elicited by either equilevel or nonequilevel stimulus protocols) for mice exposed at 2.5 months of age were closer to the pre-exposure baselines for WB (within ~2–3 dB) at 5 minutes exposure, than for CBAs (within ~6–9 dB), C57s (within 7–10 dB), or BALBs (within ~10–14 dB). However, for 6-month-old mice, there were no striking differences between the strains regardless of primary-tone test level in that, by 5 minutes postexposure, all strains were reduced from baseline levels by about the same amount (i.e. WBs = ~6–8 dB, BALBs = ~4–7 dB, CBAs = ~3–6 dB, and C57s = ~3–7 dB). In general, then, mice exposed at the older age of 6 months returned slightly closer to pre-exposure baseline DPOAE levels by 5 minutes postexposure than the younger mice at 2.5 months.

Susceptibility to permanent DPOAE level changes

Figure 2 illustrates DP-grams for the CBA (A,E), BALB (B,F), C57 (C,G), and WB (D,H) strains at the pre- (solid squares) and 28-days postexposure (open squares) intervals for the two OBN-exposure ages of 3 (A–D) and 7 (E–H) months, as measured with representative equilevel primaries at 65 dB SPL. Essentially, the average recovery patterns describing OHC function present at 28 days were established by 2 days after the OBN exposure and were similar, with slight variations, to the recovery patterns observed at 7, 14, and 21 days postexposure. Thus, only the 28-day results are illustrated in Figure 2. In each plot, the continuous gray background lines represent the variability (±1 SD) in DPOAE levels for age-matched controls at 4 (3 months + 28 days postexposure) and 8 (7 months + 28 days postexposure) months, to control for aging of the experimental animals at 28 d post-exposure. It is clear from the DP-grams of Figure 2A and E that emission levels for the CBAs, at either exposure age, were essentially at pre-exposure levels and that as expected there were no age-related changes that influence the recovery of postexposure DPOAEs. In contrast, BALBs (Figs. 2B, F) exhibited large DPOAE level decrements (open squares) as a result of the noise exposure at both ages, but particularly when exposed at 3 months of age (i.e. differences between solid and open squares in Fig. 2B).

The widely separated ±1-SD traces of the age-matched controls in Figure 2B illustrate the appreciable variability at 4 months of age. However, the large DPOAE level decreases for the 3-month-old BALBs were clearly outside the limits set by the aging-induced decreases in DPOAEs and can be essentially attributed to the results of the acoustic overstimulation episode. Figure 2F also indicates that, for the 7-month-old BALBs, the noise-induced reduction in DPOAEs was essentially outside the constraints of the aging effects indicated by the gray lines. However, it is clear from Figure 2B and F that the overall DPOAE level decrement produced by overexposure was less for the 7-

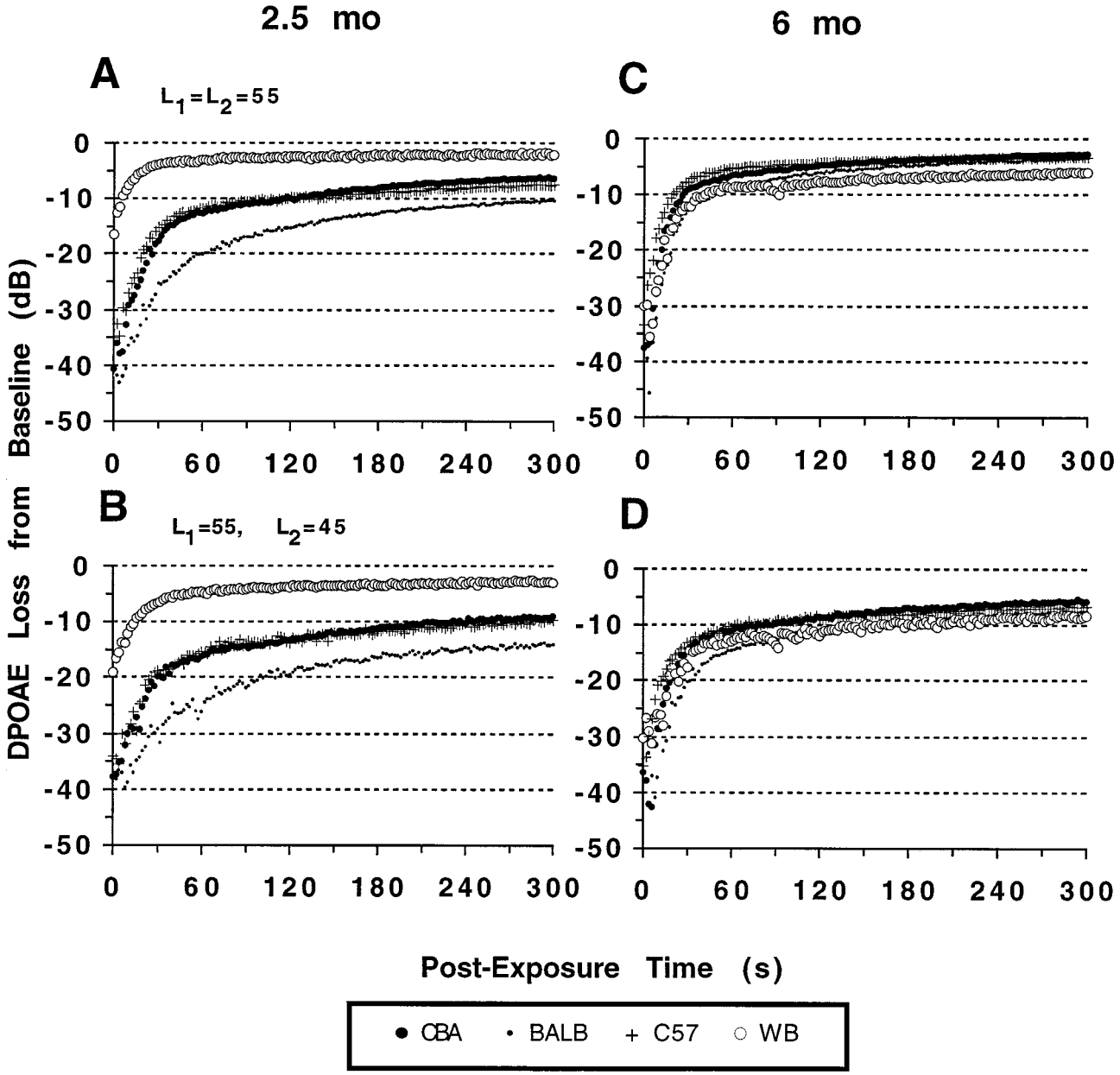

FIG. 1. Average DPOAE level differences from pre-exposure baseline at a GM frequency of 13 kHz following a 1-min, 10-kHz tonal exposure at 100 dB SPL. Data are plotted as a function of linear time (in s) at the two exposure ages of 2.5 months (**A**, **B**, $n = 5$) and 6 months (**C**, **D**, $n = 4$), elicited by two distinct primary-tone paradigms with $L_1 = L_2 = 55$ (**A, C**), and $L_1 = 55$, $L_2 = 45$ (**B, D**) dB SPL. Variability in the form of SDs and SEMs (not shown) was largest at 12 and 5.5 dB, respectively, for the 2.5-month WBs during the first 2 min of recovery. At 2.5 months (**A, B**), the WBs (○) were least affected, while the BALBs (●) showed the greatest losses. The CBA and C57 strains showed almost identical decrements that were between those of the two other strains. At 6 months (**C, D**), all four strains recovered almost alike in response to both stimulus protocols.

month-old (Fig. 2F) than for the 3-month-old (Fig. 2B) BALBs for the low frequencies that were <20 kHz. For the 3-month-old C57s, Figure 2C clearly shows that the noise-induced decrements in DPOAE levels were at the NF at the midfrequencies from about 20 to 35 kHz, while the age-related changes primarily affected higher frequencies that were >35 kHz. In contrast to the BALB findings, there were highly similar noise-induced reductions in DPOAEs in the 7-month-old C57s of Figure 2G compared with the 3-month-old mice in frequency regions with remaining DPOAEs. Finally, despite their severe, age-related, high-frequency loss, the 3-month-old WBs of Figure 2D exhibited noise-induced decrements in regions with remaining DPOAEs, i.e., over the low to midfrequencies (~12–25 kHz). Because of even more severe age-related DPOAE losses, noise-induced losses for 7-month-old WBs of Figure 2H were apparent over a

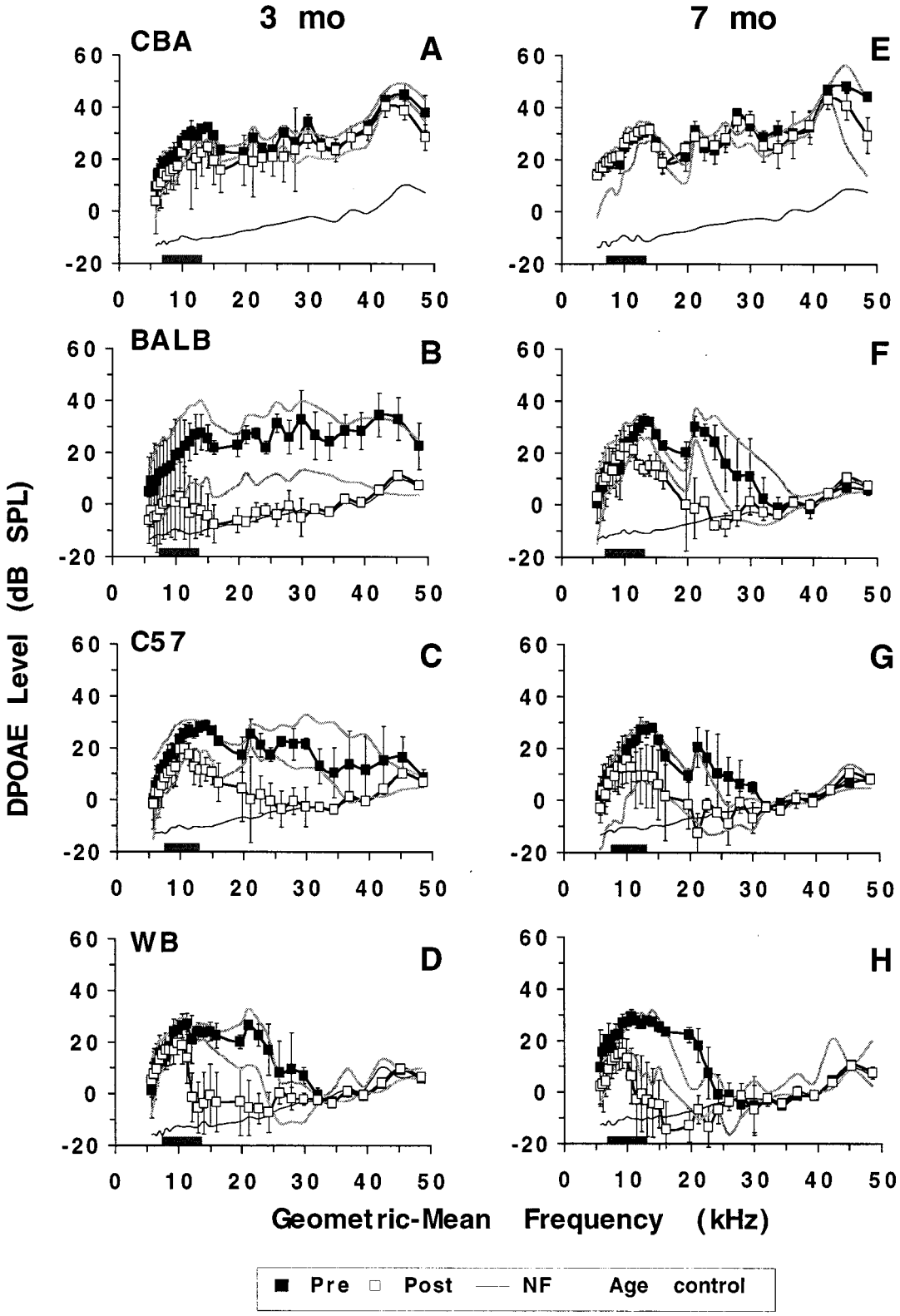

FIG. 2. Average DP-grams for CBA (**A, E**), BALB (**B, F**), C57 (**C, G**), and WB (**D, H**) strains before (■) and 28 days after (□) a 1-h, 10-kHz OBN exposure at 105 dB SPL. DPOAE level is plotted as a function of the GM frequency at a representative level of stimulation, i.e., $L_1 = L_2 = 65$ dB SPL, for the two different noise-exposure ages of 3 months (**A–D**, $n = 5$) and 7 months (**E–H**, $n = 4$) of age. The ±1 SD of age-matched DPOAE levels (solid gray lines), determined previously for other mice of the corresponding strains (Jimenez et al. 1999), are plotted in the background shown for the ages of 4 months (**A–D**) and 8 months **(E–H)**, which were equivalent to the age of the present subjects at 28 days after the OBN exposure. The gray horizontal bar around 10 kHz represents the frequency spectrum extent of the OBN exposure, while the thin solid line indicates the levels of the NF. Error bars represent ±1 SDs. The gray horizontal bar around 10 kHz represents the frequency spectrum extent of the OBN exposure for the plots of this figure and Figure 3.

slightly more restrictive frequency range, i.e., from about 12 to 21 kHz.

Figure 3 illustrates the OBN-induced reductions in DPOAEs, as described by the plots of Figure 2, in terms of change from baseline levels (open squares) at 28 days postexposure for both 3-month-old (Figs. 3A–D) and 7-month-old (Figs. 3E–H) mice for equilevel primaries at 75 dB SPL. Also noted on these "difference" plots for comparative purposes are the corresponding age-induced changes in DPOAEs (black squares). The solid bold line indicates the maximum DPOAE loss that could be produced by the noise exposure based upon the difference between the postexposure NF and the pre-exposure DPOAE level. In addition, Table 2 lists for all strains tested and at two exposure ages the statistically significant DPOAE level decrements (post minus pre) elicited by the three equilevel primary-tone protocols at 28 days post-OBN exposure that could not be attributed to the aging process (i.e. losses from aging also had to be significant from the noise-induced reductions).

For the purpose of statistical analyses, as noted in the Methods section, the tested frequency range was collapsed into seven corresponding frequency bins. It is clear from the plots of Figure 3 and the data in Table 2 that the CBAs (Figs. 3A, E) essentially were not affected by age-related or noise-induced changes at either exposure age. Interestingly, although slightly reduced DPOAEs were present at the later exposure age of 7 months, particularly for test frequencies >25 kHz, this decrement was not significantly different but rather the result of increased variability among the participating subjects. Thus, these data indicate that CBAs were not, in general, affected by either advancing age or noise overexposure, at least in response to the exposure and measurement parameters used here. Thus, for ages ≤7 months, CBAs represented reasonable control subjects for investigating age and / or noise susceptibility in mice.

The plots of Figures 3B and F and the 65- and 75-dB SPL data for BALB mice in Table 2 show that noise-exposed BALBs exhibited a significant loss in DPOAEs across the entire frequency range that was significantly different from the age-induced decreases observed, particularly for the 3-month-old animals. Thus, a comparison of the two sets of data shows that the low GM frequencies, i.e., those <15 kHz, were more susceptible to the OBN when exposed at 3 months rather than at 7 months. Note that frequencies >20 kHz for 3-month-old BALBs and the midfrequencies at 20–30 kHz for the 7-month-old mice in general were reduced to NF levels as defined by the bold solid line representing the post-NF minus pre-DPOAE levels. Thus, it is clear that the decrease in DPOAE levels for the mid- to high frequencies observed for both mouse ages resulted specifically from the noise overstimulation, since the maximum possible losses (bold solid lines) were well-separated from their aging counterparts at these frequencies. However, it is important to note that while the older mice of Figure 3F exhibited reduced DPOAEs for the midfrequencies (~20–30 kHz) that were caused by the overstimulation, the decreased high-frequency DPOAEs were most likely the result of aging. Taken together, these results indicate that if exposed at an early age BALBs were more susceptible to noise-induced DPOAE decrements before more advanced age-related cochlear dysfunction occurred, and that the decrease in DPOAE levels at 7 months observed for the mid-GM frequencies, resulted from a permanent OBN-induced dysfunction.

Comparable plots for the C57 strain are illustrated in Figures 3C and G. It is clear from these representations and from the findings noted in Table 2 that DPOAE levels for C57s were significantly decreased, primarily as a result of the OBN exposure at 3 months of age (Fig. 3C) and, in contrast, as a result of the aging process at 7 months of age (Fig. 3G). For 3-month-old C57 mice, the maximum amount of cochlear dysfunction was observed at an octave or more above the center frequency of the 10-kHz OBN exposure. In contrast, for mice exposed at 7 months of age, the OBN-induced reductions in DPOAE levels for the mid- to high frequencies were not significantly different (Table 2) from the decrements observed for the high-frequency DPOAEs associated with the aging process because at this age DPOAEs were already at NF levels. Together, these results indicate that C57s, like the BALBs, were susceptible to sound overexposure at an early age. However, after significant age-related cochlear dysfunction, determinations of the effects of noise exposure apart from the effects of aging were difficult, if not impossible (e.g., at frequencies where DPOAEs were at the NF before the exposure, as in Fig. 3G). However, it can be noted that, at the 3-month exposure, C57s appeared somewhat less susceptible to noise-induced DPOAE decrements than the BALBs, particularly at the lower frequencies that were <15 kHz.

As the plots of Figures 3D and H show, the WB strain also exhibited permanent changes in DPOAE levels as a result of the OBN exposure. For example, DPOAEs for the young WBs at the 3-month exposure were also affected over the low- to midfrequency range, where DPOAEs were still measurable. It is also clear that there was little difference between the 3- and 7-month groups, with the advancing AHL dysfunction of the WBs obscuring even more of the high-frequency effects above 20 kHz at both exposure ages.

In general, the different primary-level protocols identified similar differences between the four strains. The results noted in Table 2 also indicate that DPOAEs elicited with higher-level primaries, at $L_1 = L_2 = 65$ and

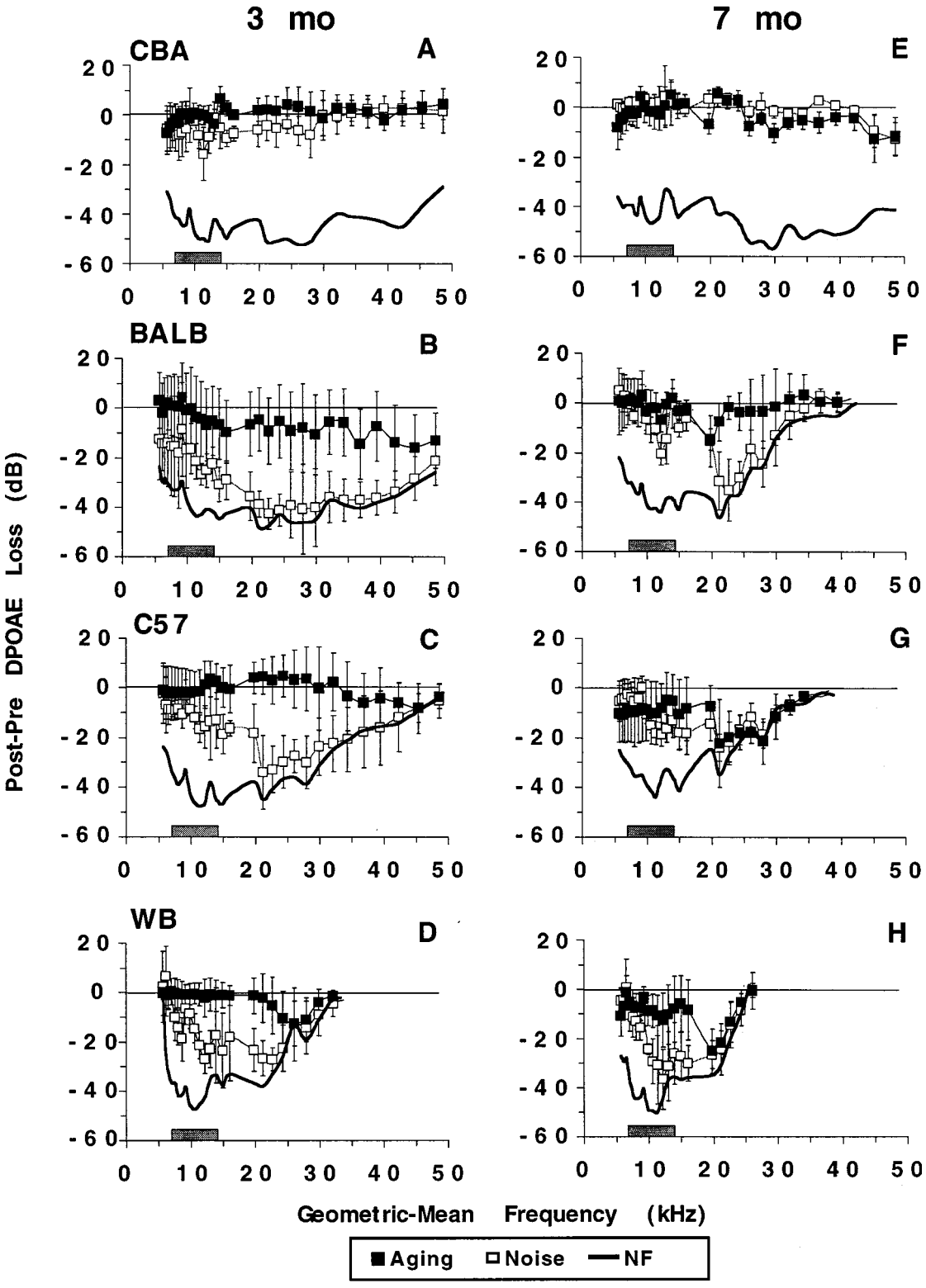

FIG. 3. Average DPOAE difference levels, i.e., post- minus pre-exposure emission levels, for CBA (**A, E**), BALB (**B, F**), C57 (**C, G**), and WB (**D, H**) strains, 28 days after the OBN noise exposure (□) and for the two exposure ages of 3 months (**A–D**, $n = 5$) and 7 months (**E–H**, $n = 4$). Also plotted are the DPOAE changes for "control"; aging mice, i.e., 4 minus 3 months and 8 minus 7 months (■). This curve indicates the amount of loss that can be attributed to aging. DPOAE level is plotted as a function of the GM frequency at the representative stimulation level of $L_1 = L_2 = 75$ dB SPL. Error bars represent ±1 SD. The horizontal line at 0 on the *y* axis represents no change from pre-exposure, baseline levels. The bold solid line indicates the maximum DPOAE difference (post-NF minus pre-DPOAE levels) that could be obtained following noise exposure, if the exposure reduced the DPOAEs to the NF. Note that, with advanced AHL (e.g., **G, H**), this line meets the aging control condition and no DPOAEs could be measured, particularly, for the highest test frequencies. When DPOAEs were absent in the pre-exposure period, the symbols were omitted.

75 dB SPL, detected slightly more statistically different changes between pre- and postexposure-related DPOAE levels because, at these levels, DPOAEs were further from the NF and less variable. For example, the 75-dB SPL primaries identified statistical differences for WBs at the low frequencies (for bin b) that were not identified by the other two primary-level protocols. Again, the identified significant differences mirrored the major findings of the plots of Figures 2 and 3 in that, whereas the CBAs showed no permanent noise-induced effects on DPOAE levels at either exposure age, the OBN reduced the remaining high-frequency emissions for the young 3-month-old BALBs.

DISCUSSION

The present findings revealed that when four mouse strains, with differential rates of AHL, were exposed to a brief tonal exposure, they were substantially affected according to postexposure measures of DPOAEs. Although by 6 months of age there were differences between the strains with respect to the amount of tonal-induced DPOAE loss when exposed at 2.5 months, all four strains reacted similarly to the brief overexposure. In contrast, for the OBN exposures that produced permanent DPOAE losses, all strains exhibiting accelerated AHL, whether exposed at 3 or 6 months, were extremely susceptible to its effects, while CBA mice remained unaffected. Although these groups experienced a brief tonal exposure prior to the permanently damaging noise, since all strains were treated identically, it is highly unlikely that the differences between strains can be attributed to this confounding variable. Together, these results suggest that the mechanisms involved in temporary and permanent noise-induced dysfunction are probably distinct, as recently suggested by the histopathological experiments of Nordmann et al. (2000). In addition, the genetic defect(s) that predisposes these mouse strains to accelerated AHL appears to render the cochlea much more susceptible to noise overexposures that are capable of producing permanent dysfunction.

Of the early-onset strains, the BALB inbred strain, which has the slowest rate of AHL as measured by DPOAEs (Jimenez et al. 1999), showed the most extensive cochlear dysfunction at 2.5 months of age as a result of the brief tonal overexposure. In contrast, the WBs, with the fastest rate of AHL, exhibited the least initial cochlear dysfunction at this age. The C57s, with a rate of AHL between the BALB and WB strains, and CBAs, with very late AHL, displayed intermediate initial DPOAE level decrements and comparable recovery responses to the reversible tonal exposure paradigm at 2.5 months of age. The DPOAE losses remaining at 5 minutes postexposure could be ordered based upon the initial loss, and, consequently, recovery toward baseline at this time was greater for the WB strain.

Surprisingly, at 6 months of age, the tonal exposure resulted in highly similar initial and final DPOAE decrements for all strains, regardless of their propensity to exhibit AHL. Because the CBA strain has been shown to be more susceptible to noise exposure at 1 vs. 3 months of age (Li 1992; Li et al. 1993), it is tempting to speculate that the differences between the four strains at 2.5 months may be due to subtle developmental effects, even though previous studies suggest that the OHCs are mature at this age. Overall, as measured by DPOAEs, the rate of developing AHL does not seem to be an important determinant in the susceptibility of OHC function to very brief acoustic overexposures.

The permanently damaging OBN paradigm, given at 3 months of age, was chosen on the basis that at this age only the WB strain exhibits severe DPOAE level decrements in the high-frequency range as a result of aging processes, while the BALBs, C57s, and CBAs show substantial DPOAE responses to all but the highest test frequencies. The paradigm was repeated at 7 months of age to allow the comparison of the effects of the OBN on the BALBs, C57s, and WBs, all of which show more advanced age-related DPOAE losses at this time, while no losses are apparent for the CBAs. The CBA strain, which exhibits a late-onset AHL characterized by decrements in high-frequency DPOAEs (Parham et al. 1999) and ABR threshold increases starting at about 17 months of age (Henry and Chole 1980; Henry 1983; Li and Borg 1991; Erway et al. 1993; Parham et al. 1999; Willott et al. 1998), was not susceptible to the permanent damaging sounds of the present study. This outcome was true for both exposure ages and in response to any of the three test-stimulation levels of $L_1 = L_2 = 55$, 65, or 75 dB SPL. Thus, the CBAs served as a control in that they retained their resistance to noise overstimulation up to the age of 7 months, which represented the oldest age that was subjected to the OBN-exposure paradigm.

In contrast, BALBs, C57s, and WBs, which exhibit differential rates of early-onset AHL (Erway et al. 1993; Jimenez et al. 1999), showed dramatically increased susceptibilities to noise overstimulation. The BALBs, which exhibit the slowest rate of age-related cochlear dysfunction of the three AHL strains (Jimenez et al. 1999), displayed large, permanent, DPOAE decrements over the entire frequency range and at all levels of stimulation when exposed at 3 months. Similarly, the C57s, with an intermediate rate of AHL, and the WBs, which have the fastest rate of AHL of the three age-sensitive strains (Jimenez et al. 1999), were both permanently affected by the OBN paradigm at this age. Because these three strains had considerably different

amounts of AHL at the time of OBN exposure, ranking them with respect to susceptibility was generally not possible. However, the results suggest that susceptibility to the adverse effects of acoustic overstimulation is not dependent on the rate of progression of AHL, at least for these three mouse strains, i.e., the BALBs, with the slowest rate, were affected as much if not more than the other two strains. When exposed at 7 months, a time at which AHL had progressed further for the three early-onset strains, their susceptibility to noise aftereffects did not increase. In addition, the BALBs' susceptibility in the low frequencies <20 kHz was decreased compared with the comparable results at 3 months. Thus, advancing AHL did not appear to increase the susceptibility to noise in the frequency regions where emissions could be measured for all strains, a finding consistent with the 3-month data in which the WBs, with the most advanced AHL, were not demonstrably more susceptible than the BALBs or C57s. In fact, it appeared that advancing AHL may have tended to make these strains more resistant to noise, possibly because the OHCs became more difficult to stimulate as the damage process related to aging progressed. Overall, the present work agrees with previous studies that have shown that the C57 strain is more susceptible to noise-induced damage than the CBA strain, regardless of the experimental noise exposure, which consisted of a number of various levels and frequencies (Shone et al. 1991; Li 1992; Li et al. 1993; Erway et al. 1996; Miller et al. 1998; Davis et al. 1999). However, the suggestion that advancing age predisposes the ear to increased susceptibility to NIHL (Miller et al. 1998) was not supported.

The results of prior studies (Erway et al. 1993; Zheng et al. 1999) suggested that several genes may be involved in the early-onset hearing losses observed in the AHL strains. However, more recent results (Johnson et al. 2000) indicate that a single gene (*Ahl*) is sufficient. Because the *Ahl* gene appears to be the essential requirement for AHL, at least for strains that exhibit early-onset aging at ages <12 months old (Johnson, personal communication), it is possible that this same gene is responsible for the increased noise-induced susceptibility observed for the C57 and BALB strains, which carry at least one form of the *Ahl* gene (Johnson et al. 1997; Willott et al. 1998), and possibly for WB, which has not yet been tested for this gene. In contrast, the *Ahl* gene has not been observed in CBAs. Other AHL modifier genes, or alleles, that may be involved in determining the rate of AHL (Johnson, personal communication) may be responsible for rendering the cochlea more susceptible to NIHL in some inbred mouse strains. However, the findings that all of the early-onset AHL strains reacted more or less the same to the permanently damaging OBN, regardless of the rate of AHL, support the notion that the defect responsible for the greatly enhanced susceptibility to noise overexposures of these strains may, in fact, have a single-gene basis.

In summary, the present findings revealed that, in response to a temporarily damaging exposure, all four strains were susceptible, especially at 6 months. The similarity across the strains of the 6-month temporary results, when compared with the permanent effect of the OBN exposure, supports the notion that the temporary versus the permanent effects of noise exposure involve significantly different mechanisms. This inference follows from the findings that all of the early-onset strains were much more susceptible than the CBA strain to permanent OBN-induced dysfunction. The eventual determination of the differences between early-onset AHL strains and the CBA strain may give some important insights into the processes that determine the susceptibility of OHCs to permanent noise damage.

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REFERENCES

CLARK WW, BOHNE BA. Animal model for the 4-kHz tonal dip. Ann. Otol. Rhinol. Laryngol. 87(Suppl 51):1–16, 1978.

CODY AR, ROBERTSON D. Variability of noise-induced damage in the guinea pig cochlea: electrophysiological and morphological correlates after strictly controlled exposures. Hear. Res. 9:55–70, 1983.

DAVIS RR, CHEEVER ML, KRIEG EF, ERWAY LC. Quantitative measure of genetic differences in susceptibility to noise-induced hearing loss in two strains of mice. Hear. Res. 134:9–15, 1999.

ERWAY LC, SHIAU Y-W, DAVIS RR, KRIEG EF. Genetics of age-related hearing loss in mice. III. Susceptibility on inbred and F1 hybrid strains. Hear. Res. 65:123–132, 1996.

ERWAY LC, WILLOTT JF, ARCHER JR, HARRISON DE. Genetics of age-related hearing loss in mice: I. Inbred and F1 hybrid strains. Hear. Res. 65:125–132, 1993.

HENRY KR. Aging and audition. In: Willott JF, (ed) The Auditory Psychobiology of the Mouse. Charles C. Thomas, Springfield, IL, 1983, pp. 470–493.

HENRY KR, CHOLE RA. Genotypic differences in behavioral, physiological and anatomical expressions of age-related hearing loss in the laboratory mouse. Audiology 19:369–383, 1980.

JIMENEZ AM, STAGNER BB, MARTIN GK, LONSBURY–MARTIN BL. Age-related loss of distortion product otoacoustic emissions in four mouse strains. Hear. Res. 138:91–105, 1999.

JOHNSON KR, ZHENG QY, ERWAY LC. The same gene on Chromosome 10 is a major contributor to age-related hearing loss (AHL) in 10 inbred strains of mice. Assoc. Res. Otolaryngol. Abstr. 23:219–220, 2000.

JOHNSON KR, ERWAY LC, COOK SA, WILLOTT JF, ZHENG QY. A major gene affecting age-related hearing loss in C57BL/6J mice. Hear. Res. 114:83–92, 1997.

LI H-S. Influence of genotype and age on acute acoustic trauma and recovery in CBA/Ca and C57BL/6J mice. Acta Otolaryngol. 112:956–967, 1992.

LI H-S, BORG E. Age-related loss of auditory sensitivity in two mouse genotypes. Acta Otolaryngol. 111:827–834, 1991.

LI H-S, HULCRANTZ M, BORG E. Influence of age on noise-induced permanent threshold shifts in CBA/Ca and C57BL/6J mice. Audiology 32:195–204, 1993.

MARTIN GK, LONSBURY–MARTIN BL, PROBST R, SCHEININ SA, COATS AC. Acoustic distortion products in rabbit ear canal: II. Sites of origin revealed by suppression contours and pure tone exposures. Hear. Res. 28:191–208, 1987.

MILLER JD, WATSON CS, COVELL WP. Deafening effects of noise on the cat. Acta Otolaryngol. Suppl. 176:1–91, 1963.

MILLER JM, DOLAN DF, RAPHAEL Y, ALTSCHULER RA. Interactive effects of aging with noise induced hearing loss. Scand. Audiol. 27 (Suppl 48):53–61, 1998.

NORDMANN AS, BOHNE BA, HARDING GW. Histopathological differences between temporary and permanent threshold shift. Hear. Res. 139:13–30, 2000.

PARHAM K. Distortion product oteacoustic emissions in the C57BL/6J mouse model of age-related hearing loss. Hear. Res. 112:216–234, 1997.

PARHAM K, SUN X-M, KIM DO. Distortion product otoacoustic emissions in the CBA/J mouse model of presbycusis. Hear. Res. 134:29–38, 1999.

SHONE G, ALTSCHULER RA, MILLER JM, NUTTALL AL. The effects of noise exposure on the aging ear. Hear. Res. 56:173–178, 1991.

SULKOSKI W. Some epidemiological data on noise-induced hearing loss in Poland, its prophylaxis and diagnosis. In: Proceedings of International Congress on Noise as a Public Health Problem. U.S. Environmental Protection Agency, Publication #550/9-73008 Washington, DC, 1973, 139–155.

SUTTON LA, LONSBURY–MARTIN BL, MARTIN GK, WHITEHEAD ML. Sensitivity of distortion-product otoacoustic emission to tonal overexposure: Time course of recovery and lowering L_2. Hear. Res. 75:161–174, 1994.

TAYLOR W, PEARSON J, MAIR A. Study of noise and hearing in jute weaving. J. Acoust. Soc. Am. 38:113–120, 1965.

WARD WD. Susceptibility to TTS and PTS. In: Proceedings of International Congress on Noise as a Public Health Problem. U.S. Environmental Protection Agency, Publication #550/9-73008 Washington, DC, 1973, 281–292.

WHITEHEAD ML, MCCOY MJ, LONSBURY–MARTIN BL, MARTIN GK. Dependence of distortion-product otoacoustic emissions on primary levels in normal and impaired ears. I. Effects of decreasing L_2 below L_1. J. Acoust. Soc. Am. 97:2346–2358, 1995.

WILLOTT JF, ERWAY LC. Genetics of age-related hearing loss in mice. IV. Cochlear pathology and hearing loss in 25 B $\times$ D recombinant inbred mouse strains. Hear. Res. 119:27–36, 1998.

WILLOTT JF, TURNER JG, CARLSON S, DING D, BROSS LS, FALL WA. The BALB/c mouse as an animal model for progressive sensorineural hearing loss. Hear. Res. 115:162–174, 1998.

ZHENG QY, JOHNSON KR, ERWAY LC. Assessment of hearing in 80 inbred strains of mice by ABR threshold analyses. Hear. Res. 130:94–107, 1999.