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Differential susceptibility of avian hair cells to acoustic trauma *

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Five groups of 10-day-old chicks were continuously exposed to either 500 or 1500 Hz pure tone at 125 dB for 4 or 12 h and killed 10 days later. The basilar papillae were fixed, embedded in plastic, sectioned, and hair cells were counted according to type: tall or short. Short hair cells were found to be more susceptible to acoustic overstimulation than tall hair cells. Further, the position of maximum short hair cell loss varied along the length of the basilar papilla as a function of the exposure frequency while the position of tall hair cell loss did not. Similarities between morphological response of short hair cells in avians after acoustic trauma and outer hair cells in mammals are discussed.

basilar papilla, short hair cell, tall hair cell, outer hair cell

Introduction

A differential susceptibility to acoustic trauma exists between inner and outer hair cells in the mammalian cochlea. Outer hair cell loss is more extensive, seen earlier and occurs at lower stimulus levels [5,11,23,24]. The position of outer hair cell loss, which occurs prior to inner hair cell loss, corresponds well with the frequency of stimulation. With more intense or longer duration stimulation the position of both cell types corresponds to the frequency of stimulation. It is as yet unknown whether the inner ear systems of non-mammalians show a similar differential susceptibility to acoustic trauma. We have previously reported [17,18,19] that the hair cells of the chick inner ear degenerate after intense auditory stimulation. As in mammals, the position and degree of hair cell loss are predictable on the basis of the frequency of the acoustic stimulus and the duration of the exposure. In our previous studies, the total population of hair cells along the basilar papilla, or inner ear, was analyzed. However, two hair cell types, tall and short, have been identified within the basilar papilla and some similarities between these and inner and outer hair cells in mammals have been drawn [6,15,22,25,27]. In the present study, we felt that by analyzing differential receptor susceptibility to trauma between systems with two receptor cell types but vastly different receptor organization (for example: in the chick both receptor cell types exist across the width of the basilar membrane without structural division, unlike the Organ of Corti arrangement in mammals, and the tall and short hair cells have a varied density ratio throughout the papilla length, unlike the fixed ratio of inner and outer hair cells in the mammalian cochlea) further information might be gained regarding this differential susceptibility.

Methods

Subjects

Five groups of Hubbard × Hubbard chicks (n = 20 chicks) were used in the present study. Eggs were incubated, hatched and reared in the laboratory. Chicks were exposed to acoustic overstimula-

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tion at 10 days post-hatch. By this age both hair cell types are fully differentiated, and the cochlea and brainstem auditory system responds in an adult manner [2,3,14,16,20].

Sound exposure

Pure tone exposure conditions varied in duration and frequency (Hz). Outer hair cells have been shown to degenerate at less intense or shorter duration noise levels than inner hair cells. In order to determine whether tall or short hair cells might show a similar pattern of response, two durations of exposure, at a constant intensity level, were chosen: 4 h and 12 h. These conditions have previously been shown to cause hair cell degeneration within the avian basilar papilla (BP) [18]. Two frequency conditions for sound exposure were chosen: 500 and 1500 Hz. These frequencies have previously been shown to cause discreet regions of hair cell loss at two distinctly different positions along the length of the BP [17,19]. Since both the density of hair cells and the ratio of tall to short hair cells increase constantly from the proximal to distal portions of the papilla these two frequencies were chosen in order to determine what effect a differing hair cell density as well as ratio of hair cell type, rather than the relatively constant density and constant ratio found in mammals, might have on differential susceptibility to pure tone overstimulation.

The procedures for pure tone sound exposure and signal calibration have been described previously [18,19]. Briefly, audio signals were generated by an audio oscillator, amplified, led to a freefield speaker and delivered at an intensity of 125 ± 5 dB at the ear canal. The acoustic environment of the chamber in which animals were confined during sound exposure was calibrated with a constant voltage input and was shown to vary by less than 10 dB through 2000 Hz. Sound pressure levels were measured at the level of the ear canal and were calibrated to 125 ± 5 dB. Levels for the experimental tone and its harmonics were then measured at ten other positions within the chamber; the experimental tone was within ± 5 dB at all positions and first and second harmonics were at least 30 dB below the fundamental.

Procedure

10-day-old chicks were placed, in pairs, in a small wire mesh tubular chamber and were continuously exposed to the appropriate frequency (500 Hz, 1500 Hz) for either 4 or 12 h. Acoustic over stimulation was provided monaurally in two chicks (one from the 500 Hz 12 h condition and one from the 1500 Hz 12 h condition) so that the other ear could be used for within-animal comparisons. The ear plugging procedure used to protect one ear from overstimulation has been described elsewhere [9] and has been shown to provide at least 40 dB attenuation from 125 to 4500 Hz. After acoustic overstimulation earplugs were removed and the chicks returned to standard brooders.

Fixation and tissue preparation

After 10 days' survival duration chicks were deeply anesthetized by intravenous injection of Nembutal. A direct intralabyrinthine perfusion of 0.07 M phosphate-buffered 1% paraformaldehyde/0.75% gluteraldehyde mixture (pH 7.3) was performed bilaterally immediately after decapitation. The entire head was then immersed in cold fixative for 8–12 hours. The basilar papillae were removed from the skull, washed in phosphate buffer and post-fixed in 2% buffered osmium tetroxide (0.12 M phosphate buffer, pH 7.3) for 1–2 h. Following osmication, the papillae were dehydrated in a graded methanol series and embedded in Epon. The Epon-embedded papillae were sectioned transverse to the longitudinal axis in the proximal to distal direction using an LKB Ultramicrotome or Paramitome. A group of three or four, 3 μm thick sections were collected at each 100 μm interval throughout the length of the papilla. The three sections were mounted together in each group such that the groups of sections were arranged in serial order from the proximal tip (base) to the apex. Sections were stained with toluidine blue and coverslipped prior to the counting of hair cells.

Quantitative analysis of number of hair cells

Tall and short. Quantitative analysis of the number of hair cells present at each sampling interval was identical to that previously described [18,19]. Briefly, hair cells were viewed under 40 × planapochromatic oil immersion objective (NA =

1.0) at a total magnification of ×640. A hair cell was counted when the following criteria were met: a normal orientation of cuticular plate and cilia with a surrounding recognizable cytoplasm. The average counts from the three sections at each 100 μm sampling interval were then grouped as a function of normalized distance (percentage of total membrane length from the proximal tip) of the basilar membrane.

Cells were classified as to cell type in accordance with the criteria of Tanaka and Smith [26]: short hair cells were defined as those cells located over the inferior or free edge of the basilar membrane and having a width greater than height; tall hair cells were defined as those cells located over the superior edge of the basilar membrane and having a height greater than width. Although it was apparent that the ratio of tall to short hair cells changed throughout the length of the BP such that more short hair cells were located at the proximal tip and more tall hair cells at the distal tip, the specific classification of cell type at these extreme tips was difficult. This difficulty was encountered since the specific shape of the hair cells, i.e. width vs. height, was less well differentiated at these two ends. This same difficulty in

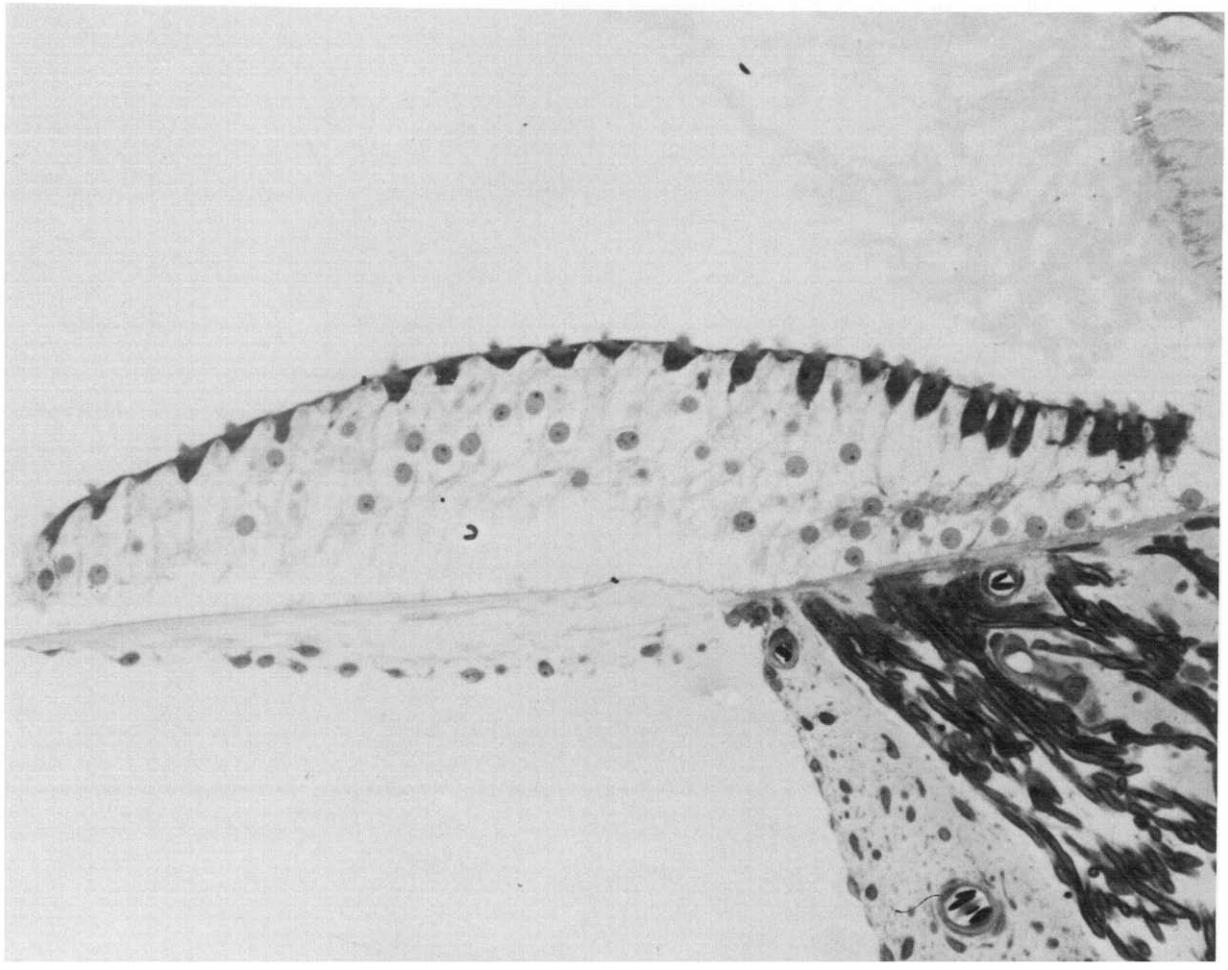
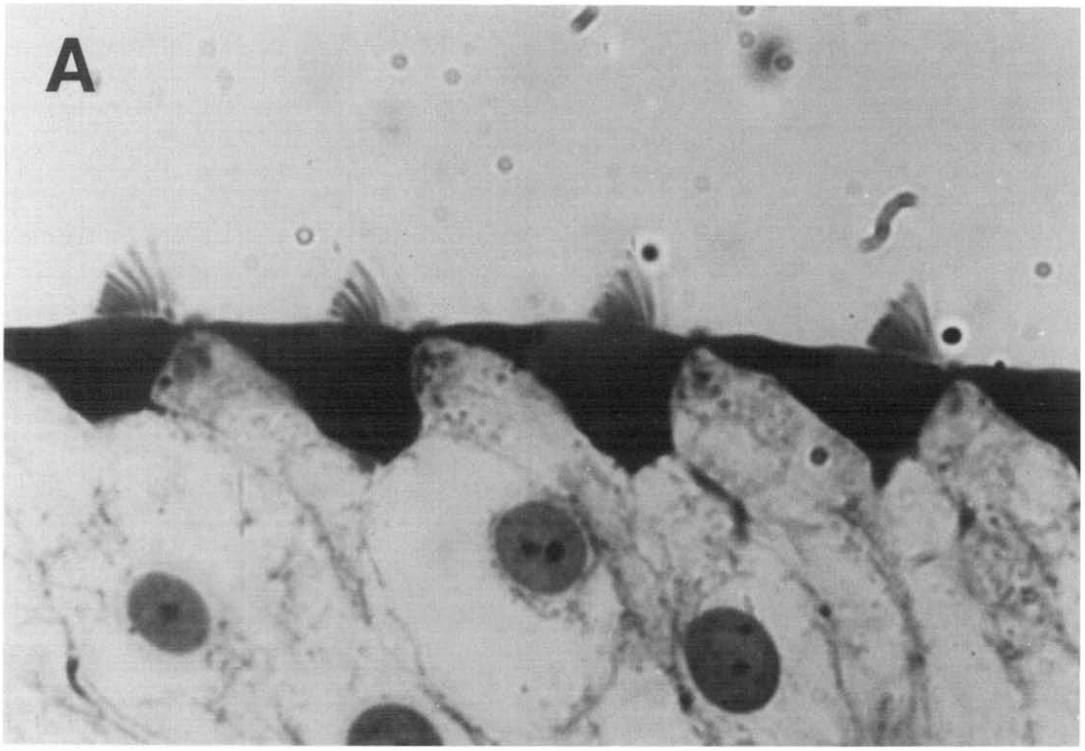


Fig. 1. Photomicrograph showing the general appearance of the basilar membrane taken from the middle of the BP. Tall hair cells are seen along the superior edge over the fibro-cartilagenous plate and short hair cells are located toward the free edge of the basilar membrane.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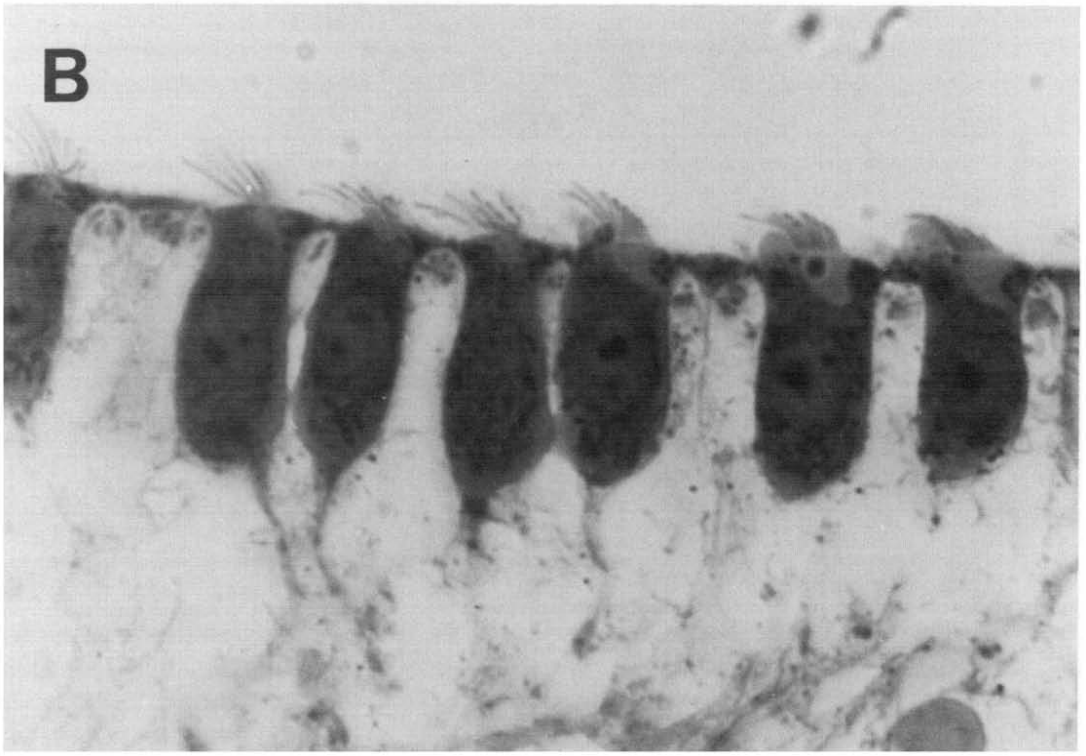
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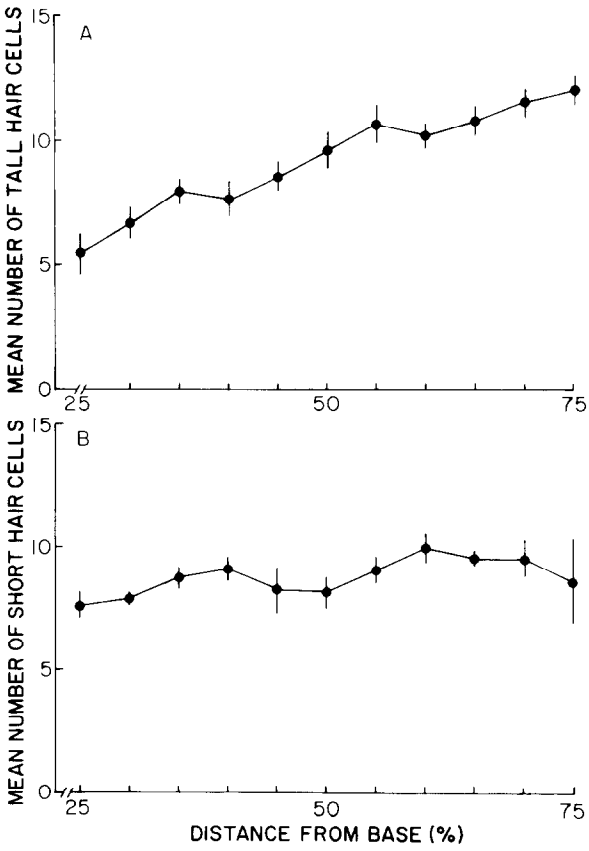


Fig. 3. Mean total tall (A) and short (B) hair cells (±1 S.E.) in normal control 20-day-old chicks ($n = 5$ chicks, 8 ears). Two control ears were taken from monaurally noise exposed chicks. No difference in cell number was seen between 'plugged ears' ($n = 2$) and non-exposed control ears ($n = 6$). Tall hair cells progressively increase in number as the distal (apical) end is approached while short hair cells maintain a relatively constant number throughout the middle of the BP.

classification along the length of the basilar membrane has been discussed by other authors [2,25,27]. Therefore, since cell types are most reliably classified along the middle 50% of the papilla, both cell types are located in this region, and hair cell loss occurs at various positions within the middle section after trauma, primary analysis of differential damage to cell type was performed only on the middle half of the BP. The average cell counts from the three sections at 100 μm intervals were plotted for the middle 50% of the length for each cell type and compared with the normative counts for each cell type.

Position. A position 55% of length from the proximal (basal) tip was chosen for analysis of hair cell loss along the width of the basilar membrane. Cells were counted along the width of the basilar membrane and plotted according to their position from superior to inferior edge of the basilar membrane (at 10% intervals of width). Cumulative percent hair cell counts from superior to inferior edge in normal controls were compared to cumulative cell counts in experimental animals.

Results

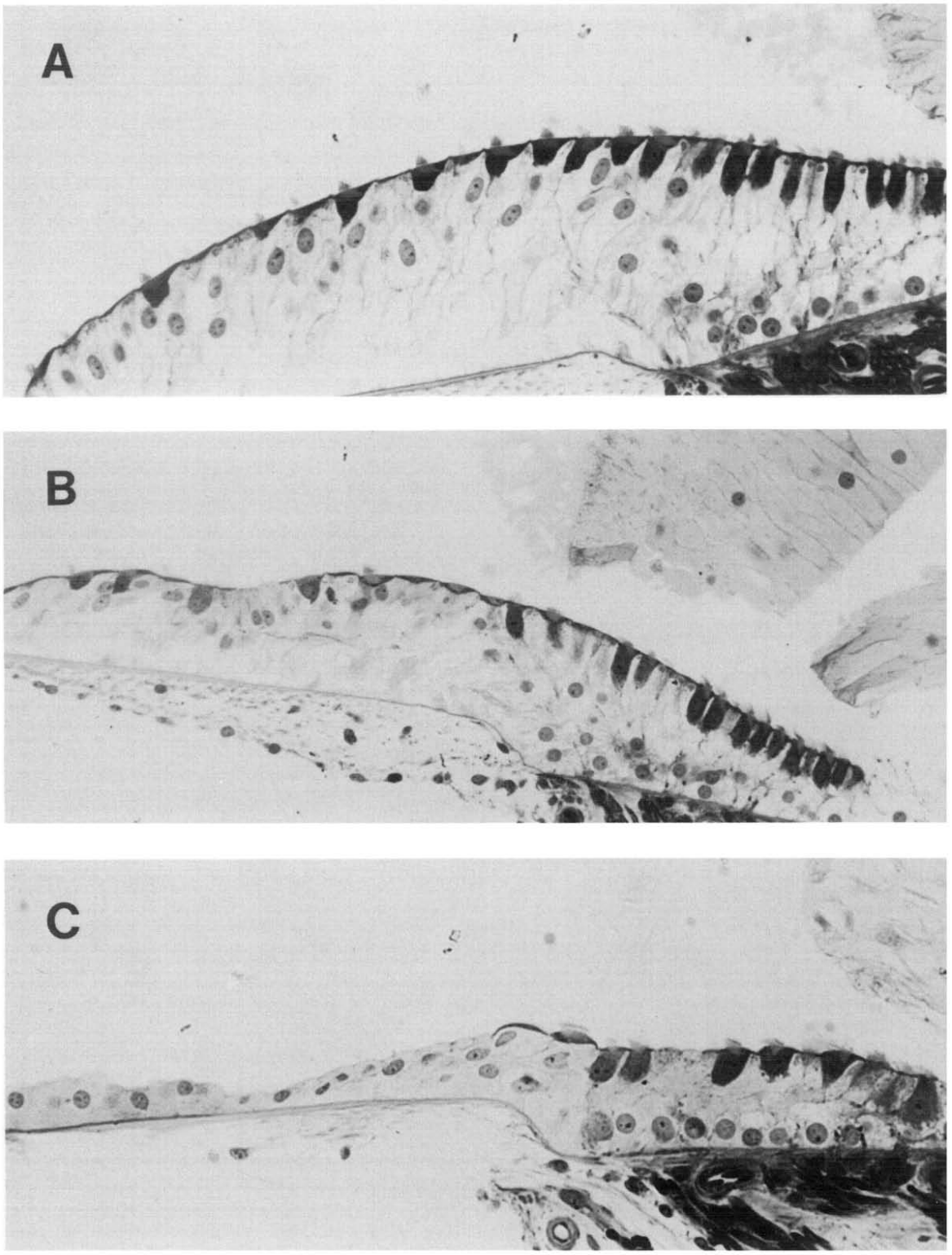
Normative baseline hair cell counts

Fig. 1 is a photomicrograph showing the general appearance of the basilar membrane seen in a 3 μm thick section taken from the middle of the BP. Tall hair cells can be seen located along the superior edge of the fibro-cartilagenous plate and short hair cells are located toward the free edge of the basilar membrane.

Figure 2 shows the hair cell types in more detail. The short hair cells are pitcher-like in shape, while tall hair cells are columnar in shape. The cuticular plate is shown by the slightly lighter stained region at the top of the cells. Cilia atop the cells are characterized by a gradation in length from tallest to shortest, oriented with the tallest cilia nearest the inferior edge of the basilar membrane and adjacent to the basal body.

Fig. 3 shows the normal distribution of tall and short hair cells throughout the middle 50% of the BP. Tall hair cells progressively increase in number as the distal end is approached while short hair cells maintain a somewhat constant number throughout the middle portion of the BP.

Fig. 2. (A) Short hair cells in the chick BP. These cells are pitcher-like in shape and are located on the inferior (free) edge of the basilar membrane. The cuticular plate is located on the upper surface of the cell with protruding cilia arranged in step-like order with tallest hairs on one side and adjacent to the basal body. (B) Tall hair cells in chick BP. These cells are located on the superior edge (fibro-cartilagenous plate) of the basilar membrane and are columnar in shape. The cuticular plate covers most of the upper surface of the cell. Cilia protrude from the cuticular plate and are arranged in step-like rows with the tallest hairs being on one side and adjacent to the basal body.


A
B
C

Qualitative analysis

Fig. 4 shows a typical region of hair cell damage in two basilar papillae, one exposed to 125 dB for 4 h (4B) and the other for 12 hours (4C) compared with the same region in a normal papilla (4A). In both sections it is apparent that fewer cells are seen on the inferior edge of the basilar membrane, the region containing short hair cells. In the animal exposed for 12 h, distortion of cells is also seen farther toward the superior edge where tall hair cells are located.

Hair cell counts

Differential effects of duration. Fig. 5 shows the mean number of tall, short, and total hair cells present after exposure to 125 dB, 500 or 1500 Hz pure tone for 4 hours compared to hair cell counts from normal control and plugged ears. (No qualitative or quantitative differences were found in cell appearance or number in control or plugged ears and therefore they were combined for normal hair cell counts). Tall hair cell loss is minimal; 96% of tall hair cells were counted as present in the middle 50% of the basilar papilla after intense pure tone stimulation. Short hair cells, however, show a broad decrease in number across the middle section of the papilla; less than 75% of short hair cells remain after intense pure tone exposure.

Fig. 6 shows the same comparison for the 12 h exposure condition. Tall as well as short hair cell loss is now present. Both frequency conditions resulted in a 28–30% decrease in tall hair cell number in the middle 50% of the papilla. For short hair cells the lower frequency tone resulted in a 53% decrease in cell number through the middle half of the papilla. The higher frequency condition produced a much more restricted area of cell loss, with a 27% decrease in cell number in the middle 50% of the papilla.

Differential effect of frequency. Position of total hair cell loss in the chick BP has previously been shown to vary as a function of the frequency (Hz) of exposure [17,19]. For a short duration of exposure (4 h) negligible or no tall hair cell loss is seen regardless of frequency. Short hair cell loss is, however, present and the position of short hair cell loss does vary with the frequency of exposure (Fig. 5). For the lower frequency tone short hair cell loss is generalized throughout the middle portion of the papilla and appears greatest in the area 35–70% of papilla length. The higher frequency tone produces a more localized region of short hair cell loss which is greatest in the area 30–50% of papilla length. Thus, the higher frequency tone produces a more restricted basal region of short hair cell loss, while the lower tone produces a broader more apical region of loss. As a result of the 12 h exposure both cell types are lost (Fig. 6). Again, the position of maximum short hair cell loss varies as a function of the frequency of exposure with a more apical region of loss for the 500 Hz exposure and a more basal region of loss for the 1500 Hz exposure. Tall hair cells, somewhat surprisingly, maintain a similar position of maximum loss regardless of the frequency of exposure. For the 500 Hz condition tall hair cells are decreased maximally in the area from 25 to 60% of length. For the 1500 Hz condition the maximal area of tall hair cell loss is from 25 to 50% of length. Thus changing the frequency of stimulation is reflected in a change in position of maximal hair cell loss only for short hair cells.

Position of hair cell loss across the width of the basilar membrane. Fig. 7 shows the cumulative cell counts taken in normal control animals at 55% of length from the proximal tip compared to animals exposed to a 500 Hz pure tone at 125 dB for either 4 or 12 h. Data from 8 and 24 h

Fig. 4. (A) Transverse section taken from the middle 50% area of a normal control BP. Tall hair cells are located on the superior edge above the fibro-cartilagenous plate and short hair cells are located over the free or inferior edge of the basilar membrane. Stereocilia, cuticular plate and cell body can be seen in cells of both types. (B) Transverse section from a similar portion of the BP in a chick exposed to a 500 Hz pure tone for 4 h at 125 dB. Tall hair cells are normal in appearance while short hair cells are often distorted, swollen, and lack stereocilia. (C) Transverse section from a similar portion of the BP in a chick exposed to a 1500 Hz pure tone for 12 h at 125 dB. Fewer short hair cells are present over the inferior edge of the basilar membrane. Distortion of cells is also seen toward the superior edge where tall hair cells are located.

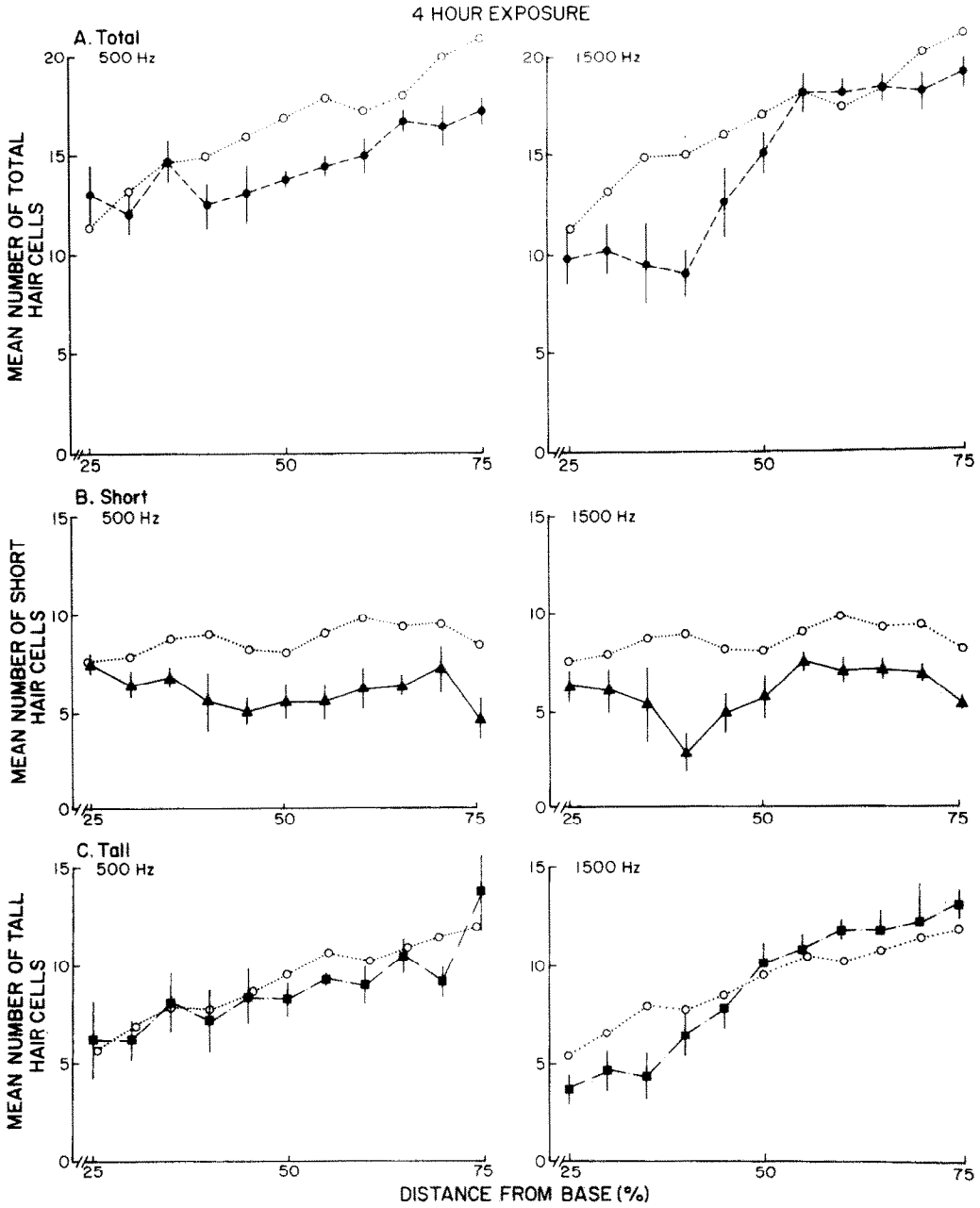


Fig. 5. (A) Mean total hair cell counts in the middle 50% of the BP in chicks exposed to 125 dB, 500 Hz ($n = 3$ chicks, 3 ears) or 1500 Hz ($n = 4$ chicks, 4 ears) pure tone for 4 h (± 1 S.E.). Open circles show mean total hair cell counts in normal control and plugged ears. (B) Mean short hair cell counts in the middle 50% of the BP for the same exposure conditions. Open circles show mean short hair cell counts in normal control and plugged ears. (C) Mean tall hair cell counts in the middle 50% of the BP for the same exposure conditions. Open circles show mean tall hair cell counts in normal control and plugged ears.

exposure groups are included to show the systematic progression of hair cell loss. These data are taken from a previous study in which animals were treated identically [18]. The slope of the cumulative percent cell counts in normals shows a systematic increase in cell number from the superior to the inferior edge of the basilar membrane. More hair cells are located toward the superior

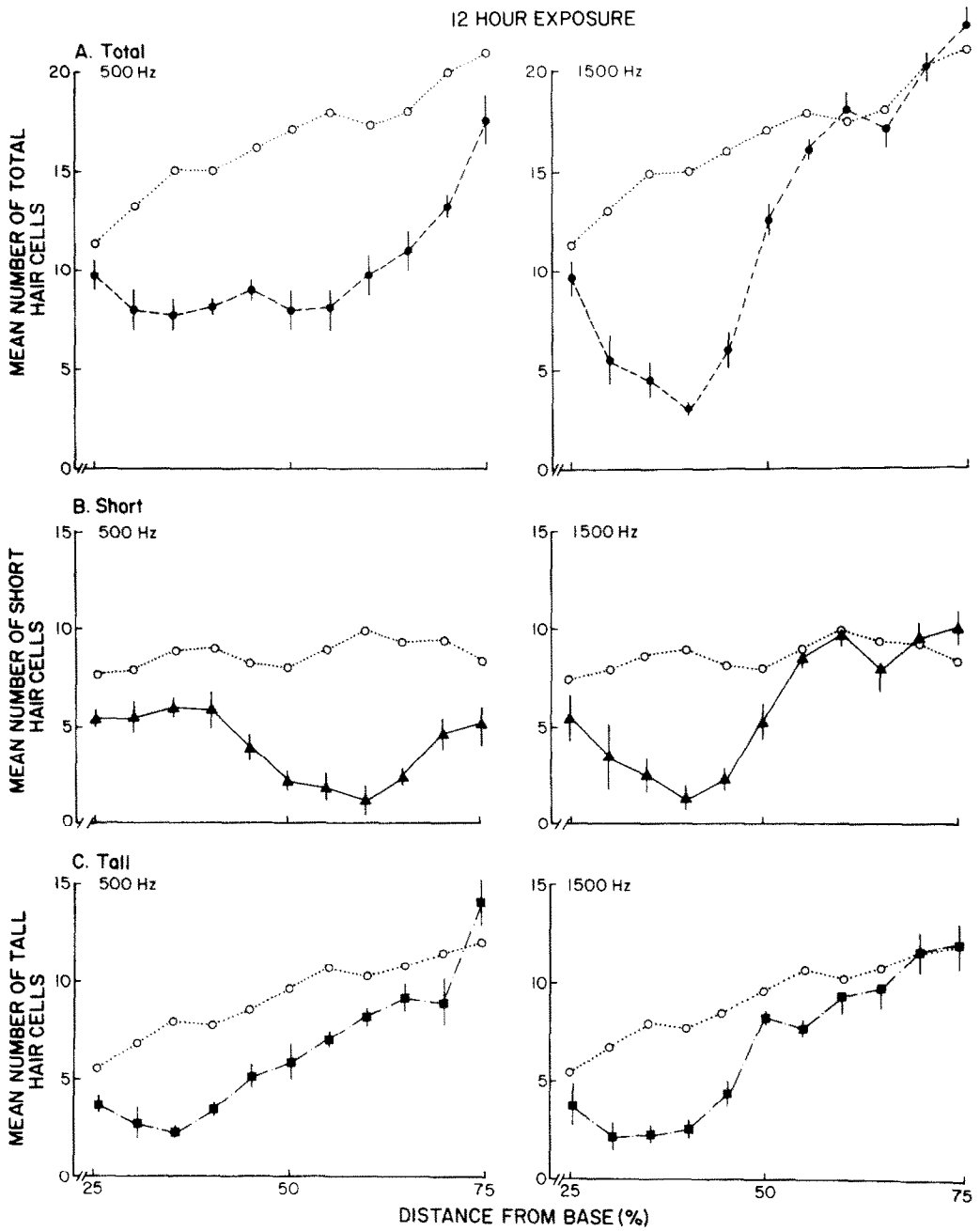


Fig. 6. (A) Mean total hair cell counts in the middle 50% of the BP in chicks exposed to 125 dB, 500 Hz ($n = 3$ chicks, 3 ears) or 1500 Hz ($n = 4$ chicks, 4 ears) pure tone for 12 h (± 1 S.E.). Open circles show mean total hair cell counts in normal control and plugged ears. (B) Mean short hair cell counts in the middle 50% of the BP for the same exposure conditions. Open circles show mean short hair cell counts in normal control and plugged ears. (C) Mean tall hair cell counts in the middle 50% of the BP for the same exposure conditions. Open circles show mean tall hair cell counts in normal control and plugged ears.

edge with cells continuing to be present to about 80% of width. The final 20% of width on the inferior edge of the basilar membrane is occupied by cuboidal cells; no hair cells are normally present. After 4 h of exposure the number of hair cells has decreased only on the inferior (outer) edge of the basilar membrane causing a slight increase in the slope of the cumulative percent counts. After

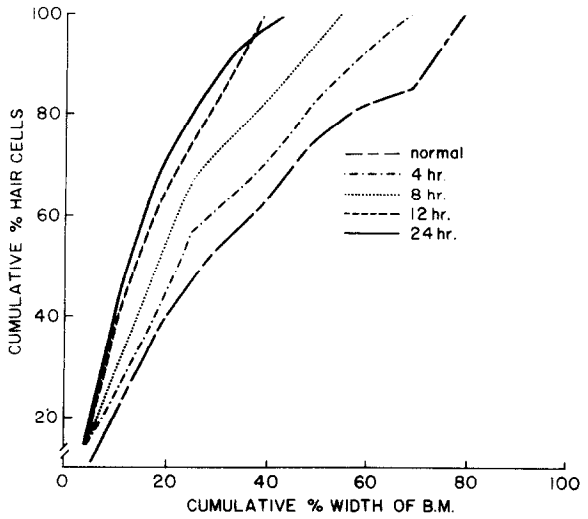


Fig. 7. Cumulative percent of hair cell counts across the width of the basilar membrane from superior to inferior edge in normal and sound exposed (500 Hz pure tone for 4, 8, 12 or 24 h at 125 dB) chicks. Counts were made at approximately 55% of length from the proximal tip of the BP.

8, 12 and 24 h of exposure the slope increases progressively and more dramatically. This increase in slope shows that the position of hair cell loss progresses from inferior to superior edge as duration of exposure increases. These data suggest that hair cell loss is systematically related to the radial position of the hair cells across the basilar membrane. Our interpretation is that this relationship is due to increasing basilar membrane deformation as one progresses from the edges to the middle of its expanse across the radial dimension.

Discussion

Short hair cells have been shown to be damaged to a greater degree by intense auditory stimuli than tall hair cells. As the duration of the stimulation increases, light-microscopic analysis indicates loss of both receptor types. The position of maximum short hair cell loss has been shown to vary with the frequency (Hz) of the acoustic stimulation. The position of tall hair cell loss was in approximately the same location for either the low (500 Hz) or high (1500 Hz) frequency condition. The correspondence between the position of short hair cell damage and the frequency of exposure stimulus was expected. It corresponds to what we and others have reported in previous papers [19,21]. We have also reported a correspondence between the position of hair cell damage and the frequency range to which N_1 potentials are elevated [13]. Similar results have been reported in mammals. Outer hair cells are more susceptible to noise damage than inner hair cells and the position of damage to outer hair cells varies systematically as a function of the exposure frequency [4,10,12]. These results demonstrate a similarity in morphological response to acoustic trauma between short hair cells of the avian basilar papilla and outer hair cells of the mammalian cochlea.

Other structural similarities have been shown between outer hair cells and short hair cells [2,6,7,22,25,27]. They are essentially located on the same area of the basilar membrane, the free or inferior edge. They also have a pattern of ontogenesis similar to outer hair cells and their innervation and synaptogenesis correspond to outer hair cells. There are, however, striking dissimilarities. Their general cellular shape is different; outer hair cells are rather tall and thin while short hair cells are, by definition, wider than they are tall. Short hair cells are not separated from other cell types by pillar cells; their cilia do not appear to be arranged in the typical W shape seen in outer hair cells and they are not organized in any fixed number or pattern of rows along the length of the basilar membrane. The results of the present study suggest that it is perhaps the similarities of location and innervation between avian short hair cells and mammalian outer hair cells that render them most susceptible to acoustic trauma.

In view of the fact that variations in acoustic trauma have similar effects on mammalian outer hair cells and avian short hair cells the absence of a systematic relationship between the frequency of intense sound exposure and the position of tall hair cell loss was unexpected. In mammals, when sound exposure is of sufficient intensity or duration to extend the damage to the inner hair cells, the position of damage appears similar to that found in the outer hair cells [4,10,12]. In the present study, loss of tall hair cells was always located in the basal half of the basilar papilla, regardless of the position of short hair cell loss. This region of tall hair cell loss, at approximately

30–35% from the proximal tip, is directly beneath the oval window and columellar footplate, suggesting the possibility of increased 'mechanical stress' due to footplate acceleration. It is noteworthy that short-term exposure (4 h) was not sufficient to cause loss of these cells, indicating that the 'mechanical stress' must occur over an extended period to result in loss of this cell type. It is not at all clear why the position of damage to this cell type was independent of frequency, especially since the traveling wave measurements of von Békésy [1] and the tonotopic organization found centrally [16] indicate that the frequency organization is similar to that found in mammals. Since we have found that N_1 threshold changes correspond to the frequency of exposure (and therefore to the position of short hair cell loss) it will be of considerable interest to determine the ganglion cell innervation ratio of short and tall hair cells as well as the specificity of innervation.

Two observations from mammals may be related to our inability to find any relationship between position of tall hair cell damage and exposure frequency. First, Liberman and Kiang [10] suggest that CF gaps (lack of fibers tuned to a particular frequency) are more closely related to the position of outer hair cell loss than inner hair cell loss. Second, these same authors, as well as others [12], find a 'second focus' of hair cell loss restricted to the basal end of the cochlea which is unrelated to the spectral properties of the exposure stimulus. As in the present study, this 'second focus' seemed unrelated to the primary focus, but was at a position most proximal to the footplate. Possibly greater attention to the factors responsible for these 'anomalous' centers of damage will yield important principles for understanding the mechanisms of noise-induced hearing loss.

Acknowledgements

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