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## Sound-induced priming of the chinchilla auditory system

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### Abstract

Exposure of the auditory system to either continuous or interrupted nontraumatic noises, often collectively referred to as priming exposures, has been shown, in a number of experimental paradigms, to reduce the susceptibility of the auditory system to noise-induced hearing and sensory cell loss from a subsequent traumatic exposure. Using auditory evoked potentials to obtain pure-tone thresholds and cochleograms to quantify sensory cell losses, the issue of priming-induced protective effects was examined in the chinchilla. Priming was accomplished with either a continuous noise or with a continuous noise followed by an interrupted noise. Trauma was induced by exposure to high-level impacts over a 5-day period that resulted in an asymptotic threshold shift. A comparison of the two groups of primed subjects with an unprimed control group showed that there were some statistically significant reductions in the asymptotic response of the primed groups to the traumatic exposure but no differences in permanent changes in thresholds among the three groups 30 days following the traumatic exposure. There were, however, some statistically significant, frequency-specific, reductions in outer hair cell loss in the primed groups. When conditioning was followed by the interrupted exposure that produced a threshold shift toughening effect, the conditioning protocol had no effect on the response of subjects to the interrupted exposure. There were also no differences in thresholds or sensory cell loss between the two primed groups 30 days post-trauma. Priming protocols may have different effects on the development of noise-induced trauma that are dependent on the nature of the traumatic stimulus, that is, long-term high-level impact noise exposure versus acute continuous noise exposure. © 1999 Elsevier Science B.V. All rights reserved.

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### 1. Introduction

Two classes of relatively low-level noise exposures, often referred to as conditioning and toughening exposures, and collectively as priming exposures, have shown some ability to reduce the susceptibility of the mammalian auditory system to noise-induced hearing loss (NIHL); that is, in some species, to protect the auditory system from a subsequent traumatic exposure. The conditioning paradigm typically uses a low-level continuous noise as a stimulus while the toughening

exposures use a higher-level interrupted noise exposure paradigm. Results from the conditioning type of exposure paradigm used by Canlon and Dagli (1996) and Canlon et al. (1988), for example, have shown that exposure to a low-level pure tone that produces no permanent change in pure-tone thresholds or in the sensory cell population can reduce permanent changes caused by an exposure to a subsequent traumatic exposure.

Interrupted noise exposure paradigms have been shown to produce a toughening effect on the auditory system (Miller et al., 1963). That is, the auditory system becomes more resistant to threshold shifts (TS) as the daily interrupted exposure continues. This toughening effect, unlike the developing resistance to noise associated with the conditioning class of priming exposures, can be quantified prior to any subsequent traumatic exposure, provided that the interrupted exposure causes some initial TS. As with the conditioning protocol,

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when the toughened cochlea is exposed to a traumatic noise a reduction in permanent effects is often measured (Campo et al., 1991; McFadden et al., 1997). However, for reasons that are not clear, a protective effect is not always found in the primed auditory system (Miller et al., 1963; Fowler et al., 1995; White et al., 1998; Skellett et al., 1998; Ahroon and Hamernik, 1998).

It seems reasonable to consider the possibility that the two priming exposure paradigms might produce their protective effects through mechanisms that have at least some elements in common. As a consequence, the two priming paradigms should interact with each other. Roberto et al. (1996) attempted to test this hypothesis by conditioning groups of chinchillas with either a 72 or 78 dB SPL pink noise, presented 24 h/day for 20 days. The conditioning exposure was immediately followed by an interrupted exposure (toughening exposure) to either 113 dB or 119 dB peak SPL, broadband impacts presented 1/s, 6h/day for 20 days. These two impact noise exposures were known to produce toughening (+TS<sub>R</sub>); where toughening is defined as the difference in the TS measured following the first day of the interrupted noise exposure and that measured on the last day (X) of the daily repeating exposure cycle (i.e. TS<sub>R</sub> = TS<sub>1</sub> - TS<sub>X</sub>). The conditioning exposures did not appear to exert any effect on the development or the magnitude of the toughening phenomenon that was produced by the interrupted exposures. Using a similar exposure sequence [i.e. conditioning followed by an interrupted noise exposure (2767 Hz pure tone at 103 dB SPL, 5 min/day for 10 days)] in the guinea pig, and cubic distortion product otoacoustic emissions as a metric of cochlear function, Dagli and Canlon (1997) showed that the conditioning exposure did affect the development of TS produced by the subsequent interrupted exposure. The sound conditioned group was less affected by the overstimulation produced by the interrupted paradigm during the initial days of the exposure compared to the unconditioned group. As the daily exposures continued, the conditioned group became more affected. The unconditioned group showed greater shifts during the initial days of the daily exposure and showed a resistance to overstimulation (toughening) as the interrupted exposure continued. Changes in the magnitude of the otoacoustic emissions, after the end of the 10-day interrupted exposure were, however, similar in both groups. Their results indicated that their conditioning stimulus did affect the response of the cochlea to an interrupted noise exposure.

Considering that: (1) The only two studies in the literature on the interaction between conditioning and toughening exposures, one of which was from our laboratories, showed divergent results and (2) other work from our laboratory on toughening-induced protection (Hamernik and Ahroon, 1998, 1999; Ahroon and Ha-

mernik, 1999) also failed to demonstrate clear and systematic protective effects in noise toughened cochleae, we have continued to explore both the issue of an interaction between conditioning and toughening mechanisms as well as the protective effects that the priming protocols have been shown to exert on a subsequent traumatic exposure.

## 2. Methods

Monaural chinchillas (between 1 and 2 years old) were used as the experimental subjects. Brainstem (inferior colliculus) auditory evoked potentials (AEPs) were used to estimate pure-tone thresholds and surface preparation histology was used to quantify sensory cell populations. Details of the experimental methods, beyond those that are presented below, can be found in Ahroon et al. (1993).

### 2.1. Noise exposures

Three different noise exposures were used. The conditioning exposure consisted of a 1.0 kHz center frequency (CF), octave band of noise (OBN) presented at 80 dB SPL, 24 h/day for 5 days. The noise was generated by passing the output of a General Radio 1382 random noise generator through a Brüel and Kjær Model 125 graphic spectrum equalizer. The output of the filter was directed to an AB International Precedent Series 900 amplifier and finally to a Electro-Voice TL550D low-frequency speaker. The output of the speaker was calibrated and measured by a Brüel and Kjær Type 4134 half-inch condenser microphone and Brüel and Kjær Type 2610 Measuring amplifier, calibrated by a Brüel and Kjær Type 4220 pistonphone. The analog output of the measuring amplifier was fed to an Apple Macintosh Quadra 840AV computer system with a 16-bit National Instruments NB-2100 audio board and sampled at 48 000 samples per second. A Wavetek Model 852 Dual HI/LO filter provided anti-aliasing. A set of virtual instruments using the National Instruments LabView graphical programming language performed the spectral and intensity analysis. The spectrum of the 1.0 kHz OBN stimulus is presented in Fig. 1(a).

The toughening noise and the traumatic noise exposure consisted of narrow-band impacts (NBI) 400 Hz wide, having a 1.0 kHz CF, presented at the rate of 1 impact/s at 115 dB peak SPL 6 h/day for 10 days for the former and at 127 dB peak SPL for 24 h/day for 5 days for the latter. The conditioning and the traumatizing exposures produced an asymptotic threshold shift (ATS) condition (Carder and Miller, 1972). The impact noise stimulus was generated digitally using a

LabView virtual instrument in which a fixed-length pulse was fed through a 4th-order, band-pass, Butterworth filter. The resulting waveform was played through the computer's (Macintosh Quadra 840 AV) sound output and fed to an AB International Precedent Series 900A amplifier. The output of the amplifier was fed to a JBL Model 2445J speaker with Model 2360H horn and Model 2360T transition piece. Fig. 1b shows the relative spectrum and temporal waveform of the 127 dB peak SPL NBI. The spectrum and waveform of the 115-dB peak SPL toughening stimulus were qualitatively similar to those of the 127-dB peak SPL stimulus.

During exposure, individual chinchillas were confined to cages (10"×11"×16") with free access to food and water. A maximum of six animals was exposed at a time. Peak and RMS SPLs in the exposure field were uniform to within 2 dB.

## 2.2. Experimental groups

The following groups of animals were exposed to the indicated noise presentation protocols.

Group 1: Conditioning/trauma group ( $n=9$ ). Conditioning Exposure: 1.0 kHz CF, OBN @ 80 dB SPL, 24 h/day for 5 days. All animals recovered to within  $\pm 5$  dB of preexposure thresholds (i.e. to within the test-retest reliability of the AEP procedure). Recovery took from 1 to 5 days. Upon recovery to preexposure thresholds, the animals were exposed to the 1.0 kHz, NBI noise at 127-dB peak SPL, 24 h/day for 5 days.

Group 2: Conditioning/toughening/trauma group ( $n=12$ ). Each animal received the same conditioning and recovery protocol as in group 1. The animals were then given the toughening exposure; 115-dB peak SPL, 1.0 kHz, NBI noise, 6 h/day for 10 days. Within 1 h after completion of the day 10 exposure the animals were put into the traumatic noise environment; 127-dB peak SPL, 1.0 kHz, NBI, 24 h/day for 5 days.

Group 3: ( $n=54$ ) Laboratory norms for AEP thresholds measured during the toughening exposure. Animals were presented with the toughening protocol during which the amount of toughening was measured for comparison with the group 2 animals. It should be noted that these animals are being used only as a reference group against which the amount of toughening in the group 2 animals is compared. The subsequent exposures of most of these animals were not related to the objectives of this paper.

Group 4: ( $n=10$ ) Unconditioned and untoughened control group. These animals were presented with only the 127-dB peak SPL traumatic exposure.

Comparisons of AEP thresholds and sensory cell losses between groups 1 and 2 established any effects of an interaction between conditioning and toughening

exposures on  $TS_R$ , while comparisons among groups 1, 2 and 4 established any protective effects that are produced as a result of the conditioning and toughening exposures.

## 2.3. Threshold testing

Thresholds for all AEP audiograms were measured at octave intervals from 0.5 to 16.0 kHz. The mean (in dB SPL) of three threshold determinations measured on different days defined each animal's preexposure audiogram. For the 10-day interrupted exposure paradigm, a complete audiogram was measured immediately prior to the first exposure and following the first and last two daily 6 h exposures in order to establish the magnitude and time course of the toughening phenomenon. The amount of toughening ( $TS_R$ ), at each audiometric test frequency, was defined as the difference between the threshold measured at a given frequency following the first day exposure and the mean of the thresholds measured following exposure on days 9 and 10.

A complete audiogram was measured once daily during each of the five exposure days of the uninterrupted exposures and the average (in dB SPL) taken over the 5 days established the mean asymptotic threshold levels. Thirty days following the complete exposure protocol for each experimental group, three AEP audiograms were measured again on different days and averaged for each animal to establish permanent postexposure threshold levels.

## 2.4. Histology

Following the last AEP test protocol, each animal was killed under anesthesia and the right auditory bulla removed and opened to gain access to the cochlea for perfusion. Fixation solution consisting of 2.5% glutaraldehyde in Veronal acetate buffer (final pH=7.3) was perfused through the cochlea. After 12 to 24 h of fixation the cochlea was postfixed in 1%  $OsO_4$  in Veronal acetate buffer. Surface preparation mounts of the entire organ of Corti were prepared and inner and outer hair cell (IHC, OHC) populations were plotted as a function of frequency and location using the frequency-place map of Eldredge et al. (1981). Missing cells were identified by the characteristic phalangeal scars which form in the 30-day period prior to killing. For purposes of this presentation, sensory cell population data are presented as group averages (in percent missing) taken over octave band lengths of the cochlea centered on the primary AEP test frequencies.

## 2.5. Statistical analysis

The dependent variables reported in this paper are

(1) AEP thresholds, before, during and following noise exposure(s), and (2) sensory cell losses in octave-band lengths of the cochlea. Comparisons of groups of animals receiving different treatments were accomplished by mixed model analyses of variance with repeated measures on one factor (frequency). The probability of a Type I error was set at 0.05 for all analyses. Analyses of thresholds within groups of animals, where necessary, were performed using completely within-subjects analyses of variance. Statistically significant main effects of frequency are expected in most of the following analyses because of the frequency-specific nature of not only the chinchilla audiogram (Fay, 1988), but also of the noise stimuli. For this reason main effects of frequency will not be repeatedly discussed in the following presentation of results. Analysis of variance summary tables may be obtained from the authors.

### 2.6. Animal care

The care and use of the animals used 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

In an experimental paradigm that entails a sequence of several exposures there is always a question of the status of the auditory system following any one of the exposures. In the case of conditioning with an ‘innocuous’ noise or toughening with the higher SPL exposures, the system, based upon results in the literature, has clearly been altered. Thus, for reasons that go back to the idea of Davis et al. (1950), the ‘damaged ear theory’, and some of the subsequent experiments that it generated (e.g. Mills, 1973, 1992; see also the review by Humes, 1984), much of the presentation of results in this paper is given in terms of shifted thresholds (dB SPL) rather than the more conventional threshold shifts.

In some of the following figures the shaded region on the AEP audiograms represents the mean normative AEP audiogram ( $\pm$  S.D.) based on a population of 924 chinchillas. The bars on the data points in each figure represent the S.E.M.; where no bar is shown the standard error was less than the size of the datum symbol.

### 3.1. The effects of the conditioning exposure on a subsequent traumatic exposure; a comparison of groups 1 and 4

Fig. 2 shows the mean AEP thresholds for the group 1 animals; (1) prior to any noise exposure, (2) at their asymptotic levels produced over the course of the 5 day, 80-dB SPL conditioning exposure and (3) after a 1- to 5-day recovery from the conditioning exposure. The preexposure thresholds are well within laboratory norms. The conditioning exposure produced a 9 to 23 dB group mean ATS from 0.5 through 2.0 kHz which recovered within 5 days after the termination of the conditioning exposure. There were no statistically significant differences between the pre-conditioning thresholds and those measured after the 1- to 5-day recovery from the conditioning exposure.

Fig. 3 shows the group mean AEP thresholds of the conditioned (group 1) and unconditioned (group 4) animals immediately prior to their exposure to the 127-dB peak SPL, trauma-inducing exposure. While the preexposure thresholds are within laboratory norms, statisti-

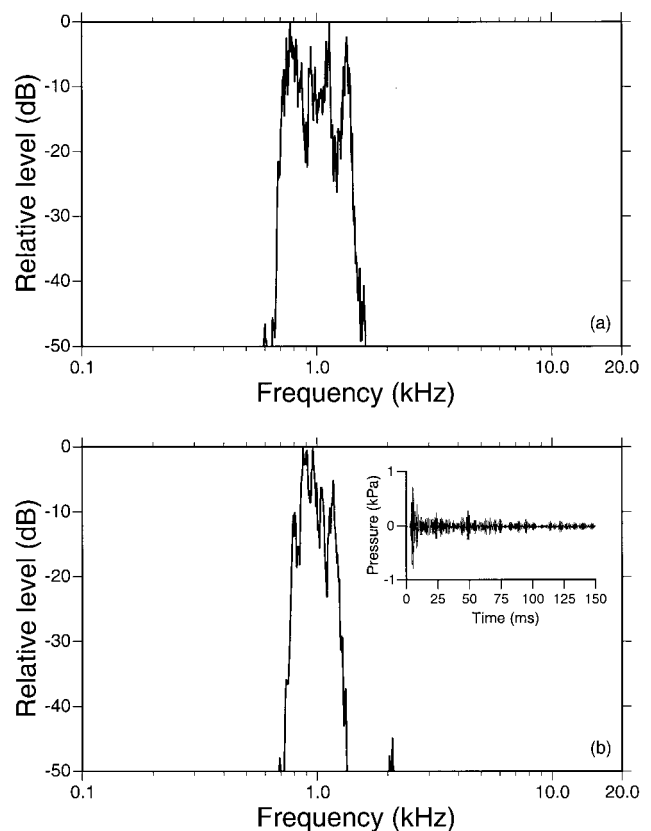


Fig. 1. a: The relative spectrum of the 80 dB SPL, 1 kHz octave band of noise used as the conditioning stimulus. b: The spectrum and temporal waveform of the 127-dB peak SPL impact used as the traumatizing stimulus. The 115-dB peak SPL, narrow band impact that was used as the toughening noise had a spectrum and waveform that was qualitatively similar to the 127-dB peak SPL impact.

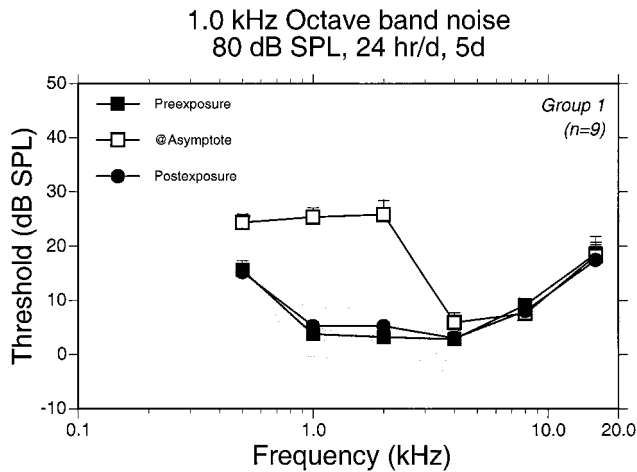


Fig. 2. The mean group 1 preexposure AEP audiogram (■) and the audiogram measured after recovery from the conditioning noise exposure (●). Recovery to preexposure thresholds required from 1 to 5 days. Also shown is the group mean thresholds (□) at their asymptotic levels, measured during the course of the 5-day conditioning exposure.

cal analyses indicated that there was a significant effect of group and an interaction between group and frequency. That is, the preexposure thresholds of the unconditioned group 4 animals are up to 6 dB better at some frequencies than they are in the conditioned group 1 animals. Extremely small standard errors are responsible for this result. Also shown in this figure are the thresholds for groups 1 and 4 at asymptote during the 127-dB peak SPL, 5 days exposure. While the conditioned group shows systematically lower thresholds at asymptote (by up to 10 dB at 0.5 and 16 kHz) across the audiometric test frequency range, these differences are not statistically significant (no effect of group and no interaction). However, if these same data are analyzed as ATS rather than shifted thresholds a statistically significant main effect of group is found as a result of the slightly lower (~6 dB) preexposure thresholds and the systematically higher asymptotic thresholds in the unconditioned group 4 animals.

Permanent changes in thresholds, measured 30 days following the traumatic exposure, for the conditioned and unconditioned groups are also shown in Fig. 3. The 127-dB exposure produced on the order of 12 to 23 dB of PTS in both groups. There were no statistically significant differences between these two sets of thresholds regardless of whether shifted thresholds or threshold shifts were analyzed.

Fig. 4 shows the group mean percent OHC and IHC losses measured over adjacent octave band lengths of the basilar membrane for the group 1 and 4 animals. The 127-dB peak SPL exposure produced a broad loss of OHCs along the length of the organ of Corti with a maximal loss of both IHCs and OHCs in the 1-kHz

region. There were no statistically significant effects of group but there was a significant group by frequency interaction for the OHC loss. That is, there was no significant difference in the OHC loss between the two groups collapsed across frequency, but there were significant differences at some frequencies. Subsequent *t*-tests revealed that these differences were significant only at 0.25 and 8.0 kHz. (Note the small standard errors in the group 1 OHC measures.) There were no significant group effects nor a group by frequency interaction for the analyses of IHC loss for the two groups.

Thus, based on the above and within the confines of the experimental parameters, there was no protective effect on permanent changes in threshold metrics as a consequence of the conditioning exposure. There was also no difference in the total OHC loss between the two groups but there was a frequency-specific (at 0.25 and 8.0 kHz) reduction of OHC loss in the conditioned subjects.

### 3.2. The effects of a conditioning exposure on the toughening phenomenon; a comparison of groups 2 and 3 prior to the traumatic exposure

Fig. 5a shows the pre- and post-conditioning AEP thresholds for the group 2 animals along with the preexposure thresholds of group 3. The group 3 thresholds represent a normative preexposure data base for animals receiving the 1.0 kHz toughening exposure. For the group 2 animals there was no statistically significant main effect of treatment but there was a statistically

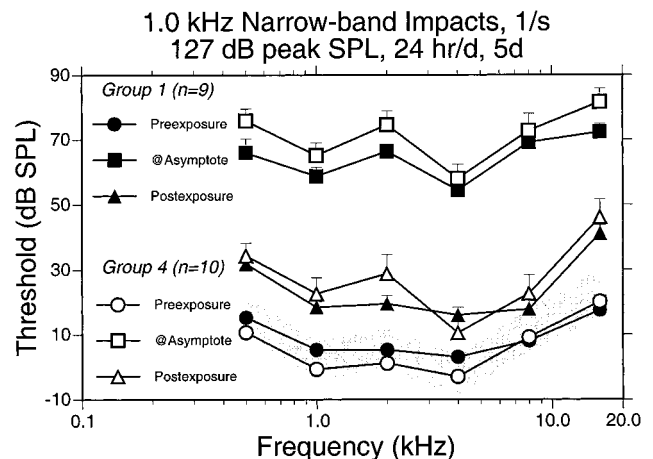


Fig. 3. The mean AEP thresholds measured just before exposure to the 127-dB peak SPL traumatizing noise in the group 1 conditioned animals (●) and in the group 4 (○) unconditioned animals. Also shown are the AEP thresholds for these two groups at their asymptotic values recorded over the course of the 5-day traumatic exposure [group 1 (■), and group 4 (□)]; and at 30 days after the traumatic exposure [group 1 (▲), and group 4 (△)].

significant interaction of exposure and frequency, presumably because of the small standard deviations in the group 2, pre- and post-conditioning thresholds. This interaction reflected a 3-dB or less difference in thresholds. As with the group 1 animals discussed above, the conditioning exposure in the group 2 animals had no substantial effect on AEP thresholds. Thresholds returned to normal (taken to be within  $\pm 5$  dB of each animal's initial values which is typically about the test-retest reliability of the AEP procedure) within 1 to 5 days after removal from the conditioning noise. There were no statistically significant differences in the thresholds for groups 2 and 3 prior to the toughening exposure.

The asymptotic threshold levels resulting from the conditioning exposure of the group 2 animals are shown in Fig. 5b along with corresponding thresholds for the similarly conditioned group 1 animals. There was no statistically significant effect of group but an interaction between group and frequency. Despite the approximately 8-dB difference in asymptotic thresholds at 2 kHz, which was responsible for the significant interaction, both groups exhibited a very similar response to the conditioning exposure.

As soon as each of the group 2 animal's thresholds were within 5 dB of preexposure values the animal was placed into the interrupted (toughening) noise environment. The effect of the toughening exposure on AEP thresholds is shown in Fig. 5c where thresholds for group 2 and 3 animals, measured after day 1 and the mean thresholds measured after day 9 and 10 are compared. The vertical distance between corresponding pairs of data points at each test frequency is a measure of the amount of toughening,  $TS_R$ . There were no statistically significant differences between the amount of  $TS_R$  found in groups 2 and 3. All test frequencies except 16 kHz showed some toughening which varied in

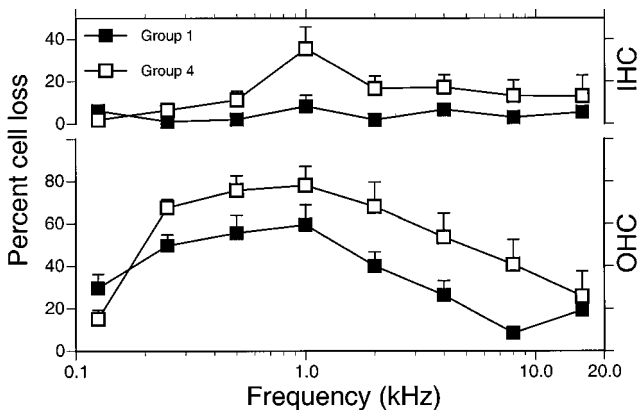


Fig. 4. The mean percent OHC loss and IHC loss for the conditioned group 1 animals (■) and the unconditioned group 4 animals (□).

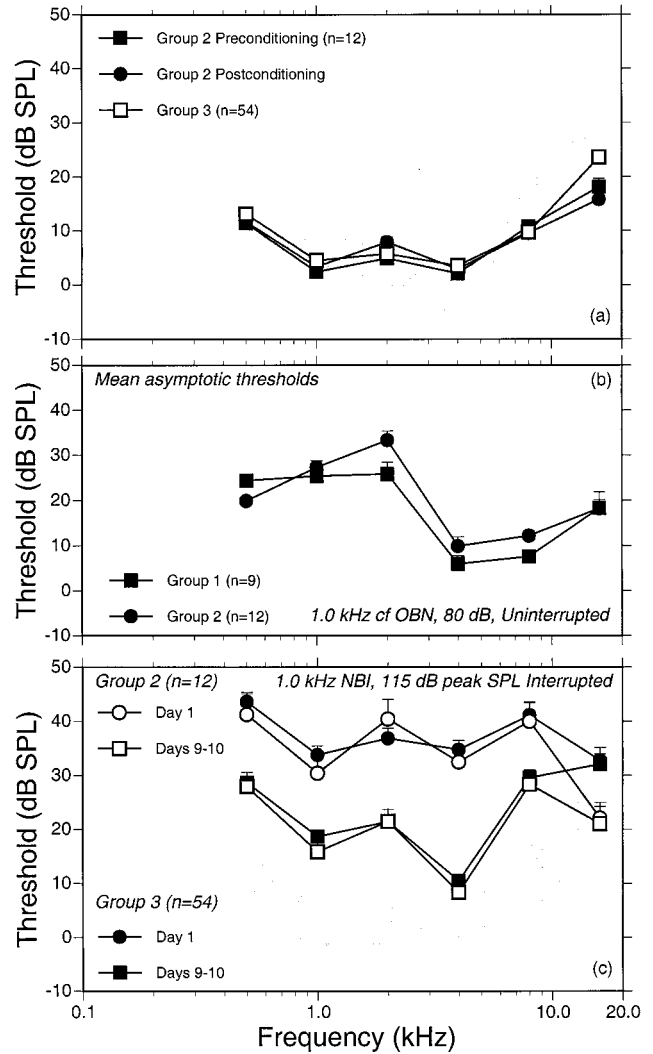


Fig. 5. The mean AEP thresholds measured; a: for the group 2 animals before the conditioning exposure (■) and just before the toughening exposure (●). Also shown are the preexposure thresholds for the reference group 3 animals (□) that were only toughened by the 1-kHz, 115-dB peak SPL interrupted stimulus. b: The mean AEP thresholds measured at their asymptotic values over the course of the 5-day conditioning exposure in group 1 (■) and group 2 (●). c: The mean AEP thresholds measured in group 2 (○, □) and 3 (●, ■) after the day 1 and day 9,10 exposures to the toughening noise.

each group from 12 to as much as 24 dB at 4 kHz. There were no statistically significant differences in the thresholds measured following day 1 of the toughening exposure between the two groups. A comparison of the mean thresholds measured following day 9 and 10 in groups 2 and 3 showed no effect of group but a significant group by frequency interaction. This is the result of the differences in thresholds at 16 kHz, a frequency which showed no  $TS_R$  in either group. Thus the conditioning protocol had no effect on the magnitude or development of a  $TS_R$  in the group exposed to the interrupted noise paradigm.

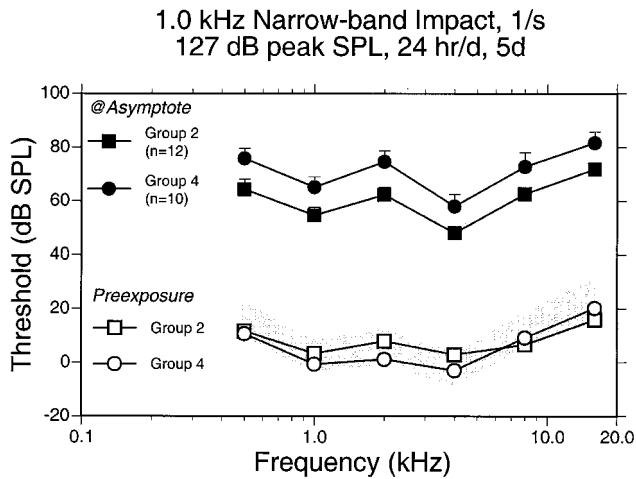


Fig. 6. The mean thresholds for the group 2 (□) and group 4 (○) animals measured just prior to exposure to the 127-dB peak SPL trauma-producing noise and the thresholds measured at their asymptotic levels produced by the traumatic exposure; group 2 (■) and group 4 (●).

3.3. *The effects of the conditioning and interrupted exposures on the outcome of a subsequent traumatic exposure; a comparison of the response of groups 2 and 4 to the 127-dB peak SPL traumatizing noise exposure*

The preexposure thresholds measured before and the asymptotic thresholds measured during the 127-dB peak SPL exposure for groups 2 and 4 are shown in Fig. 6. While the preexposure thresholds for both groups are within a standard deviation of laboratory norms, there is a statistically significant effect of group and a group by frequency interaction for preexposure thresholds of groups 2 and 4. This reflects 4 to 7 dB better preexposure thresholds at some frequencies in the group 4 animals (it should be noted that there were no significant differences between the preexposure thresholds of the group 1 and 2 animals). During the 127-dB peak SPL, 5 days exposure, thresholds at asymptote for groups 2 and 4 were significantly different. Analyses of the two sets of thresholds at asymptote showed a statistically significant effect of group but no interaction between group and frequency. The group effect is the result of the systematically lower (by up to 11 dB at 2 kHz) asymptotic thresholds in the group 2 animals. Thus, at asymptote the animals that were conditioned by the continuous OBN and toughened by the interrupted noise exposure showed statistically significant better thresholds than the unprimed group 4 animals. A similar conclusion follows when ATS between the two groups is compared. There were no statistically significant differences in the asymptotic threshold levels produced by the 127-dB peak SPL traumatic noise exposure between groups 1 and 2; that is, between

the animals that were only conditioned (group 1) and those that were both conditioned and toughened (group 2).

The permanent effects of the traumatic noise exposure on AEP thresholds for groups 2 and 4 are shown in Fig. 7. Despite the significantly better preexposure thresholds in the group 4 animals, when compared to the primed (group 2) animals, and their higher thresholds at asymptote, there are no statistically significant differences between the 30-day postexposure AEP audiograms shown in Fig. 7a. These analyses were repeated twice; once using threshold shift (i.e. PTS) with the post-conditioning thresholds of group 2 as a reference and once with the pre-conditioning thresholds as a reference. Both of these analyses showed no statistically significant differences among the two sets of PTSs. Similar analyses of the 30-day postexposure thresholds and PTSs in groups 1 and 2 revealed no significant differences.

The sensory cell loss profile 30 days after the traumatic 127-dB peak SPL exposure for the unprimed group 4 animals and the conditioned and toughened group 2 animals is shown in Fig. 7b. There are no

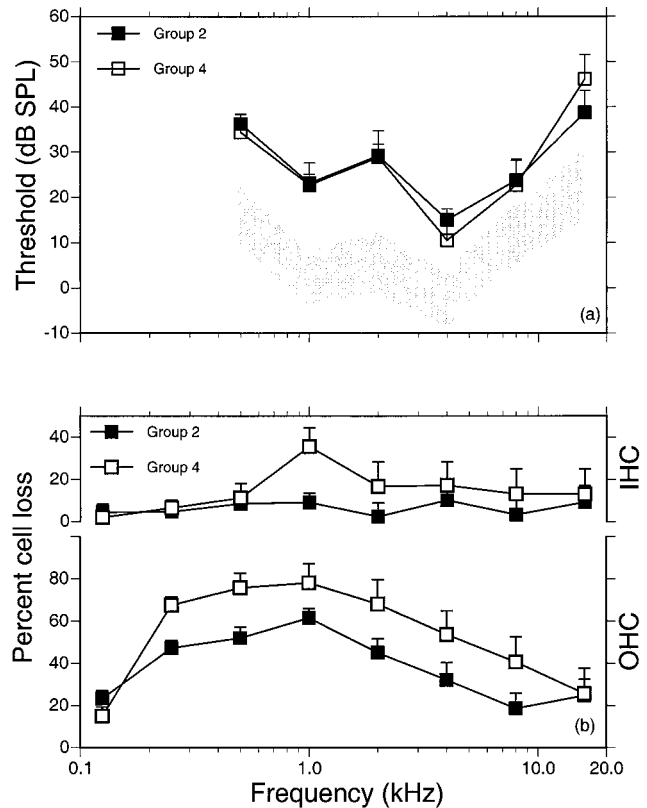


Fig. 7. a: The mean thresholds measured 30 days after the 127-dB peak SPL traumatic exposure in group 2 (■) and group 4 (□). b: The mean percent OHC loss and IHC loss for the conditioned/toughened group 2 animals (■) and the unprimed group 4 animals (□).

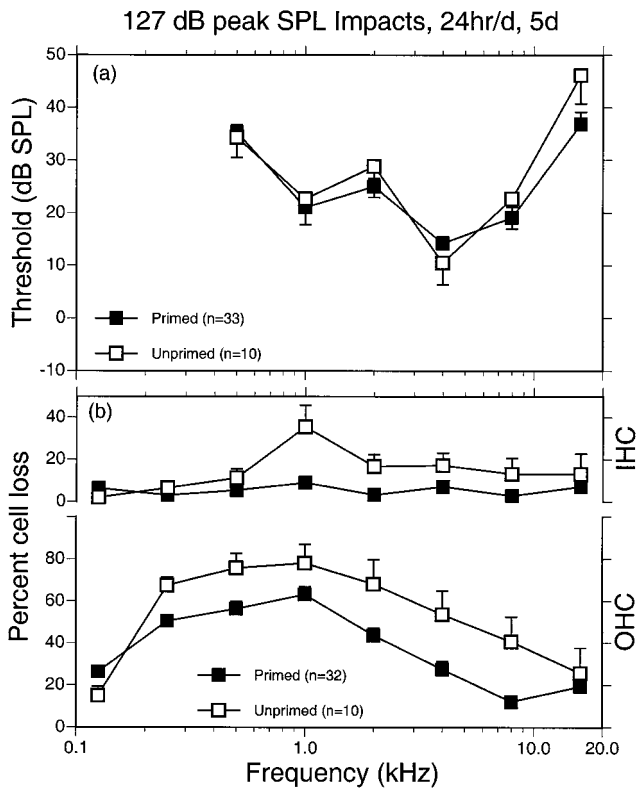


Fig. 8. a: Comparison of the 30-day postexposure thresholds and b: IHC and OHC losses in the unprimed group 4 animals (■) with the collapsed groups 1, 2 and group 7 animals (□), the latter are taken from Ahroon and Hamernik (1999). The group 7 animals received the same toughening exposure as group 2 and following an 18-h recovery period were exposed to the same 127-dB peak SPL traumatizing noise.

statistically significant differences in OHC loss between the two groups (although the differences approach statistical significance) and no significant effect of group for the IHC loss. There is, however, a group by frequency interaction for the IHC losses. That is, there are no differences in the losses of IHCs between the two groups collapsed across frequency but there are differences at some frequencies (i.e. at 1.0 kHz). An analysis of the sensory cell losses for the conditioned group 1 and the conditioned/toughened group 2 animals reveals no significant differences between these two groups.

#### 4. Discussion

Conditioning with an 80-dB SPL, 1-kHz CF, OBN for 5 days resulted in a statistically significant reduction in ATS when groups 1 and 4 were compared, but there were no differences in the shifted thresholds at asymptote. Whether this difference in statistical significance is an actual effect of the conditioning protocol or only the effect of differences in preexposure thresholds between the conditioned and unconditioned groups cannot be

determined. Conditioning of the auditory system with the stimulus that we selected did not have any effect on the permanent changes in threshold produced by the 127-dB peak SPL, NBI traumatic exposure when compared to the unconditioned control group. Based on the ANOVA analysis, our conditioning stimulus did result in a frequency-specific reduction in OHC losses but *t*-tests revealed that these differences were significant only at 0.25 and 8.0 kHz; frequencies widely separated on the basilar membrane and far removed from the frequencies of noise stimulation. Thus, while the data plotted in Fig. 4 suggest that conditioning did reduce OHC losses, the effect is statistically significant only at frequencies that are difficult to explain given the CFs and band widths of the noises that primed the system or caused trauma. These results and analyses indicate that the conditioning protocol used in these experiments will not produce a significant degree of protection from a traumatic exposure.

Conditioning with low-level pure tones followed by a traumatic exposure to the same pure tone has typically resulted in a clear reduction in permanent effects in a guinea pig model (Canlon, 1996; Canlon and Fransson, 1995; 1998; Canlon et al., 1988, 1992). Use of noise bands as a conditioning stimulus has produced mixed results. Roberto et al. (1996) used a low level (72- or 78-dB SPL) pink noise as a conditioning stimulus for the chinchilla. Their results showed statistically significant conditioning-induced protection from a relatively mild traumatic exposure but no significant protective effects when the trauma-inducing exposure was more severe. Fowler et al. (1995) showed, in the mouse model, either no effect of conditioning or an exacerbation of permanent effects compared with an unconditioned control. White et al. (1998) conditioned the gerbil with a 2-kHz OBN and showed reductions in compound threshold shift, PTS and sensory cell losses while Skellett et al. (1998) could not evoke a protective effect with their 89-dB SPL and 1–2-kHz OBN conditioning stimulus. Species differences and differences in exposure parameters might contribute to the variety of results to be found in the literature (Canlon et al., 1999). Conditioning with pure tones, which produce relatively localized effects in both mechanical and neural activity may be a significant factor in inducing a consistent protective effect from conditioning.

We approached the issue of conditioning-induced protection from an applied perspective, that is, we choose stimuli that had at least some features in common with industrial noises such as broad band noises and in this paper a 1.0-kHz OBN (many industrial noises have spectra that peak around this frequency region). While there may be ideal conditioning stimuli for the chinchilla model that will produce a protective effect on acute exposures to a continuous Gaussian

noise, clearly neither the low-level broad-band stimulus used by Roberto et al. (1996) nor the OBN used in this study are effective when trauma is produced by a long-term high-level impact noise exposure.

An alternate measure of the effects of our conditioning stimulus is through its effects on the amount of toughening produced by an interrupted noise exposure paradigm. If the conditioning exposure that we used does have a protective effect on the cochlea, as shown in some of the referenced literature, one might expect to see some of that effect manifested in the TSs measured during the interrupted exposure of the group 2 animals. As seen in Fig. 5, our conditioning exposure had no effect on toughening produced by an interrupted noise exposure. The amount of toughening, despite the prior conditioning, was the same as that developed in the unconditioned but toughened group 3 subjects. Roberto et al. (1996) also concluded that conditioning using broad-band noise did not have an effect on the amount of  $TS_R$  produced by a subsequent interrupted-noise exposure. Dagle and Canlon (1997), however, using narrow-band stimuli and otoacoustic emissions as an index of function concluded that their pure-tone conditioning stimulus affected the response of the guinea pig to an interrupted noise that produced toughening (i.e. some resistance to overstimulation). When the conditioned and toughened subjects of group 2 were exposed to the 5-day traumatic noise there was a reduced loss of threshold at asymptote but no difference in permanent effects on threshold or OHC loss.

A comparison of the animals that were conditioned and then exposed to the traumatic noise with those that were conditioned and toughened (groups 1 and 2) shows that there were no differences in the asymptotic thresholds or threshold shifts produced by the 5-day traumatic exposure. Since the two primed groups showed the same asymptotic response to the traumatic noise the two groups were collapsed into a group of 20 subjects and then this expanded group compared to the unprimed group 4 animals. This exercise showed that there was a statistically significant reduction in both ATS and threshold at asymptote, suggesting that priming, in general, had the effect of reducing the loss of threshold during the asymptotic response to the traumatic noise. A similar reduction in asymptotic thresholds in the primed auditory system in response to a 121-dB, 5-day, NBI exposure was shown by Ahroon and Hamernik (1999). Despite this reduction, they did not show (as in this present study) a protective effect on the permanent changes in threshold or sensory cell loss.

The Ahroon and Hamernik (1999) paper presented data on a group (identified as group 7) that was toughened with the same paradigm as the group 2 animals and 18 h following the toughening protocol were ex-

posed to the same 5-day, 127-dB traumatic NBI presentation (the toughening exposure alone was shown by Ahroon and Hamernik (1999) to produce no PTS and very little OHC loss). When the data from their group 7 are compared to the present group 1 and 2 responses to the traumatic exposure there were no differences in permanent effects on threshold or on sensory cell loss among these three groups. Thus it did not seem to matter what the original priming paradigm was (conditioning, toughening or a combination of both), the permanent effects of the traumatic exposure in primed subjects were the same. Since there were no significant differences in the response of groups 1, 2, and 7 to the traumatic exposure, these groups were collapsed into a single primed group and this new group with its increased number of subjects (and hence statistical power) was compared to the unprimed group 4. The audiometric and sensory cell loss data for this exercise are shown in Fig. 8. There were no statistically significant differences in permanent effects on thresholds but a significant reduction in OHC and IHC loss in the primed group. While audiometric results do not reflect the reduced sensory cell loss in the primed animals, this is not unusual in the noise literature in general (Hamernik et al., 1989) or in the literature on toughening (Campo et al., 1991; Subramaniam et al., 1993; Boettcher et al., 1992).

The above results, coupled with the lack of any effect of the conditioning paradigm on the response of the subjects to the toughening paradigm suggests that not just any innocuous exposure is capable of producing some protective effect, but that there may be very specific noise parameters that are necessary for generating a protective effect (Canlon et al., 1999). However, the sizable toughening produced by the interrupted exposures coupled with the lack of a protective effect is more difficult to explain. If the protective effects of priming are small, large sample sizes may be required to demonstrate positive effects as in Fig. 8b. Finally, the diversity of results in the literature on priming and the frequent lack of consistency in the experimental data raise the possibility of an uncontrolled variable influencing the results. For example, the results of Liberman and Kujawa (1998) suggest that a whole body stress response may have a substantial effect on the results of priming experiments.

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