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Noise-induced threshold shift dynamics measured with distortion-product otoacoustic emissions and auditory evoked potentials in chinchillas with inner hair cell deficient cochleas

Roger P. Hamernik *, William A. Ahroon, Brenda M. Jock, Jessie A. Bennett

Auditory Research Laboratory, State University of New York at Plattsburgh, 107 Beaumont Hall, 101 Broad Street, Plattsburgh, NY 12901, USA

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Abstract

Chinchillas ($n=6$) were treated with carboplatin and, following a 30-day recovery period, were exposed to a 115 dB peak SPL impact noise presented at a rate of 1/s for 6 h/day for 10 days. A second group ($n=6$) received only the noise treatment. Cubic distortion product otoacoustic emissions ($2f_1-f_2$) and auditory evoked potential (AEP) detection thresholds in response to tone bursts were measured before and 30 days after drug treatment and following the first and 10th day of the noise exposure. Thirty days after the final exposure day, permanent changes in AEP detection thresholds and emissions were measured and cochleograms constructed. The drug treatment eliminated over 80% of the inner hair cells (IHC) in the cochlea, leaving the outer hair cell (OHC) population essentially intact prior to the interrupted noise exposure. The drug treatment alone had very little or no effect on AEP detection thresholds and emission metrics. Following the noise exposure, the IHC-deficient animals showed clear 'toughening' effects in the AEP and emission measures which were the same as measured in the group receiving only the noise. After a 30-day post-exposure recovery period, AEP thresholds were elevated about 10 dB at the low frequencies in the drug-noise group whereas emissions returned to near normal despite the massive IHC losses. These results are consistent with the idea that an intact OHC population is required for toughening. However, sound-evoked efferent pathways activated by the few remaining IHCs ($\sim$20%) which, in this preparation, are distributed throughout the cochlea, may still contribute significantly to the toughening phenomena.

Key words: Carboplatin; Otoacoustic emission; Threshold shift dynamics; Noise effect

1. Introduction

A variety of noise exposure paradigms have the effect, in the exposed animals, of enhancing the cochlea's resistance to noise exposure as measured by temporary and permanent threshold shifts, otoacoustic emissions and sensory cell losses (Clark et al., 1987; Canlon et al., 1988; Boettcher et al., 1992; Subramaniam et al., 1994). There are essentially two broad classes of noise experiments that are commonly used to study the development of resistance to noise. (1) Priming exposures, in which a high-level noise is preceded by a low-level exposure that produces very little or no threshold shift (TS). The effect of the low-level priming noise is to reduce the amount of trauma produced by the high-level exposure (i.e., to toughen the ear; Canlon et al., 1988). (2) Interrupted noise exposures such as those of Clark et al. (1987), Clark and Bohne (1992) and others that showed an improvement in pure-tone thresholds over time despite the continued, regularly repeated (interrupted) exposures. The extent of this improvement in threshold or the developed resistance to noise as reflected in TS measures is referred to as 'toughening' and is affected by the level, spectrum, and temporal variables of the noise stimulus (Franklin et al., 1991; Subramaniam et al., 1991a,b; Henderson et al., 1992; Hamernik et al., 1994). The development of resistance to noise is, in part, influenced by the efferent system as

* Corresponding author.
Tel.: +1 (518) 564-7700; Fax: +1 (518) 564-7827;
E-mail: AhroonWA@SPLAVA.CC.PLATTSBURGH.EDU

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well as by processes inherent in the sensory cells themselves (Rajan, 1996; Zheng et al., 1997).

Previous experiments have shown that carboplatin administration in the chinchilla has its effect primarily on the inner hair cells (IHCs) leaving the outer hair cells (OHCs) essentially unaffected (Takeno et al., 1994a,b; Wake et al., 1993, 1994). The viability of the remaining OHCs was inferred from the normal $2f_1-f_2$ distortion product otoacoustic emissions (DPOAEs) that were recorded from the IHC-deficient cochleas (Jock et al., 1996; Trautwein et al., 1996). Also in the Jock et al. (1996) study the animals' thresholds, estimated from the AEP recorded from the inferior colliculus (IC), were shown to be normal, and the response of the animals to an asymptotic threshold shift-producing noise was also essentially normal despite severe losses of IHCs. These results indicate that the intact OHC system is primarily responsible for the generation of the $2f_1-f_2$ DPOAEs. In addition, they also suggest that the first approximately 50 dB of changes in AEP thresholds induced by noise are either controlled by the OHC system or that very few IHC are required to elicit normal quiet AEP thresholds and TS responses following an uninterrupted noise exposure. In this paper evidence is presented that is consistent with the idea that the OHC system is responsible for the developing resistance to TS (toughening) produced by repeated (interrupted) noise exposures.

2. Methods

Six chinchillas were treated with carboplatin (Bristol Laboratories, Paraplatin-AQ™). A 75 mg/kg dosage of Paraplatin-AQ was administered via intravenous (i.v.) injection using a vein in the pinna. The animals were allowed to recover for 30 days, following which they were exposed to an interrupted 115 dB peak SPL impact noise. Six additional chinchillas that received no drug treatment were exposed to the same impact noise in order to define the normal pattern of TS dynamics produced by the noise. Detection thresholds for tone burst stimuli were estimated using the AEP recorded from the IC and cochlear changes were estimated from the DPOAEs and surface preparation histology.

2.1. Surgical preparation

Each animal was anesthetized [intramuscular (i.m.) injection of ketamine (35 mg/kg body weight) and xylazine (1 mg/kg body weight)] and made monaural by the surgical destruction of the left cochlea. A bipolar, platinum electrode with electrode lengths of 7.5 mm (probe) and 2.5 mm (ground) was implanted into the left IC under stereotaxic control for single-ended recordings of the AEP (Henderson et al., 1973; Salvi et al., 1982). A xylazine-reversing agent [yohimbine, Lloyd Laboratories (2 mg/kg body weight, i.m.)] was administered after the surgical procedure. The animals were allowed to recover for at least 2 weeks before AEP testing began. Additional details of the AEP procedures and surgery can be found in Ahroon et al. (1993).

2.2. Threshold testing

The animals were awake during AEP testing and restrained in a yoke-like apparatus to maintain the animal's head in a constant position within the calibrated sound field (Blakeslee et al., 1978). AEPs were collected to 20 ms pure-tone bursts with 5 ms rise/fall times, presented at a rate of 10/s. A general purpose computer was used to acquire the AEP data and control the frequency, intensity, and timing of the stimulus. The electrical signal from the implanted electrode was amplified (50 000×), filtered (30 Hz to 3000 Hz), and sampled using an analog-to-digital converter at 20 000 samples/s (50 μs period) over 500 points to obtain a 25 ms sampling window. Each digitized waveform was analyzed, on line, for large amplitude artifacts, and if present, the sample was rejected from the average and another sample taken. Averaged AEPs were obtained from 250 presentations of the 20 ms signal. Each waveform was stored to be used in threshold determination following the completion of the test stimulus intensity series.

Thresholds were estimated from each tone-burst intensity series using 5 dB steps at octave intervals from 0.5 to 16 kHz and at 11.2 kHz, resulting in seven test frequencies. Threshold was determined to be one half step size (2.5 dB) below the lowest intensity that showed a response consistent with the responses seen at higher intensities. The average of at least three separate threshold determinations at each frequency obtained on different days was used to define the pre-exposure audiogram. Thirty days following the drug injection, thresholds were tested again using the pre-treatment protocol and the average of three threshold measurements at each frequency was used to construct the post-drug-injection audiogram. This AEP audiogram also represents the pre-noise exposure audiogram. Permanent threshold shift (PTS) was defined as the difference between the post-treatment and pre-treatment thresholds at each test frequency. During the 10-day noise exposure a complete AEP threshold series was measured daily immediately prior to exposure and immediately following exposure in order to establish the amount of the toughening effect produced by the 10-day interrupted noise exposure. The amount of toughening was defined as the difference between the immediate post-exposure TS measured on day 1 and day 10.

For those animals that received the drug and the

noise, the noise exposure began immediately following the 30-day post-drug-injection test protocol. At least 30 days after the noise exposure, final AEP audiograms were constructed for all animals using the average of three separate threshold determinations at each of the pre-exposure frequencies using the pre-exposure AEP protocol. Noise-induced PTS was defined as the difference between the 30-day post-exposure and pre-exposure AEP thresholds at each individual test frequency.

2.3. Otoacoustic emissions testing

DPOAEs were measured in the ear canal of the awake but restrained animal (Hargett et al., 1986) with the Etymotic ER-10C probe assembly using CUB^{e}DIS v2.40 software. The $2f_1-f_2$ DPOAE was measured at 32 points per octave. The set of 81 DPOAEs collected at 81 different frequencies for a fixed set of primary levels is referred to as the DPgram. This set of 81 emissions was divided into one-third octave band bin widths and the emissions within that third-octave band averaged to obtain a single datum point representative of the DPOAE within that frequency band. This resulted in a DPgram with 12 data points. The following parameters were used in collecting the DPOAEs: 1.0 kHz $\leq f_2 \leq$ 10 kHz, where f_2 is the higher frequency primary tone; $f_2/f_1 = 1.22$; $L_1 = L_2+10$ dB, where L_1 and L_2 are the levels of f_1 and f_2 respectively. The averaging time was constant at 2 s. The level of L_1 was varied between 35 and 65 dB in 10 dB steps to produce four DPgrams. All DPOAE data points were plotted as a function of the geometric mean frequency of the primaries. The average of the three pre- and three post-treatment DPOAE measurements was used to establish permanent treatment effects. During the interrupted noise exposure a single set of DPgrams (i.e., one DPgram for each primary level) was acquired at the end of the first and tenth day of exposure. These two measures established the amount of toughening based on the DPOAE metric.

2.4. Noise exposure

The spectrally narrow noise impacts were synthesized using a Macintosh 840AV computer system and the LabVIEW™ graphical programming language. A digital pulse was synthesized and passed through a 400 Hz wide digital Butterworth filter centered at 1.0 kHz. The impulse was played at 1 impulse/s through an AB International amplifier and an Altec/Lansing Model 299-8A high frequency driver with Model 84666 transition piece and MR 94B horn. The pressure-time waveform and spectrum of the 115 dB peak SPL impact are shown in Fig. 1. Animals were individually exposed, 6 h/day for 10 consecutive days.

2.5. Histology

All animals were killed under sodium pentobarbital anesthesia 32 days after the noise exposure. Cochleas were immediately removed and perfused with 2.5% glutaraldehyde and post-fixed in 1% osmium tetroxide, both with veronal acetate buffer at 7.3 pH. Following complete removal of the basilar membrane with the attached sensory epithelium, the tissue was mounted in glycerin on glass slides. The sensory cell population was assessed using differential interference contrast microscopy at 400× magnification. Cochleograms were constructed by computing the percent IHC and OHC loss across adjacent 0.24 mm segments of the sensory epithelium over the entire length of the organ of Corti. Cell loss as a function of basilar membrane position was plotted as a function of frequency using the chinchilla frequency-place map constructed by Eldredge et al. (1981).

2.6. Analysis

The data were analyzed using mixed model analyses of variance with repeated measures on at least one factor (frequency). The probability of a type I error was set at 0.05. Throughout the remainder of this manuscript, F ratios, degrees of freedom and associated probabilities are indicated for these analyses by superscript numbers referring to Table 1. Complete analysis of variance summary tables are available upon request. The error bars in the figures represent one standard error of the mean. If the error bar is not present, it was less than the size of the symbol depicting the group mean.

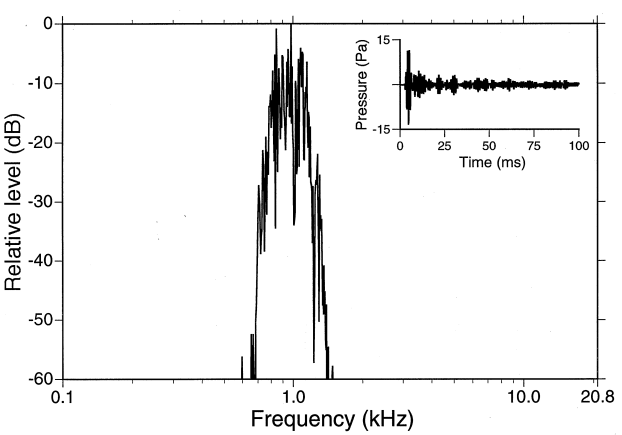

Fig. 1. The spectrum and pressure-time history of the narrow band impact used for the interrupted noise exposure. Exposure: 1.0 kHz narrow band impact; 115 dB peak SPL, 1 impact/s, 6 h/day for 10 days.

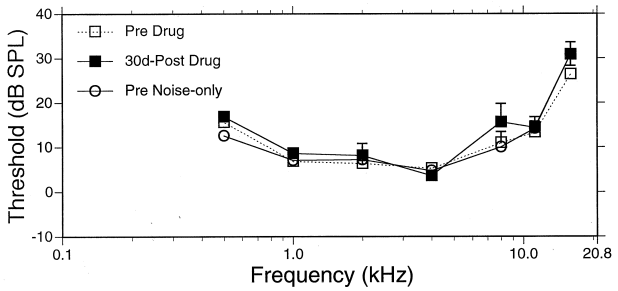

Fig. 2. Group mean AEP thresholds measured before and at least 30 days following administration of carboplatin (squares). Also shown are the group mean pre-noise-exposure AEP thresholds for the noise-only group.

2.7. Animal care

The care and use of the animals reported on in this study were approved by the Plattsburgh State University of New York Institutional Animal Care and Use Committee. In conducting the research described in this report, the investigators adhered to the Guide for Care and Use of Laboratory Animals, as promulgated by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources Commission on Life Sciences, National Academy of Sciences-National Research Council, revised 1985.

3. Results

3.1. Carboplatin treatment effects

3.1.1. AEP thresholds

Fig. 2 shows the group mean AEP thresholds for the drug-noise group prior to any drug treatment and 30 days following injection with carboplatin. Also shown in this figure are the pre-noise-exposure mean AEP thresholds for the group that was given only the interrupted noise exposure (i.e., the noise-only group). An analysis of variance performed on the thresholds measured before and 30 days following the administration of the drug established the drug-treatment effects. There was no statistically significant main effect of drug treatment[1] on AEP thresholds nor was the interaction between frequency and drug treatment statistically significant[2]. The main effect of frequency was statistically significant[3] but this is expected based upon our knowledge of the chinchilla audiogram (Fay, 1988). Statistically significant main effects of frequency are expected in most of the following analyses. For this reason, specific F ratios and degrees of freedom for this main effect are not given. From the statistical analyses of the data in Fig. 2 it is clear that the administration of carboplatin had no effect on the group mean pure-tone AEP thresholds.

Table 1
Results of mixed model analyses of variance

1		$F = 2.345$, df = 1/5, $P = 0.1863$
2		$F = 0.839$, df = 3/60, $P = 0.5497$
3		$F = 20.218$, df = 6/30, $P < 0.0001$
4	65 dB:	$F = 11.883$, df = 1/5, $P = 0.0183$
	55 dB:	$F = 20.395$, df = 1/5, $P = 0.0063$
	35 dB:	$F = 18.392$, df = 1.5, $P = 0.0078$
5	65 dB:	$F = 4.394$, df = 11/55, $P = 0.0001$
	55 dB:	$F = 3.932$, df = 11/55, $P = 0.0003$
6		$F = 0.791$, df = 11/55, $P = 0.6475$
7	Main effect:	$F = 5.524$, df = 1/5, $P = 0.0655$
	Interaction:	$F = 1.007$, df = 11/55, $P = 0.4525$
8		$F = 0.026$, df = 1/10, $P = 0.8743$
9		$F = 1.183$, df = 6/60, $P = 0.3277$
10		$F = 1.843$, df = 1/10, $P = 0.2044$
11		$F = 0.736$, df = 6/60, $P = 0.6225$
12	65 dB:	$F = 2.060$, df = 1/10, $P = 0.1817$
	55 dB:	$F = 0.006$, df = 1/10, $P = 0.9390$
	45 dB:	$F = 1.832$, df = 1/10, $P = 0.2057$
	35 dB:	$F = 0.301$, df = 1/10, $P = 0.5953$
13	65 dB:	$F = 8.808$, df = 1/10, $P = 0.0141$
	55 dB:	$F = 2.272$, df = 1/10, $P = 0.1627$
	45 dB:	$F = 0.538$, df = 1/10, $P = 0.4803$
	35 dB:	$F = 2.706$, df = 1/10, $P = 0.1310$
14		$F = 7.055$, df = 5/45, $P = 0.0001$
15		$F = 0.009$, df = 1/9, $P = 0.9281$
16		$F = 0.868$, df = 5/45, $P = 0.5101$
17	65 dB:	$F = 0.001$, df = 1/9, $P = 0.9703$
	55 dB:	$F = 0.324$, df = 1/9, $P = 0.5833$
	45 dB:	$F = 0.808$, df = 1/9, $P = 0.3921$
	35 dB:	$F = 2.863$, df = 1/9, $P = 0.1249$
18	65 dB:	$F = 1.880$, df = 11/99, $P = 0.0510$
	55 dB:	$F = 1.813$, df = 11/99, $P = 0.0615$
	45 dB:	$F = 1.145$, df = 11/99, $P = 0.3351$
	35 dB:	$F = 0.588$, df = 11/99, $P = 0.8342$
19		$F = 8.336$, df = 1/9, $P = 0.0180$
20		$F = 4.265$, df = 6/54, $P = 0.0008$
21		$F = 2.708$, df = 6/54, $P = 0.0362$
22	65 dB:	$F = 0.444$, df = 1/9, $P = 0.5219$
	55 dB:	$F = 8.542$, df = 1/9, $P = 0.0170$
	45 dB:	$F = 2.166$, df = 1/9, $P = 0.1752$
	35 dB:	$F = 2.653$, df = 1/9, $P = 0.1378$
23	65 dB:	$F = 1.582$, df = 11/99, $P = 0.1157$
	55 dB:	$F = 0.962$, df = 11/99, $P = 0.4860$
	45 dB:	$F = 0.965$, df = 11/99, $P = 0.4833$
	35 dB:	$F = 1.031$, df = 11/99, $P = 0.4253$
24	% IHC loss:	$F = 1293.622$, df = 1/10, $P = 0.0001$
	Total IHC loss:	$F = 4270.746$, df = 1/10, $P = 0.0001$
25	% IHC loss:	$F = 4.945$, df = 7/70, $P = 0.0001$
	Total IHC loss:	$F = 19.900$, df = 7/10, $P = 0.0001$
26	% OHC loss:	$F = 6.449$, df = 1/10, $P = 0.0294$
	Total OHC loss:	$F = 5.887$, df = 1/10, $P = 0.0357$
27	% OHC loss:	$F = 1.216$, df = 7/70, $P = 0.3059$
	Total OHC loss:	$F = 1.159$, df = 7/70, $P = 0.3375$
28	% OHC loss:	$F = 2.687$, df = 7/70, $P = 0.0159$
	Total OHC loss:	$F = 3.450$, df = 7/70, $P = 0.0031$

3.1.2. Otoacoustic emissions

The group mean DPOAEs (for $L_1 = 65$ and 35 dB) collapsed across third-octave bands are shown in Fig. 3 prior to any drug treatment and 30 days following injection with carboplatin. Also shown in this figure are the pre-noise-exposure DPOAEs for the group that re-

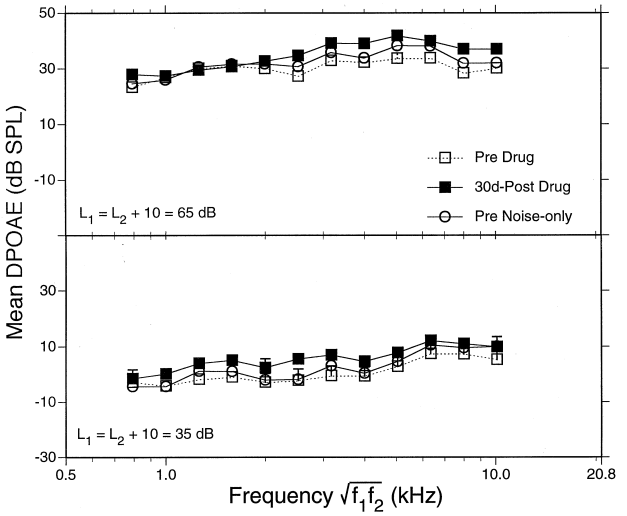

Fig. 3. Group mean DPOAEs measured over third-octave band intervals before and at least 30 days following administration of carboplatin. Also shown are the group mean pre-noise-exposure emissions measured in the noise-only group.

ceived only the interrupted noise exposure. Statistical analyses were performed on DPOAEs individually at each of the four L_1 primary level data sets. The main effect of drug treatment was statistically significant for the DPOAEs evoked by L_1 levels of 65, 55, and 35 dB[4]. In the first two of these analyses (65 and 55 dB L_1 levels) the interaction of treatment and frequency was also statistically significant[5]. The interaction of treatment and frequency was not statistically significant in the analysis of 35 dB L_1 levels[6]. For the 45 dB L_1 test, neither the main effect of drug treatment nor the interaction of treatment and frequency was statistically significant[7]. The main effects of frequency were statistically significant for all analyses. The statistically significant main effect of the carboplatin, seen in Fig. 3, amounts to a slight increase (~5–10 dB) in the DPOAE output across the range of test frequencies 30 days after administration of the drug.

3.2. Pre-exposure audiometric and emissions measurements

3.2.1. AEP thresholds

The pre-exposure thresholds shown in Fig. 2 also were compared using two-way analyses of variance with repeated measures on one factor (frequency). (Note: The 30d-post-carboplatin thresholds represent the pre-exposure thresholds for the drug-treated group.) The first analysis compared the initial thresholds measured before any drug or noise exposure treatments in the two experimental groups. The results indicated that there were no statistically significant main effects of group[8] nor interaction between group and frequency[9]. Thus, the two experimental groups had the same initial AEP thresholds prior to any experimental manipulation.

A similar analysis compared the pre-noise-exposure thresholds (i.e., 30d-post-drug treatment thresholds in the drug-noise group vs. the pre-exposure thresholds for the noise-only group). The results of this analysis showed no statistically significant main effects of treatment group[10] nor interaction between group and frequency[11]. The main effects of frequency were statistically significant for both analyses. Thus, there was no difference in the mean pre-noise-exposure AEP thresholds for the two groups.

3.2.2. Otoacoustic emissions

Similar analyses were performed on the set of DPOAEs for each of the L_1 primary levels used for emissions testing (Fig. 3). Each comparison of pre-exposure DPOAEs was performed using a two-way analysis of variance with repeated measures on one factor (frequency). The first set of analyses compared the initial DPOAEs in the two experimental groups before any drug or noise exposure treatments. All main effects of group were not statistically significant[12]. A parallel analysis of pre-exposure emissions was also run. A statistically significant main effect of treatment group was revealed at only the 65 dB, L_1 level[13], where, as indicated below, the group treated with carboplatin had slightly higher (~5 dB) DPOAE output at a number of test frequencies. In summary, except for the slightly elevated DPOAEs mentioned above, the basic trends in

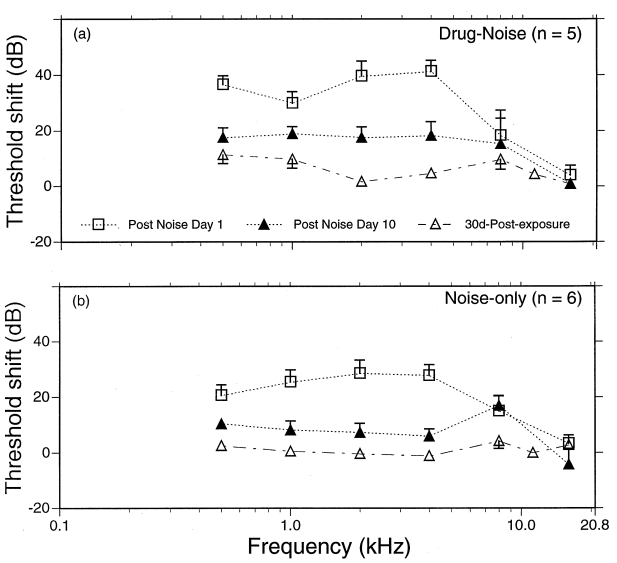

Fig. 4. The group mean TSs for AEPs measured at the indicated test frequencies in animals exposed to the interrupted impact noise: (a) TSs in the drug-noise group; (b) TSs in the noise-only group. Thresholds were measured immediately after the first day's exposure (i.e., after 6 h exposure to impact noise) (□); after the 10th day of exposure (▲), and at 30 days post-noise exposure (△).

the group mean data, shown in Fig. 3, were similar for the two groups.

3.3. Effects of the interrupted noise exposure

3.3.1. AEP threshold shift recovery

Fig. 4 presents the group mean TSs measured following the first 6 h exposure, the tenth 6 h exposure, and 30 days after termination of the exposure for the two experimental groups. (Note: One animal in the drug-noise group developed a defective AEP plug during exposure. The plotted noise effects data for this group indicate an *n* equal to 5. The animal, however, completed the noise exposure protocol and was included in the mean cochleogram data base.) Following the first day of the exposure, both groups showed large TSs through the 4.0 kHz test frequency. After the first 6 h exposure, the drug-noise group showed about a 7–10 dB greater TS than the noise-only group across those test frequencies that were affected by the noise. For the drug-noise group, TSs amounted to between 30 and 40 dB while the noise-only group showed a similar profile of TS but TSs varied from about 20 to 30 dB. The TSs measured in both groups after the tenth day of the noise exposure had decreased (recovered) by about 20–25 dB in the mid-frequencies and, as with the first day's TSs, the group with the drug treatment continued to show about a 10 dB greater TS across the 0.5–4.0 kHz range. Other than the greater TS in the drug-noise group, there were no other transient TS features that differed between the two groups. Both groups showed a clear and consistent decrease in TS with increasing exposure time (toughening) for frequencies from 0.5 through 4.0 kHz.

The TS recovery, or toughening effect produced by the interrupted noise exposure, defined as the difference between the TS following the first day's 6 h exposure and the TS following the tenth 6 h exposure, is presented in Fig. 5 for the two groups. The data presented in Fig. 5 were analyzed using a two-way analysis of variance with repeated measures on one factor (frequency). The main effect of frequency was statistically

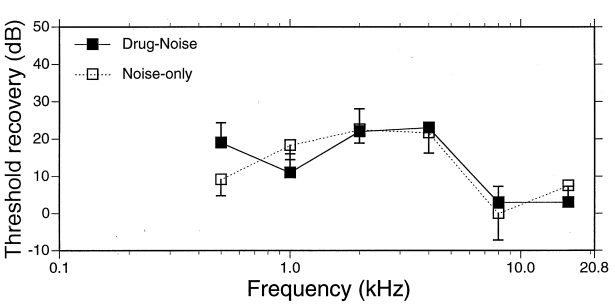

Fig. 5. Group mean AEP TS recovery (toughening) measured as the difference between the first and last day's post-exposure TSs.

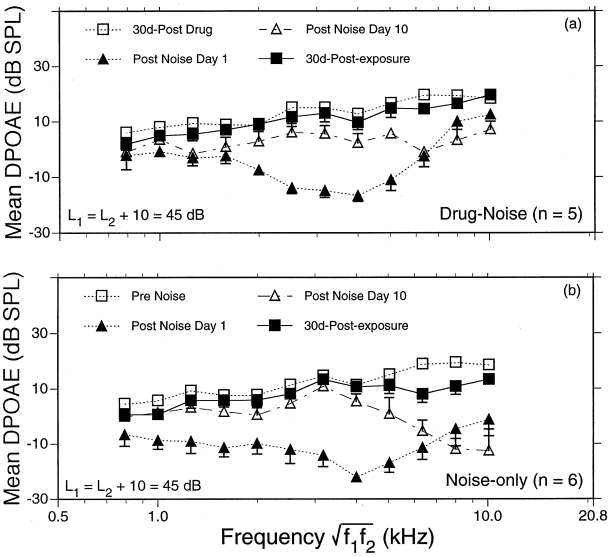

Fig. 6. The group mean DPOAEs measured at the indicated test frequencies in animals exposed to the interrupted impact noise: (a) the drug-noise group; (b) the noise-only group. DPOAEs were measured immediately after the first day's exposure (i.e., after 6 h exposure to impact noise) (▲); after the 10th day of exposure (△), and at 30 days post-noise exposure (■).

significant[14]. The main effect of carboplatin treatment[15] and the interaction between treatment and frequency were not statistically significant[16]. This result indicates that there were no differences in the magnitude of TS recovery (i.e., in toughening) between the group treated with carboplatin and the noise-only group.

3.3.2. Otoacoustic emissions

The interrupted noise effects, as reflected by the DPOAE metric, are shown in Figs. 6 and 7. Immediately following the first day of the 6 h exposure, emissions were depressed across the test frequency range with 30–35 dB reductions of DPOAE output in the mid-frequencies (2.0–5.0 kHz). After the tenth day of the exposure, the DPOAEs in both groups had recovered 20–25 dB in the mid-frequencies. This recovery of emissions output by the tenth day of the exposure protocol is a clear manifestation of the toughening phenomena reflected in the DPOAE metric.

The magnitude of the toughening effect as reflected in DPOAEs, defined as the difference between the level of the emissions following the tenth exposure day and the emissions following the first 6 h exposure, is shown for two of the four primary levels in Fig. 7. The main effect of carboplatin treatment was not statistically significant[17] for any of the levels of L_1. The main effect of frequency was statistically significant for all analyses at all four L_1 levels and the interactions of drug treatment and frequency were not statistically significant[18] at any of the four L_1 levels.

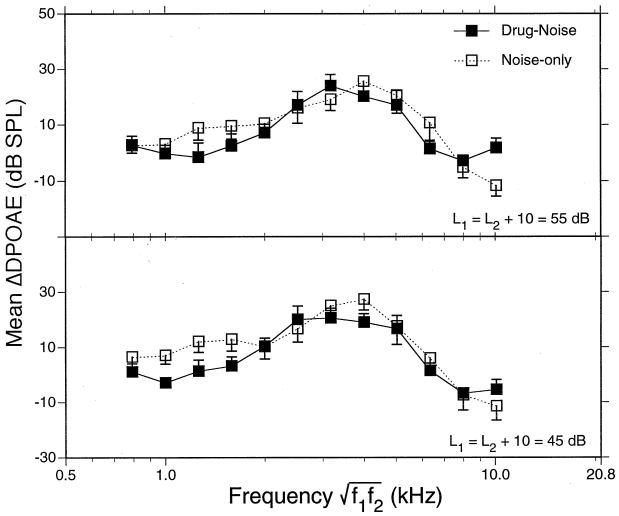

Fig. 7. The change in group mean third-octave band DPOAE (ΔDPOAE) measured as the difference between the tenth and the first day's post-exposure emissions for each of the two experimental groups.

3.4. Permanent effects

3.4.1. Noise-induced permanent threshold shifts

The permanent AEP TSs caused by the 10-day interrupted noise exposure are shown in Figs. 4 and 8. A comparison of the PTS measured in the two groups (Fig. 8) was performed using a two-way analysis of variance with repeated measures on one factor. The results indicated that there were statistically significant main effects of drug treatment[19] and frequency[20] as well as a statistically significant interaction of drug treatment and frequency[21]. The analysis showed that the group treated with carboplatin had significantly more noise-induced hearing loss at most frequencies than did the noise-only group which did not receive the drug treatment prior to noise exposure. The difference between PTS in the drug-noise group and the noise-only group amounted to a maximum of about 10 dB at 0.5 and 1.0 kHz; in the noise-only group PTS was essentially zero.

3.4.2. Otoacoustic emissions

Figs. 6 and 9 summarize the DPOAEs measured for $L_1 = 55$ dB and/or 45 dB in the two groups measured at least 30 days following noise exposure. Analyses of permanent changes (Fig. 9) in emissions were performed for each of the four primary levels using two-way analyses of variance with repeated measures on one factor (frequency). The main effects of frequency were statistically significant for all analyses. The main effect of drug treatment was statistically significant for only the emissions evoked by the 55 dB L_1 level[22]. In this case, the group treated with carboplatin prior to the noise exposure showed slightly greater emissions output than the

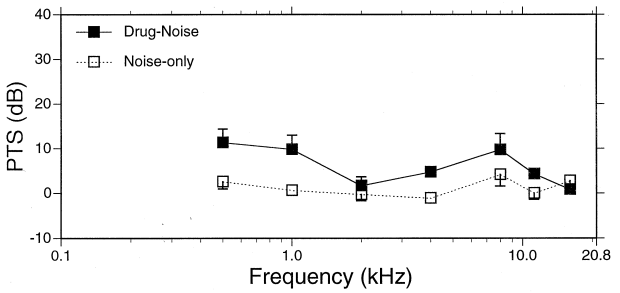

Fig. 8. Group mean AEP PTS in the two experimental groups measured at least 30 days following noise exposure.

group not treated with the drug. None of the interactions between treatment and frequency were statistically significant[23].

3.5. Percent and total cell loss

The purpose of this study was to evaluate the effect of deficient IHC populations on the toughening effect produced by daily 6 h exposures to impact noise. The carboplatin treatment was designed to produce IHC-deficient cochleas prior to noise exposure. The profile of the group mean percent IHC and OHC loss averaged over octave band lengths of the cochlea in the two groups is shown in Fig. 10. The group exposed to only the interrupted noise showed very little IHC loss averaging only 32 missing cells throughout the cochlea, and a nominal scattered loss of OHCs that, on average, was less than 10% in any octave band. The group that first received the carboplatin injection followed 30 days later by the same noise exposure showed a similar profile of OHC loss but about twice the total number of OHCs were missing (i.e., an average total of 321 versus

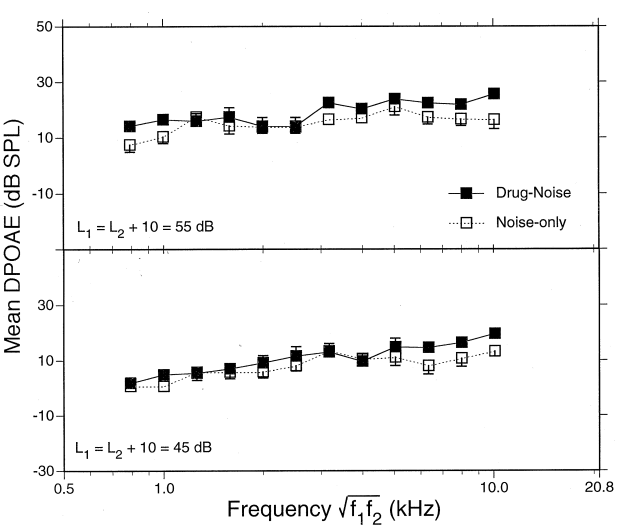

Fig. 9. Group mean third-octave band DPOAE measured at least 30 days following noise exposure for the group treated with carboplatin 30 days before noise exposure (solid symbols) and for the group which received no drug treatment (open symbols).

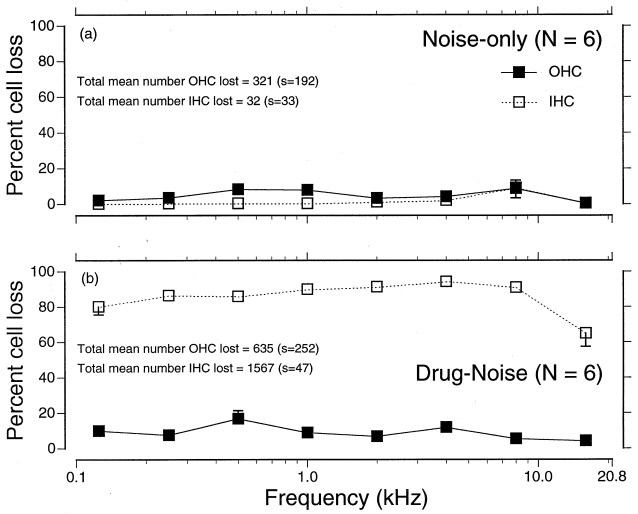

Fig. 10. The group mean percent IHC and OHC losses for the two experimental groups exposed to 1.0 kHz narrow-band impacts at 115 dB peak SPL, 1/s, 6 h/day for 10 days. The top panel (a) represents the group that received no drug treatment. The bottom panel (b) represents the group that was treated with carboplatin at least 30 days prior to the noise exposure (s = standard deviation).

603 missing OHCs). The most striking difference between the two groups was the severe losses of IHCs in the drug-noise group; amounting to an average 84% loss of IHCs throughout the entire cochlea. Analyses of variance confirmed this observation[24]. In addition, the main effects of frequency and frequency-group interactions were statistically significant[25].

A similar analysis of the total OHC losses demonstrated a statistically significant main effect of drug treatment[26]. Unlike the IHC analyses, these differences were relatively small and the frequency-group interactions were not statistically significant[27], indicating that the differences between the groups were not dependent on the location on the basilar membrane. The frequency effects were statistically significant[28].

4. Discussion

Based on the results of Takeno et al. (1994a, Takeno et al., 1994b), Trautwein et al. (1996), Jock et al. (1996), and Wang et al. (1997) and the data shown in Fig. 10, the IHC loss seen in the drug-noise group is clearly the result of the carboplatin treatment. Thus, the animals in the drug-noise group had, on average, more than an 80% IHC loss scattered throughout the extent of their cochleas prior to their introduction into the noise. The lack of any substantive changes in the DPOAE output (Fig. 3) and TS measured 30 days after the drug treatment (Fig. 2) would indicate that the OHC system in these animals was essentially normal, a conclusion also reached by Trautwein et al. (1996), Jock et al. (1996), and others. The statistically significant increase in the DPOAE output in the carboplatin-treated animals, which was in the order of a 5–10 dB effect, may reflect test-retest reliability, which amounted to about 6 dB in a large sample of normal animals (Hamernik et al., 1996), or it may reflect a reduced efferent feedback resulting from a diminished IHC afferent input (Liberman et al., 1996).

The results showing that normal AEP detection thresholds can be measured in animals having severe losses of IHCs but a normally functioning OHC population is also in agreement with the results of Jock et al. (1996) and others. In cochleas whose IHC population was severely reduced, Qiu et al. (1996) reported normal compound action potential thresholds although suprathreshold potentials were reduced. AEP detection thresholds from the IC and the auditory cortex were also unchanged. The magnitude of the suprathreshold IC potentials were, however, reduced and surprisingly the cortical potentials actually increased for several weeks following the drug treatment. Similar IC results were reported by Burkard et al. (1997). At the periphery, Wang et al. (1997) showed that the thresholds of many of the remaining VIII nerve fibers were also within the normal range. Thus the normal AEP thresholds, reported in this paper following carboplatin treatment, are consistent with recently published data.

The average total OHC loss in the drug-noise group is approximately 9% while in the noise-only group it is about 4%. Animals administered only carboplatin in a companion study (Jock et al., 1996) using a similar drug administration protocol showed average total OHC losses of about 5%. The statistically significant increased average total OHC loss in the drug treated group following the noise exposure basically reflects an additive incremental loss of OHCs that is the result of carboplatin treatment. This loss is relatively small compared to the OHC loss reported in the Trautwein et al. (1996) and Wang et al. (1997) papers that showed average OHC losses in the basal third of the cochlea that exceeded 40%. The differences in OHC losses between these two studies and the present study simply reflect differences in the amount of drug administered, that is, two intraperitoneal injections of 38 mg/kg of carboplatin separated by 24 h in the two referenced studies versus a single 75 mg/kg i.v. injection of Paraplatin-AQ (i.e., a single 37.5 mg/kg i.v. dose of carboplatin) in this study.

As seen in Figs. 4 and 8, the drug-noise group generally showed a 10–15 dB greater TS across the affected frequencies during the interrupted exposure and about a 10 dB greater PTS at 0.5 and 1.0 kHz. In both these cases these threshold loss differences were statistically significant. Noise-induced OHC losses of the magnitude seen in Fig. 10 in the low frequency region of the cochlea are typically not reflected in a low-frequency PTS. The DPOAEs, measured at the same times that the TS

and PTS data were collected, indicate that despite the small increase in OHC loss in the drug-noise group the functional status of the OHC systems is about equivalent in the two experimental groups. This suggests the involvement of the small remaining population of IHCs in the additional TS and PTS seen in the drug-noise group perhaps as a result of a diminished (protective) efferent feedback (Liberman, 1992; Liberman et al., 1996).

Both Figs. 5 and 7 are clear in showing 20–30 dB toughening effects in the TS metric and in the DPOAE metric, respectively. A consideration of the stark differences in the IHC population in the two groups; the lack of any statistically significant difference between the two groups in the amount of toughening as reflected in both TS and DPOAE data, and the results discussed in the preceding paragraph suggest that mechanisms associated with the OHC system are responsible for the observed toughening. Since the efferent system has a strong effect on the toughening phenomenon (Zheng et al., 1997) the 80% reduction in the IHC population in the drug-noise group may have been insufficient to produce an efferent mediated effect on toughening. However, an explanation relying only on IHC mediated, efferent-induced protective effects is not supported by the results that showed no differences in toughening but increased TSs and PTS between the drug-noise group and the noise-only group.

In summary, the data from this study indicate that despite severe losses of IHCs: (1) a relatively normal functioning OHC system is responsible for most of the DPOAE amplitude; (2) a cochlea with severe losses of IHCs can still present normal IC AEP detection thresholds, and (3) the toughening phenomena resulting from interrupted noise exposures is most likely associated with the normally functioning OHC system. On the basis of the Jock et al. (1996) study, in which animals having a severely reduced IHC population demonstrated normal TS response to an asymptotic threshold shift-producing impact noise exposure paradigm, and the above results, using an interrupted noise exposure paradigm, the first 40–50 dB of noise-induced TS dynamics would appear to be primarily controlled by an intact OHC system.

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