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Noise-induced hearing loss in the noise-toughened auditory system

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Abstract

The auditory system, toughened by an interrupted noise exposure, has been shown in several reports to be less affected by (or protected from) a subsequent high-level noise exposure. Exposure to 115 dB peak SPL, 1 kHz narrow band (400 Hz) transients presented 1/s, 6 h/day, to four groups of chinchillas produced a 10–28 dB toughening effect across the 0.5–8.0 kHz test frequency range. Following either a 30 day or an 18 h recovery period the animals were exposed to the same impulses but presented at 121 or 127 dB peak SPL for five uninterrupted days, thus producing an asymptotic threshold shift (ATS) condition. Comparisons between toughened and untoughened control subjects showed: (1) During the 121 dB exposure there was a statistically significant reduction of 10–25 dB in ATS across the entire test frequency range. Thirty days following the 121 dB exposure there were no significant differences in the postexposure permanent effects on thresholds and sensory cell loss. (2) During the 127 dB exposure only the group with the 30 day interval between the toughening and traumatic exposures showed a small ($\sim$10 dB), statistically significant, frequency-specific (8 kHz), reduction in ATS. Thirty days following the 127 dB exposure a statistically significant protective effect on threshold was measured only at 16.0 kHz. However, both toughened groups showed less inner hair cell loss at and above 1.0 kHz, while only the group with the 18 h interval between the toughening and traumatic exposures showed less outer hair cell loss at and above 1.0 kHz. There were no systematic differences in the response of the toughened animals that could be attributed to the 30 day or 18 h post-toughening interval.

Key words: Noise-induced hearing loss

1. Introduction

Under some experimental conditions a noise exposure that has no permanent effect on the auditory system has been shown to render the system less susceptible to the effects of a subsequent traumatic exposure. That is, the auditory system has been toughened. Interrupted exposure paradigms used by, for example, Clark et al. (1987), Subramaniam et al. (1991), and Hamernik et al. (1994) or low-level uninterrupted (conditioning) exposures such as those used by Canlon et al. (1988) and Canlon (1996) have been shown to toughen the auditory system. In the former procedure, threshold shifts (TS), measured following each daily noise exposure, have been shown to decrease up to as much as 35 dB despite the continuing exposure. The decreased TS produced by an interrupted noise exposure is one measure of the amount of toughening. An alternative indication of the amount of toughening can be obtained by measuring the effects of a subsequent noise exposure on the noise-toughened cochlea. The toughening produced by the conditioning type of an exposure, however, has only been quantified by its effects on a subsequent exposure.

A fundamental issue associated with both of these approaches to the toughening phenomena is: To what extent or under what conditions is a toughened cochlea, which is subsequently exposed to a more traumatic noise, protected from that noise? That is, this paper as well as the referenced papers test the following hypothesis: The auditory system that exhibits a recovery of threshold shift during the course of an interrupted

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noise exposure (i.e., is toughened) will be less affected by a subsequent noise exposure and that this protective effect persists for up to 30 days following the toughening exposure.

This issue is addressed in a number of papers where the answer is equivocal. The earliest reference to the issue of just such a protective effect can be found in the Miller et al. (1963) paper in which the noise-toughened cat cochlea was shown to be affected by a high-level noise to the same extent as the untoughened cochlea. More recently, Subramaniam et al. (1993) showed that, under some conditions, the cochlea toughened by a low frequency, interrupted noise did not provide any protection from a subsequent high frequency [4 kHz cf octave band noise (OBN)] exposure. Instead the 4 kHz OBN exposure produced an exacerbation of both PTS and outer hair cell (OHC) loss.

Protection from a low-frequency noise exposure in the chinchilla was, however, reported by, for example, Campo et al. (1991) and McFadden et al. (1997). In both of these studies permanent threshold shifts (PTS) were about 10–15 dB less in the toughened group than in the untoughened controls. A confounding factor in both of these papers was that when statistically less PTS was found in the toughened ears there was no protective effect on the OHC population as measured by conventional cochleograms. The effect of a traumatic impact noise exposure on the noise-toughened cochlea of the chinchilla was explored by Roberto et al. (1996) and by Henselman et al. (1994). In the former paper, where both interrupted and low-level uninterrupted noise exposures were used to toughen the cochlea, the protective effects were inconsistent, while in the latter paper, although there was considerable variability, very large, statistically significant protective effects were observed in both the PTS and sensory cell loss data. Canlon et al. (1988) and Canlon and Fransson (1995, 1998), using a low-level (uninterrupted) continuous noise to toughen (condition) the cochlea of the guinea pig, consistently showed a protective effect, while in CBA/Ca mice, neither continuous nor interrupted toughening paradigms produced a protective effect (Fowler et al., 1995). Rather a number of conditions in that study produced an exacerbation of PTS.

White et al. (1998) and Skellett et al. (1998) also used both interrupted and continuous protocols to toughen the cochlea prior to subjecting the experimental animals to a high level of noise. The former showed that, in the gerbil, toughening produced by the continuous protocol reduced the amount of compound threshold shift (CTS) and PTS while the animals toughened by the interrupted protocol showed a considerable reduction of CTS but no reduction of PTS compared to the untoughened control animals. However, both of the toughened groups showed less sensory cell loss than the untoughened control group. Skellett et al. (1998) also indicated that in the guinea pig there were significant differences between the effects of the two toughening protocols. They concluded that the interrupted protocol produced a protective effect while the continuous protocol failed to do so. Unfortunately, the protective effects reported by Skellet et al., while statistically significant, amount to only about a 3–5 dB effect and sensory cell evaluations, an alternative index of protection, were not performed. A more parsimonious interpretation of their data might simply be that there was essentially no protective effect and thus no difference in the effect produced by the two different toughening paradigms. Also in the Skellet et al. report there was no clear index of the amount of toughening produced by the interrupted exposure, making comparisons with other work difficult.

In a study of the effects of the olivocochlear bundle on the protective effect in noise-toughened animals, Liberman and Kujawa (1998) found that sham surgery in control animals had the effect of reducing permanent effects; that is, the animals appeared to be protected from a traumatic noise exposure despite not receiving the toughening noise protocol. They suggested that toughening-related protection might be the result of a general stress-mediated response.

It is interesting that in most of the papers on toughening-elicited protective effects, the work of Miller et al. (1963) is referenced as the first demonstration of a toughening effect being elicited by an interrupted noise exposure, yet most of the published work is silent on Miller et al.'s finding that despite toughening there was no protection from a subsequent traumatic exposure.

Also germane to the issue of protection is a study by Hamernik and Ahroon (1998) that explored the extent to which the noise-toughened cochlea is protected from the very noise that produced the toughening. On the basis of their large-sample ($n = 266$) study, they concluded that there was no correlation between the amount of toughening and the extent of the permanent noise-induced changes (AEP thresholds and sensory cell populations) produced by the interrupted noise that caused the toughening.

It is clear from this brief review that threshold shifts following a severe noise exposure can be consistently modulated by up to 30 dB or more by a variety of toughening-inducing noise paradigms while the permanent effects on threshold measures, emissions measures, and sensory cell populations can be extremely variable and not necessarily consistent with what would be expected from a toughened cochlea. The objective of the experiments reported in this paper were to use an interrupted noise exposure, that is known to produce a large toughening effect, to determine if consistent protective effects could be elicited on threshold metrics (temporary and permanent) and sensory cell losses following a more severe exposure.

2. Methods

Seventy-six chinchillas, randomly divided into seven groups with 9–12 animals/group, were exposed to a toughening noise or to one of two traumatizing noises or to a toughening noise followed 18 h or 30 days later by one of the two traumatizing noises. Brainstem (inferior colliculus) auditory evoked potentials (AEPs) collected from monaural chinchillas 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

All the exposures consisted of narrow band impacts (NBI) 400 Hz wide, having a 1 kHz center frequency, presented at the rate of 1 impact/s. All noise stimuli were generated digitally using a virtual instrument developed using the LabView™ software package. A fixed-length pulse was fed through a fourth-order, band-pass Butterworth filter. The resulting waveform was played though 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. 1 shows the relative spectrum and temporal waveform of the 115 dB peak SPL NBI. Waveforms and spectra at other levels were qualitatively similar.

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 SPLs in the exposure field were uniform to within 2 dB. The seven groups of animals were exposed to one of the following impact presentation protocols:

Group 1 ($n=9$). The toughening exposure; 115 dB peak SPL, 6 h/day for 10 days.
Group 2 ($n=9$). A traumatic exposure; 121 dB peak SPL, 24 h/day for 5 days.
Group 3 ($n=10$). A traumatic exposure; 127 dB peak SPL, 24 h/day for 5 days.
Group 4 ($n=12$). The toughening exposure followed after 30 days by the 121 dB traumatic exposure.
Group 5 ($n=12$). The toughening exposure followed after 18 h by the 121 dB traumatic exposure.
Group 6 ($n=12$). The toughening exposure followed after 30 days by the 127 dB traumatic exposure.
Group 7 ($n=12$). The toughening exposure followed after 18 h by the 127 dB traumatic exposure.

2.2. Threshold testing

Thresholds for all AEP audiograms were measured at octave intervals of 0.5–16.0 kHz. The mean (in dB SPL) of three threshold determinations measured on different days defined each animal's pre-exposure audiogram. A complete audiogram was measured once daily during each of the five exposure days of the uninterrupted traumatic exposure paradigms and the average (in dB SPL) taken over the 5 days established the mean asymptotic threshold levels. For the 10 day interrupted exposure paradigm a complete audiogram was measured immediately following the first and last two daily

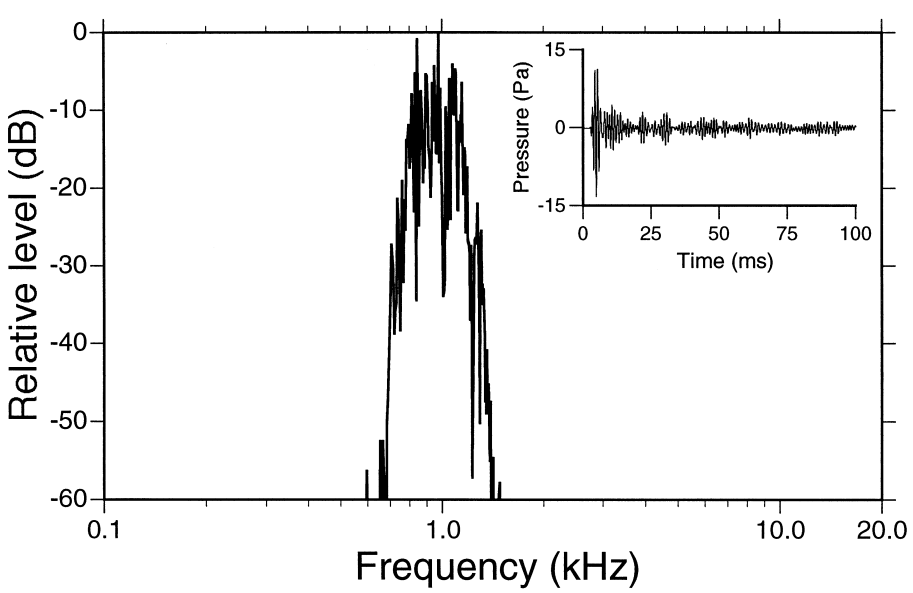

Fig. 1. The spectrum and pressure-time history of the 115 dB peak SPL, narrow band impact that was used in the interrupted noise exposures. The spectra and waveforms of the 121 and 127 dB peak SPL impacts used in the uninterrupted noise exposures were qualitatively similar.

6 h exposures in order to establish the magnitude and time course of the toughening. The amount of toughening 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. Groups 4 and 6 were allowed to recover from the interrupted (toughening) noise for 30 days and a complete AEP audiogram was measured on each animal prior to exposure to the traumatic noises. Groups 5 and 7 were allowed to recover for only 18 h after the toughening noise exposure and AEP threshold levels were not measured prior to the traumatic exposure. Thirty days following the complete exposure protocol for each group, AEP audiograms were measured again on three different days and averaged for each animal to establish permanent postexposure threshold levels.

2.3. 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 12–24 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). For purposes of this presentation, sensory cell population data are presented as group averages taken over octave band lengths of the cochlea centered on the primary AEP test frequencies.

2.4. 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). Analyses of thresholds within groups of animals was performed using completely within-subjects analyses of variance (Figs. 2, 5 and 6). The probability of a type I error was set at 0.05 for all analyses. Analysis of variance summary tables may be obtained from the authors.

2.5. Animal care

The care and use of the animals used in this study were approved by the SUNY Plattsburgh 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 all of the following figures the shaded region on the AEP audiograms represents the mean normative AEP audiogram (±1 S.D.) based on a population of 924 chinchillas. The bars on the data points in each figure represent 1 S.E.M.; where no bar is shown the standard error was less than the size of the datum symbol.

Fig. 2a shows the mean thresholds of the group 1 animals measured at the indicated times before and after the toughening exposure. There were no statisti-

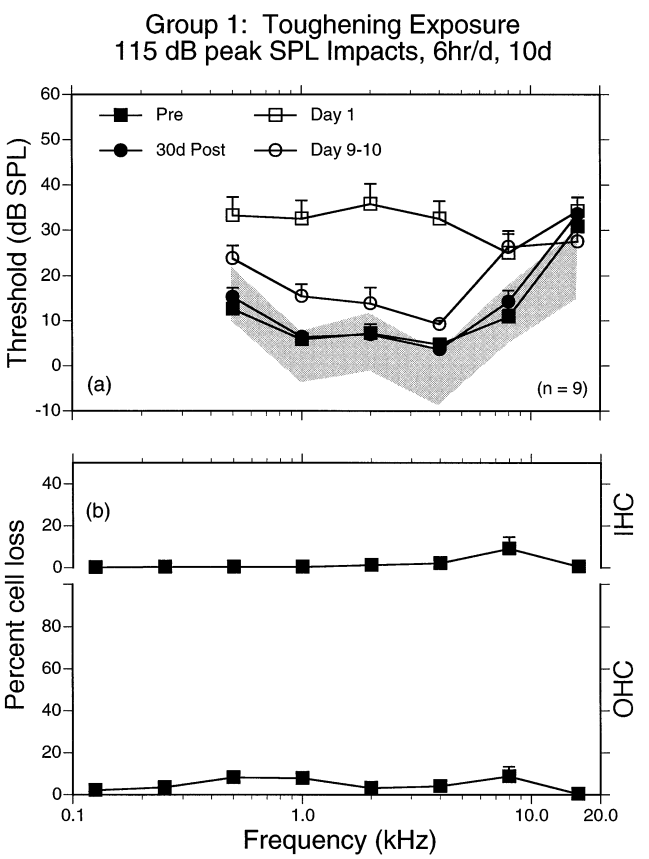

Fig. 2. a: Group mean AEP thresholds measured before (■) and 30 days after (●) the toughening exposure. Also shown are the thresholds measured after the first 6 h exposure (□) and the mean of the thresholds measured after the day 9 and 10 (○) exposures. The vertical distance between the open symbols is a measure of the amount of toughening produced by the interrupted noise exposure. b: The mean percent IHC and OHC losses produced by the interrupted exposure.

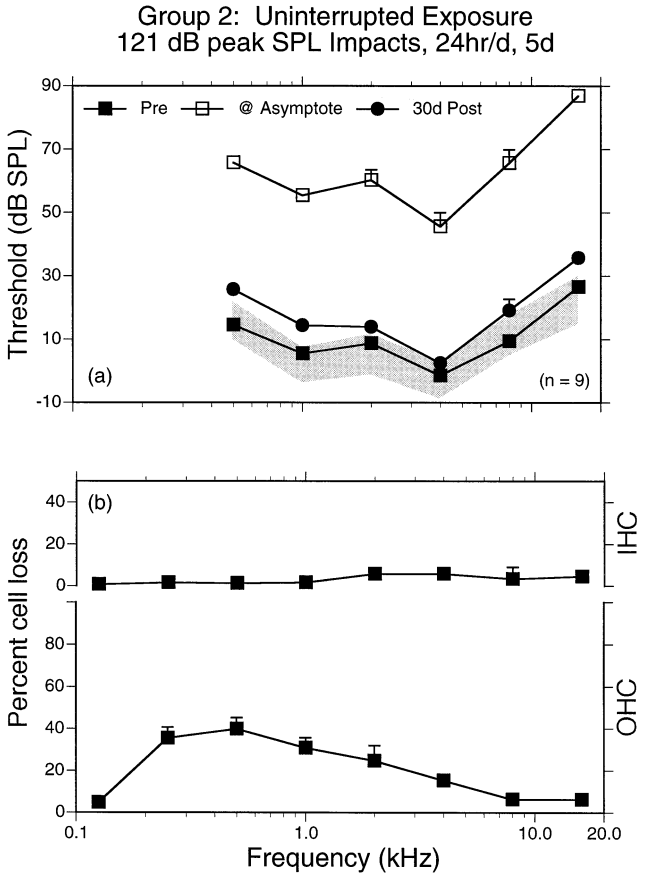

Fig. 3. a: Group mean AEP thresholds measured before (■) and 30 days after (●) the 121 dB uninterrupted traumatizing exposure. Also shown are the asymptotic thresholds measured during the 5 day exposure (□). b: The mean percent IHC and OHC losses produced by the uninterrupted 121 dB exposure.

cally significant differences between the thresholds measured before and 30 days following the toughening noise exposure. Thresholds were within normal limits indicating that the toughening exposure had no lasting effect on AEP thresholds. Immediately following the first 6 h (day 1) of the toughening exposure thresholds were shifted about 18–28 dB between 0.5 and 4.0 kHz. By the 10th day of the interrupted exposure thresholds have recovered 10–23 dB between 0.5 and 4.0 kHz; i.e., the auditory system was, by definition, toughened 10–23 dB as a result of the interrupted noise exposure. There was no statistically significant toughening at 8.0 or 16.0 kHz. Fig. 2b shows the group mean OHC and IHC loss averaged over octave-wide bin lengths of the basilar membrane for the group 1 animals. Both IHC and OHC losses were less than 10% and localized to the 8 kHz bin for IHCs and to the 0.5, 1.0 and 8.0 kHz bins for the OHCs. Losses of this amount are usually not reflected in either behavioral or AEP threshold measures (Hamernik et al., 1989).

Figs. 3 and 4 show the group mean thresholds and sensory cell loss from the animals exposed to one of the two traumatic noise exposures; i.e., the 121 dB (group 2) and 127 dB (group 3) peak SPL, uninterrupted 5 day exposures. During the exposure an ATS is produced, which in the 121 dB group amounted to between 45 and 60 dB across the test frequency range. In the 127 dB group ATS varied from about 62 dB to 76 dB. Thirty days after the exposure there were statistically significant elevations in thresholds; in the former group they were elevated 11 dB or less while in the latter group PTS varied between about 13 and 28 dB. Sensory cell losses were commensurate with the PTS; in the 121 dB group there was a broad loss of OHCs centered at 0.5 kHz with a maximum loss of 40%. In the 127 dB group nearly 80% of the OHCs and 30% of the IHCs were missing in the 1.0 kHz region. In both groups the OHC loss extended into the basal regions of the cochlea.

Figs. 5 and 6 present the threshold shift dynamics, measured prior to the traumatic exposure, in the four groups of animals that were toughened with the 115 dB peak SPL, interrupted noise. These animals were then exposed, following either an 18 h or a 30 day quiet interval, to either the 121 or 127 dB traumatic noise. In each of the four groups thresholds were shifted fol-

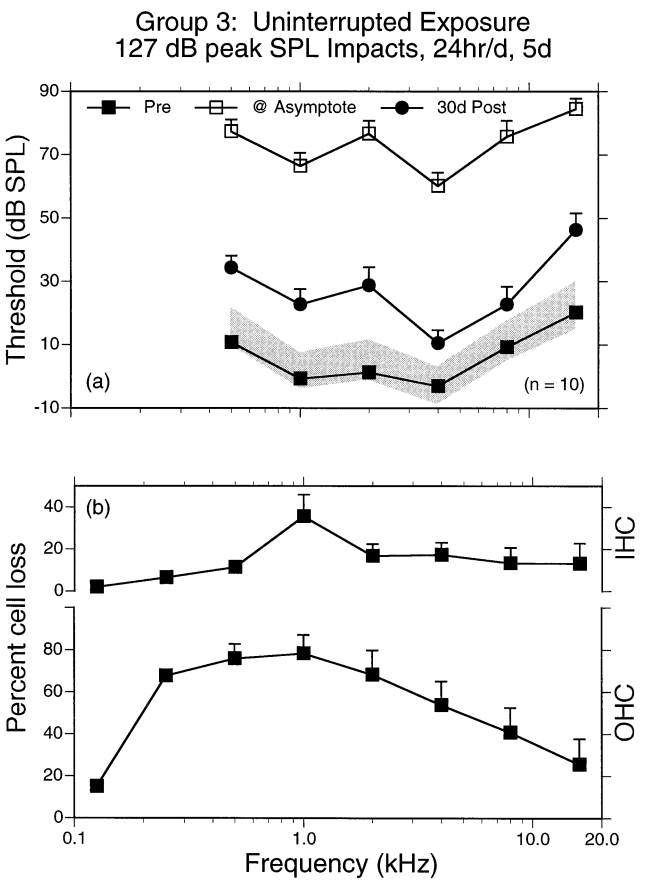

Fig. 4. a: Group mean AEP thresholds measured before (■) and 30 days after (●) the 127 dB uninterrupted traumatizing exposure. Also shown are the asymptotic thresholds measured during the 5 day exposure (□). b: The mean percent IHC and OHC losses produced by the uninterrupted 127 dB exposure.

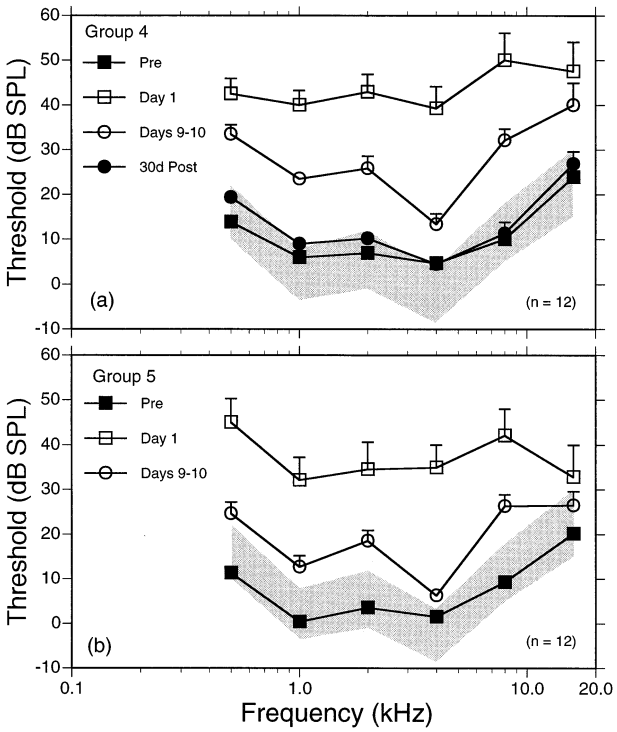

Fig. 5. a: Group mean AEP thresholds measured before (■) and 30 days after (●) the toughening exposure in animals that will be exposed to the 121 dB traumatizing noise 30 days after the toughening exposure. Also shown are the thresholds measured after the first 6 h exposure (□) and the mean of the thresholds measured after the day 9 and 10 (○) exposures. The vertical distance between the (□) and (○) symbols is a measure of the amount of toughening produced by the interrupted noise exposure. b: A similar presentation of threshold data for the group 5 animals that will be exposed to the 121 dB traumatizing noise 18 h after the toughening exposure. Thresholds were not measured in this group 18 h after the toughening exposure.

lowing the first 6 h exposure from 20 to 40 dB at and below 8 kHz. The greatest amount of toughening was measured at 4 kHz, and amounted to between about 22 and 28 dB across the four groups while at other test frequencies below 8 kHz it varied between 10 and 20 dB. Thus the four groups of animals showed fairly large toughening effects across most of the audiometric test frequency range (16 kHz being the notable exception) prior to being exposed to the higher level noises. Note that unlike the toughened group 1 animals the toughened groups 4–7 animals showed significant toughening at the 8 kHz test frequency.

Figs. 5a and 6a also show the group mean audiograms for the toughened groups 4 and 6 prior to the traumatic exposure. These two groups were allowed to recover from the toughening exposure for 30 days prior to their respective traumatic exposures. For the group 4 animals there were no statistically significant differences between the pre- and 30 day post-toughening thresholds, while the group 6 animals showed a small but statistically significant difference that amounted to 2 dB at 8 kHz and −5 dB at 16 kHz. For groups 5 and 7, which were allowed to recover for only 18 h after the interrupted noise exposure, thresholds were not measured immediately prior to the traumatic exposure. The thresholds of the group 5 animals upon removal from the toughening noise were within 5–17 dB of their pre-exposure values while the group 7 animals were within about 9–22 dB of their pre-exposure values. Thus, after an 18 h recovery, these two groups of animals, on average, probably entered the 121 or 127 dB traumatic exposures with some residual temporary threshold shift.

Figs. 7 and 8 show the response of the toughened groups 4 and 5 and groups 6 and 7 during exposure, to the 121 and 127 dB peak SPL, uninterrupted 5 day exposures respectively. Group 4, which recovered for 30 days following the toughening exposure, showed a statistically significant decrease in ATS of 16–25 dB when compared to the response of the untoughened group 2 animals. The group 5 animals, which were allowed to recover for only 18 h following the toughening exposure, showed a response to the 121 dB exposure

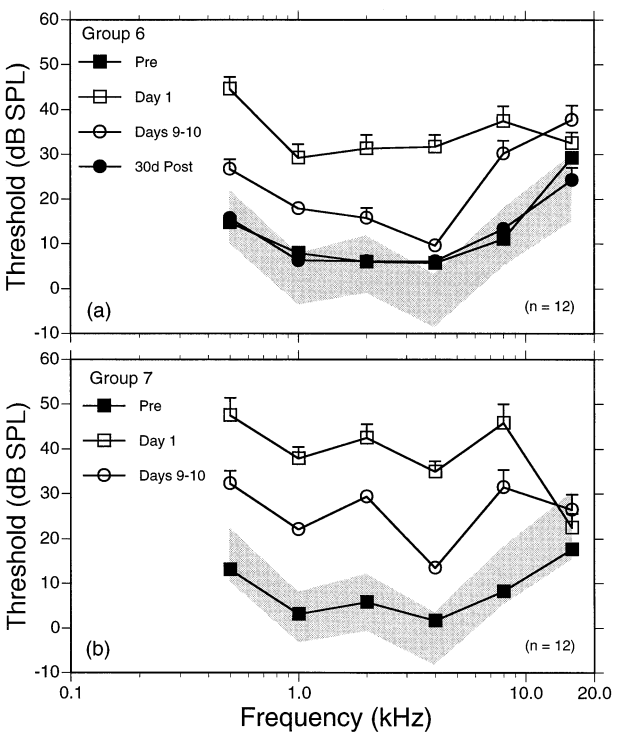

Fig. 6. a: Group mean AEP thresholds measured before (■) and 30 days after (●) the toughening exposure in animals that will be exposed to the 127 dB traumatizing noise 30 days after the toughening exposure. Also shown are the thresholds measured after the first 6 h exposure (□) and the mean of the thresholds measured after the day 9 and 10 (○) exposures. The vertical distance between the (□) and (○) symbols is a measure of the amount of toughening produced by the interrupted noise exposure. b: A similar presentation of threshold data for the group 7 animals that will be exposed to the 127 dB traumatizing noise 18 h after the toughening exposure. Thresholds were not measured in this group 18 h after the toughening exposure.

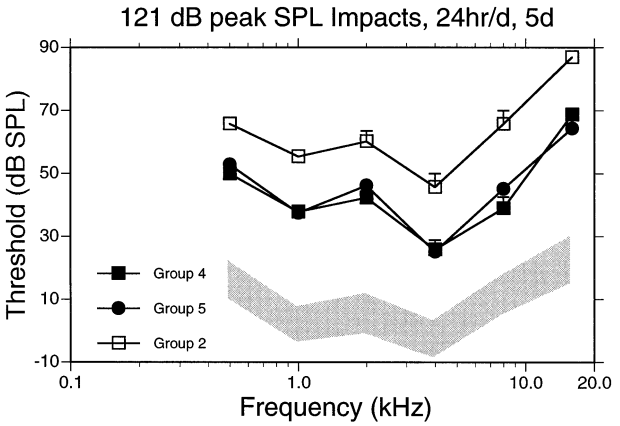

Fig. 7. Group mean asymptotic thresholds measured during the 5 day uninterrupted 121 dB exposure in groups 2, 4 and 5. Group 2 animals (□) were not toughened, while group 4 (■) and 5 (●) animals were toughened by the interrupted exposure paradigm.

that was virtually identical to that of the group 4 animals. Analysis of variance confirmed the effect of group and also indicated that there was no statistically significant difference between the response of groups 4 and 5. That is, the interval of time (18 h versus 30 days) between toughening and noise trauma had no effect on thresholds at asymptote.

When the level of the traumatizing noise was increased to 127 dB (Fig. 8), the analysis of variance revealed no statistically significant differences between groups 3 and 7. Comparisons between threshold at asymptote for groups 3 and 6 indicated no main effect of group but a significant interaction between group and frequency, indicating that the toughened group showed lower thresholds (~10 dB) at only the 8.0 kHz test frequency. There was no statistically significant effect of group in the analysis of the asymptotic threshold levels for the two toughened groups (groups 6 and 7) exposed to the 127 dB noise, but there was a

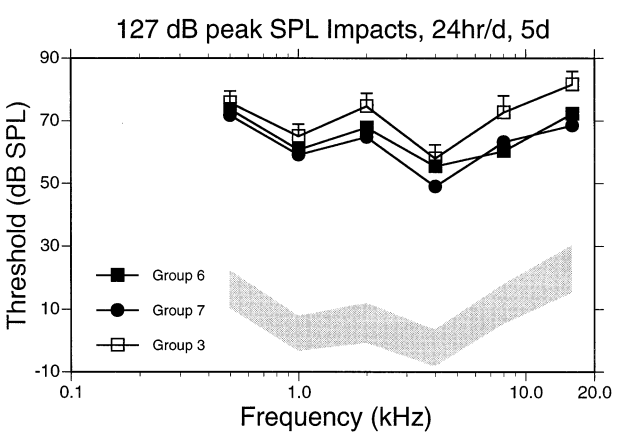

Fig. 8. Group mean asymptotic thresholds measured during the 5 day uninterrupted 127 dB exposure in groups 3, 6 and 7. Group 3 animals (□) were not toughened, while group 6 (■) and 7 (●) animals were toughened by the interrupted exposure paradigm.

statistically significant interaction as a result of the 6 dB difference in thresholds at the 4 kHz test frequency. Based on the above, one could conclude that the interval of time (18 h versus 30 days) between toughening and noise trauma had a neither substantial nor a systematic effect on thresholds at asymptote.

The permanent effects of the two traumatic noise exposures on the toughened and untoughened animals are shown in Figs. 9 and 10. Thirty days following the 121 dB exposure the analysis of variance of AEP thresholds revealed no statistically significant main effect of group nor a statistically significant interaction between group and frequency among the three groups. That is, the toughened and untoughened animals showed the same thresholds measured 30 days after the 121 dB exposure. The IHC and OHC losses for these three groups of animals are shown in Fig. 9b. As in the AEP threshold measures, there were no statistically significant differences in sensory cell losses among the three groups. In all three groups the IHC population was very near normal while the OHCs showed a broad loss of about 20–40% between 0.25 and 2.0 kHz. Thus, on the basis of the above, the toughened cochlea, regardless of the interval between the toughening and traumatic exposures, clearly showed

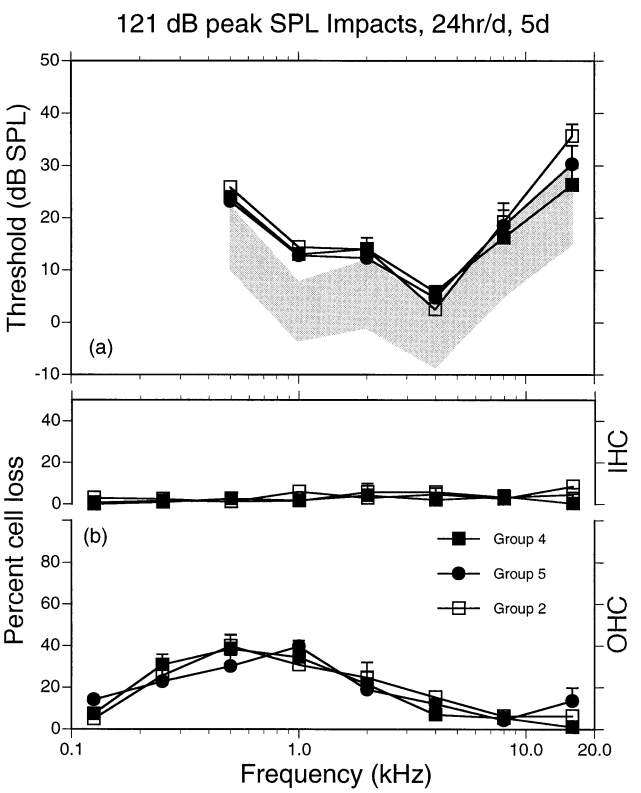

Fig. 9. a: Group mean AEP thresholds measured 30 days after the 121 dB uninterrupted traumatizing exposure in the group 2, 4 and 5 animals. Group 2 animals (□) were not toughened, while group 4 (■) and 5 (●) animals were toughened by the interrupted exposure paradigm. b: The mean percent IHC and OHC losses produced by the uninterrupted 121 dB exposure in the group 2, 4 and 5 animals.

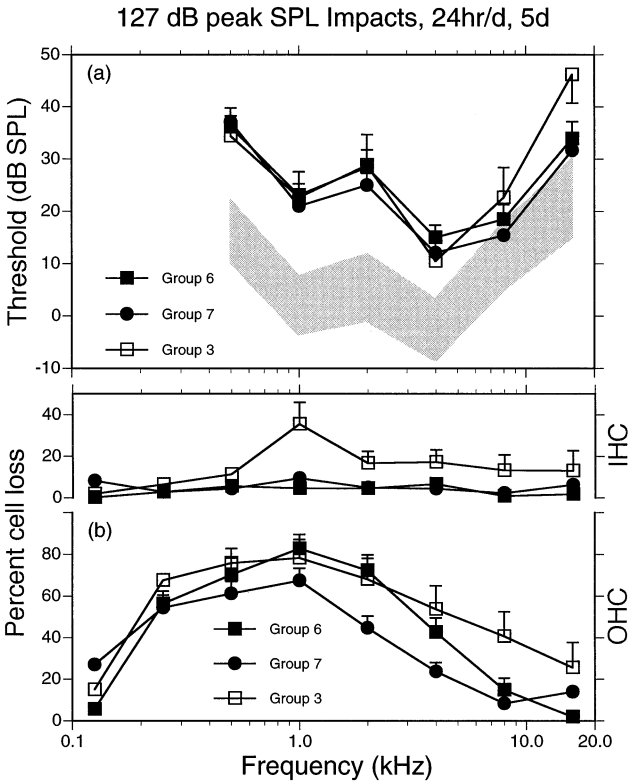

Fig. 10. a: Group mean AEP thresholds measured 30 days after the 127 dB uninterrupted traumatizing exposure in the group 3, 6 and 7 animals. Group 3 animals (□) were not toughened, while group 6 (■) and 7 (●) animals were toughened by the interrupted exposure paradigm. b: The mean percent IHC and OHC losses produced by the uninterrupted 127 dB exposure in the group 3, 6 and 7 animals.

substantially less ATS than the untoughened cochlea as a result of the 121 dB exposure but this savings is not manifested in the permanent changes in AEP thresholds or sensory cell losses.

The results of the 127 dB exposure are not as straightforward as those of the 121 dB exposure. Thirty days following exposure to the 127 dB impacts there were no statistically significant differences in the thresholds of the two toughened groups, groups 6 and 7. That is, the recovery interval between the toughening and traumatic exposures had no effect on the final thresholds of these two groups. Comparison of the two toughened groups with the untoughened group 3 animals showed no statistically significant effect of group and only a group by frequency interaction as a result of the 14 dB lower thresholds at 16 kHz in the toughened group 7 animals and about a 12 dB lower 16 kHz threshold in the group 6 animals. Note that at this frequency in these two groups there was no toughening measured as a result of the interrupted noise exposure. Sensory cell losses for groups 3, 6 and 7 are shown in Fig. 10b. The IHC analysis for the two toughened groups 6 and 7 showed a main effect of group and an interaction of group and frequency. However, since the difference in the mean loss of IHCs between these groups is only 2%, the difference, despite the statistical result, is not particularly meaningful. More significantly, both toughened groups show statistically less IHC losses than the untoughened group 3 animals that showed a large IHC loss in the 1.0 kHz octave band and a consistent 15–20% loss above 1.0 kHz. Analysis of OHC losses in groups 7 and 3 showed that there was a significant main effect of group and an interaction of group and frequency. Compared to the untoughened group 3 animals, OHC losses were 20–30% less in the toughened group 7 animals above 1.0 kHz. Analysis of OHC losses in groups 3 and 6 indicated no statistically significant differences despite the approximately 25% difference in OHC losses at 8.0 and 16.0 kHz. Thus, for this exposure condition, a protective effect on the sensory cell population which correlates with the reduced 16 kHz high-frequency threshold in the toughened groups was found only in group 7 animals that were exposed to the traumatic noise 18 h after the toughening exposure. In the four toughened groups this is the only condition under which the interval between the toughening and traumatic exposures showed some substantive effect.

4. Discussion

A review of the papers referenced in Section 1 of this report indicates that, during the course of many different interrupted noise exposure paradigms, threshold shift dynamics can be altered. This alteration often takes the form of making the system less susceptible to subsequent threshold shifts. Since the declining threshold shifts associated with the intermittently repeating noise exposure have been referred to as a toughening effect, the natural expectation is that a toughened system is going to be less susceptible to (or protected from) the effects of a subsequent exposure. The analysis of a large data base acquired from a large variety of intermittent noise exposures led Hamernik and Ahroon (1998) to conclude that 30 days after the toughening exposure the toughened cochlea is essentially no different from the untoughened cochlea that received an equal-energy uninterrupted exposure. Any savings in PTS or cell loss associated with the toughening exposure was shown to be what would be anticipated as a result of the recovery intervals between daily exposure sessions and not the result of toughening. Their results suggest that there may be a component of CTS that is susceptible to modulation but that the modulation has no effect on the permanent effects of the toughening exposure. It is also clear, from the references noted in the introduction, that the toughened cochlea's response to a subsequent exposure can be altered. This alteration takes the form of making the system either less, more, or equally susceptible to

CTS, PTS and sensory cell loss depending, in as yet unclear ways, on the experimental conditions.

The series of experiments reported here provide a clear estimate of the amount by which the cochlea has been toughened prior to the traumatic exposure. All groups exposed to the 115 dB interrupted noise showed large amounts of toughening. For two of the groups (4 and 5) the toughening manifested itself in large reductions in ATS during the 121 dB traumatic exposure. For the 127 dB exposure the toughening effect on threshold at asymptote is not as evident and achieves statistical significance only at one test frequency (8 kHz).

The permanent effects of the 121 and 127 dB traumatic exposures in the noise-toughened groups bear some similarities to, and differences from other related published data. For the 121 dB exposure there were no statistically significant differences in permanent threshold levels among the three groups. Likewise, the sensory cell data analysis showed no group or interaction effects, i.e., the sensory cell losses across groups 2, 4 and 5 were not significantly different. Thus, while there was a substantial (16–25 dB) reduction in ATS in the toughened groups that was not related to the 18 h or 30 day post-toughening interval, this reduced ATS did not resolve to less PTS. That is, there was no protection. This result is in agreement with Miller et al. (1963) in demonstrating that TS dynamics can be modulated without any subsequent protective effects. This result is also similar to that reported by White et al. (1998), where an intermittent toughening paradigm produced large TS reductions across the test frequency range following a traumatic exposure but no protection against PTS. In the White et al. paper however, there was some reduction in the OHC loss in the toughened group. Unfortunately, while their Figure 5 appears to present group mean OHC data, there is no indication of variability or whether the reported differences are statistically significant.

In the Subramaniam et al. (1993) paper a low-frequency (0.5 kHz octave band) interrupted noise was used to toughen the cochlea prior to exposure to a 4.0 kHz octave band of noise that caused permanent losses. They found that despite a clearly measured toughening at the high audiometric test frequencies there was an exacerbation of the effect of the traumatic exposure at these frequencies; i.e., the toughened frequencies showed more trauma than found in untoughened animals. In this paper we show that while there is no statistically significant toughening at the 16.0 kHz test frequency, there is a systematic protective effect at 16.0 kHz for the two toughened groups exposed to the more intense traumatic exposure which extends, in group 7, to a strong effect on the OHC population. The obvious lack of correlation between the AEP thresholds and the sensory cell populations in the group 7 data, while problematic, is frequently reported in the noise effects literature. Furthermore considering the reports of Jock et al. (1996), Hamernik et al. (1998), and Trautwein et al. (1996) on cochlear function in the absence of IHCs one might not expect the reduced IHC losses in the group 6 or 7 animals to be reflected in the AEP thresholds.

Thus, considering all the above, several dilemmas are posed by the accumulating data bases that clearly demonstrate toughening produced by interrupted exposure paradigms. (1) A toughened cochlea can exhibit substantial reductions in ATS or CTS during a traumatic exposure without any commensurate effect on PTS or cell loss. (2) Frequencies that are not toughened can develop a statistically significant reduction of PTS and cell loss (i.e., be protected). (3) The traumatic noise can have an exacerbating effect on PTS and sensory cell loss at frequencies that have been clearly toughened.

5. Conclusions

The results described above indicate that while groups of animals are clearly toughened by interrupted noise exposure paradigms, a systematic protective effect on the permanent noise-induced changes from a subsequent exposure is not, in general, elicited by this toughening phenomenon. The reduction in sensory cell losses shown in Fig. 10 represents the largest protective effect that was found. While this may be a real effect we must also consider, in light of the lack of protective effects under the other experimental conditions, that variability in the group response may play a role. Only increasing the sample size will resolve this issue.

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