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Cryoprobe-induced apical lesions in the chinchilla. II. Effects on behavioral auditory thresholds

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Lesions of the hair cells in the cochlear apex were produced by a miniature cryoprobe and changes in behavioral auditory thresholds were measured. Monauralized adult chinchillas were behaviorally trained using operant procedures to produce pure-tone audiograms at frequencies from 63 Hz to 40 kHz. Following collection of baseline thresholds, the apical and middle turns of the experimental ear were visualized through a hole drilled in the bulla and a copper cryoprobe that had been cooled in liquid nitrogen was placed on the apical turn of the cochlea. Post-lesion threshold shifts from two subjects showed a flat loss of approximately 20 dB restricted to frequencies below either 710 Hz or 1 kHz; thresholds were normal at higher frequencies. The cytochleograms, prepared from the ears following completion of threshold testing, show an almost complete loss of both inner and outer hair cells in the apical-most 20% of the cochlea with an abrupt transition region to areas of normal-looking hair cell populations. The relationship between the frequencies at which hearing was impaired and the location of missing hair cells along the basilar membrane is in agreement with the frequency-place map for the chinchilla of Eldredge et al. [(1981) *J. Acoust. Soc. Am.* 69, 1091–1095]. The magnitude of the loss, however, is less than might be expected based on comparison with threshold shifts produced by similar pathology in the basal turns.

Apical hair cell lesion; Low frequency hearing loss; Cryoprobe

Introduction

Theories of afferent encoding on the basilar membrane are based, in part, on a frequency-to-place transformation (Von Békésy, 1960). The functional analysis of the tonotopic organization of the basal and middle turns of the organ of Corti has typically involved procedures in which hair cells in those regions are selectively damaged by either noise or aminoglycoside poisoning, and the subsequent effects on hearing noted (Stebbins and Moody, 1979; Eldredge et al., 1981; Bohne and Clark, 1982). Damage to basal-turn hair cells is associated with an impairment in high-frequency hearing (Clark et al., 1974; Prosen et al., 1978). When drug treatment or noise exposure is varied

to include damage to middle-turn hair cells, hearing loss occurs as well at mid-range frequencies and continues to be correlated with the location of missing hair cells (Dolan et al., 1975; Prosen et al., 1978; Stebbins and Moody, 1979; Bohne and Clark, 1982; Clark and Bohne, 1986).

In the apical turn of the cochlea, however, this selective lesioning strategy has been difficult to apply for it has not been possible to produce lesions restricted to that region using either noise or ototoxic drugs. Aminoglycoside ototoxicity is always characterized by a progressive loss of hair cells beginning in the base. Apical turn damage due to intense low-frequency noise exposure is variable and generally produces only partial loss of hair cells (Ades et al., 1974; Stephenson et al., 1984; Clark and Bohne, 1986). Given the inability to produce discrete apical lesions, and the variability in the extent of the destruction in different subjects with the same exposure, it is not surprising that the correlations between hearing loss and

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histopathology, such as have been reported for basal regions, have not been found in the cochlear apex. Furthermore, Sutton and Schuknecht (1954) showed that mechanically induced lesions of inner and outer hair cells restricted to the apical turn produce a loss of sensitivity restricted to low frequencies as measured by cortical recording electrodes. However, using the same ablation technique in an earlier study, Schuknecht and Neff (1952) showed that similar apical lesions yielded behavioral hearing losses of higher frequencies located in regions not corresponding to those areas of restricted hair cell loss.

Several reasons may explain the poor correlation between damage to apical hair cells and changes in low-frequency hearing. First, with apical turn damage, adjacent areas of normal hair cells may be used to detect the presence of low-frequency stimulation thereby underestimating the extent of the hearing loss. However, Clark and Bohne (1986) demonstrated in chinchillas with up to 50% loss of apical outer hair cells (OHCs) from exposure to intense low-frequency noise that, by masking adjacent normal hair cell populations with high-pass noise, high-frequency thresholds were significantly elevated, yet the detection of low-frequency tones was not altered and therefore must be based on processing by the damaged apical turn. Clark and Bohne (1986) suggest that a full complement of apical OHCs is not necessary for the simple detection of low-frequency sound and that pure-tone thresholds are therefore not sensitive to significant loss of apical turn OHCs. Under these conditions, it might be possible to detect a functional deficit using auditory tasks which require more sophisticated, or complete, processing mechanisms. Indeed, Clark and Bohne (1986) showed that, following low-frequency noise exposures in chinchillas, thresholds for the detection of frequency modulation were increased, while low-frequency pure-tone thresholds were unaffected.

In order to determine more accurately the nature of the tonotopic organization of the cochlear apex, it is necessary to use a method that produces well defined and more complete lesions in this area. Brown and Nuttall (1985, 1986) have described the use of a miniature cryoprobe that produces damage to the hair cells and supporting

structures of the organ of Corti in areas limited to the apical turn. While the exact extent of the lesion was variable, a complete destruction of the organ of Corti within a well-defined region of the apical turn was reported. The objectives of the current study were to determine the functional effects of such lesions on pure-tone thresholds.

Methods

Subjects

The subjects were adult male chinchillas (*Chinchilla laniger*) that weighed between 400 and 500 g during this study. They were obtained as culls from a local breeder and housed in individual cages in a reversed light cycle, sound insulated colony. The animals were maintained on a restricted diet to facilitate the use of food in an operant reinforcement paradigm for training and testing. The subjects were monauralized prior to training by both disarticulation of the ossicular chain and cryoprobe obliteration of the right cochlea. All monauralized subjects had hearing within normal limits from 63 Hz to 40 kHz, as determined by behavioral threshold procedures prior to experimental lesioning of the intact ear.

Apparatus

Experimental sessions were conducted in a hardware cloth cage (26 cm × 26 cm × 30 cm) mounted on a 90-cm tall stand and housed in a double-walled sound attenuating chamber (IAC, 60). A food pellet dispenser was also mounted on the stand. The front wall of the cage was fitted with a response lever, food trough, and indicator light. The particular speaker used in each session depended upon whether high or low frequencies were to be tested. The low-frequency transducer (Radio Shack, 40-1331B) was suspended from the booth ceiling 5 cm above the center of the cage and was used for stimuli from 63 Hz to 2.8 kHz. The high-frequency transducer (Motorola 71905a piezoelectric), used for stimuli from 4 to 40 kHz, was mounted directly on the cage in a position above the response key.

Stimuli

The stimuli were pure tones generated by a Krohn-Hite (model 4030R) programmable oscilla-

tor. The tones were 2.5 s in duration, were gated with random phase, and had 20-ms rise-fall times.

The sound field was calibrated in 6 locations in the area which approximated that of the animal's head while responding. These calibrations were performed using a Brüel and Kjaer 1/2 inch condenser microphone (type 4133) and Hewlett-Packard wave analyzer (3590A). The arithmetic mean of the microphone output voltages was used to calculate average SPL values. The sound field varied by less than 2 dB at frequencies below 2 kHz, and by less than 10 dB at frequencies up to 22.4 kHz.

Behavioral procedures

The subjects were trained using positive-reinforcement procedures, as described by Clark et al. (1974). A trial consisted of the subject making an observing response by pressing down the lever in the presence of a flashing cue light. The light then remained on without flashing while the lever was held down. Following a variable period (1–8 s), a 2.5-s pure tone was presented. If the subject released the lever during the tone presentation, a 45-mg food pellet reinforcer (Noyes, formula 'N') was delivered. A response at any other time resulted in a 5-s 'time-out' during which the cue light was extinguished and further responses postponed light onset. The time-out was mildly aversive and served to decrease inappropriate responding. Twenty percent of the trials were 'catch trials' where all test parameters were identical to test-tone trials except the tone was not presented. These catch trials were used to assess the subject's guessing rate. Data from sessions in which the subject responded to more than 20% of the catch trials were not used in summary data calculation.

A staircase or tracking procedure was used to vary the level of the tone from trial to trial. Correct detection of the test tone resulted in a 10-dB decrease in the tone level on the subsequent trial, making the tone more difficult to detect. When the subject failed to respond to a test tone, the tone was increased 10 dB on the subsequent trial. This change, from a detection to miss, or vice versa, was called a transition. A threshold at a given frequency was defined as the average sound pressure level over six such transitions. Nine frequencies were each measured twice during any

session, and the two determinations averaged to produce that day's threshold. Daily sessions were alternated between high and low frequency testing. The criterion for threshold stability was that 5 of 6 consecutive daily threshold values at any frequency had to be within 10 dB. Both pre- and post-lesion summary data presented here are the average of the 5 sessions that met that criterion. Post-lesion data collection continued for 30 days following the last systematic change in the data.

Stimulus presentation and experimental session parameters were under computer control (PDP 8/E).

Surgical procedure

The surgical procedure has been described in detail elsewhere (Brown and Nuttall, 1985, 1986). In these studies, the tip of the probe was placed on the apical turn of the cochlea for 1.5 min. The probe was then removed allowing the cochlea to rewarm for approximately 3 min, recooled, and reapplied to the same location for an additional 1.5 min. An additional animal was lesioned using the same protocol except that the lesion times were 3 and 2 min. In general, the animals recovered sufficiently to begin retesting within 24–48 h.

The histological procedures varied slightly from those used in non-behavioral subjects (Brown and Nuttall, 1986). Following the conclusion of behavioral testing, the animals were killed and cochleae taken for histological evaluation. The cochleae were fixed by perfusion with 0.1 M phosphate buffered Karnovsky's solution (4% paraformaldehyde, 5% glutaraldehyde), dissected in alcohol, and mounted as surface preparations in Medcast-araldite. Complete cytochleograms were prepared under light microscopy.

Results

The initial effects of the cryolesions were seen as a 40–60 dB increase in thresholds at all frequencies. For example, Fig. 1 presents audiograms from C-47 obtained at various times following cryolesioning. A broad threshold shift was evident the day immediately following cryosurgery. Partial recovery began approximately 72–96 h after surgery, and the hearing loss stabilized within 10–14 days.

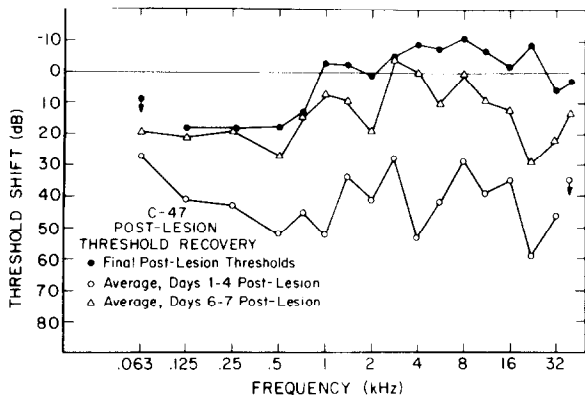


Fig. 1. Threshold shifts measured at various times following cryolesioning showing recovery pattern for C-47.

The final results for C-47 are shown in Fig. 2. The top portion of the figure is the cytochleogram and the lower portion is the corresponding final audiogram. The alignment of the frequency and distance coordinates is based on the frequency-place map for the chinchilla from Eldredge et al. (1981). Unfortunately, that map does not account for hearing at frequencies below 125 Hz, and above 20 kHz, both of which we routinely measure. The hair cell count showed a spotty retention of outer hair cells in the apical-

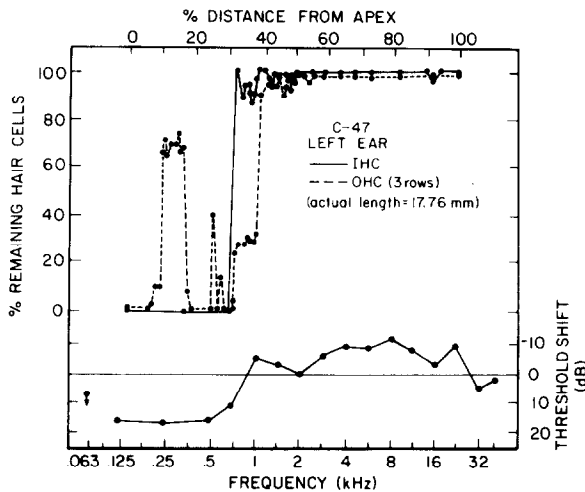


Fig. 2. Threshold shift (lower panel) and corresponding cytochleogram (upper panel) for C-47. Alignment of distance and frequency coordinates based on that of Eldredge et al. (1981).

most 40% of the cochlea: a 60–70% survival from 10 to 15% from the apex, and an approximate 0–30% retention from 20 to 40%. All OHCs were present from 40 to 100% of the distance from the apex. The inner hair cells were completely absent from the apical-most 30% of the cochlea, but present from 40 to 100%. The corresponding audiogram showed a 15–20 dB shift at frequencies of 710 Hz and below, and normal or slightly better than pre-exposure thresholds at frequencies of 1 kHz and above. The threshold shift at 63 Hz was greater than 10 dB but could not be measured with the available equipment.

Fig. 3 presents the cytochleogram and the corresponding final threshold shifts for C-83. Both inner and outer hair cells were missing in the apical-most 28% of the cochlea, but all were present from 35 to 100% distance from the apex. There was a sharp transition between these two regions. As with C-47 the specific frequencies of the threshold shifts were in good agreement with the location of the hair cell loss. A loss of 16–22 dB was evident at frequencies of 500 Hz and below, with normal or slightly better than pre-exposure thresholds at 710 Hz and above. As with C-47 the shift at 63 Hz was in excess of 10 dB and not measurable.

Fig. 4 presents the cytochleogram and final

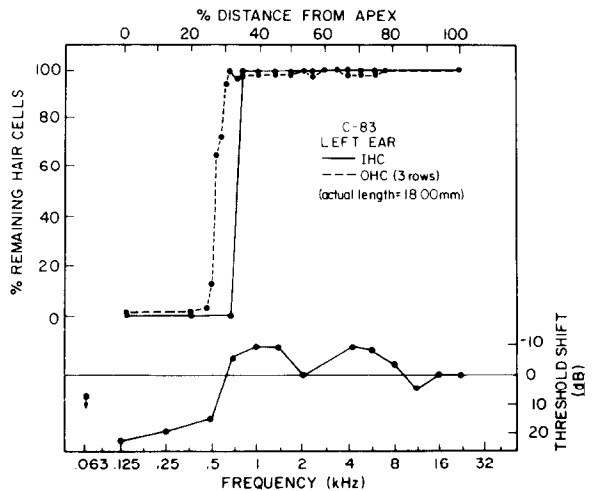


Fig. 3. Threshold shift (lower panel) and corresponding cytochleogram (upper panel) for C-83. Alignment of distance and frequency coordinates based on that of Eldredge et al. (1981).

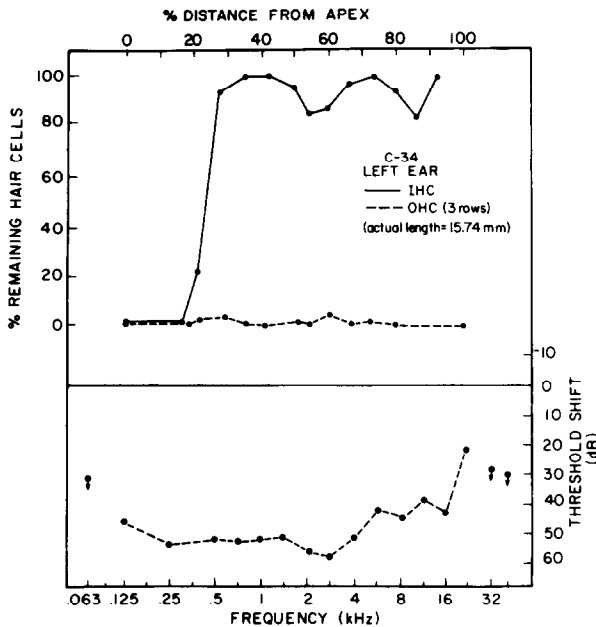


Fig. 4. Threshold shift (lower panel) and corresponding cytochleogram (upper panel) for C-34. Alignment of distance and frequency coordinates based on that of Eldredge et al. (1981).

threshold shifts for C-34, the animal in which the probe was applied for 2 and then 3 min. No IHCs were present from 0 to 20% of the distance from the apex, yet 90–100% were retained in the 25–100% region. There were no OHCs throughout the entire organ of Corti. The corresponding final behavioral threshold shifts are well correlated with the histopathology, showing a 40–60 dB loss of sensitivity at all frequencies except 22.4 kHz, where a 22-dB shift was evident. Thresholds at 63 Hz, 32 and 40 kHz were elevated by more than 30 dB and were not measurable. The pattern of hearing loss and histopathology is similar to that observed in dihydrostreptomycin-treated Patas monkeys and kanamycin-treated guinea pigs and cats (Hawkins et al., 1977; Stebbins and Moody, 1979) with selective loss of OHCs. The short length of this basilar membrane, 15.74 mm, is most likely explained by the extensive destruction found post-lesion. Dissection and evaluation were difficult, and the 16.7 mm length of the basilar membrane from the control ear suggests a loss of approximately 1 mm of tissue from either the apical or basal end. However, given the pattern of hair cell loss and

accompanying threshold shifts, displacement of the ordinates to compensate for the 6% change in length would have little effect.

While not within the specific aims of the present study, a general characterization of myelinated nerve cell survival within the spiral osseous lamina following cryolesioning suggests a good correlation with IHC survival (Brown and Nuttall, 1986).

Cytochleograms from the ears destroyed prior to training show a varying degree of damage between subjects. A comparison of these ears from animals C-47 and C-83 yields a markedly different extent of pathology. The cytochleogram from C-83 showed an average of 20% remaining IHCs present throughout the upper 50% of the organ of Corti, and no OHCs present throughout the ear. However, the hair cell count for the monauralized ear of C-47 showed a normal complement of inner and outer hair cells in the apical-most 10% of the organ of Corti, a punctate lesion at 25% distance from the apex with the region from 30 to 60% from the apex showing approximately 60% of IHCs and 3rd row OHCs, and less than 20% of 1st and 2nd row OHCs surviving. There was 100% retention of both inner and outer hair cells in the basal-most 40% of the organ of Corti.

Clark and Bohne (1985) determined that disarticulation of the ossicular chain in the chinchilla provides an average of 60 dB of hearing loss from 125 Hz to 4.8 kHz. With the maximum losses in hearing of 33 dB evident after cryolesioning the experimental ear, it is reasonable to assume that even in the case where all hair cells in the disarticulated ear were not destroyed, the detection of test-tones in the free-field testing paradigm must have been based on stimulation of the experimental left ear. Moreover, if the disarticulation was not effective in monauralizing the subjects, one might reasonably assume that the low-frequency thresholds for C-47 to have been lower than those of C-83 because of the normal region of hair cells found in the apex of C-47. Yet the threshold shifts evident were the same for both subjects.

Discussion

These results suggest that the relation between the frequency-specific loss in hearing and the loca-

tion of the lesion in the basal and middle turns of the cochlea is also found in the apex. In two subjects there was a 15–20 dB increase in pure-tone thresholds at those frequencies which, based on the frequency-place map of Eldredge et al. (1981), correspond to regions of the cochlea with a complete, or nearly complete, loss of hair cells. Only C-47 showed a retention of any hair cells in the apical 20% of the cochlea, where a small patch of OHCs was present. Based on the afferent innervation pattern of the cochlea (Spoendlin, 1972) and current discussions of the role of the two different hair cell sub-systems (Khanna, 1984; Kim, 1984), these hair cells are probably of little functional significance in this threshold procedure. A comparison of the threshold changes for C-47 and C-83, where both inner and outer hair cells are absent, supports this assumption.

The elevation in pure-tone thresholds with complete loss of hair cells is less than might be expected when compared to the magnitude of the threshold shifts with comparable lesions in the basal and middle turns. A comparison of the magnitude of the low-frequency threshold shift in C-34 with that in C-47 and C-83 suggests that the residual low-frequency hearing in the latter two subjects may be the result of responses of normal hair cells from adjacent regions of the basilar membrane which are lacking in C-34 but present in C-47 and C-83. Additional studies with high-pass masking noise should serve to elucidate further the dependence of low-frequency hearing in apically lesioned ears on normal hair cell populations in more basal regions.

These results, in conjunction with the findings of Clark and Bohne (1986), suggest that loss of apical hair cells has remarkably little effect on absolute sensitivity. Those authors showed that loss of up to 50% of apical OHCs had no effect on threshold, and the present study demonstrates that, with total loss of both inner and outer hair cells, a loss in sensitivity occurs that is only approximately 20 dB in magnitude, but that is frequency specific.

Conclusions

(1) With complete and discrete lesions of apical hair cells, the correlation between the location of

histopathology on the basilar membrane and the frequencies at which thresholds are increased is similar to those observed in the basal and middle turns following comparable hair cell lesions. The apical turn exhibits a pattern of tonotopic organization similar to that found in other regions of the basilar membrane.

(2) The magnitude of the threshold shift is smaller than what might be predicted based on similar lesions of basal and middle turn hair cells, suggesting the use of hair cells from normal, more basal regions in the detection of low frequency stimulation.

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