Conditioning-Related Protection From Acoustic Injury: Effects of Chronic Deafferentation and Sham Surgery

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Kujawa, Sharon G. and M. Charles Liberman. Conditioning-related protection from acoustic injury: effects of chronic deafferentation and sham surgery. J. Neurophysiol. 78: 3095–3106, 1997. The demonstrated protective role of the OC bundle (OCB) in acute acoustic overexposure has led to speculation that the OC system also might play a role in the reduction of threshold shifts seen in variety of chronic noise-exposure protocols, collectively referred to as “conditioning” or “toughening” of the ear (for reviews, see Canlon 1996; Subramaniam et al. 1996). These protective effects of acoustic-exposure history have been studied in a number of different laboratories, with a number of different paradigms; however, the protocols fall into two main classes. In one type of experiment, animals are conditioned by daily exposure (for 10–21 days) to a moderate-level acoustic stimulus, usually a mildly traumatic one, which is delivered in daily doses (e.g., 6 h on, 18 h off) with some threshold measure obtained immediately before and immediately after the daily noise dose (e.g., Subramaniam et al. 1991a, b). The toughening observed in this paradigm is that thresholds measured each day, immediately postexposure, improve as the number of daily doses increases. However, in most such studies, the thresholds measured each day immediately before the exposure progressively deteriorate, and the animals demonstrate PTS (and/or permanent cochlear damage) weeks after the daily exposures are terminated (Boettcher et al. 1992; Subramaniam et al. 1991b). Thus the toughening consists of an observed decrease in a compound threshold shift (CTS) consisting of a small, increasing PTS and a larger, decreasing TTS component.

These two paradigms differ in a number of important ways and may involve significantly different mechanisms. As described above, the first paradigm, the condition/trauma paradigm, measures a protective effect in PTS, whereas the second, the toughening paradigm, measures protection as a decrease in a CTS that is largely temporary in nature. A second

INTRODUCTION

Activation of the olivocochlear (OC) efferent system in anesthetized animals has been shown in a number of laboratories to minimize the acute and temporary threshold shifts (TTSs) seen after acoustic overstimulation (Rajan 1991; Reiter and Liberman 1995). The role of olivocochlear activation in protecting the ear in awake animals (or humans) from the permanent effects of acoustic overexposure is much less well studied, and the existing literature is somewhat contradictory (Handrock and Zeisberg 1982; Liberman and Gao 1995; Trahiotis and Elliott 1970; Zheng et al. 1997a). Nevertheless, a number of studies have suggested that chronically deafferented animals can show greater permanent threshold shifts (PTSs) than identically exposed control animals (Handrock and Zeisberg 1982; Liberman and Gao 1995; Zheng et al. 1997a).

The demonstrated protective role of the OCB in acute acoustic overexposure has led to speculation that the OCB system also might play a role in the reduction of threshold shifts seen in variety of chronic noise-exposure protocols, collectively referred to as “conditioning” or “toughening” of the ear (for reviews, see Canlon 1996; Subramaniam et al. 1996). These protective effects of acoustic-exposure history have been studied in a number of different laboratories, with a number of different paradigms; however, the protocols fall into two main classes. In one type of experiment, animals are conditioned by daily exposure (for 10–21 days) to a moderate-level acoustic stimulus, which, by itself, causes no damage or minimal damage to the auditory periphery. Then, after a variable rest period (with no experimental sound exposure), the animals are given a traumatic exposure of shorter duration to an acoustic stimulus; usually, the same spectrum as the conditioning stimulus is applied at a higher sound pressure (e.g., Canlon and Fransson 1995; Canlon et al. 1988). The protection observed is that the condition/trauma animals show less PTS (and/or cochlear damage) than a trauma-only group exposed to the same traumatic exposure without the conditioning exposures. In a second type of experiment, there is only one type of exposure stimulus, usually a mildly traumatic one, which is delivered in daily doses (e.g., 6 h on, 18 h off) with some threshold measure obtained immediately before and immediately after the daily noise dose (e.g., Subramaniam et al. 1991a, b). The toughening observed in this paradigm is that thresholds measured each day, immediately postexposure, improve as the number of daily doses increases. However, in most such studies, the thresholds measured each day immediately before the exposure progressively deteriorate, and the animals demonstrate PTS (and/or permanent cochlear damage) weeks after the daily exposures are terminated (Boettcher et al. 1992; Subramaniam et al. 1991b). Thus the toughening consists of an observed decrease in a compound threshold shift (CTS) consisting of a small, increasing PTS and a larger, decreasing TTS component.

These two paradigms differ in a number of important ways and may involve significantly different mechanisms. As described above, the first paradigm, the condition/trauma paradigm, measures a protective effect in PTS, whereas the second, the toughening paradigm, measures protection as a decrease in a CTS that is largely temporary in nature. A second
important difference is that, in the condition/trauma paradigm, the inner ear is functionally normal at the time of the trauma (even in the conditioned group), whereas in the toughening paradigm, the ear is accumulating permanent injury as the daily TTS component decreases. This accumulating chronic injury could be changing the response of the inner ear to trauma in different ways than the nontraumatic conditioning exposure.

The mechanisms underlying both of these protective effects are understood poorly. A significant role for the middle ear muscles (MEMs) has been ruled out by several studies of the condition/trauma paradigm in which animals with MEMs cut have been shown to develop protection in the same way as normal animals (Henderson et al. 1994; Ryan et al. 1994). One recent study has examined the role of the OC system in protection by comparing the behavior of normal and chronically deafferented animals in a hybrid paradigm involving repeated exposure to a mildly traumatic stimulus followed by a single exposure to a highly traumatic stimulus (Zheng et al. 1997a). The study’s conclusions were limited by the fact that only three successfully deafferented animals survived. The conclusions regarding an OC role in toughening were mixed. First, they showed that the decrease in CTS during the repeated exposure disappeared at some test frequencies, but not others, in the deafferented animals. Second they showed that PTS after the final exposure was greater in the deafferented animals; however, this latter result speaks to the issue of a protective role for the OC system in acoustic trauma, in general, rather than to the issue of an OC role in toughening, per se.

The purpose of the present study was to further investigate the role of the OC system in conditioning-related protection and in the overall protection of the ear from permanent effects of acoustic overstimulation. The experimental design was a condition/trauma paradigm in which the conditioning stimulus does not, by itself, cause any permanent deleterious threshold shifts and in which the ultimate metric of protection is one of PTS only (as seen in alterations of compound action potentials and distortion product otoacoustic emissions). We also were interested in whether any OC-mediated effects involved primarily the medial olivocochlear (MOC) system [the efferent supply to the outer hair cells (OHCs)] or the lateral olivocochlear (LOC) system, which contacts mainly the unmyelinated dendrites of afferent fibers in the inner hair cell area. Toward this end, the experimental design included two different lesion sites in the floor of the IVth ventricle: one positioned at the midline, which interrupts most of the MOC system to both ears while leaving the LOC system largely intact, and a second positioned laterally at the sulcus limitans, which eliminates both LOC and MOC systems unilaterally (Robertson et al. 1987).

METHODS

Experimental groups and experimental design

Experimental animals were albino guinea pigs of either sex. Animals entered the surgery/conditioning/exposure protocol weighing between 325 and 350 g. Animals were assigned randomly to one of eight groups, as described below. Exactly 39 days after entering the protocol, the auditory function of each animal was tested via measurement of compound action potentials (CAPs) and ear-canal distortion-product otoacoustic emissions (DPOAEs) in a terminal experiment (Fig. 1, Final Test), which was followed immediately by fixation and harvesting of the cochleas and brain stem. The timing, for the different groups, of the surgical treatments (if any), conditioning noise exposure (if any) and traumatic noise exposure (if any) are schematized in Fig. 1. The number of ears in each group is given at the left. The timing of experimental procedures was designed so that the animals would all be the same age at the time of acoustic overexposure and final physiological testing. All procedures were approved by the Animal Care and Use Committee of the Massachusetts Eye and Ear Infirmary.

Deefferentation surgery

Animals to undergo chronic lesions of the OCB were anesthetized with pentobarbital sodium (17.5 mg/kg ip) and ketamine/xylocaine (3.5 mg/kg im). After surgical levels of anesthesia were achieved, the skin and muscle layers over the occiput were reflected, and a small piece of the skull overlying the central portion of the cerebellar cortex was removed with rongeurs. The cerebellum was elevated gently, revealing the floor of the IVth ventricle, and an anterior-to-posterior cut was made in the dorsal surface of the brain stem with a small sickle-shaped knife. In some animals (Fig. 1, Midline OCB), the cut was positioned so as to sever the crossed component of the OCB to both ears. For other animals, the lesion was positioned at one side of the dorsal brain stem (Fig. 1, Unilateral OCB), to sever the entire OCB to one side (Liberman 1990). The side of the cut (right vs. left) was randomized among the animals in this group. In some animals, the landmarks on the brain stem surface were not sufficiently clear (due to bleeding), and no brain stem cuts were made; in others, the brain stem cuts did not section the intended pathway (see further text): both of the latter groups constitute the sham surgery groups.

Pretest

As illustrated in Fig. 1, all animals except those in the unilateral OCB lesion group were pretested on day 8 of the protocol, to ensure that no animal with a preexisting threshold shift was entered into the protocol. Animals to be pretested were sedated with pentobarbital (25 mg/kg ip) to allow insertion of the acoustic assembly into the ear canal. The pretest comprised the measurement of growth functions for the DPOAEs at 2f1–f2 in each ear. Pairs of equal-level primaries were used, with f2 (the higher of the 2 primary tones) set at each of eight logarithmically spaced values between 2.78 and 12.14 kHz (f1/f2 = 1.2). (Details of the DPOAE measurement techniques are described below: see Final testing of CAP and DPOAEs). Animals passed the pretest only if the 2f1–f2 DPOAEs to 40 dB SPL primaries were present (3 dB above the surrounding noise floor) at all eight f2 test frequencies.

Conditioning and traumatization

For both conditioning and traumatization, the stimulus was an octave band of noise (2.0–4.0 kHz) delivered in the free-field to unanesthetized and unrestrained animals suspended in cages (1 animal per cage division) within a small reverberant sound-exposure box (Liberman and Gao 1995). The acoustic stimulus was generated by a custom-made white-noise generator, filtered (Brickwall Filter with a 60 dB/octave slope), amplified (Crown power amp), and delivered (JBL compression driver) through an exponential horn fitted securely to a hole in the top of the reverberant box.

For the conditioning period, each animal was exposed to the octave band of noise at 85 dB SPL, on a 6-h on:18-h off schedule for 10 consecutive days, the last of which was 6 days before the
acoustic overexposure. The acoustic trauma consisted of a 4 h exposure to the same noise band presented at 109 dB SPL.

Sound pressure levels were measured at several positions within each cage using a 1/4-in Bruel and Kjaer condenser microphone. The sound pressure was found to vary by <1 dB across these measurement positions. Sound pressure was calibrated daily by positioning the microphone at the approximate position of the animal’s head without animals in the cages.

**Final testing of CAP and DPOAEs**

For the final test, animals were anesthetized with sodium pentobarbital (25 mg/kg ip) and fentanyl and droperidol (0.2 and 10 mg/kg im, respectively). Surgical levels of anesthesia were maintained with booster injection of pentobarbital (1/2 of original dose every 6 h) and fentanyl and droperidol (1/4 of original dose every 2 h). Surgical preparation for the terminal experiment involved insertion of a tracheostomy tube, exposing the bullas bilaterally, and severing the ear canals near the tympanic ring. The bullas were opened by shaving the bone with a scalpel blade.

The CAP was recorded from both ears of each case via a silver wire on the bone ventral to the round window referred to the tongue. The response was amplified (10,000 times), filtered (300 Hz to 3 kHz), and averaged with an A-D board in a LabVIEW-driven data acquisition system. CAP thresholds were measured under computer control in response to 5-ms tone pips (0.5-ms rise-fall with a cos² onset envelope) and were defined as the sound pressure required to produce a peak-to-peak response of 10 μV. In each animal, two separate measures of the threshold at each test frequency were obtained and the results averaged. For the CAP measurements, the acoustic stimuli were produced and monitored with a closed system consisting of a 1-in Bruel and Kjaer condenser microphone as the sound source and a 1/4-in condenser microphone to monitor sound pressure near the tympanic membrane (Kiang et al. 1965).

The DPOAEs were measured from both ears of each case using an ER-10C (Etymotics Research) acoustic system consisting of two sound sources and one microphone. The sensitivity of the microphone (dB volts/dB SPL) was measured on each experimental day by coupling a calibrated Bruel and Kjaer condenser microphone to the output port of the ER-10C system. Stimuli consisted of two equilevel primary tones (f₂ :f₁ ≈ 1.2). The tones were generated by a D-A board in a Macintosh Quadra 950. Attenuation was provided with external analog attenuators. The ear canal sound pressure was filtered (high pass at 1,000 Hz) and digitized by a D-A board, a FFT was computed, and the sound pressures at f₁, f₂, and 2f₁ − f₂ were extracted after spectral averaging from serial waveform traces. The noise floor also was measured (defined as the average of 6 points in the FFT on either side of the 2f₁ − f₂ frequency) and ranged between −20 and −10 dB SPL, depending on frequency.

**Histological preparation and analysis of deafferentation**

After the final test, the cochleas of each animal were harvested for processing as plastic-embedded surface preparations. Before removal from the skull, the ears were fixed by intralabyrinthine perfusion of a buffered solution of paraformaldehyde and glutaraldehyde. After overnight postfixation, the cochleas were osmicated, dehydrated, and infused with epoxy resins. The cochleas were dissected into a series of pieces, each containing ~1 mm of the organ of Corti, re-embedded in plastic, thinned with sanding disks, and mounted on microscope slides.

In one series of animals (i.e., those with unilateral OCB lesions), the animals were perfused intravascularly with a buffered aldehyde

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**FIG. 1.** Schematic illustrating the timing of experimental manipulations for different groups. Number of ears in each group is indicated at the left; data were obtained from both ears in all cases. Shorthand used to describe the groups is given to the right of the colon within each name-box. Initial letters (C, T, or CT) indicate the noise-exposure protocol (condition-only, trauma-only, or condition/trauma, respectively). Letters within the brackets indicate the surgical procedure and the success or failure of the OCB cut: X+ and X− for successful and unsuccessful cuts, respectively (either midline or unilateral); O+ and O− for opposite (control) sides of successful and unsuccessful unilateral lesions, respectively. Asterisks indicate either X+ or X−. Thus for example, the CT[X*] group consists of those ears from animals with intended OCB cuts (successful or not) in the condition/trauma group, whereas T[O−] comprises those ears contralateral to the brain stem cut in those cases for which the cut was unsuccessful from animals in the trauma-only noise-exposure group.
solution, and both the cochleas and brain stems were harvested. The cochleas were processed as described above. The brain stems were stained for acetylcholinesterase (AChE) activity to allow visualiza-
tion of the OCB (Osen and Roth 1969) and the success of the cut within the brain.

To assess the degree of deafferentation, the organ of Corti of each case was examined with high-power Nomarski optics. In such an examination of the osmium-stained cochlea, fascicles of MOC fibers can be seen in the tunnel of Corti as they cross to the OHCs. To quantify the degree of deafferentation, an observer (blinded to the physiological data and animal groupings) measured the diameters of all the MOC fascicles in the tunnel. The afferent innervation to the OHCs travels in the floor of the tunnel, enveloped by the feet of the pillar cells, whereas the efferent innervation crosses through the middle of the tunnel, entering the tunnel from the inner hair cell (IHC) area directly through the inner spiral bundle. Thus our measurements were made near the tunnel spiral bundle; such a metric gives reproducible results in control animals and provides a reliable index of the volume of MOC terminals remaining under the OHCs in deafferented animals (Liberman and Gao 1995).

In each animal, the measurements were made over one complete dissected piece of the organ of Corti (corresponding to ~1 mm of cochlear length). In each case, the piece was chosen from the cochlear region located ~25% of the distance from the cochlear base, where the volume of efferent fascicles is greatest in control animals. Results from a previous study (Liberman and Gao 1995) show that, in cases of incomplete OC lesion, the degree of deafferentation is uniform across the entire cochlea; thus a single sample (placed at the point of maximal normal innervation density) can estimate effectively the overall success of the lesion.

To assess the nature, severity, and extent of the structural damage from the sound exposures, cytocochleograms were prepared for a subset of the animals in the present study (see Liberman and Beil 1979 for technical details). These analyses of hair cell loss also were performed by an observer blind to the physiological results or animal groupings.

RESULTS
Histological assessment

The cochlear material allowed assessment of the degree of deafferentation in the animals with brain stem lesions and also allowed assessment of the nature and extent of the hair cell and neuronal lesions.

DEGREE OF DEAFFERENTATION. In a previous study of chronic OCB lesions (Liberman and Gao 1995), it was shown that the degree of deafferentation could be estimated effectively by measuring the summed diameters of all the fiber fascicles crossing to the OHCs through the tunnel of Corti. This metric of OC innervation is highly reproducible in normal ears: as shown in Fig. 2 (A and B), the normal values for the 12-KHz region of the cochlea (where the density of MOC innervation is greatest) are clustered tightly between values of 130 and 160 μm/mm.

These normal values for OC fascicle density are compared with values obtained in animals with unilateral brain stem lesions and those with midline brain stem lesions in Fig. 2, A and B, respectively. Data from the unilateral lesions (Fig. 2A) are further divided on the basis of an independent assessment of lesion success (by a different observer) based on analysis of the AChE-stained brain stems. In the brain stem tissue, the normal OCB is a clear and compact bundle of AChE-positive fibers running from the floor of the IVth ventricle through the facial genu ventrally to exit the brain with the vestibular nerve root. In the “successful” unilateral lesion cases, the knife cut is visible at the normal location of the bundle beneath the sulcus limitans, and the absence, or diminution in the size of, the bundle on the cut side can be seen clearly. In the unsuccessful cases, the knife cut was usually too superficial to reach the OCB, and an AChE-positive bundle, of normal size, appeared on both sides of the lesion. On the basis of this brain stem analysis, the unilateral lesion cases were classified as successful or unsuccessful (with partial cuts included in the successful group). As shown in Fig. 2A, there is good agreement between the brain stem analysis and the cochlear analysis, in that all unsuccessful cases show MOC fascicle densities

FIG. 2. Assessment of the success of the deafferentation surgery by measuring olivocochlear (OC) fascicles in the tunnel of Corti after unilateral lesions (A) or midline lesions (B). Each point represents the average summed diameter of OC fascicles (in micrometers) seen per millimeter of cochlear length, as averaged over a ~1-mm piece of the organ of Corti. Control data (from ears with no brain stem lesions) are shown in both panels. Cochlear location is converted to frequency via a cochlear map (inset) that also allows assessment of the nature and extent of the hair cell and neuronal lesions 1997.

A: cases are classed as successful or unsuccessful based on an independent assessment of acetylcholinesterase (AChE)-stained brain stem (see text for further details). B: brain stems were not processed in these cases, thus successful (X+) vs. unsuccessful (X−) cases are differentiated based on the medial olivocochlear (MOC) fascicle analysis only.
damage to the OHC stereocilia extending apical and basal to the lesion focus as defined by the cytocochleogram (plotting hair cell loss only).

Physiological assessment

CAP thresholds. Animals without chronic surgery. The conditioning exposure in our experimental paradigm was designed to be one that, by itself, does not cause cochlear damage. Indeed, as indicated in Fig. 4, average CAP thresholds for animals that received only the conditioning exposure (group C: no brain stem surgery and no traumatic exposure) were slightly lower than CAP thresholds for the control group, which received neither brain stem surgery, conditioning nor traumatic exposure. Note that these condition-only animals were tested 6 days after the end of the conditioning period, i.e., at exactly the time when the condition-trauma animals would have been exposed to the high-level noise. The possible significance of this response enhancement due to conditioning is discussed elsewhere (Kujawa and Liberman 1996).

The conditioning exposure, in our experimental paradigm, also was designed to be one that, when presented in advance of the traumatic exposure, provided a significant protective effect. This conditioning-related protection is illustrated in Fig. 5A, which compares the mean CAP thresholds in the three groups of animals that did not undergo chronic brain stem surgery: control animals (without conditioning or traumatic noise exposure); trauma-only (T) animals, which were exposed to the 4-h traumatic exposure at 109 dB SPL without any prior conditioning; and condition/trauma (CT) animals, which were exposed to the 10-day conditioning at 85 dB SPL before the 4-h traumatic exposure at 109 dB SPL. The PTS due to the traumatic exposure is the difference between the control and the T data; the difference between the T and CT curves is the conditioning-related protection. That difference, plotted as the gray squares in Fig. 8, ranges from 8 to 20 dB, peaking at test frequencies near 6 kHz.

Animals with midline lesions. The average CAP thresholds for different groups of animals with midline brain stem...

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1 For the midline lesion cases, efferent fascicle density was only measured in one of the two ears. A previous study of this lesion in guinea pigs showed that the degree of deafferentation suggested by this metric was always symmetrical on the two sides of one case (Liberman and Gao 1995).
The important conclusions from these animals undergoing unilateral brain stem cuts are as follows. First, the loss of efferent innervation dramatically increases the ear’s vulnerability to noise exposure: throughout much of the test frequency range all the successfully deefferented ears (open circles and triangles in Fig. 6A) show significantly more threshold shift than the unsuccessful cuts; this increased vulnerability is seen for both the trauma only (T) and the condition/trauma group (CT). The magnitude of this OC-mediated effect on PTS is illustrated in Fig. 7, in which the difference between the CAP mean threshold curves are plotted. The PTS difference between the successful and unsuccessful deefferentations peaks at ~20 dB for the condition/trauma group and 15 dB for the trauma-only group. The maximum OC-mediated protection appears at test frequencies of ~8 kHz. The apparent “anti-protective” effect seen at high frequencies among the trauma-only ears arises from a bilateral threshold elevation at high frequencies; see curves labeled T[X−] and T[O−] in Fig. 6. It could be a statistical anomaly due to the small sample size (only 3 animals in this group).

Second, the conditioning exposure has a significantly dele-
COCHLEAR DEEFFERENTATION AND CONDITIONING

FIG. 7. Measures of the protection afforded by an intact OCB, expressed as the difference in average CAP thresholds between successful [X+] and unsuccessful [X−] defferentations for both the condition/trauma (CT) groups and the trauma only (T) group. Mean CAP data for each of the four groups used to compute these 2 difference functions are shown in Fig. 6A.

The only possible exception was the CT[O] group, i.e., the uncut sides of condition/trauma cases in which the cut was successful. This group may show a small anti-protective effect analogous to that described above. Note finally, that all other groups of animals that underwent the chronic surgery and were not defferentiated appear protected whether or not they were exposed to the conditioning noise exposure.

DPOAE THRESHOLDS. For all of the ears in the present study, measures of DPOAE growth functions at 2f1-f2 also were performed as part of the final test. These growth functions, obtained at a number of f2 frequencies, were transformed into iso-response contours, analogous to the CAP iso-response contours analyzed earlier, and those DPOAE contours were

FIG. 8. Measure of the effect of conditioning on the PTS from the traumatic exposure for normal (gray squares) vs. defferentiated (black squares) ears, demonstrating that, whereas conditioning provides protection in normal ears, the same conditioning exposure increased the PTS in defferentiated, traumatized animals. The normal data in this figure represent the difference between the trauma only (T) and condition/trauma (CT) groups, as shown in Fig. 5A; the defferentiated data represent the difference between the analogous exposure groups from the successful unilateral defferentations (T[X+] - CT[X+]), as shown in Fig. 6A.

Effect, either protective or deleterious, on the PTS magnitude in the successfully defferentiated group. Instead of protecting the ears against subsequent trauma, the CAP thresholds in the defferentated condition/trauma (CT[X+]) group are higher than those in the defferentated trauma-only (T[X+]) group. This anti-protective effect is illustrated graphically in Fig. 8, where the conditioning-related protection described above for the no-surgery groups (T-CT from Fig. 6) is compared with the conditioning-related effect in the successful defferentations (T[X+] - CT[X+] from Fig. 6). In the unsuccessful defferentations (filled symbols in Fig. 6A) and among three of four groups contralateral to the brain stem lesion (all symbols in Fig. 6B), the conditioning exposure has no significant

FIG. 6. Average CAP thresholds for all animals exposed to the traumatic stimulus after unilateral brain stem lesion. Ears are divided into 8 groups according to whether they were ipsilateral ([X]: A) or contralateral ([O]: B) to the OC bundle (OCB) cut. Within each panel, they are further divided according to the noise-exposure history, condition/trauma (CT) vs. trauma only (T), and according to the success ([+]) or failure ([-]) of the lesions. Data from successful defferentations are shown as open symbols. Data from A are reproduced in gray to facilitate comparison of threshold shifts. Error bars correspond to standard errors of the mean. Numbers of ears in each group are shown in the legend.
effect varies with exposure frequency: it is most robust for exposures that involve cochlear frequency regions near 15 kHz (Rajan 1995; Reiter and Liberman 1995). A recent study from our laboratory suggested that these protective effects were related causally to the slow effects of OC stimulation (Reiter and Liberman 1995); these effects are thought to involve slow increase in intracellular Ca$^{2+}$ in the OHCs (Sridhar et al. 1995, 1997).

Much less is known about the chronic protective effects of the OCB in awake animals for exposures producing PTSS. One study (Handrock and Zeisberg 1982) showed that cutting the OCB in guinea pig (by cutting the inferior vestibular nerve) greatly increased the PTS seen after an exposure to a 4-kHz octave band noise at 125 dB SPL for 30 min, although there was no effect on the TTS from the same noise at 120 dB for 5 min. The PTS increased from a mean of 16 dB to a mean of 40 dB in deafferented animals. Two studies of unilateral deafferentation in chinchillas (Zheng et al. 1997a,b), performed by cutting the inferior vestibular nerve, also showed a dramatic increase in PTS after exposure to an octave band of noise centered at 4 kHz presented at 95 dB for 48 h, although the number of successful deafferentations was extremely small (see further). Previous work in our laboratory showed that lesions to the OCB at the midline IVth ventricle produce modest increases in vulnerability of guinea pigs to a noise band centered at 10 kHz presented at 112 dB SPL for 2 h (Liberman and Gao 1995).

One of the most clear-cut results of the present study was the large increase in vulnerability to acoustic injury associated with the complete transection of the OCB. As shown in Fig. 6A, for both the condition/trauma group and the trauma-only group, there was a very large and systematic increase in the PTS for the successful versus the unsuccessful lesions: i.e., $PTS_{CT(X^+)} > PTS_{CT(X^-)}$ and $PTS_{T(X^+)} > PTS_{T(X^-)}$. Furthermore, a comparison of the data in Fig. 6, A and B, shows that for both noise-exposure groups, the successful unilaterally deafferented ears showed significantly more PTS than the opposite control sides: i.e., $PTS_{CT(X^+)} > PTS_{CT(X^-)}$ and $PTS_{T(X^+)} > PTS_{T(X^-)}$. Indeed, the presence of an asymmetrical pattern of PTS in a particular animal (higher thresholds on the cut side) was an excellent physiological predictor of the success of the unilateral lesion (as verified histologically). The results from the midline lesions were much less dramatic. Indeed, even those animals with successful midline cut (CT[X+] and T[X+]) showed no more PTS than their counterparts with an intact (CT[X−] and T[X−]) OC system.

Thus, all existing studies agree that cutting the crossed OCB (COCB) at the midline in the brain stem has, at most, modest effects on cochlear vulnerability; whereas, cutting the entire OCB, either in the brain stem or in the internal auditory meatus, leads to large increases in vulnerability to noise bands in the mid- to high-frequency range. In interpreting these results, it is important to review the differences between the effects of OC lesions in the different sites. According to current understanding of the OC pathways, a successful complete transection of the COCB should eliminate roughly two-thirds of the MOC pathway to both ears and leave the LOC pathway largely intact (Guinan et al. 1983; Robertson et al. 1987). Consistent with this prediction is the observation, shown in Fig. 2, that the maximum loss

![Fig. 9. Average distortion-product otoacoustic emission (DPOAE) thresholds for the all animals exposed to the traumatic stimulus after unilateral brain stem lesion, i.e., the same 8 groups of animals shown in Fig. 6. DPOAE threshold is derived from growth functions for 2f − f; and is defined as the sound pressure of the equilevel primaries required to produce a DP of −5 dB SPL. Error bars correspond to standard errors of the mean.](image-url)
of OHC efferents in our midline lesion cases was roughly two-thirds. In contrast, a cut at the side of the brain stem or in the inferior vestibular nerve should interrupt completely both the MOC and LOC systems to one ear because all fibers are intermingled in a reasonably compact bundle at these loci (Arnesen and Osen 1984; Terayama et al. 1969).

In light of these anatomic observations, there are three main interpretations of the existing data. The first is that the protection seen is mediated mainly by the uncrossed component of the MOC system, i.e., those fibers not transected by the midline cut. We know from a variety of sources that the uncrossed OCB contains MOC neurons responding to sound in the contralateral ear (Warren and Liberman 1989). The idea that the contralaterally responsive (contra) OC neurons contribute a larger protective effect than the ipsilaterally responsive (ipsi) fibers does not directly follow from any known aspect of the physiology or anatomy of the MOC system. For example, existing evidence (Brown 1989; Liberman 1988) suggests that the sound-evoked discharge patterns of contra and ipsi fibers are very similar and that there are no fundamental differences in the nature or extent of their cochlear projections, except that the COCB (ipsi units) projections, as a whole, are slightly skewed toward basal cochlear regions compared with the uncrossed OCB (UCOB) (contra units) projections (Guinan et al. 1984).

Nevertheless, the suggestion that the contralaterally responsive UOCB has a larger protective role than the ipsilaterally responsive COCB has some precedent in the literature from OC-mediated protection from TTS. For example, Rajan and colleagues have shown repeatedly that activation of the UOCB via contralateral sound decreases ipsilateral TTS, whereas cutting the COCB, which should interrupt all sound-evoked activation of the ipsilateral OC reflex, does not increase the TTS from a monaural exposure (Rajan 1991). Of course, other interpretations of the latter result (and the present result) include the hypothesis that some threshold level of OC activation is necessary to provide protection and that activation of both contra and ipsi MOC reflexes are necessary to cross that threshold.

The second main interpretation of the results is that the protection seen is mediated mainly by the LOC system, the major peripheral targets of which are the unmyelinated dendrites of auditory nerve afferents in the area under the inner hair cell (Smith 1961; Smith and Rasmussen 1963). The LOC system should be relatively unaffected by the midline cuts but should be completely eliminated by a successful cut at the side of the brain stem or in the internal auditory meatus. We only assessed the tunnel-crossing fascicles, which consist, mainly or exclusively, of MOC neurons to OHCs; however, a number of lines of evidence suggest that the LOC system is equally affected. First, in cats with unilateral OCB lesions in the brain stem, we have shown, via electron-microscopy of the cochlea, that the efferent system in the inner hair cell area, (i.e., the inner spiral bundle) is missing in ears judged successfully deafferented based on tunnel-crossing MOC fascicles (Liberman 1990). Second, the absence of an inner spiral bundle also has been noted via AChE staining of chinchilla cochleas deafferented by cutting the vestibular nerve (Zheng et al. 1997b) and with anti-neurofilament immunohistochemistry in guinea pigs deafferented with a surgical procedure identical to that used in the present study (unpublished observations). There are no other data relevant to a possible role of the LOC system in protecting the ear from acoustic overstimulation because nothing is known about the functional effects of activating this system on the inner ear. The fact that the LOC system does not directly target the OHCs, at least not in the basal half of the cochlea, is not consistent with the notion that its activation could decrease PTS in the present study, because the DPOAE data (Fig. 9) strongly suggest that the protection involves decreasing damage to the OHCs. Nevertheless, the possibility that some of the protective effects seen here are mediated by the LOC system is impossible to rule out.

An additional possibility to consider is that the increased vulnerability seen in all the above-mentioned studies are actually due to the changes in MEM function rather than in OC function. The facial nerve, which supplies the stapedius muscle, runs close to the lateral wall of the IVth ventricle where the OC bundle is lesioned for the unilateral cuts, and it also runs very close to the inferior vestibular nerve in the internal auditory meatus. A number of observations from other studies argue against a strong MEM contribution to the observed effects. First, a study of the magnitude of sound-transmission changes elicited by sound-evoked MEM contractions in awake guinea pigs has been shown to be very small (Avan et al. 1992). For example, for a white-noise elicitor presented at levels up to 100 dB SPL (i.e., 30 dB re acoustic reflex threshold), there was no measurable attenuation at frequencies >2 kHz and mean attenuation for frequencies <2.5 kHz was 1 dB. Second, a study of the acoustic injury in gerbils showed that surgical removal of the MEM tendons did not change the mean PTS in animals exposed, awake and unrestrained, to a two-octave noise band (1.4–5.6 kHz) at 110 dB for 1 h (Ryan et al. 1994). Further argument for a MOC role rather than a MEM role is the observation that the magnitude of the protective effects versus frequency (i.e., Fig. 7) is reminiscent of the density distribution of MOC terminals on OHCs as a function of cochlear location: both show a pronounced peak in the upper basal turn at cochlear regions corresponding to the 6–12 kHz regions (Liberman et al. 1990).

Conditioning-mediated protection: an OC role or a generalized stress response?

The protective effect of repeated daily exposure to moderate-level sounds now has been demonstrated in a number of species (including rabbit, guinea pig, chinchilla, gerbil, and rat). In the present study, we have found a number of different exposure paradigms (for review, see Canlon 1996). The paradigm we have chosen is a variant of the condition / trauma paradigm, in which the chronic conditioning stimulus, delivered 6 h/day for 10 days, does not by itself produce any permanent threshold elevations, at least in normal animals (Kujawa and Liberman 1996). Yet when this conditioning exposure is presented in advance of a traumatic exposure, it can reduce significantly the PTS from the latter. A form of protection is also demonstrable in a very different paradigm, the repeated-exposure paradigm, in which animals are exposed on a daily basis to a mildly traumatic stimulus. In the repeated-exposure paradigm, protection is seen as a daily decrease in the acute TS measured immediately after the end of each day’s exposure.
However, as the daily exposures continue, the animals develop a slowly growing residual TS, as seen in from the deterioration of thresholds measured before each daily exposure, which ultimately becomes a PTS, as documented many days post exposure (Boettcher et al. 1982). Thus the protection measured in the repeated-exposure paradigm appears to be a compound threshold shift, consisting of relatively large TTS and a smaller accumulating PTS.

Given the many known differences between the mechanisms underlying TTS and PTS, it is possible that the mechanisms underlying the protection seen in these two paradigms are different in important ways. For example, the slowly progressing PTS in these animals might involve slowly progressing damage to the stereocilia on IHC and/or OHCs (Boettcher et al. 1992). Cells with damaged stereocilia would be expected to have decreased ion fluxes during the daily noise exposure and, as such, might undergo progressively less TTS each day if TTS involves acute changes in hair cell homeostasis brought on by abnormally high potassium fluxes through the transduction channels. Furthermore, the practical value of protecting the ear by inducing a mild PTS is questionable.

Little is known about the mechanisms underlying the protection seen in either of these paradigms. As for the condition-trauma paradigm, a number of studies have shown that the middle-ear muscles are not involved (Henderson et al. 1994; Ryan et al. 1994). There have been a few studies of the morphological changes seen in ears that have been conditioned but not yet traumatized. Canlon suggested that conditioned ears showed elaboration of membranous tubules/vesicles in the basal pole of OHCs (Canlon et al. 1991, 1993); however, no other structures within the cochlear duct have been examined systematically at the ultrastructural level. Our own functional study of conditioned animals, which have not been traumatized, suggested that the conditioning exposure used in this study enhances the amplitudes of the distortion-product (DP) otoacoustic emissions at 2f1-f2 (Kujawa and Liberman 1996). Given that these DPs, at least at low sound pressure levels, primarily reflect OHC function (Mountain 1980; Schmiedt 1984; Siegel et al. 1982), this observation is also consistent with the hypothesis that changes in the OHCs are induced by the conditioning.

The only other study of a possible OC role in protection involved a hybrid paradigm, combining aspects of the repeated exposure and condition-trauma approaches (Zheng et al. 1997b). Each animal was exposed daily to a mildly traumatic stimulus, and DPs were measured before and after each exposure. Then, after the last of these daily exposures, each animal was exposed to the same stimulus at a much higher SPL, and final PTS was measured several days later. In one group of animals, the OCB was cut in the inferior vestibular nerve; however, there were only three successful deafferentations, and only two were carried all the way through the protocol. Nevertheless, the results are of interest, although not very clear cut. During the daily repeated exposure, control animals showed reducing CTS (i.e., protection) at three of the test frequencies, as this group has shown in numerous other studies (Subramaniam et al. 1996). In contrast, the three deafferented animals showed reducing CTS at one only of the three test frequencies: i.e., less protection, but protection was not abolished, even though the deafferentation was essentially complete. As for the final PTSs after the high-level traumatic exposure, the two deafferented animals that completed this part of the protocol showed significantly higher PTSs than the control animals with the same noise exposure history. However, this difference probably reflects the protective effects of OC activity per se (see further text), rather than a role of the OCB in conditioning-mediated protection. To assess the latter, the experimental design would have to have included a trauma-only group; it did not.

The results of the present study also do not provide an unambiguous answer as to whether the OC system is involved in conditioning-related protection, although its role in generally protecting the ear from acoustic injury is supported strongly. The demonstration that the loss of the OCB leads to loss of conditioning-related protection (Fig. 6) is consistent with an OC role in conditioning. However, the apparent increase in PTS in the deafferented condition/trajma group (Figs. 6 and 8) suggests that the deafferentation has rendered the ear vulnerable to the moderate-level conditioning exposure itself. Thus if the degree of OC-mediated protection is large enough, the effects of deafferentation may mask a remaining conditioning-related protection in these animals.

One unexpected result from the present study was the observation that animals that underwent the sterile surgery procedure (yet maintained an intact OCB) were protected from acoustic overexposure as effectively as if they had been conditioned with the moderate-level noise: e.g., from Fig. 6, $PTS_{TX} = PTS_{TX} = PTS_{T(O-D)} = PTS_{T(O-D)} = PTS$. Analogous results are visible in the midline-lesion group (Fig. 5), except that there is more variability in the PTS at low frequencies. One interpretation of these results is that the changes brought on by conditioning actually are mediated through a more generalized stress response to the noise and that any other treatment (including the chronic brain surgery used in the present study) that elicits the same stress response also will have a protective effect. The stressful effects of noise exposures are well known, and it has been reported recently that emotional stress decreases noise-induced TTS in guinea pigs (Muchnik et al. 1992). Based on the existing literature on stress and noise, the types of conditioning exposures used in this and other studies could have significant effects on circulating levels of a number of hormones including epinephrine and glucocorticoids (e.g., Raey et al. 1995).

Similarly, noise exposure is only one of many noxious stimuli that can elicit upregulation of heat-shock proteins (Lim et al. 1993), and expression of heat-shock proteins can have a protective effect on numerous body functions (e.g., Lindquist and Craig 1988).

Given that the OC system clearly has a protective function in general, i.e., that deafferented animals are more vulnerable to acoustic injury regardless of their noise-exposure history, it is possible that stress/noise upregulates sound-evoked OC feedback and thus helps to protect the ear. We recently tested this hypothesis by recording single-fiber activity from MOC neurons in control and conditioned (but not traumatized) animals. We found modest elevations in MOC activation, suggesting that excitability in the MOC circuitry is influenced by noise history and that this upregulation of MOC reflex strength may contribute to conditioning-related protection (Brown et al. 1998). Given the present results sug-
ggesting that the moderate-level exposures typically used to condition the ear become dangerous in the absence of the OCB, teasing out the contribution of the OC system to the conditioning phenomenon will be difficult unless the conditioning effect also can be elicited by nonnois stressors. To our knowledge, no study has addressed explicitly this possibility. Clearly this should be an area for future investigations.

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REFERENCES


