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## CORRELATION OF AUDIOMETRIC DATA WITH CHANGES IN COCHLEAR HAIR CELL STEREOCILIA RESULTING FROM IMPULSE NOISE TRAUMA

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**Abstract.** In a previous experiment, after chinchillas had been exposed to impulse noise trauma, plastic-embedded surface preparations of the organ of Corti were examined with the light microscope. A consistent relationship between cochlear hair cell loss and hearing loss was not found (Hamernik et al., 1980). In the present study, four cochleas from that experiment were sectioned and examined with the transmission electron microscope to determine if there were consistent patterns of damage to the sensory cells at the ultrastructural level that would more closely correlate with the audiometric data. Alterations of the outer hair cell stereocilia were found when threshold was elevated 15 to 30 dB. The membranes of the stereocilia appeared loose and wrinkled and the stereocilia were no longer erect. In some cases, predominantly in the first row of outer hair cells, stereocilia were missing and in other cases, stereocilia were fused. Within these giant stereocilia, the rootlets of the individual stereocilia had disintegrated. Other alterations in sensory cell ultrastructure, though present, had no consistent pattern and could not be related to changes in hearing thresholds. Only the changes in the outer hair cell stereocilia appeared to correlate with hearing loss and the degree of damage was reflected in the amount of threshold elevation.

**KEY WORDS:** acoustic trauma; cochlear pathology; hair cell; stereocilia.

Acoustic trauma does not always produce a consistent relationship between cochlear hair cell loss and hearing loss in experimental animals. Some studies have shown normal hearing thresholds in the presence of substantial hair cell loss (Hunter-Duvar & Elliot, 1972; Henderson et al., 1974; Ward & Duval, 1971) while others have shown a loss of hearing when hair cells were present (Spoendlin, 1971; Ades et al., 1974; Hunter-Duvar & Bredberg, 1974). In noise-damaged cochleas, it has also been observed that the response properties of auditory nerve fibers may not correlate with

the loss of hair cells (Kiang et al., 1976; Salvi et al., 1979). Detailed light microscopic analysis of the cochleas used for the eighth nerve recordings has shown, however, that there were changes within the organ of Corti other than missing sensory cells that could account for the discrepancies between unit thresholds and hair cell loss (Lieberman & Beil, 1979; Salvi et al., 1979).

In a recent experiment designed to assess the effects of impulse noise on hearing thresholds in the chinchilla (Hamernik et al., 1980) no consistent relationship between hair cell loss and hearing loss was found in several of the animals. When plastic-embedded surface preparations of the organ of Corti were studied by light microscopy, in three of the cochleas there were areas where there was a large hearing loss in the absence of any significant hair cell loss. In one cochlea, there was no hearing loss and little hair cell loss. Although normal animals were processed and examined along with the experimental animals, this one animal could be considered to be an internal control. In all animals, the hair cells and supporting cells in the area studied appeared normal when viewed with the light microscope.

Since the information obtained with the light microscope did not explain the changes in hearing thresholds, these plastic-embedded cochleas were sectioned for observation with the transmission electron microscope. We were interested in determining if, in the regions of the cochlea where the hair cells were present and normal in appearance, there was

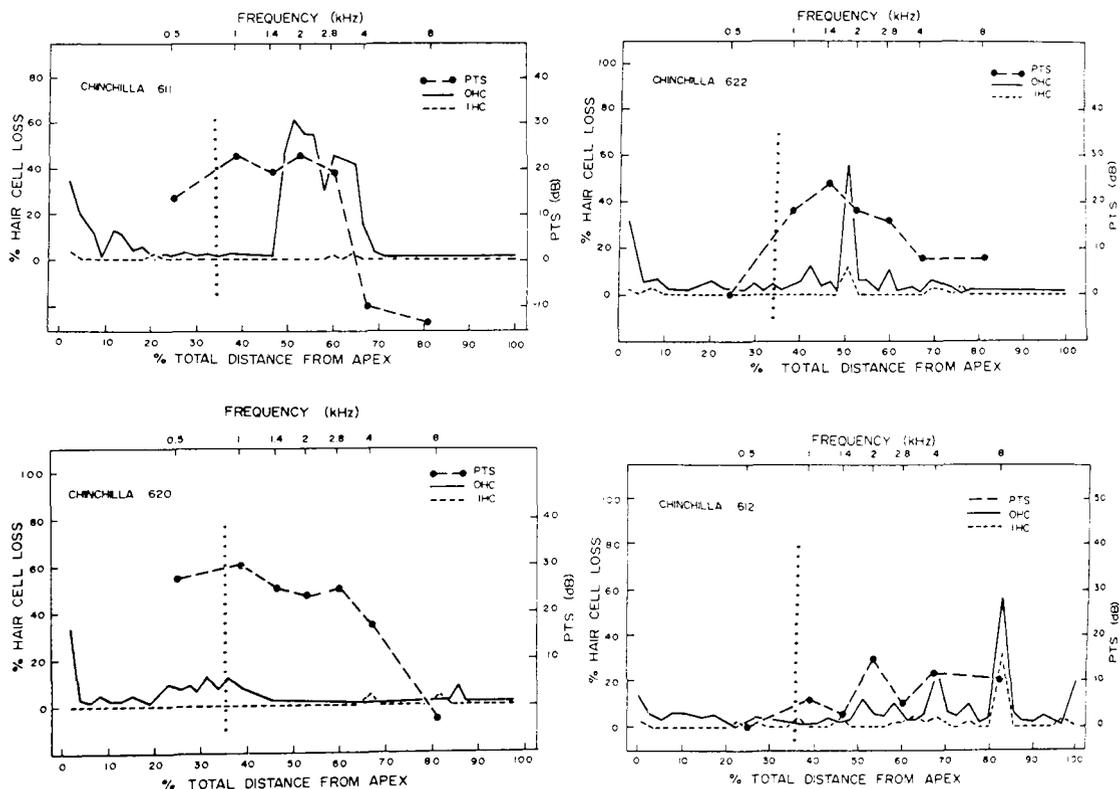


Fig. 1. Cochleograms and auditory evoked response audiograms from chinchillas exposed to impulse noise. Percent inner (---) and outer (—) hair cell loss was plotted as a function of percent total distance from the apex. Auditory evoked response audiograms (---) were ex-

pressed as permanent threshold shift in decibels. Vertical dotted lines indicate the region of each cochlea that was sectioned for examination with transmission electron microscopy.

damage to the sensory cells at the ultrastructural level that would correlate with the changes in hearing reflected in the audiometric data.

### METHODS

Four chinchillas were exposed to impulse noise from a compressed air driven source. Fifty impulses were presented, one per minute, at an intensity of 155 dB peak sound pressure level. Hearing was measured before and after exposure using the evoked response technique. Thirty days after exposure a final audiogram was obtained, after which the animals were killed. The cochleas were removed and perfused with 1% osmium tetroxide in

Zetterqvist veronal acetate buffer, pH 7.0 at 0°C, and fixed for one hour. They were washed in Tyrode solution, dehydrated through ethanol and propylene oxide, and embedded in Araldite. Cochleas were dissected, trimmed, and prepared as surface preparations for examination with the light microscope (Bohne, 1972). Percent inner and outer hair cell losses were plotted as a function of percent distance from the apex. Auditory evoked-response audiograms showing hearing loss expressed as permanent threshold shift (PTS), in decibels (dB) were superimposed on the cochleograms, aligning cochlear distance and frequency according to the place-frequency map for the chinchilla (Eldridge et al., 1977). The noise exposure and method for measuring

hearing thresholds have been described previously (Hamernik et al., 1980).

After examination of cochlear surface preparations with the light microscope, specimens of the organ of Corti, taken from a point along the basilar membrane 35–40% of the distance from the apex were selected for electron microscopic analysis. In this region of the cochlea, although hair cells were present, there was a variation in the extent of hearing loss from animal to animal. Pieces from each cochlea were coded so that specimens could be examined with no knowledge of audiometric data, re-embedded and thin sectioned on a Porter Blum 2B microtome. Silver sections were stained with uranyl acetate and lead citrate and observed with a Siemens Elmiscop 1A.

## RESULTS

The 30-day post-exposure cochleograms and audiograms for the animals used in this study are shown in Fig. 1. All of the cochleas had only scattered outer hair cell loss in the apical half of the cochlea, yet in the region sectioned (vertical dotted lines in Fig. 1) three of the chinchillas (chin. 611, 620 and 622) displayed a PTS of 15–30 dB, and one (chin. 612) had normal hearing.

The ultrastructure of cochlear inner hair cells in this region was remarkably similar. In all inner hair cells from the four cochleas exposed to impulse noise, lysosomes and multivesicular bodies were present (Fig. 2). Synaptic bodies were present in inner hair cells opposite afferent nerve terminals (Fig. 3). Possible signs of damage were more variable in outer hair cells (Fig. 4). Outer hair cells from 2 of the 4 animals (chin. 612 and 622) contained a large number of lysosomes. Outer hair cells from 3 of the 4 animals (chin. 612, 620 and 622) displayed a proliferation of the subsurface cisternae and contained many Hensen bodies. Only hair cells from one animal (chin. 611) contained multivesicular bodies.

The micrographs of sensory cells from the

animals exposed to impulse noise showed that signs of possible sensory cell pathology were present in inner hair cell somata from all four cochleas sectioned, yet only 3 chinchillas (chin. 611, 620 and 622) displayed a hearing loss corresponding to frequencies located in this region of the basilar membrane. Although possible signs of damage were found in outer hair cells, their presence was variable from animal to animal, and there appeared to be no consistent pattern to the changes in soma ultrastructure that would correlate with hearing loss. However, the degree of damage to the outer hair cell stereocilia in the different animals did appear to be reflected in the amount of threshold elevation. In radial sections, the stereocilia of normal hair cells appeared erect with the cell membrane smooth and closely apposed to the stereocilia (Fig. 5*a*). In sections parallel to the reticular lamina, the stereocilia from each hair cell appeared as a discrete bundle. There were no stereocilia missing, and the stereocilia within each bundle were separated one from the other by a slight space (Fig. 5*b, c*).

In the animals exposed to impulse noise there were changes in the stereocilia only when there was a hearing loss. In chinchilla 612, in the area of the cochlea sectioned, there was only slight and scattered outer hair cell loss and there was little if any significant PTS. The stereocilia of both the inner and outer hair cells appeared normal (Fig. 6*a, b*). The stereocilia remained erect and had only a slight wrinkling of the surrounding membrane.

In chinchilla 622, the spread of hearing loss was considerably greater than would be expected on the basis of the cochleogram. In the area sectioned, there was no inner hair cell loss and only a scattered outer hair cell loss. There was an 18 dB PTS at the region of the cochlea corresponding to 1 kHz but hearing was normal at 0.5 kHz. The stereocilia of the inner hair cells appeared normal, but stereocilia from outer hair cells appeared altered. In some cases the stereocilia were no longer erect but were flaccid (Fig. 7*a*). In other cases

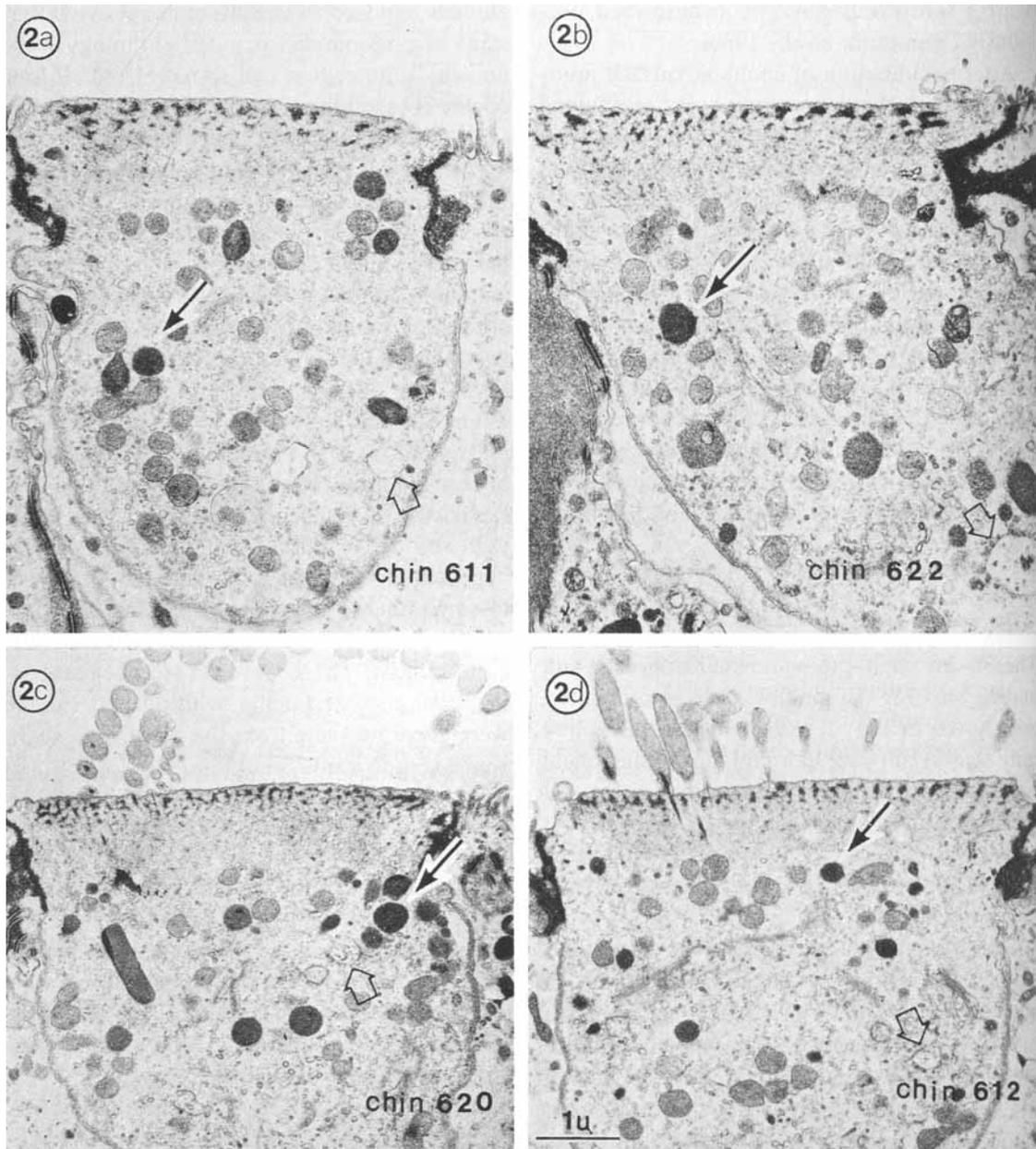


Fig. 2. Electron micrographs of the apical region of inner hair cells from the chinchillas exposed to impulse noise. There were lysosomes (closed arrows) and multivesicular bodies (open arrows) present.

the membranes surrounding the stereocilia were loose and very wrinkled and the stereocilia were surrounded by debris (Fig. 7b, c).

In chinchilla 611, in the area sectioned there were no inner hair cells missing and few outer hair cells missing, yet the hearing loss spread

to frequencies associated with the apical end of the cochlea. PTS ranged from 22 dB at 1 kHz to 15 dB at 0.5 kHz. Stereocilia from inner hair cells in this region appeared normal (Fig. 8a). They were erect and the membranes were close to the stereocilia. The outer hair

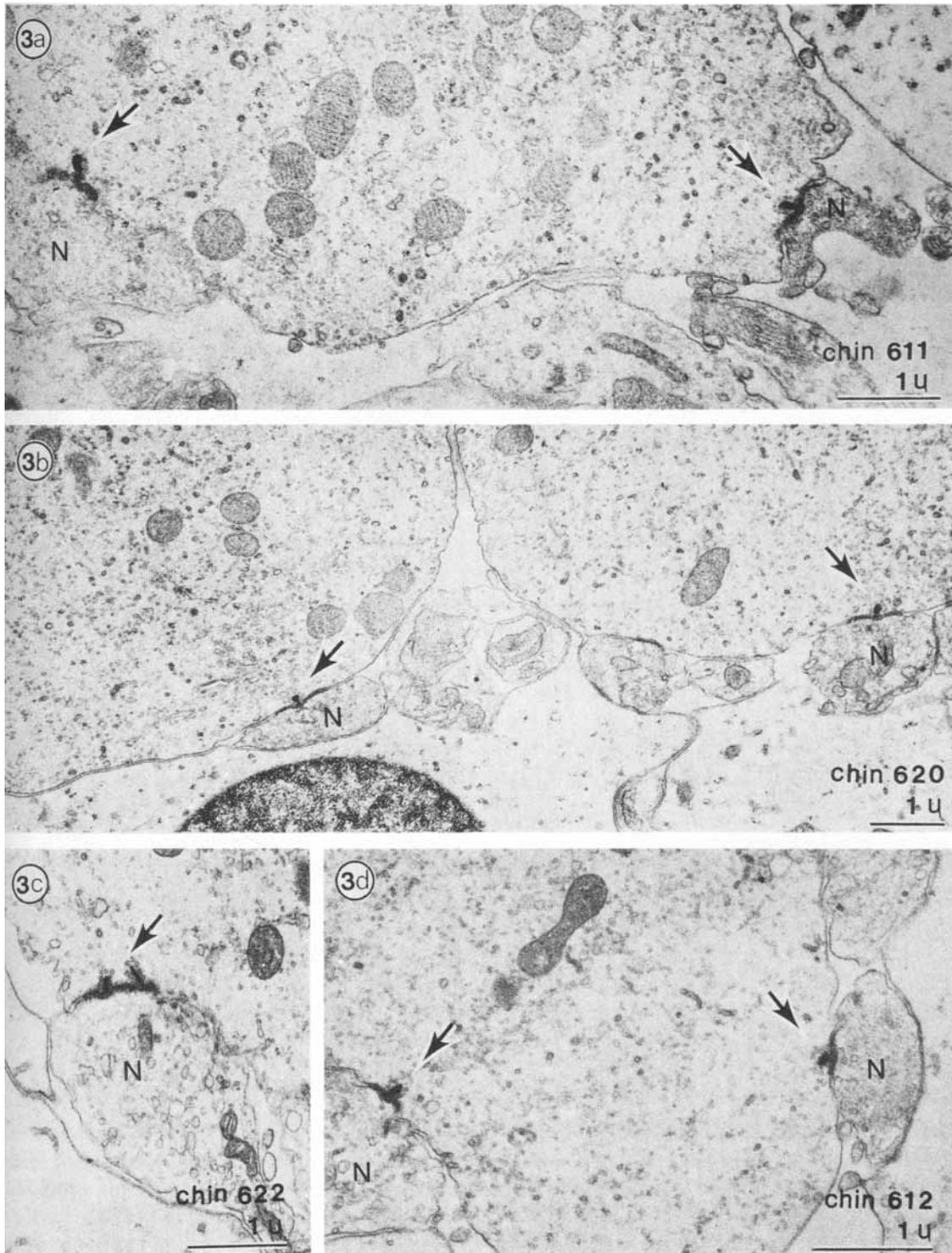


Fig. 3. Electron micrographs of the synaptic region of inner hair cells from the chinchillas exposed to impulse noise. There were synaptic bodies present (arrows) at afferent nerve terminals (N).

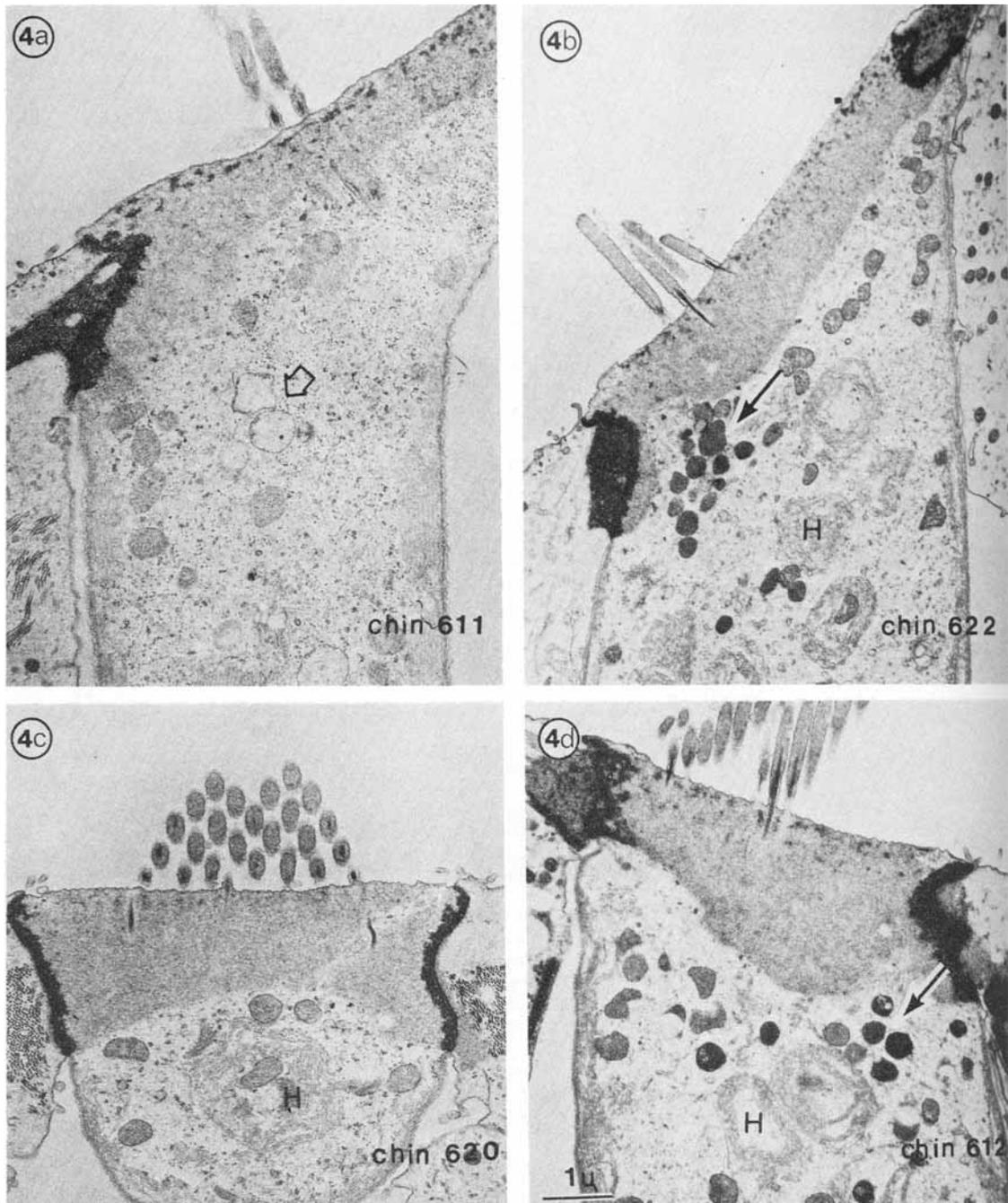


Fig. 4. Electron micrographs of the apical region of outer hair cells from the chinchillas exposed to impulse noise. The presence of lysosomes (closed arrows), multivesicular bodies (open arrow) and Hensen bodies (H) varied from animal to animal.

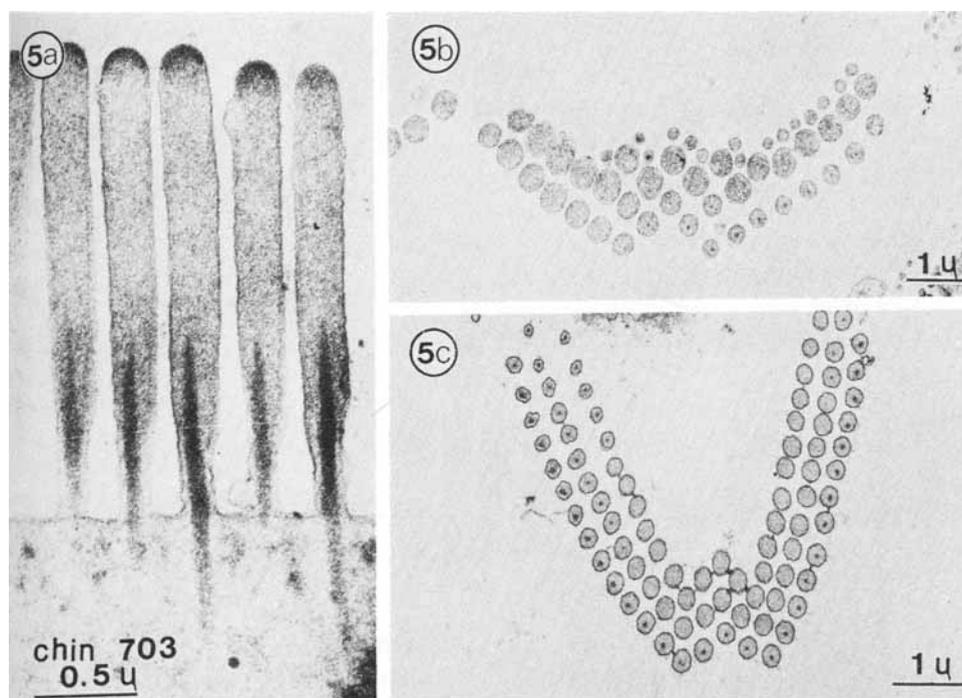


Fig. 5. Electron micrographs of stereocilia from a chinchilla not exposed to impulse noise. (a) A radial section of the organ of Corti showing the outer hair cell stereocilia. The membranes appeared smooth and the stereocilia were erect. (b) A section of the organ of Corti parallel to the

reticular lamina showing inner hair cell stereocilia. (c) A section of the organ of Corti parallel to the reticular lamina showing outer hair cell stereocilia. The membrane surrounding the stereocilia was smooth and the stereocilia were separated one from the other by a slight space.

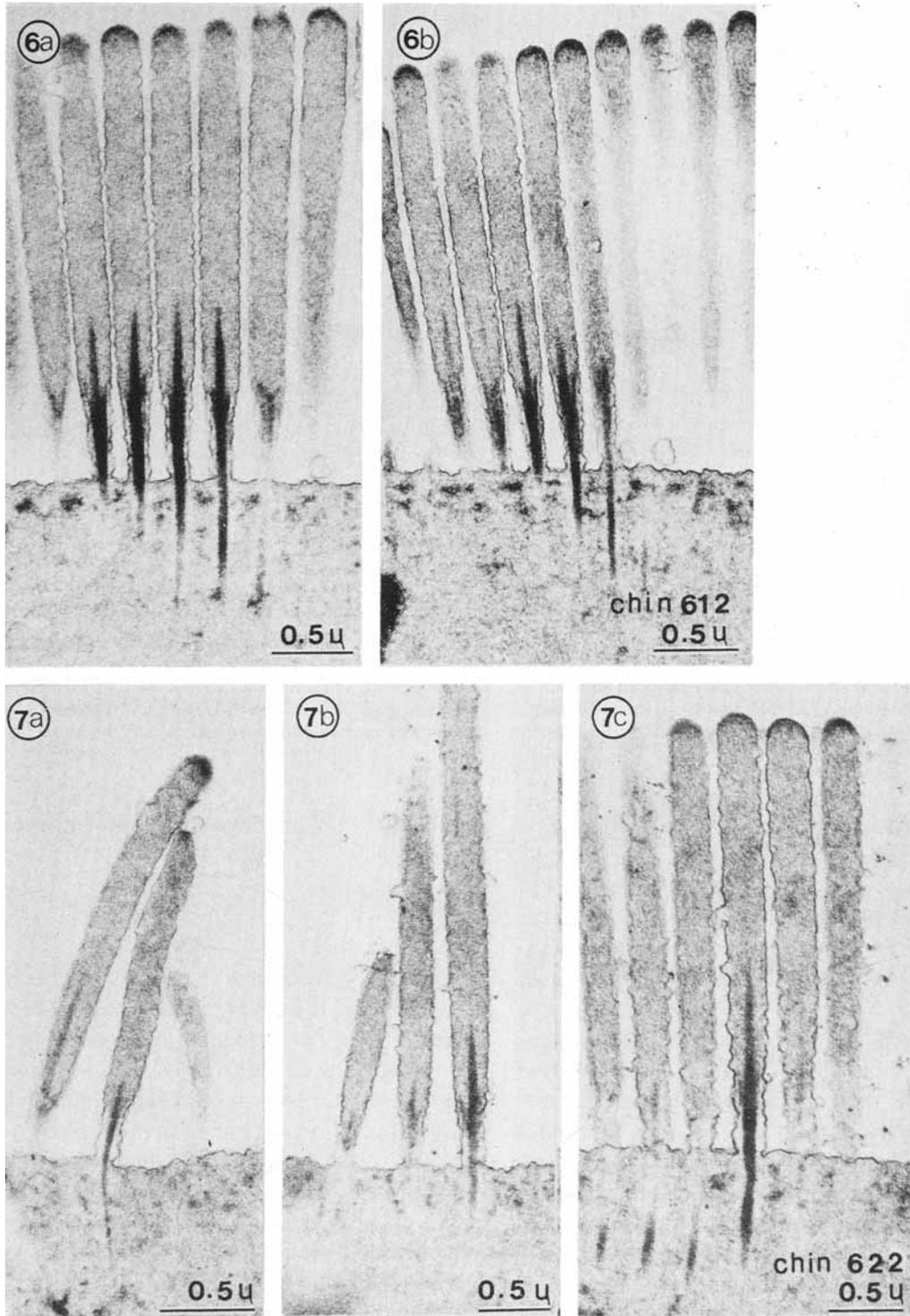
cell stereocilia showed marked alterations. The membranes surrounding the stereocilia were loose and wrinkled in all three rows of outer hair cells. In addition, particularly in the first row of outer hair cells, the stereocilia were in some cases missing and in other cases fused (Fig. 8 b).

Chinchilla 620 was particularly interesting because it had only scattered hair cell loss throughout the cochlea yet it had a PTS that ranged from 25 to 30 dB between 0.5 and 4 kHz. In the area of the cochlea sectioned, inner hair cell stereocilia appeared normal (Fig. 9 a). Outer hair cell stereocilia appeared abnormal. In some cases, again predominantly in the first row of outer hair cells, stereocilia were missing, and in other cases they were fused. When the stereocilia had fused to form giant stereocilia, some of the rootlets, the darkly staining core of the stereocilia that pen-

etrates into the cuticular plate, had disintegrated (Fig. 9 b).

## DISCUSSION

Since loss of hearing has been observed in cases where hair cells are present, one can no longer depend on cochleograms alone to predict the amount of hearing loss resulting from trauma to the cochlea. It has been demonstrated by various investigators that after exposure to noise, although some cells degenerate, the remaining cochlear hair cells can show a variety of changes both at the light and electron microscopic levels. Although it is not yet known which of these changes may be responsible for changes in sensory cell sensitivity and the resulting hearing loss, there are specific examples following noise trauma where at the light microscope level, changes in sensory



*Figs. 6 & 7. Electron micrographs of outer hair cell stereocilia from chinchillas exposed to impulse noise. Fig. 6a, b. The stereocilia looked normal, with only a slight wrinkling of the surrounding membrane. The chinchilla (chin. 612) displayed no significant PTS. Fig. 7a, b, c. The stereocilia showed signs of damage. The chinchilla (chin. 622) had a PTS of 0–18 dB in the region of the cochlea sectioned.*

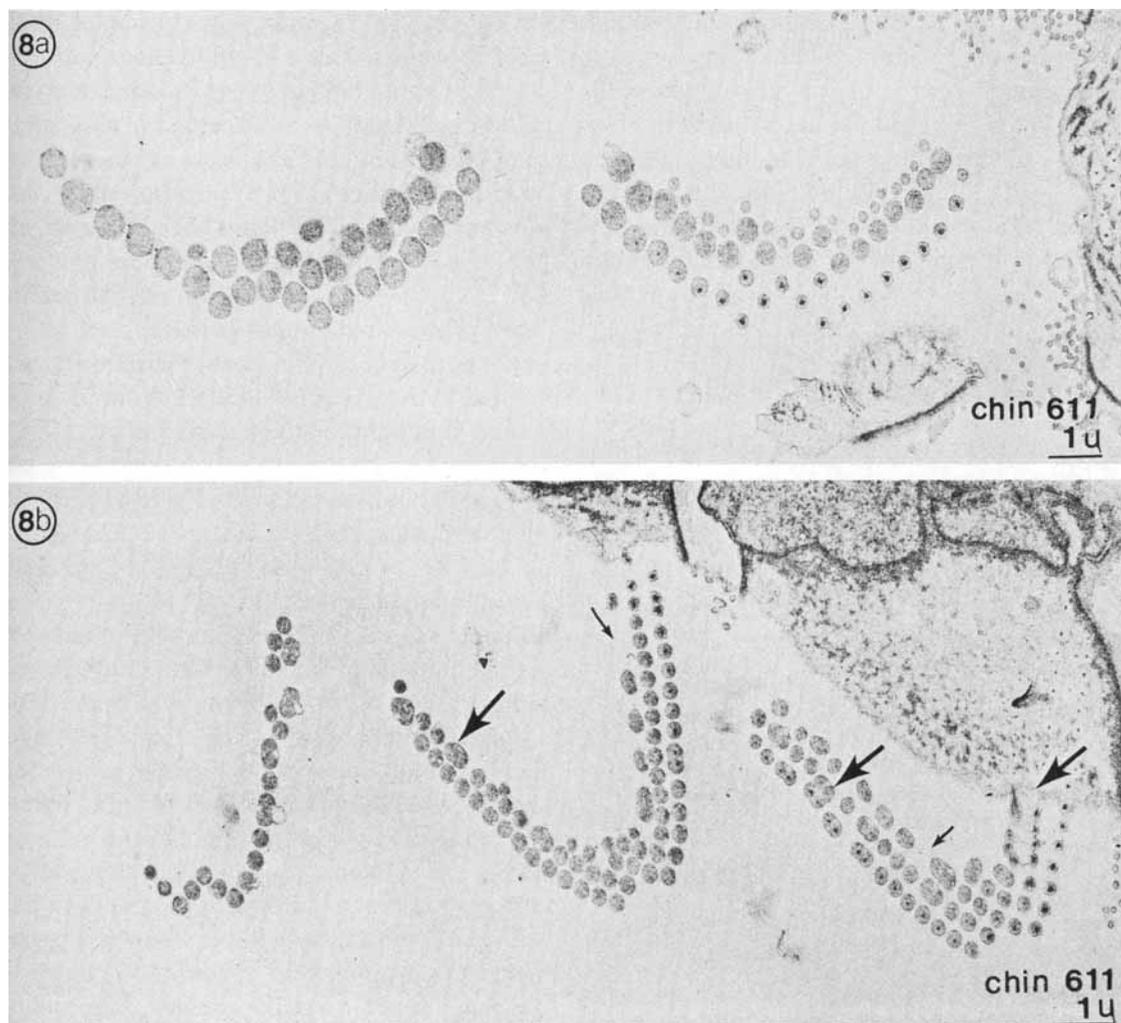


Fig. 8. Electron micrographs of inner and outer hair cell stereocilia from a chinchilla exposed to impulse noise. (a). Inner hair cell stereocilia appeared normal. (b) Outer hair cell stereocilia were in some cases missing (*small arrows*)

and in other cases fused (*large arrows*) to form giant stereocilia. The chinchilla (chin. 611) showed a PTS of 15–22 dB in the region of the cochlea sectioned.

cell shape, inner and outer pillar defects (Salvi et al., 1979) and 'floppy' stereocilia (Liberman & Beil, 1979) have been associated with changes in auditory nerve fiber recordings. Scanning electron microscopy has shown that after mechanical trauma to the organ of Corti, fusion of stereocilia can be associated with changes in spiral ganglion cell recordings (Robertson et al., 1980). However, as yet there has been no information on the ultrastructure of the sensory and supporting cells

of these cochleas to demonstrate that only the changes documented could be responsible for the physiological changes.

Our own findings at the ultrastructural level, 30 days after noise exposure, show the presence in hair cells of lysosomes and multivesicular bodies, vacuolization and disorganization of subsurface cisternae, and proliferation of Hensen bodies (Slepecky et al., 1981). Although these structures are normally present in sensory cells, previous authors have

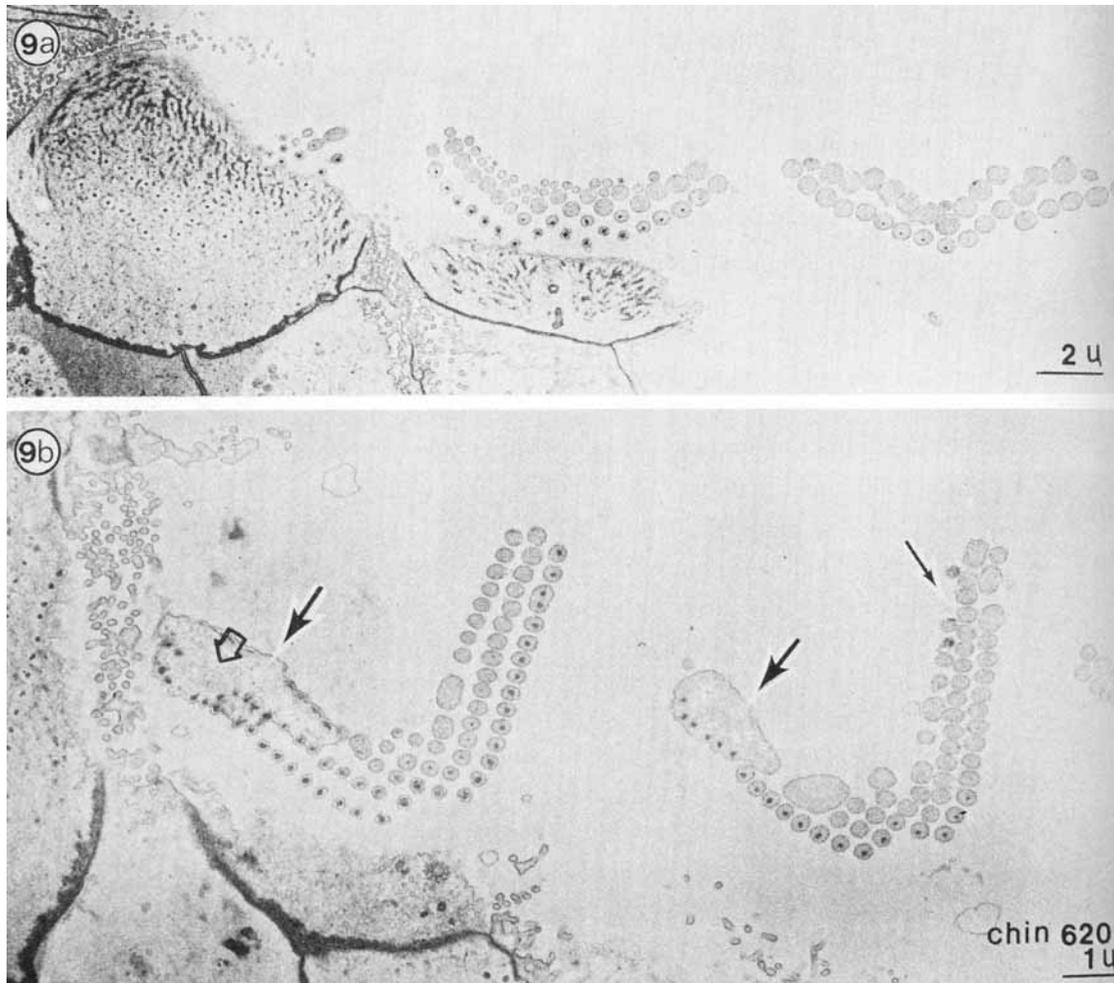


Fig. 9. Electron micrographs of inner and outer hair cell stereocilia from a chinchilla exposed to impulse noise. (a) The inner hair cell stereocilia appeared normal. (b) The outer hair cells were in some cases missing stereocilia (small arrows) and in other cases the stereocilia were

fused (large arrows) to form giant stereocilia. The rootlets of the fused stereocilia were disintegrating (open arrow). The chinchillas (chin. 620) displayed a PTS of 25–30 dB in the region of the cochlea sectioned.

claimed that variations in their number may be a typical sign of acoustic trauma and may be responsible for noise-induced hearing loss. Our results do not support this.

In the regions of the cochleas sectioned (35–40% of the distance from the apex) the changes in the ultrastructure of the inner hair cell somata in the four cochleas were similar while the variation in threshold shift ranged from no change to a 30 dB change in sensitivity. Changes in the outer hair cell somata varied in the different animals yet the presence of

lysosomes, multivesicular bodies and Hensen bodies still could not be correlated with a loss in hearing. Because we have observed similar ultrastructural changes in regions of cochleas whose corresponding threshold shifts were as little as 0 or as great as 30 dB, we conclude that such alterations to hair cell ultrastructure are not critical to the decrease in hair cell sensitivity.

However, in the cochleas studied damage to the stereocilia always accompanied noise-induced PTS and the degree of damage could be

correlated with the amount of change in threshold sensitivity. As the amount of PTS increased the damage to the outer hair cell stereocilia progressed from loosening of the membranes, to large wrinkles occurring along the length of the stereocilia, to deformation of the stereocilia and finally to fusion of the outer hair cell stereocilia and disintegration of the stereocilia rootlets.

Previous experiments on mechanically damaged (Robertson et al., 1980) and drug damaged (Ryan & Dallos, 1975; Dallos & Harris, 1978) cochleas have demonstrated that loss of outer hair cells causes threshold shifts of from 30 to 40 dB. Our results indicate that only changes in the stereocilia of outer hair cells may be the primary determinant of PTS at least up to a 30 dB change in sensitivity. Since the major afferent output from the cochlea comes from the inner hair cells (Spoendlin, 1973), our data suggest that at low stimulus levels the outer hair cells in some way influence inner hair cell activity and that the interaction may be mediated by the stereocilia, perhaps through the tectorial membrane.

The actual changes observed in the outer hair cell stereocilia may indicate that there are changes in their membrane properties resulting from noise trauma, both in terms of permeability and surface charge. These changes could affect the relationship of the stereocilia with each other causing fusion of the stereocilia (Flock et al., 1977) as well as influencing the movement of ions around and into the sensory cells. In addition, observed deformation of the stereocilia may indicate that the actin filaments within the stereocilia (Flock & Cheung, 1977; Tilney et al., 1980) have depolymerized and may no longer be arranged in the stiff conformation. The fact that the rootlets penetrating the cuticular plate disappear in the fused stereocilia may also indicate that conformational changes in actin result from noise trauma.

In summary, the results of this study indicate that there is a relationship between the amount of noise-induced PTS and the condi-

tion of the stereocilia. The ultrastructural changes observed by transmission electron microscopy may indicate that the impulse noise trauma used in this experiment has caused alterations in outer hair cell stereocilia membrane permeability, surface charge and actin conformation. These changes by themselves may be sufficient to cause a PTS of at least up to 30 dB.

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#### ZUSAMMENFASSUNG

In einem früheren Experiment, in dem Chinchillas mit hoher Lärmintensität traumatisiert worden waren, wurde das Cortische Organ in Plastik gebettet und mit dem Lichtmikroskop untersucht. Eine übereinstimmende Beziehung zwischen Verlust von Cochlear-Haarzellen und Gehör wurde nicht festgestellt (Hamernik et al., 1980). In der vorliegenden Untersuchung wurden Dünnschnitte von vier Cochleas des vorhergehenden Experiments hergestellt und mit dem Elektronenmikroskop auf übereinstimmende ultrastrukturelle Schädigungen der Sinneszellen untersucht, um eine engere Beziehung zu den akustischen Ergebnissen herzustellen; Veränderungen der Stereocilien der äußeren Haarzellen wurden bei Erhöhung der Schwelle auf 15 bis 30 dB gefunden. Die Membranen der Stereocilien waren lose und faltig und die Stereocilien nicht mehr aufrecht. Die Stereocilien fehlten in einigen Fällen, vorwiegend in der ersten Reihe der äußeren Haarzellen, und waren in anderen Fällen verschmolzen. Innerhalb dieser Riesenstereocilien hatten sich die Würzelchen der individuellen Stereocilien aufgelöst. Andere Veränderungen der Ultrastruktur der Sinneszellen waren nicht mit den Veränderungen der Hörschwellen in Beziehung gebracht worden. Nur die Veränderungen in den Stereocilien der äußeren Haarzellen schienen mit dem Hörverlust in Beziehung zu stehen und der Grad der Schädigung von der Erhöhung der Schwelle abzuhängen.

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