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Regeneration of hair cell stereociliary bundles in the chick cochlea following severe acoustic trauma

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Examination of pure-tone acoustic damage in the chick cochlea revealed a significant amount of hair cell recovery over a 10 day period following the exposure. The recovery included both a regeneration of stereociliary bundles to replace those that were lost and a reshuffling of the mosaic pattern of the hair cell surfaces that survived. Ten-day-old chicks were exposed to a 1500 Hz pure tone at 120 dB SPL for 48 h and their cochleae were processed for scanning, transmission and light microscopy at 0 h, 24 h, 48 h, 4 d, 6 d and 10 d after exposure. Immediately after exposure the damaged region exhibited two types of hair cell trauma. The first was a defined area of complete hair cell loss and the second was an area where the hair cells survived but exhibited varying amounts of stereocilia injury. After 48 h of recovery, new hair cells were identifiable in the region of hair cell loss and with time they underwent a progressive maturation of their stereociliary bundles. The surviving hair cells showed a dramatic rearrangement and expansion of their surfaces but exhibited no repair of the damaged stereociliary bundles. These results suggest that the chick cochlea is capable of a significant amount of recovery and regeneration following acoustic trauma.

Stereocilium; Hair cell; Acoustic trauma; Regeneration; Chick

Introduction

It has been well established that severe acoustic overstimulation causes a broad spectrum of damage to hair cells in the cochlea which can include the complete loss of cells from the epithelium (Bohne, 1971; Lim, 1976; Hunter-Duvar, 1978). This damage to the organ of Corti is thought to result in a permanent hearing loss because studies have shown that once the hair cells are lost they are not regenerated but instead are replaced by phalangeal scars (Engstrom et al., 1966; Bohne, 1976; Hawkins and Johnsson, 1976; Bohne and Rabbit, 1983). We have been conducting noise damage studies in the chick ear in which the condition of hair cells in the basilar papilla was analyzed with scanning electron microscopy (SEM) immediately after exposure to an intense pure tone in order to examine the initial effects of acoustic trauma on the structure of the stereociliary bundle (Cotanche et al., 1987). Our studies have shown that acoustic trauma at intensities of 120 dB SPL or greater produced large, tonotopically-positioned areas of hair cell damage which included both stereocilia injury and hair cell loss. These findings were in general agreement with other reports on noise damage in the chick ear which had utilized a 10 day recovery period following the exposure (Rubel and Ryals, 1982, 1983; Ryals and Rubel, 1982, 1985; Cousillas and Rebillard, 1985). However, in order to accurately compare our results on the location and extent of the damage with those of other reports, we repeated our SEM analyses on animals that had been allowed to recover from the stimulus for 10 days.

To our surprise, it was found that the region of damage had not stabilized or expanded over the 10 day recovery period, instead it exhibited a considerable amount of structural recovery which included what appeared to be a population of hair cells with newly-formed stereociliary bundles (Cotanche et al., 1986). In order to examine this

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recovery process in greater detail and to identify the origin of the apparently newly-formed hair cells, we have studied a series of intermediate stages of recovery between 0 and 10 days after the exposure. Our results have indicated that a significant number of new stereociliary bundles are regenerated in the chick basilar papilla in a manner which is equivalent to that seen during the embryonic development of the cochlea (Cotanche and Sulik, 1984; Tilney et al., 1986).

Materials and Methods

Organisms

Fertilized eggs were obtained from Pee Dee Hatchery (Hartsville, SC) on embryonic day 0 and were placed in a laboratory incubator at 38°C and 40% humidity. Upon hatching (embryonic day 21), the chicks were relocated to a small heated brooder, and maintained in a noise-free environment until they were used for noise exposure experiments on post-hatching day 10.

Noise exposure and recovery

Pairs of 10-day-old chicks were placed in a small, cylindrical chicken-wire cage inside a sound-attenuated chamber and were supplied with food and water. A 100 W loudspeaker was suspended from the ceiling of the chamber and was centered over the chicken-wire cage. Each pair of chicks was exposed to a 1500 Hz pure tone for 48 h at an intensity of 120 dB SPL. Calibration of stimulus intensity (expressed in dB re 20 μPa) and analysis of the harmonic content of the stimulus were carried out for several locations throughout the chamber using a Hewlett Packard model no. 3561A spectrum analyzer. The size of the chicken-wire cage was designed so that the change in intensity of the primary tone was no more than ± 1 dB at all locations within the cage. For all exposures, the secondary and tertiary harmonics were at least 40 dB below the primary tone.

After the 48 h exposure period, the chicks were removed from the chamber. One of the pair was sacrificed immediately and its ears processed for either SEM or transmission electron microscopy (TEM). The other chick was allowed to recover for a period ranging from 1 to 10 days at which time it was sacrificed and its ears processed for either SEM or TEM.

Preparation for SEM

The chicks were sacrificed by an i.p. injection of saturated ethyl carbamate (Urethane). The birds were decapitated, each temporal bone was isolated, the columella was removed and the bony wall of the scala vestibuli dissected away. This procedure, which exposed the vestibular surface of the tegmentum vasculosum, took less than 4 min. The cochlear ducts were fixed for 45 min in 1% osmium tetroxide in 0.1 M sodium phosphate buffer at pH 6.4 and 4°C. The fixed tissues were dehydrated in ethanol up to 70%, at which point final dissections were carried out in order to expose the luminal surface of the basilar papilla. For the final dissection, the tegmentum vasculosum, lagena and lagenar otolith were removed and the tectorial membrane was lifted off the sensory epithelium with a pair of hand-sharpened EM forceps. A stream of 70% ethanol was squirted over the surface of the BP with a syringe fitted with a 27-gauge needle. This procedure removed any remains of the tectorial membrane and also any free otoconia which were dispersed when the lagenar otolith was pulled out.

After the final dissection, the cochleae were dehydrated to 100% ethanol and critical point dried from CO_2. The dried specimens were mounted on aluminum stubs and sputtercoated with gold/palladium. The tissues were viewed on a Jeol JSM-35C scanning electron microscope at an accelerating voltage of 25 kV.

Preparation for LM and TEM

The chicks were sacrificed as above and the isolated temporal bones were dissected in oxygenated Hepes-buffered Hanks' balanced salt solution (Hanks and Wallace, 1949). The columella was removed and the lateral wall of the scala vestibuli was dissected away. This procedure, which exposed the vestibular surface of the tegmentum vasculosum, took less than 4 min. The cochlear ducts were fixed for 45 min in 1% glutaraldehyde and 1% osmium tetroxide in 0.1 M sodium phosphate buffer at pH 6.4 and 4°C (Tilney et al., 1980). The fixed tissues were dehydrated in ethanol up to 70%, at which point final dissections were carried out in order to free the cochlear duct from the surrounding bony tissue. After the final dissection, the cochleae were dehy-

drated to 100% ethanol and embedded in a Spurrs/Epon resin. Thick (1 μM) and thin sections were cut on a Sorvall MT-2B ultramicrotome and the thin sections were examined with a Jeol 100S transmission electron microscope at 60 kV.

Assessment of hair cell damage and recovery

In a previous publication (Cotanche et al., 1987), the variation in hair cell damage which resulted from pure-tone overstimulation at several frequencies and stimulus intensities was examined with scanning electron microscopy. From these studies it was possible to identify a stimulus frequency and intensity that produced a clearly-defined region where the normal mosaic pattern of the hair cells was disrupted. This region included areas where hair cells were lost from the epithelium as well as areas where the cells remained in place but their stereociliary bundles were severely damaged. Outside the borders of the damaged region, hair cells showed no signs of acoustic trauma.

In order to assess the amount of hair cell damage and recovery in our experiments, we exposed birds to the stimulus in pairs. One member of the pair was sacrificed immediately after the exposure so that the initial extent of damage could be determined. The second bird was allowed to recover for 1 to 10 days and then the extent of hair cell damage was compared to the cochleae sacrificed immediately after exposure. So although we could not determine what the initial extent of damage was for each recovering basilar papilla, it was possible to obtain a general idea of the extent of damage for each exposure.

Accurate assessments of the damage done to specific stereociliary bundles and the extent of their recovery was made possible by the detailed studies of Tilney and co-workers on the normal gradients in stereocilia structure in the adult and embryonic chicken basilar papilla (Tilney and Saunders, 1983; Tilney et al., 1983, 1986; Tilney and DeRosier, 1986). The position-specific gradients in the length, width and number of stereocilia per cell allowed for the determination of how the acoustic trauma affected the morphology of the stereociliary bundles at certain locations within the damaged region. In addition, the studies on developing hair cells permitted detailed comparisons between embryonic stereociliary bundles and those which were regenerated in the recovering sensory epithelium.

Analysis of the changes in the lengths and numbers of stereocilia per bundle were made by taking high-magnification micrographs of the bundles at various times during recovery, photographically enlarging them and either measuring the lengths or counting the number of stereocilia present on the cells. Changes in hair cell surface areas during recovery were measured by converting the SEM video output to a normal TV scan rate and recording the SEM images on a standard video cassette recorder. The videotapes were played back at a slow speed and the outlines of the cells were drawn onto acetate sheets. The areas of the cells were computed on an Apple IIe microcomputer with a digitizing pad and a standard graphics analysis program.

Results

No recovery

When the basilar papillae of chicks sacrificed immediately after exposure were examined with SEM, two regions of hair cell damage could be identified. The first region comprised a longitudinal strip of missing hair cells located close to the superior edge of the basilar papilla. The second region was a semicircular patch of hair cell damage which radiated out from the inferior edge of the basilar papilla distal to the location of the strip (Figs. 1a, 2b). A more detailed description of the relationship between the strip and patch regions of damage and their dependence on stimulus intensity was presented in a previous paper (Cotanche et al., 1987).

Within the patch region of damage, the hair cells exhibited two distinct types of pathological response to the acoustic trauma. The most severe of the two types involved a complete loss of the hair cell from the epithelium. In this instance, the hair cell apical surface was initially bloated out of the sensory epithelium and eventually the entire cell was ejected into the scala media (Fig. 1d). The hole left in the surface of the basilar papilla by the vacating hair cell surface was sealed over by an expansion of the adjacent supporting cell surfaces (Figs. 1b, 2b). The ballooning of the cells prior to

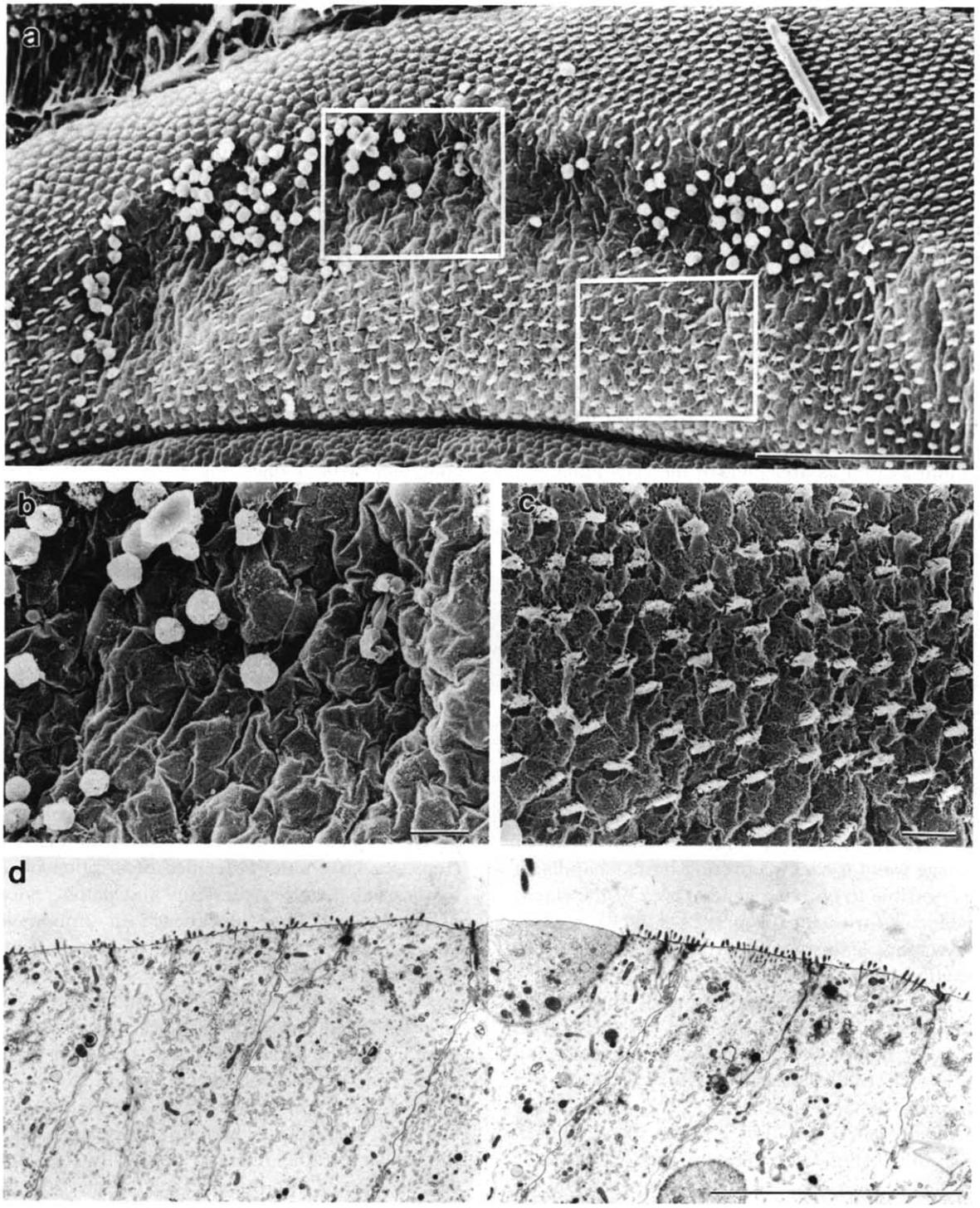

Fig. 1. Hair cell damage produced by acoustic trauma. (a) The patch-like region of hair cell damage produced by a 1500 Hz pure tone at 120 dB SPL for 48 h with no recovery. Boxes indicate regions shown in b and c below. Bar, 100 μm. (b) The region of hair cell loss within the patch (upper box in a). (c) The region of hair cell injury within the patch (lower box in a). (d) A transmission electron micrograph in the region of hair cell loss. Bars in b–d, 10 μm.

ejection from the basilar papilla was distinctly different from the blebbing seen on some hair cells after acoustic trauma (Lim and Melnick, 1971; Lindeman and Bredberg, 1972). The ballooning involved the entire hair cell surface and led to a disruption of the stereociliary bundle, whereas blebbing only included the cell surface in front of the tallest row of stereocilia, a cuticular plate-free region of the apical surface which overlies the basal body.

The second type of hair cell damage in the patch region involved only the stereociliary bundles of the cells, leaving the apical surfaces of the cells intact (Fig. 1c). In some of the bundles only the tallest rows of stereocilia were lost, while in other bundles all of the rows were damaged or disrupted. Interestingly, the surfaces of the hair cells which remained in the basilar papilla were reduced in area compared to cells in control ears (Fig. 6). In contrast, the adjacent supporting cell surfaces had expanded to compensate for the decrease in hair cell surface area (Figs. 1c, 3b). However, the reduction in the hair cell surface area was not accompanied by a bloating of the cell surface.

The two types of hair cell damage within the patch region occurred in different locations in the patch (Fig. 1). The region where the hair cells were lost from the basilar papilla was located along the superior curved border of the patch region. Occasionally there were laterally-oriented wedges of hair cell loss which extended down from the superior edge of the patch toward the inferior edge. The region of the patch in which hair cells showed only injury to their stereociliary bundles was located along the inferior edge of the patch and extended up toward the superior edge until it encountered the region of hair cell loss (Fig. 1a). Often the superior-most border of the area of hair cell injury was serrated as it interdigitated with the laterally-oriented wedges of hair cell loss described above.

Immediately after exposure, the hair cells which were located along the superior edge of the basilar papilla, superior to the patch region, showed no evidence of cell loss or stereociliary damage (Fig. 4a). These hair cells, which have a prominently afferent innervation (Takasaka and Smith, 1971), are thought to be equivalent to the inner hair cells of the mammalian organ of Corti.

In the strip region of damage, located along the superior edge proximal to the patch, only a few hair cells were lost from the epithelium and these were found in a longitudinal line which was located directly above the junction of the basilar membrane and the superior fibrocartilaginous plate (see Cotanche et al., 1987).

24 hours of recovery

After 24 h of recovery from the acoustic trauma, the mosaic pattern of the hair cells in the patch region remained severely disrupted (Fig. 2c). The extent of hair cell loss and stereocilia injury in the damaged region was equivalent to that at 0 hours of recovery. However, the one significant difference in the injured hair cells after 24 h of recovery was that their apical surfaces had once again expanded (Figs. 3c, 6). Concurrently, the surfaces of the adjacent supporting cells had contracted to form thin, microvillus-covered rings around the hair cells. Moreover, the supporting cells appeared to be secreting a new ground substance for the tectorial membrane. This aspect of recovery is examined in greater detail in the accompanying paper (Cotanche, 1987b).

Hair cells along the superior edge of the basilar papilla, which showed no signs of damage at 0 h of recovery, exhibited cells with damaged stereocilia and several bloated cells or gaps between cells which suggested that some cells had been lost from the epithelium during the first 24 h of recovery (Fig. 4b). These lost cells were not in large groups, as was seen in the patch region, but were dispersed all along the superior edge. Although this was not as extensive a loss as seen in the patch region, none of the ears examined immediately after the exposure showed as much damage in this area. Presumably, then, this loss of cells along the superior edge of the basilar papilla occurred during the first 24 h of recovery.

48 hours of recovery

When the basilar papillae were examined after 48 h of recovery, the disruption in the mosaic pattern of the hair cells in the patch region was much reduced compared to earlier time points. There were only a few bloated hair cells left projecting from the surface of the epithelium and

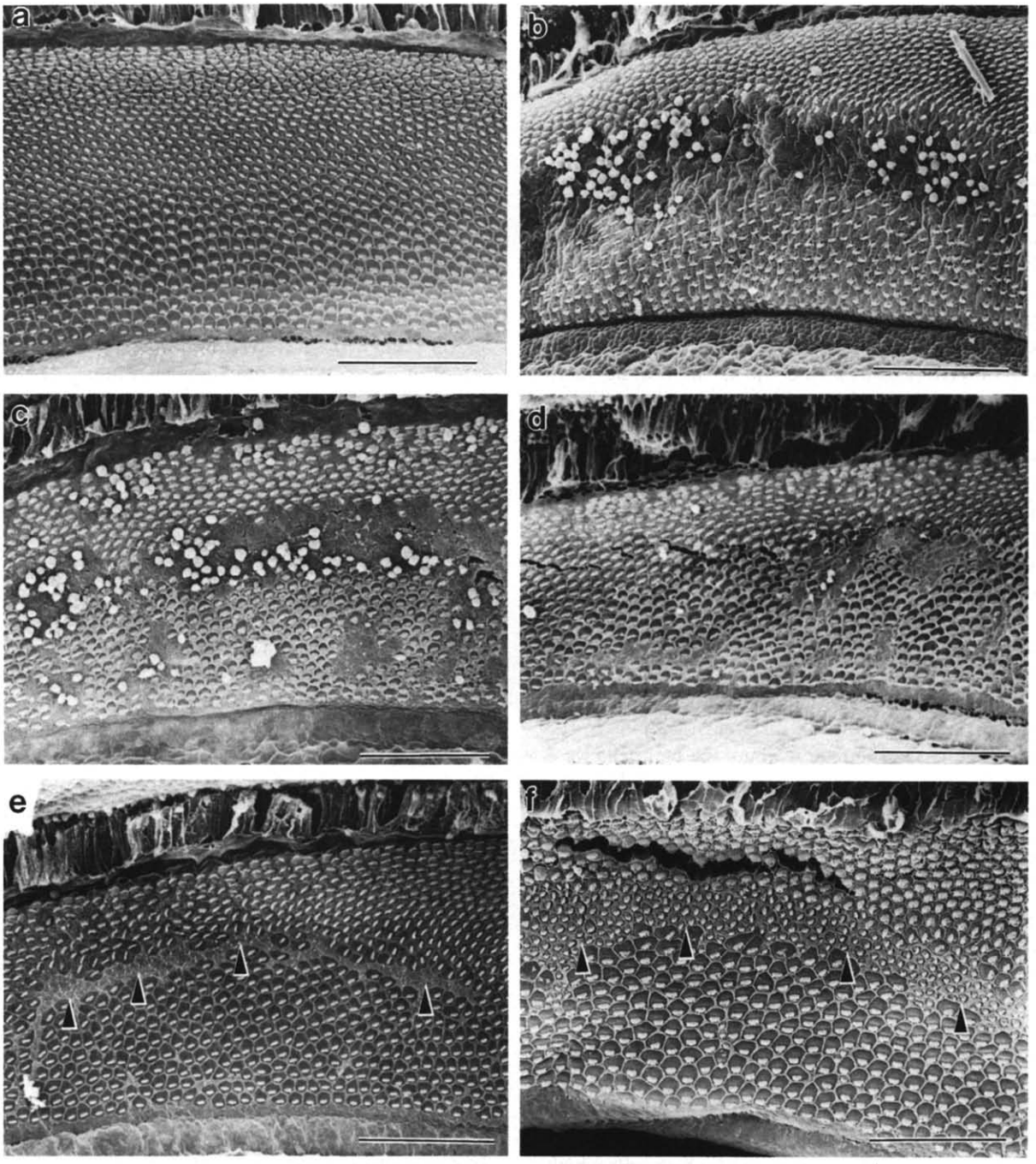

Fig. 2. Recovery of the basilar papilla from acoustic overstimulation. (a) 1500 Hz region of control basilar papilla. (b–f) Same region as in a following exposure for 48 h to 1500 Hz pure tone at 120 dB SPL. (b) No recovery; (c) 24 h of recovery; (d) 48 h of recovery; (e) 6 days of recovery; (f) 10 days of recovery. Bars, 100 μm; arrowheads, new stereociliary bundles.

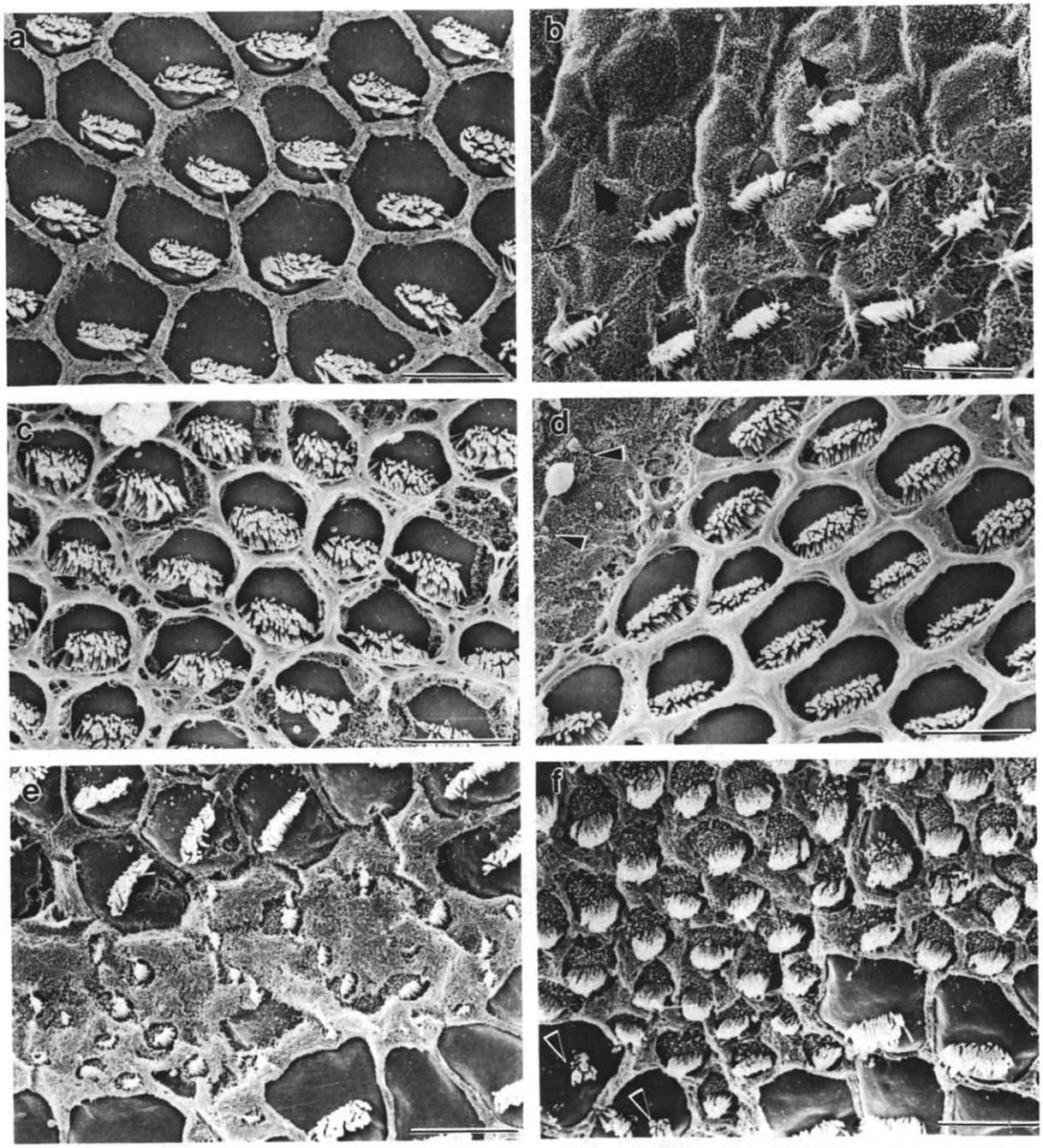

Fig. 3. Hair cell recovery in the basilar papilla. (a) 1500 Hz region of control basilar papilla. (b–f) Same region as in a following exposure for 48 h to 1500 Hz pure tone at 120 dB SPL. (b) No recovery – arrow, expanded surfaces of supporting cells; (c) 24 h of recovery; (d) 48 h of recovery – arrowheads, regenerating stereociliary bundles; (e) 6 days of recovery; (f) 10 days of recovery – arrowheads, injured stereocilia. Bars, 10 μm.

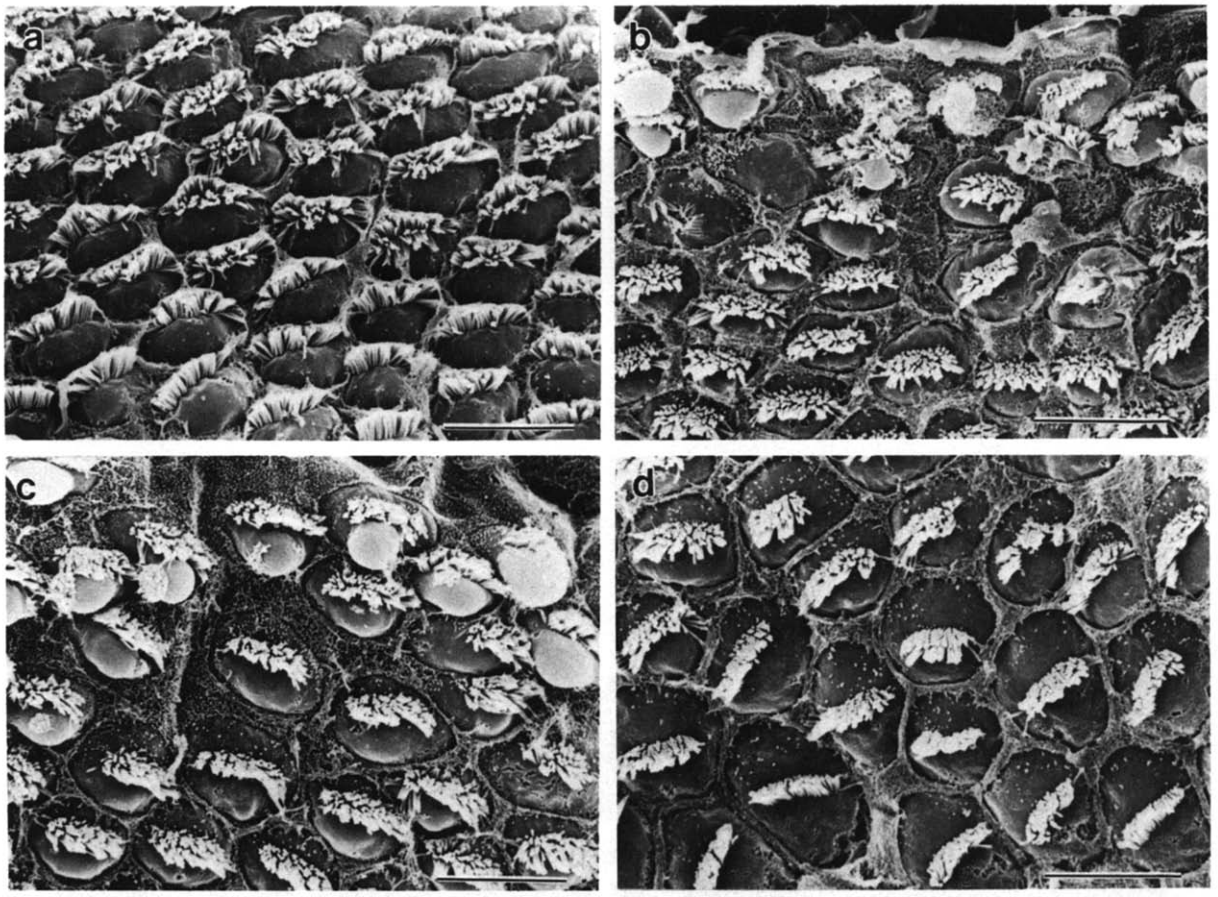

Fig. 4. Hair cell damage along the superior edge of the basilar papilla. (a) No recovery; (b) 24 h of recovery; (c) 48 h of recovery; (d) 6 days of recovery. Bars, 10 μm.

the regions of hair cell loss were much smaller than at 24 h of recovery (Fig. 2d). Most of the patch region was filled with the hair cells which had survived the acoustic trauma but had suffered varying amounts of damage to their stereociliary bundles.

Even though the areas which showed hair cell loss were much smaller than at earlier recovery times, they were still covered by the expanded surfaces of the supporting cells. However, there were several small, condensed groups of microvilli sparsely distributed among the supporting cells (Figs. 3d, 5a). These groups of microvilli were distinct from the microvilli of the supporting cells and expressed an organization which was reminiscent of the earliest stages of stereocilia differentiation on hair cells in the embryonic basilar papilla (Cotanche and Sulik, 1984). These presumptive stereociliary bundles were found only in the regions where the hair cells had been lost from the epithelium at 0 and 24 h of recovery. Examination of the epithelium with TEM showed that the presumptive stereocilia were projecting from the apical surface of small, darkly-staining cells which resembled immature hair cells (Fig. 5b).

After 48 h of recovery, the hair cells along the superior edge of the basilar papilla did not show any increase in damage or cell loss from that at 24 h of recovery (Fig. 4c).

4 days of recovery

The mosaic pattern of hair cells in the patch region after 4 days of recovery was similar to that

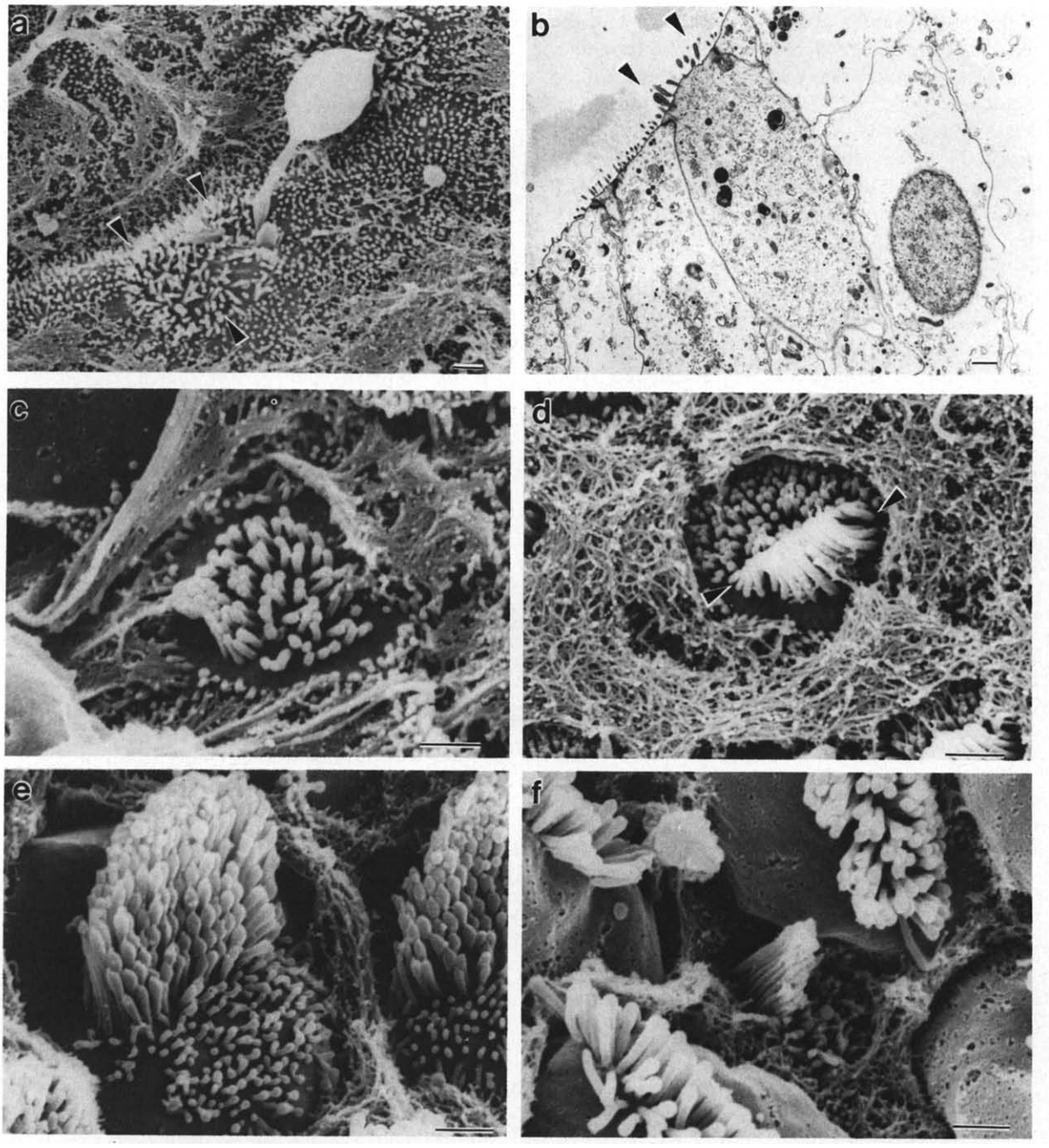

Fig. 5. Regenerating stereociliary bundles in the recovering cochlea. (a) Newly-forming bundle at 48 h of recovery (arrowheads); (b) TEM of newly-forming hair cell at 48 h of recovery – arrowheads, stereocilia; (c) regenerating bundle after 4 days of recovery; (d) new stereociliary bundle at 6 days of recovery – arrowheads, tallest row of stereocilia; (e) stereociliary bundle after 10 days of recovery; (f) newly-regenerating hair cell in the strip region of damage at 10 days of recovery. Bars, 1 μm.

of the basilar papilla after 48 h of recovery. In the regions of the patch where hair cells had been lost, numerous immature stereociliary bundles could be identified (Fig. 5c). The bundles were located in the same region of the patch as the differentiating stereocilia which were identified at 48 h of recovery. After 4 days of recovery, the newly-forming bundles were circular and separated from the microvilli of the supporting cells by a thin ring of free cell surface. The immature stereocilia were taller and thicker than the supporting cell microvilli but had a uniform height throughout the bundle. Thus, the stereociliary bundles which were first identified at 48 h of recovery appeared to have continued developing to a slightly more mature state by 4 days of recovery.

6 days of recovery

After 6 days of recovery, the mosaic pattern of the hair cells in the inferior half of the patch region appeared fairly similar to that of the control basilar papillae (Fig. 2a, e). However, along the superior border of the patch region there was a prominent band of small, developing hair cells. In addition, there were a few spots of developing hair cells dispersed throughout the inferior half of the patch. The developing hair cells exhibited the onset in the growth of the staircase pattern of stereocilia heights, with the first few rows being much taller than the rest of the bundle (Fig. 5d). Moreover, there appeared to be a single kinocilium in the center of the tallest row, although this will have to be confirmed with transmission electron microscopic examinations. The surface areas of the newly-forming hair cells were much larger than at 48 h of recovery, yet they were still separated from one another by the broad apical surfaces of the supporting cells (Fig. 3e).

Even though the mosaic pattern of the hair cells in the inferior half of the patch region was re-established, the stereociliary bundles on these cells did not recover or regenerate. Many of the cells had damaged bundles or small, aberrant remnants of stereocilia. The hair cell closest to the region of the newly-forming cells had surface areas which were larger than those in the same region of a control basilar papilla (Figs. 3a, 6). Similarly, the hair cells close to the superior edge of the basilar papilla, superior to the region of developing cells, had larger than normal surface areas and aberrant stereociliary bundles (Fig. 4d).

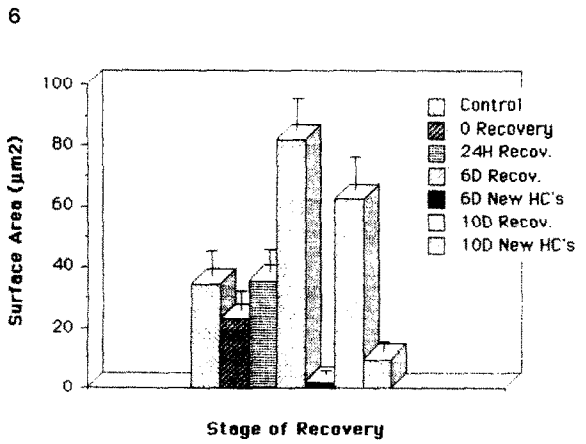

Fig. 6. Changes in hair cell surface area during recovery. This graph represents the changes in the surface area of the surviving hair cells and the newly generated hair cells in the damaged region of the basilar papilla. Error bars indicate the standard deviation for each group. Control, $n = 38$; 0 recovery, $n = 12$; 24 h recovery, $n = 12$; 6 d recovery, $n = 12$; 6 d new hair cells, $n = 10$; 10 d recovery, $n = 13$; 10 d new hair cells, $n = 18$.

10 days of recovery

After 10 days of recovery, the region of newly-developing hair cells occupied a wide, arched band across the superior half of the patch region (Fig. 2f). The new cells had increased in surface area compared to those at 6 days of recovery and were separated by thin rings of supporting cell surfaces (Fig. 3f), but were still smaller than hair cells at this position in control basilar papillae (Figs. 3a, 6). In contrast, the hair cells which were located superior and inferior to the newly-forming cells had surface areas which were larger than those of control cochleae (Fig. 6). These cells, which survived the acoustic trauma but had severely damaged stereocilia, have presumably had to increase their surface areas during the recovery period in order to gradually fill in for the large areas of hair cell loss along the superior border of the patch region. The stereociliary bundles on the surviving hair cells continued to exhibit evidence of damaged or lost stereocilia. Even after 10 days of recovery the tallest row of stereocilia on these cells was shorter than that of control cells or

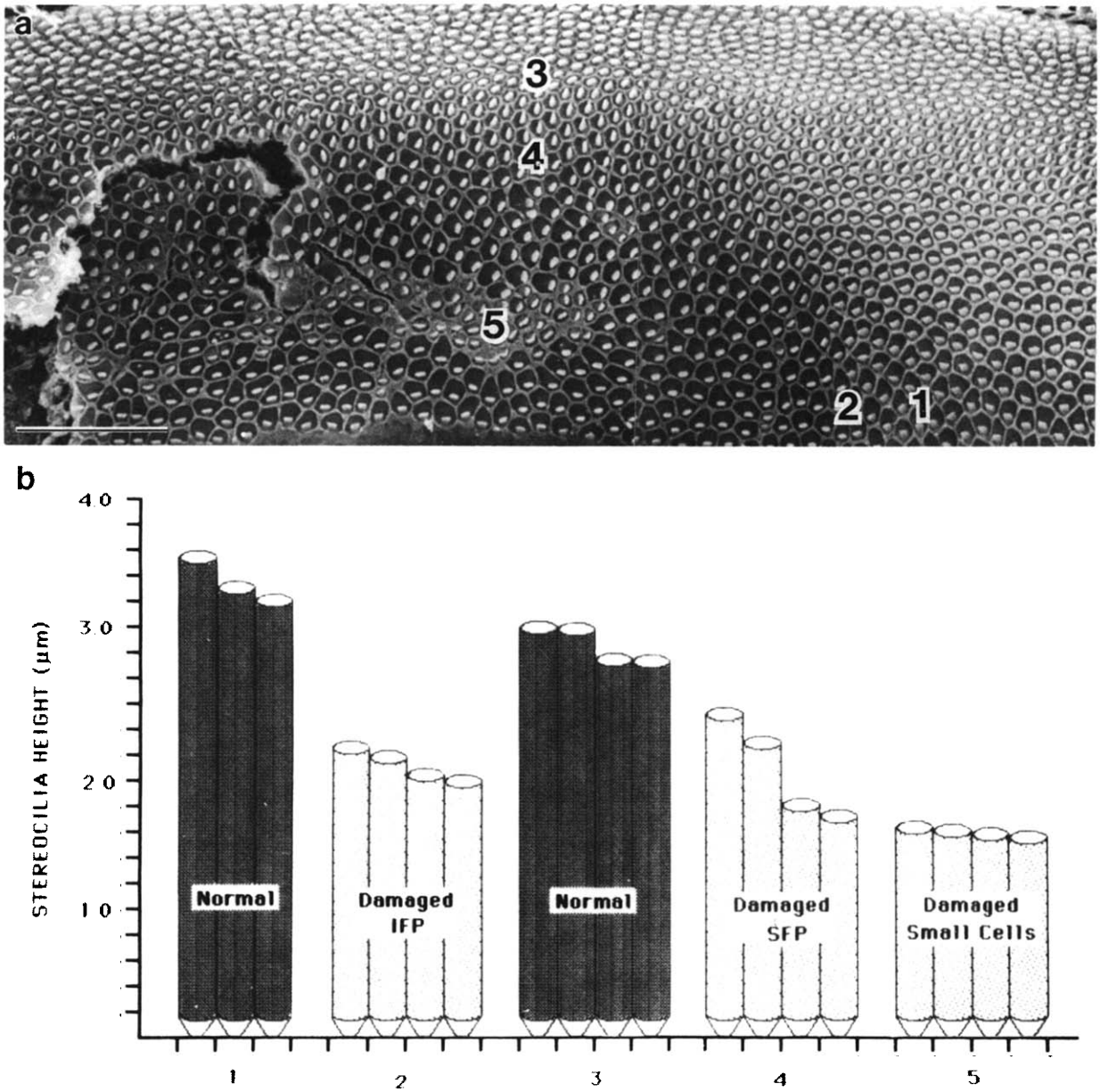

Fig. 7. Stereocilia damage in surviving cells. (a) A scanning electron micrograph of the damaged region of the basilar papilla after 10 days of recovery. Numbers on the micrograph correspond with the numbers in the graphs below. Bar, 100 μm. (b) Graphs of the heights of the tallest rows of stereocilia at various positions within and around the damaged region. Each bar in the graph represents the average height of the tallest row of stereocilia on an individual hair cell. Numbers below each group correspond to the same numbered locations in the above SEM micrograph. SFP, superior fibrocartilaginous plate; IFP, inferior fibrocartilaginous plate.

neighboring cells outside of the damaged area (Fig. 7), suggesting that the tallest row was lost as a consequence of the trauma and was not replaced during recovery.

The stereociliary bundles on the regenerating hair cells had matured considerably by 10 days of recovery. The stereociliary bundle no longer occupied the entire cell surface, instead it was

segregated into the inferior half of the cell surface (Fig. 5e). Numerous small stereocilia covered the superior half of the cell surface, but these were probably in the process of being resorbed, a process which has been described for normally developing hair cells (see Tilney et al., 1986). Moreover, the developing bundle already contained the appropriate number of stereocilia for the cell's position on the basilar papilla (~ 150/cell). The stereocilia in the regenerating bundle were organized into rows of gradually increasing height and several of them had prominent Pickles' filaments (Pickles et al., 1984; Furness and Hackney, 1985) which attached their tips to the lateral side of the next tallest row (Fig. 5e).

The strip region of damage, which had shown no signs of hair cell regeneration through the first 6 days of recovery, exhibited a few newly-regenerating stereociliary bundles after 10 days of recovery (Fig. 5f). The new bundles were very small compared to surrounding cells and only two or three were found throughout the whole strip region. The stereocilia in these bundles were not as developed as those in the patch region of the same ear and appeared to be closer to the stage of development of the bundles in the patch region after 6 days of recovery. The existence of the regenerating cells in the strip region indicates that an extensive region of hair cell loss such as that seen in the patch region is not required to stimulate the production of new bundles.

Discussion

Hair cell damage and the regeneration of stereocilia

Disruption of the normal mosaic pattern of hair cells in the basilar papilla following acoustic overstimulation occurred in a tonotopically defined patch-like region in which some hair cells exhibited injury to their stereociliary bundle while others were completely ejected from the epithelium. While most of the hair cell damage in the patch region was evident immediately after exposure, a small amount of additional hair cell loss did occur throughout the first 24 h of recovery. By 48 h of recovery, however, hair cell loss appeared to stop and from that point on the damaged region underwent a considerable amount of structural recovery which included the expansion of existing hair cell surfaces and the regeneration of stereociliary bundles in areas of hair cell loss.

It is interesting to note that hair cells along the superior edge of the basilar papilla, superior to the patch region, did not show any signs of damage immediately after exposure but did exhibit some evidence of stereocilia injury and hair cell loss during the first 24 h of recovery. The extent of this damage reached a peak by 48 h and it was never as extensive as that seen in the patch region. The hair cells along the superior edge have a primarily afferent innervation and are thought to be equivalent to mammalian inner hair cells (Tanaka and Smith, 1978). Their delayed response to acoustic trauma could indicate that they are more resistant to noise and take longer to be affected or that they are adversely affected by a loss of input from the cells on the inferior half of the epithelium, which are thought to be equivalent to the mammalian outer hair cells. At this time, however, the reason for the delayed appearance of damage in the cells along the superior edge of the basilar papilla is unclear.

In the extensively damaged patch region, the area of hair cell loss was localized predominantly along the arched superior border of the patch. The spaces left vacant by the departed hair cells were filled in by the expansion of the supporting cell surfaces during the early stages of recovery. It was in this area of the patch that the regenerating hair cells were first identifiable after 48 h of recovery. Between 48 h and 10 days of recovery, the newly-differentiated bundles went through the same sequence of developmental steps as normally developing hair cells in the embryonic cochlea (Tilney et al., 1986; Cotanche, 1987a). This included the selective growth in length of the rows of stereocilia needed to produce the staircase formation of the bundle and the reorganization of the bundle from a circular to a rectangular shape. Moreover, the stages of stereocilia development in the regenerating hair cells followed the same time course as those in the embryonic ear. After 10 days of recovery, the regenerated cells still had not reached a completely mature state, but they did contain a bundle with the correct number of stereocilia for their position on the basilar papilla (Tilney and Saunders, 1983).

Production of new hair cells

If stereociliary bundles are being regenerated during the recovery period, as our results indicate, then it needs to be determined whether they are being assembled on the tops of hair cell bodies which survived the trauma by discarding their former bundles or, instead, are forming on a new population of hair cells which have been generated in the sensory epithelium. Light and transmission electron microscopic analyses of the damaged region immediately after exposure have suggested that the whole hair cell and not just the apical portion of the cell is ejected from the epithelium. This was concluded because there were no cell nuclei or cells with darkly-staining cytoplasm in the upper part of the epithelium in the region of hair cell loss and because cellular debris which included nuclei was seen in the scala media above the damaged basilar papilla. It is possible that when the hair cells eject their apical portions they no longer stain as densely but this does not explain the absence of nuclei in the hair cell layer. Thus, our evidence suggests that new hair cells are being produced in the epithelium to replace those that were lost. If so, then these new cells must be arising from a population of stem cells in the basilar papilla which have retained the ability to produce new hair cells but have been dormant until stimulated by the acoustic trauma. The most obvious source of these stem cells would be the supporting cells which lie in the basal layer of the sensory epithelium and which expand their apical surfaces after the trauma to compensate for the lost hair cells. Evidence for the supporting cells continually dividing and functioning in a stem cell capacity has been shown in the macula neglecta of sharks (Corwin, 1981), the sacculus of toads (Corwin, 1985a) and the lateral line epithelia of amphibians (Corwin, 1985b).

If the supporting cells are acting as a source for the new hair cells during the recovery, then what is the stimulus that causes them to divide and produce new hair cells? It obviously requires the loss of hair cells from the epithelium, since no regeneration was seen in the areas where the hair cells suffered only injury to their stereocilia. However, it does not require the loss of a large number of hair cells since regeneration was seen in the strip region where only a few hair cells were lost across a long longitudinal distance. It is possible that the hair cells have some type of inhibitory effect on the adjacent supporting cells which prevents them from differentiating and when the hair cells are lost this inhibitory influence is also lost and the supporting cells can begin to produce new hair cells. However, this assumption would require that there be some other, more constant inhibitory factor in cells around the edges of the basilar papilla which would prevent continuous production of hair cells at the edges of the sensory epithelium. Indeed, a recent study on the development of tissue boundaries in the embryonic chick cochlear duct has shown that the binding specificities of primary cell adhesion molecules (CAMs) form distinct borders between the basilar papilla and the non-sensory epithelium surrounding it (Richardson et al., 1987).

When chick cochleae were exposed to acoustic trauma at higher intensities (125 dB SPL) the hair cells were lost from the entire patch region and the epithelium in the center of the patch collapsed down to a single thin layer of cells on the surface of the basilar membrane. In this instance, regeneration of stereociliary bundles was not seen or was present only at the edges of the patch region. Thus, it appears that some portion of the sensory epithelium must remain intact to generate new cells, again suggesting that the supporting cells play a role in this process.

If a stem cell population is responsible for the production of new hair cells in the chick basilar papilla, then it must be stimulated into dividing and differentiating into hair cells during the early stages of recovery, since the first evidence of regenerating stereocilia can be seen 48 h after the exposure. This suggests that there must be a period of rapid cell division early in the recovery process. We are currently examining whether these dividing cells and the newly-generated hair cells can be labeled by exposure to radioactive precursors of DNA synthesis.

Regeneration and functional recovery

Our results indicate that the basilar papilla of the chick is capable of regenerating hair cells after acoustic trauma but this does not necessarily mean that there is an associated recovery of auditory function for this region of the sensory epithelium.

However, functional recovery following severe noise exposure (third-octave-band at 111 dB SPL for 48 h) has been demonstrated in the chick cochlea (Saunders and Tilney, 1982; Saunders and Coppa, 1986). The initial threshold shifts in these experiments were on the order of 50 dB and were associated with a 35% loss in the number of hair cells. After a 10 day recovery period, the thresholds had returned to normal but the basilar papilla still showed a disruption of the normal mosaic pattern of the hair cells similar to that described in our studies. On the other hand, studies by Cousillas and Rebillard (1985) showed no recovery of threshold shifts after a 10 day recovery from a 125 dB SPL pure tone exposure, but SEM examination of the basilar papilla showed a complete collapse of the sensory epithelium in the damaged region. Thus, it appears that regeneration of hair cells can lead to a functional recovery as long as the initial damage is not severe enough to produce a complete loss of the sensory epithelium in the traumatized region.

One aspect of the recovery process that we have not yet examined in our studies is the re-establishment of synaptic connections between the cochlear nerve fibers and the newly-differentiating hair cells. If hair cells are completely lost from the epithelium immediately after acoustic trauma then the afferent and efferent nerve terminals associated with the lost cells must be snapped off and extruded into the scala media along with the basal regions of the hair cells or they must remain in the basilar papilla but without synaptic connections. When the new hair cells are generated to replace the lost cells do they make connections with existing nerve terminals in the epithelium or do they establish synapses with new fibers? These questions will be addressed in further studies of the regeneration process.

The implication that the chick basilar papilla retains the ability to regenerate hair cells after a fairly intense exposure to acoustic trauma suggests that the sensory epithelium contains a population of stem cells which can produce new hair cells to replace those that have been lost. If the signal which stimulates the stem cell population to undergo proliferation and differentiation could be identified then it could be possible to explore the nuclear mechanisms in the cell which control the production of a new sensory cell. Once these mechanisms have been determined then it may be possible to reproduce these signals in the mammalian cochlea and induce the production of new hair cells to replace those that have been lost because of noise damage or ototoxic poisoning.

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