Journal of Neurocytology **26**, 121–131 (1997)

Presynaptic terminals in hyaline cells of normal and overstimulated chick inner ears

JUAN C. FRISANCHO, LYNNELL FRITSMA and YEHOASH RAPHAEL*

Kresge Hearing Research Institute, The University of Michigan Medical School MSRB III, Room 9303, 1150 West Medical Center Drive, Ann Arbor, MI 48109-0648, USA

Received 4 September 1996; revised 22 November 1996; accepted 2 December 1996

Summary

Hyaline cells are non-sensory epithelial cells of the vibrating part of the basilar membrane of chicks; they receive an extensive efferent innervation. Although these anatomical features suggest roles in auditory transduction, very little is known about the function of these cells. One possible way to understand function is by lesion experiments. We used synapsin-specific antibodies to study changes that occur in the pattern of efferent innervation in hyaline cells after lesion of the sensory epithelium induced by acoustic overstimulation. We found only small changes in hyaline cells after such trauma. These included a small increase in size and a small decrease in density of nerve terminals on hyaline cells. This suggests that hyaline cells and their nerve terminals are less susceptible to acoustic trauma than hair cells. Using neurofilament-specific antibodies we found little or no trauma-induced change in the density of nerve fibres that cross the basilar papilla and reach the hyaline cell region. This finding suggested that trauma to the hair cells does not necessarily lead to changes in the efferent fibres that cross the papilla and extend into the hyaline cell region. Using the trauma and the morphological parameters studied here, it appears that a moderate lesion in the hair cell region in the avian inner ear does not influence the hyaline cells or their innervation.

Introduction

The primary auditory mechano-transducing cells are sensory epithelial cells that activate auditory nerve fibres. These sensory epithelial cells are the inner hair cells in mammals and the tall hair cells in birds (see Tanaka & Smith, 1978, for birds; Slepecky, 1996, for mammals). Inner hair cells and tall hair cells, despite being the primary sensory cells, do not actually rest on the vibrating part of the basilar membrane. Rather, other types of cells reside on the part of the basement membrane that is free to vibrate in the fluid of the inner ear. In mammals these cells include outer hair cells and several types of supporting cells (see Kimura, 1984, for review). In birds the vibrating part of the basement membrane is lined by short hair cells, supporting cells which surround the short hair cells, and several cell types residing adjacent to the inferior (abneural) edge of the short hair cell region (Held, 1926; Takasaka & Smith, 1971). The two cell types that reside inferior to the basilar papilla are the border cells and hyaline cells. In both mammals and birds, the static and dynamic mechanical features of the vibrating portion of the basilar membrane depend to a large extent on the cellular and extracellular components of this tissue. These mechanical features are likely to contribute significantly to the quality and quantity of transduction in the primary auditory sensory cells.

In addition to the passive mechanics of the mass which consists of the basilar membrane and the cells that vibrate along with it, an active component in cochlear mechanics has been demonstrated in mammals (Davis, 1983; Kemp, 1986). Motility of outer hair cells, otoacoustic emissions, and non-linearity in cochlear responses are among the manifestations related to the active component which are believed to be under cholinergic efferent control in the mammalian cochlea (Dallos, 1992; Ashmore & Kolston, 1994). The active functions of the auditory periphery are generally less pronounced in birds than in mammals (Klinke & Smolders, 1993; Froymovich *et al*., 1995). Nevertheless, an extensive array of efferent innervation is found throughout the length (proximal to distal) of the avian cochlea (Held, 1926; Takasaka & Smith, 1971; Hirokawa, 1978a,b; Ofsie & Cotanche, 1996). These nerve fibres extend from the basilar papilla and reach

*To whom correspondence should be addressed.

0300–4864/97

further into the layer of hyaline cells (Held, 1926; Takasaka & Smith, 1971; Ofsie & Cotanche, 1996).

Hyaline cells were originally named by Held (1926) because of their transparent glass-like appearance in his histological sections of the avian inner ear. Held demonstrated neurons extending beyond the inferior edge of the basilar papilla, traversing through border cells (which border the edge of the basilar papilla) and entering into the hyaline cell region (Held, 1926). These neurons were later shown to be part of the efferent bundle (Takasaka & Smith, 1971; Keppler *et al*., 1994). Using EM, these efferent nerve fibres were shown to form synapses on the lateral membrane of hyaline cells (Odinokova & Prokof'eva, 1975). The network of synaptic contacts between hyaline cells and efferent neurons, and the ultrastructure of the hyaline cell region in the avian inner ear were extensively described by Oesterle and colleagues (1992). The role of these neurons is especially interesting considering that hyaline cells do not appear to be auditory mechano-electro transducing cells.

Another specialized structure in hyaline cells is an extensive array of actin bundles, anchored into the basal pole of the cells (Cotanche *et al*., 1992, 1995). It is thought that the degree of contractility of these bundles may actively influence the mechanical features of the basilar membrane in birds (Cotanche *et al*., 1992; Keppler *et al*., 1994). Actin and several other proteins that are related to force generation have also been found in hyaline cells of the caiman auditory organ along with nerve terminal endings (Düring *et al*., 1974; Drenckhahn *et al*., 1991). These findings led Drenckhahn and colleagues (1991) to suggest that the activity of hyaline cells may be controlled by neural input.

The role of hyaline cells and their innervation in the process of auditory transduction is not presently clear. Since these cells are located on the vibrating part of the basilar membrane, mass and stiffness changes in hyaline cells may influence the mechanical characteristics of the basilar papilla, thereby modulating auditory transduction in tall hair cells. One way to determine the role of hyaline cells may be to characterize changes that occur in these cells after trauma. Recent observations of the responses of hyaline cells to severe acoustic trauma have suggested that (1) hyaline cells migrate into the basilar papilla and (2) once situated in the papilla these cells may inhibit hair cell regeneration (Cotanche *et al*., 1995).

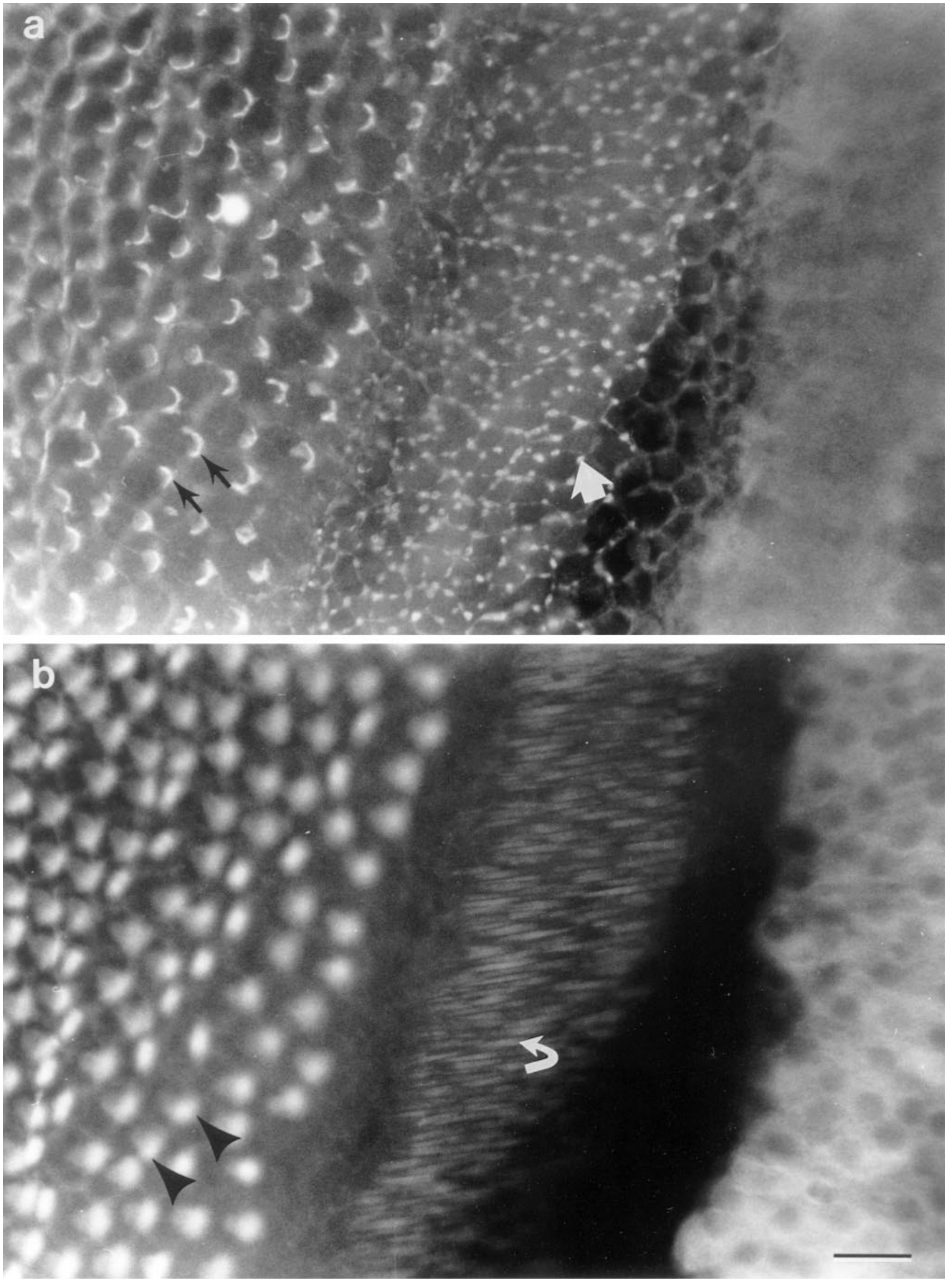
The organ of hearing and the auditory function in birds can be restored after acoustic trauma. The extent of lesion to the hyaline cells or their innervation, and its relationship with the threshold of hearing after trauma are not known. The aim of this study was to determine the normal distribution of efferent terminals in the hyaline cell region and to characterize the effects of acoustic trauma on the size and number of presynaptic terminals in this region. To determine the location, size and number of presynaptic terminals in the hyaline cells region we used antibodies to synapsin I.

Synapsins are a family of proteins associated with the membrane of synaptic vesicles (Ueda *et al*., 1977; Goldenring *et al*., 1986; Greengard *et al*., 1993, 1994). These proteins participate in regulation of vesicle traffic and neurotransmitter release (Llinas *et al*., 1991; Benfenati *et al*., 1992; Rosahl *et al*., 1993; Greengard *et al*., 1994). Synapsin-specific immunocytochemistry is a valuable tool for studying innervation dynamics after acoustic trauma in the avian inner ear (Wang & Raphael, 1996; Zidanic *et al*., 1996). Since synaptic vesicles are distributed throughout the efferent nerve terminals in the basilar papilla (Takasaka & Smith, 1971; Hirokawa, 1978a; Oesterle *et al*., 1992) labelling of the vesicle membranes is likely to be spread throughout each terminal. Such distribution would provide a measurable image of size and shape of efferent terminals and has been used here to determine the changes in hyaline cell innervation following acoustic overstimulation.

Materials and methods

White Leghorn chicks (1–3 weeks of age) were exposed to a 116 dB SPL octave-band noise (1.5 kHz centre frequency) for 8–14 h. Thirty-five chicks were killed on post-exposure day 0–4 ($n = 4$ on each day), or 5–9 ($n = 3$ on each day). Three birds of a similar age served as unexposed controls. The chicks were decapitated and the skull was sagitally cut. The cerebrum, cerebellum, and brainstem were removed, and both middle and inner ears excised from the skull. The middle ear was then opened, and the columella was removed. The inner ears were subsequently placed in 4% paraformaldehyde for approximately 30 min. Under stereomicroscopic visualization the bone was partially dissected free from the basilar papilla and again placed in 4% paraformaldehyde for 30 additional min. Finally, the basilar papilla

Fig. 1. A whole mount of control basilar papilla double-labelled with antibodies to synapsin (a) and phalloidin (b) and photographed with epifluorescence illumination. (a) Fluorescence signal is at focal plane near basal part of short hair cells (black arrows) and in hyaline cell region (white arrow). Each short hair cell is associated with 2–3 boutons connected and positioned abneurally. A large number of small labelled boutons are evenly distributed throughout hyaline cell region. (b) Phalloidin label helps identify and localize short hair cells (arrow heads point at same two cells marked by arrows in (a)) and demonstrates that synapsin-specific immuno-label is associated with each hair cell. Bundles of actin labelled with phalloidin traverse the hyaline cell region in a neural to abneural orientation, parallel to the basilar membrane (curved arrow). Scale bar = 20 μm.


a
b

was dissected free from bone, tegmentum vasculosum, and nerve bundle.

For immunocytochemistry, each ear was washed in PBS three times and then incubated in 10% normal goat serum in 0.15% Triton X-100 for 30 min to reduce background. For synapsin immunocytochemistry the basilar papillae were then incubated in rabbit anti-synapsin antibody (G-304, a gift from Dr Andrew J. Czernik, The Rockfeller University, NY) diluted 1:500 in PBS, for 2 h at room temperature. This affinity-purified antibody recognizes type Ia and IIa isoforms of synapsin (Greengard *et al*., 1993). The tissue was rinsed in PBS, then incubated in a mixture of rhodamine conjugated goat anti-rabbit secondary antibody (1:50 dilution, Jackson ImmunoResearch Laboratories, Inc. Pennsylvania) and 1:100 FITC-phalloidin (Molecular Probes, OR) for 30 min. For neurofilament immunocytochemistry, tissues were permeabilized in Triton X-100, then incubated for 90 min in the anti-neurofilament antibody (a mixture of 160 kDa- and 200 kDa-specific monoclonal antibodies from Boeringer Mannheim, Indianapolis, IN) diluted each to a final concentration of 1:15 in PBS. Secondary antibodies were goat-anti mouse rhodamine (Jackson, 1:100 dilution) mixed with FITC-phalloidin.

After a final rinse in PBS, the tissues were further dissected by clipping off the proximal end of the auditory organs ($\sim$ 20% distance from the basal end), and then mounted on microscope slides. As a result, the data reported in this work cover the distal 80% of the chick cochlea. Tissues were mounted in 60% glycerol in sodium carbonate buffer (pH 8.5) with p-phenylenediamine as an anti-bleach agent and then examined with a Leitz Orthoplan microscope equipped for epifluorescence. Preparations were photographed at 50 $\times$ and 100 $\times$ magnifications on Kodak T-max 400 film exposed at 1600 ASA.

The number of synapsin-specific boutons in the region adjacent to the lesion were counted directly from the image while viewing it through the microscope. The counted region was centred approximately 35% distance from the proximal end of the papilla (Fig. 2). The raw data was averaged between the ears of every experimental group and the average figure was used to determine if statistically significant changes occurred. For this purpose, we used the Sigma Stat software to calculate Student's *t* (unpaired, two-tailed) values, comparing each of the post-trauma groups with control values.

Results

In control animals, synapsin-specific immunolabelling was found at a focal plane corresponding to the subnuclear area of short hair cells and in the hyaline cell region (Fig. 1). Each short hair cell was associated with

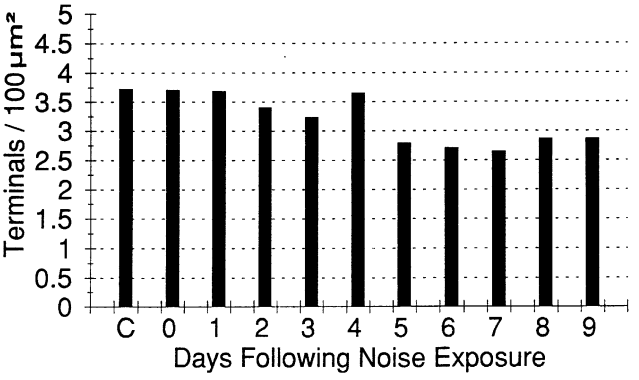
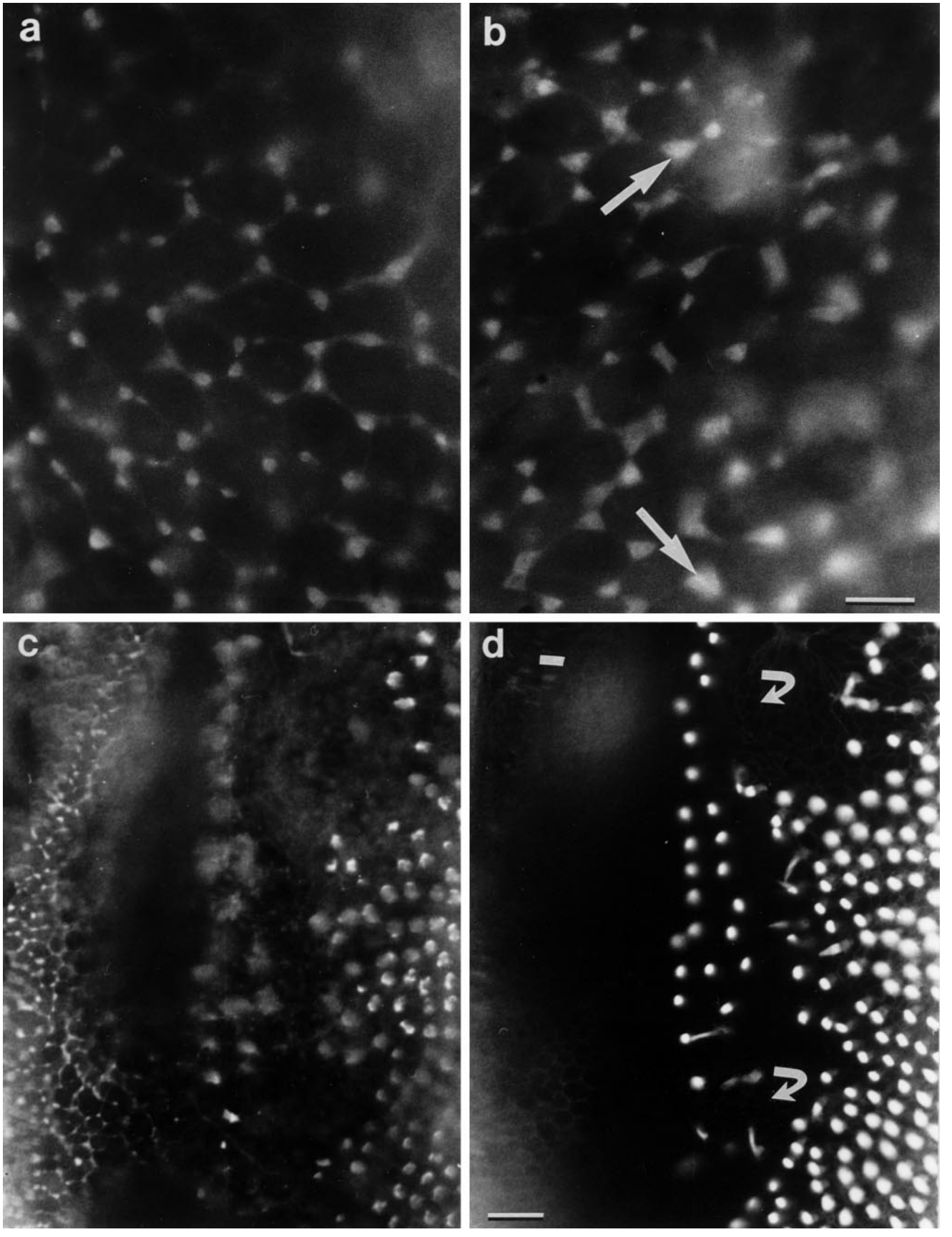


Fig. 2. A histogram presenting the density of synapsin-labelled boutons in hyaline cell area located 35% distance from the proximal end of the papilla, in controls and at days 0–9 after noise exposure. Three ears were used for each time group. When compared to controls, the differences are not statistically significant in data from days 1–4 and day 6. On days 5, 7 and 9 after trauma, the number of terminals was significantly lower than control.

2–3 boutons that were connected together and positioned around the abneural side of the tall hair cells. Synapsin-specific immunoreactivity was also detected in the hyaline cell region. The localization and shape of the staining resembled that of efferent nerve terminals described previously in both short hair cell and the hyaline cell regions using TEM (Jahnke *et al*., 1969; Takasaka & Smith, 1971; Whitehead & Morest, 1985; Oesterle *et al*., 1992). This strongly suggested that the staining we observed corresponded specifically to efferent presynaptic terminals on short hair cells.

Three to six bouton-shaped areas with distinctive synapsin-specific immunoreactivity surrounded each hyaline cell (Fig. 1). Synapsin-specific boutons were found in the hyaline cells throughout the length of the basilar papilla. Phalloidin label of the same preparation (Fig. 1b) helped to identify the location of the synapsin labelling shown in Fig. 1a. Using phalloidin stain it was possible to observe the circumferential ring associated with the adherens junction complex surrounding the apical surface of each hyaline cell (as shown in Fig. 3d). The shape of hyaline cells appeared pentagonal or hexagonal and the diameter of the approximated circle was $\sim$6 µm (not shown). Phalloidin label was also instrumental in demonstrating the actin bundles which traverse the basal portion of

Fig. 3. Epifluorescence micrographs of papillae labelled for synapsin (a and b) and a basilar papilla double labelled for synapsin and actin (c and d, respectively). Boutons labelled with synapsin-specific antibodies are mostly similar in size in controls and exposed animals, although some larger terminals were noted in exposed papillae (arrows in b, 4 days after noise exposure; compare to non-exposed control in a). (c, d) Synapsin-specific labelling shows normal size, shape and distribution of terminals in hyaline cells adjacent to the lesion (c, hyaline cell terminals are seen as crescent along the left side of the micrograph). In contrast, trauma caused lesion in region of hair cells (arrows in d). Scale bars = 5 µm in (b) for a and b and 25 µm in (d) for c and d.


a
b
c
d

.

hyaline cells, as described previously (Cotanche *et al*., 1992, 1995).

A lesion was observed in the basilar papilla immediately after the noise exposure. Among the pathological signs in the lesion were expanded supporting cells, and injured and missing hair cells. The location and the extent of the lesion were similar to those previously described with similar regimens of acoustic overstimulation (Raphael, 1993; Raphael *et al*., 1994; Wang & Raphael, 1996) and therefore not presented here. The qualitative pathological changes were similar to those described previously after acoustic overstimulation (Cotanche, 1987; Cotanche & Dopyera, 1990; Marsh *et al*., 1990; Raphael, 1992, 1993), namely, extensive hair cell loss in the centre of the lesion, numerous damaged hair cells and expanded supporting cells.

Quantification of synapsin-specific boutons in the region adjacent to the lesion ($\sim$ 35% distance from the proximal end of the papilla) is presented in (Fig. 2). In normal animals there were approximately 3.72 synaptic terminals 100 μm^{-2} in this area. Quantification of terminals in the hyaline cell region 2 days after noise exposure revealed a density of 3.41 terminals 100 μm^{-2}, similar to that in normal tissue. The density of terminals 4 days after the noise exposure was calculated as 3.65 100 μm^{-2} (Fig. 2). The density of nerve terminals was also calculated for the other post-exposure period (Fig. 2). Statistical analysis revealed no significant change in the density of presynaptic terminals in the hyaline cell region during the first four days after the noise exposure. However, significant differences were found on day 5 ($t=6.67$ with 6 degrees of freedom, $p=0.0005$), day 7 ($t=5.50$ with 4 degrees of freedom, $p=0.0053$) and day 9 ($t=4.11$ with 7 degrees of freedom, $p=0.0045$).

The size of nerve terminals associated with hyaline cells did not seem to be influenced by trauma in most cases. Nevertheless, in some cases several presynaptic terminals in the hyaline cell region were slightly larger than those of the control (Fig. 3a,b). The noise exposure used in this study did not lead to major changes in the shape and size of hyaline cells or the region which they occupy (Fig. 3c,d).

The distribution of neurofilaments traversing the hyaline cell area and the organization of actin filaments at the base of these cells were also studied. In the normal chick inner ear nerve fibres are seen to extend from the abneural (inferior) basilar papilla into the hyaline cell region (Fig. 4). The number, organization, and label intensity for neurofilaments did not perceptibly change in traumatized inner ears (Fig. 5A). Phalloidin label of the region at the basal aspect of hyaline cells revealed an extensive array of actin bundles, as previously described (Cotanche *et al*., 1992, 1995). The orientation and orderly organization of these filaments were not changed by the noise exposure we have induced (Fig. 5B). The density of neurons crossing the basilar papilla into the hyaline cell region did not appear to differ between traumatized and control tissues (data not shown).

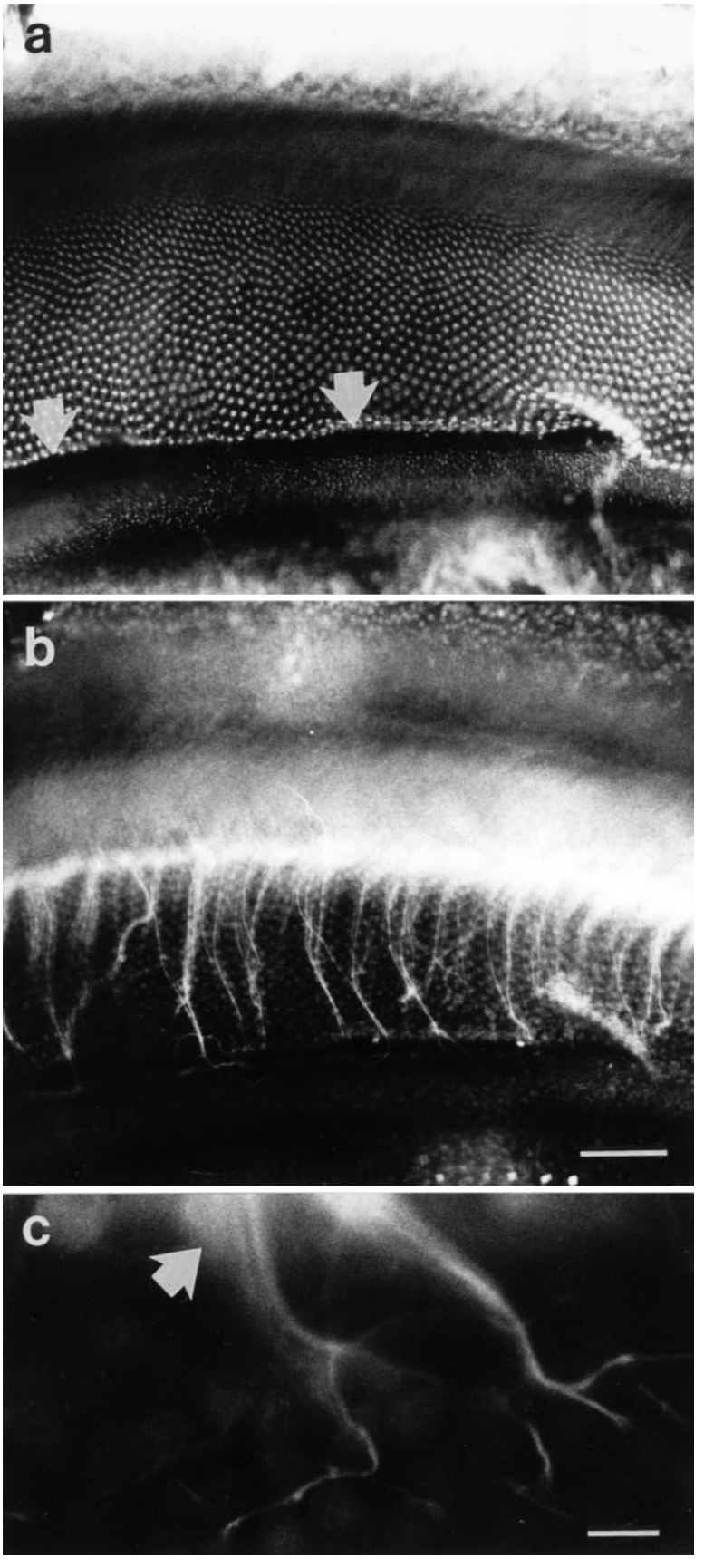
Discussion

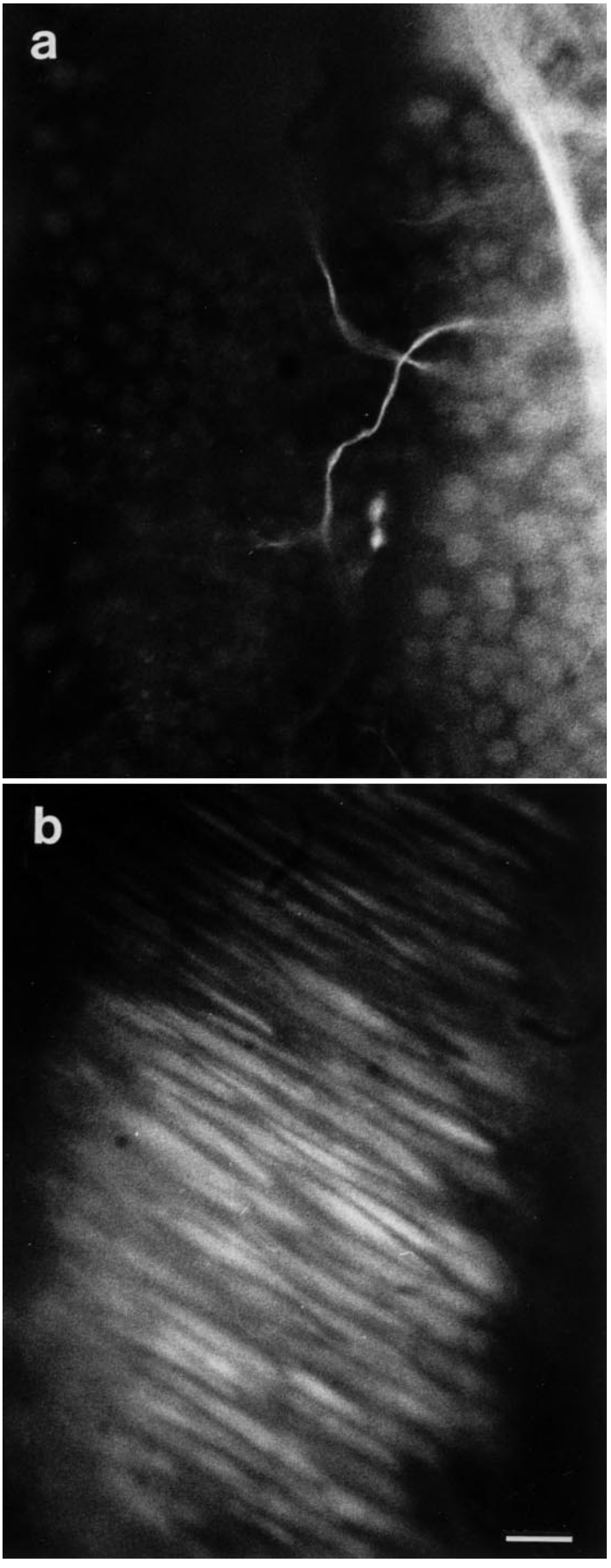
Our data demonstrate that a moderate acoustic overstimulation leading to trauma in the basilar papilla has only limited morphological impact on the nerve terminals that associate with hyaline cells. We found no correlation between the time course of changes in the nerve terminals and the time course of functional recovery known to occur during the first four days following acoustic trauma (Niemiec *et al*., 1994). The absence of correlation indicates that structures other than hyaline cell terminals are responsible for the rapid functional recovery reported during the first 2–4 days after noise exposure in birds (McFadden & Saunders, 1989; Adler *et al*., 1992; Niemiec *et al*., 1994).

Synapsin antibodies as a research tool

Synapsin-specific antibodies are a relatively new tool for studying the inner ear, so it is necessary to verify that labelling in the basilar papilla is specific for synapsin. The presence of synapsin proteins in chick tissues was demonstrated biochemically by Zidanic & Fuchs (1996). They studied several synapsin antibodies raised in rabbits against mammalian synapsins and demonstrated that these antibodies cross react with chick synapsin. Moreover, we have found that synapsin antibodies also label quail tissues (Raphael & Moody, unpublished observations). The labelling we observed in the chick ear was restricted to the region of nerve terminals, mostly beneath short hair cells and hyaline cells. Furthermore, the pattern of labeling was identical to that described in reconstructed images of chick inner ear neurons (Fischer, 1992;

Fig. 4. Whole-mounts of a normal basilar papilla double-labelled for actin and neurofilaments (a and b) and neurofilaments at higher magnification (c). (a, b) The abneural edge of the papilla is clearly marked by the last line of hair cells labelled in the stereocilia (arrows in a). Nerve fibres traverse the papillae in the direction of the abneural edge, but fibres extending from the papilla to the hyaline cell region are small and cannot be easily distinguished at this magnification (b). (c) With higher magnification it is possible to observe fibres extending into the hyaline cell region (arrow shows point of entry to the hyaline cell area). Scale bars = 50 μm in (b) for a and b and 10 μm in (c).


a
b
c


a
b

Keppler *et al*., 1994) and to that observed with histochemical stains (Takasaka & Smith, 1971). These observations lend strong support to the specificity of the staining to synapsin. Since synaptic vesicles were shown to be distributed uniformly throughout the cross section of presynaptic terminals in the basilar papilla and the hyaline cell region, there is high likelihood that the area of each bouton labelled with synapsin-specific antibodies truly represents the entire area of each terminal.

We also consider the extent to which changes in immunolabelled area reflect changes in the actual size of the terminal. If acoustic trauma were to result in redistribution of synaptic vesicles or a significant decrease in their number, terminal size would have decreased. In such a case, it would have been difficult to determine whether changes were in synaptic vesicles (number and molecular organization) or terminal size. However, our results show a slight increase (rather than decrease) in the labelled area, most likely reflecting growth (expansion) of the terminal size. Although more work is necessary on the distribution of synapsin proteins in the normal and traumatized auditory epithelium in the basilar papilla, we can conclude that antibodies to synapsins are a valuable tool for localizing and measuring size of presynaptic neural terminals in this organ.

Structure/function correlation

The correlation between the anatomical recovery from trauma in the basilar papilla and the recovery of auditory function is not a simple one. Regenerated hair cells can first be identified as early as 3–4 days following acoustic overstimulation (Cotanche, 1987; Girod *et al*., 1989; Raphael, 1992, 1993; Stone & Cotanche, 1992). A substantial recovery of hearing threshold has been found as early as 2 days after acoustic trauma in chick and quail (McFadden & Saunders, 1989; Niemiec *et al*., 1994). The rapid recovery of thresholds occurring prior to the appearance of new hair cells in these previous studies suggests that the recovery is not dependent on the regeneration of new hair cells. Rather, it may result from repair of tall hair cells or their afferent terminals, repair of the damaged tectorial membrane (McFadden & Saunders, 1989; Adler *et al*., 1992; Saunders *et al*., 1992), or repair of other components of the avian inner ear such as the endocochlear potential (Poje *et al*., 1995) and the tegmentum vasculosum (Ryals *et al*., 1995). It should be emphasized that in response to a severe noise exposure hyaline cells in the chick basilar papilla are among the earliest cells to proliferate (Girod *et al*., 1989) and migrate (Cotanche *et al*., 1995). Nevertheless, the results we now report suggest that changes in hyaline cells during the first 4 days following a moderate acoustic trauma are minor and do not play a dominant role in threshold shift and recovery.

We found that the density of nerve terminals in the hyaline cell area does not significantly change during the first 4 days following a moderate acoustic trauma. Moreover, the size change of the presynaptic terminals on hyaline cells following acoustic overstimulation appeared subtle. The data suggest that hyaline cells are more resistant to noise overstimulation than hair cells. Additional quantitative studies are needed to determine the relationship between the severity of trauma and the size changes in hyaline cells. Based on the present morphometric data alone we could not correlate the structural changes in hyaline cells with trauma-induced functional changes known to occur in chicks during the first few days after acoustic overstimulation. The small (but nevertheless significant) decrease in the number of nerve terminals on the fifth post trauma day (and later, on days 7 and 9) is also difficult to interpret as thresholds have been shown to further improve during these stages of the recovery from trauma (Niemiec *et al*., 1994).

The function of hyaline cells

The avian efferent nerve system has been described in detail and was initially thought to come in close proximity to but not innervate hyaline cells (Takasaka & Smith, 1971). It was later established that hyaline cells are indeed innervated by efferent nerves (Odinokova & Prokof'eva 1975; Oesterle *et al*., 1992; Keppler et al., 1994). Moreover, hyaline cells in the caiman (*Caiman crocodilus*) also receive innervation from cochlear efferents (von During *et al*., 1974). Cytoskeletal fibres with force generating capacity were found in the caiman hyaline cells (Drenckhahn *et al*., 1991) and in the hyaline cells of the chick basilar papilla (Cotanche *et al*., 1992, 1995). Activation of the efferent neurons may induce contractile activity in hyaline cells, which would translate into modulation of the tensile properties of the basilar papilla cells (Drenckhahn *et al*., 1991; Cotanche *et al*., 1992). Considering that hyaline cells are located on the vibrating

Fig. 5. Whole mounts of a noise exposed basilar papillae labelled for neurofilaments (a) and actin (b). (a) Two days after noise exposure, neurons labelled with neurofilament-specific antibodies extend from the lesion area in the basilar papilla (on the right hand side of the micrograph) to the hyaline cell region. (b) Phalloidin label in hyaline cells 2 days after noise exposure reveals actin bundles oriented in the neural to abneural axis. The focal plane is slightly above the basilar membrane. Scale bar = 10 μm.

part of the basilar membrane, it is very likely that changes in size or in mechanical properties in this layer of cells would directly influence the physical properties of the transduction apparatus.

Our results demonstrate that acoustic overstimulation sufficient to injure and kill hair cells does not result in major changes to the shape of hyaline cells and their efferent innervation. It therefore appears that we do not yet have a regimen of trauma that will specifically injure hyaline cells. Rather, it appears that a moderate lesion to the basilar papilla, as the one used in this study, causes only minor changes in hyaline cells, whereas a severe lesion in the papilla results in migration of hyaline cells into the region of the papilla that is denuded of hair cells (Cotanche *et al*., 1995). Developing a specific hyaline cell lesion should facilitate identifying their contribution to inner ear function. When a selective way to injure or eliminate hyaline cells is found, it will also be possible to study the fate of neurons that extend into the hyaline cell area.

Our finding that nerve terminals remain in the hyaline cell area after trauma in the basilar papilla corroborate the results obtained with the anti-neurofilament antibodies. Specifically, efferent nerve fibres that cross the basilar papilla and exit it on the abneural side to provide innervation to the hyaline cells, do not change their number and location even if they travel through a lesion in the basilar papilla. These findings confirm another recent report (Ofsie & Cotanche, 1996) which demonstrated intact efferent fibres in regions of lesion in the basilar papilla and the hyaline cell area. Together, these data suggest that efferent neurons do not regress from the basilar papilla after trauma and that their innervation of hyaline cells is maintained independently from that of the hair cells, at least in the short term.

In summary, the experiments we describe demonstrate that synapsin-specific antibodies are a valuable tool for studying the pattern of efferent innervation in the basilar papilla and the hyaline cell region. The data demonstrate only small changes in density and size of efferent nerve terminals in the hyaline cell region. The results therefore suggest that hyaline cells are less susceptible to acoustic trauma than short hair cells and that changes in hyaline cells are unlikely to account for the rapid initial recovery of thresholds after noise exposure. To better understand the role of hyaline cells it will be necessary to develop a way to induce a selective lesion restricted to these cells.

Acknowledgments

We would like to thank Henry Adler, Doug Cotanche, Jackie Kaufman and Donna Martin for their constructive comments on our study, and Dr Andrew J. Czernik (The Rockefeller University, NY) for providing the synapsin antibody. This work was supported by research grant 5 R01 DC01634-03 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health.

References

ADLER, H. J., KENEALY, J. F. X., DEDIO, R. M. & SAUNDERS, J. C. (1992) Threshold shift, hair cell loss, and hair bundle stiffness following exposure to 120 and 125 dB pure tones in the neonatal chick. *Acta Otolaryngologica* **112,** 444–54.

ASHMORE, J. F. & KOLSTON, P. J. (1994) Hair cell based amplification in the cochlea. *Current Opinion in Neurobiology* **4,** 503–8.

BENFENATI, F., VALTORTA, F., RUBENSTEIN, J. L., GORELICK, F. S., GREENGARD, P. & CZERNIK, A. J. (1992) Synaptic vesicle-associated Ca^{2+}/calmodulin-dependent protein kinase II is a binding protein for synapsin I. *Nature* **359,** 417–20.

COTANCHE, D. A. (1987) Regeneration of hair cell stereociliary bundles in the chick cochlea following severe acoustic trauma. *Hearing Research* **30,** 181–96.

COTANCHE, D. A. & DOPYERA, E. J. (1990) Hair cell and supporting cell response to acoustic trauma in the chick cochlea. *Hearing Research* **46,** 29–40.

COTANCHE, D. A., HENSON, M. M. & HENSON, O. W. Jr. (1992) Contractile proteins in the hyaline cells of the chicken cochlea. *Journal of Comparative Neurology* **324,** 353–64.

COTANCHE, D. A., MESSANA, E. P. & OFSIE, M. S. (1995) Migration of hyaline cells into the chick basilar papilla during severe noise damage. *Hearing Research* **91,** 148–59.

DALLOS, P. (1992) The active cochlea. *Journal of Neuroscience* **12,** 4575–85.

DAVIS, H. (1983) An active process in cochlear mechanics. *Hearing Research* **9,** 79–90.

DRENCKHAHN, D., MERTE, C., VON DURING, M., SMOLDERS, J. & KLINKE, R. (1991) Actin, myosin, and α-actinin containing filament bundles in hyaline cells of caiman cochlea. *Hearing Research* **54,** 29–38.

DÜRING, M. VON, KARDUCK, A. & RICHTER, H. G. (1974) The fine structure of the inner ear in Caiman crocodilus. *Zeitschrift für Anatomie und Entwicklungs Geschichte* **145,** 41–65.

FISCHER, F. P. (1992) Quantitative analysis of the innervation of the chicken basilar papilla. *Hearing Research* **61,** 167–78.

FROYMOVICH, O., REBALA, V., SALVI, R. J. & RASSAEL, H. (1995) Long-term effect of acoustic trauma on distortion product otoacoustic emissions in chickens. *Journal of the Acoustical Society of America* **97,** 3021–9.

GIROD, D. A., DUCKERT, L. G. & RUBEL, E. W. (1989) Possible precursors of regenerated hair cells in the avian cochlea following acoustic trauma. *Hearing Research* **42,** 175–94.

GOLDENRING, J. R., LASHER, R. S., VALLANO, M. L., UEDA, T., NAITO, S., STERNBERGER, N. H., STERNBERGER, L. A. & DELORENZO, R. J. (1986) Association of synapsin I with neuronal cytoskeleton. Identification in cytoskeletal preparations *in vitro* and immunocytochemical localization in brain of synapsin I. *Journal of Biological Chemistry* **261,** 8495–504.

GREENGARD, P., VALTORTA, F., CZERNIK, A. J. & BENFENATI, F. (1993) Synaptic vesicle phosphoproteins and regulation of synaptic function. *Science* **259,** 780–5.

GREENGARD, P., BENFENATI, F. & VALTORTA, F., (1994) Synapsin I, an actin-binding protein regulating synaptic vesicle traffic in the nerve terminal. *Advances in Second Messenger Phosphoprotein Research* **29,** 31–45.

HELD, H. (1926) Die cochlea der sauger und der vogel, ihre entwicklung und ihr bau. In *Handbuch der normalen und patholgischen physiologie, Vol. 11,* p. 493. Berlin: Springer.

HIROKAWA, N. (1978a) The ultrastructure of the basilar papilla of the chick. *Journal of Comparative Neurology* **181,** 361–74.

HIROKAWA, N. (1978b) Synaptogenesis in the basilar papilla of the chick. *Journal of Neurocytology* **7,** 283–300.

JAHNKE, V., LUNDQUIST, P. G. & WERSALL, J. V. (1969) Some morphological aspects of sound perception in birds. *Acta Otolaryngologica* **67,** 583–601.

KEMP, D. T. (1986) Otoacoustic emissions, traveling waves and cochlear mechanisms. *Hearing Research* **22,** 95–104.

KEPPLER, C., SCHERMULY, L. & KLINKE, R. (1994) The course and morphology of efferent nerve fibres in the papilla basilaris of the pigeon (*Columba livia*). *Hearing Research* **74,** 259–64.

KIMURA, R. S. (1984) Sensory and accessory epithelia of the cochlea. In *Ultrastructural Atlas of the Inner Ear* (edited by FRIEDMANN, I. & BALLANTYNE, J.) pp. 101–32. London: Butterworths & Co.

KLINKE, R. & SMOLDERS, J. W. (1993) Performance of the avian inner ear. *Progress in Brain Research* **97,** 31–43.

LLINAS, R., GRUNER, J. A., SUGIMORI, M., MCGUINNESS, T. L. & GREENGARD, P. (1991) Regulation by synapsin I and $Ca^{(2+)}$-calmodulin-dependent protein kinase II of the transmitter release in squid giant synapse. *Journal of Physiology* **436,** 257–82.

MARSH, R. R., XU, L., MOY, J. P. & SAUNDERS, J. C. (1990) Recovery of the basilar papilla following intense sound exposure. *Hearing Research* **46,** 229–38.

MCFADDEN, E. A. & SAUNDERS, J. C. (1989) Recovery of auditory function following intense sound exposure in the neonatal chick. *Hearing Research* **41,** 205–16.

NIEMIEC, A. J., RAPHAEL, Y. & MOODY, D. B. (1994) Return of auditory function following structural regeneration following acoustic trauma: behavioral measures from quail. *Hearing Research* **75,** 209–24.

ODINOKOVA, G. V. & PROKOF'EVA, L. I. (1975) Innervation of posterior hyaline cells in the bird cochlea. *Biologicheskie Nauki* **6,** 24–6.

OESTERLE, E. C., CUNNINGHAM, D. E. & RUBEL, E. W. (1992) Ultrastructure of hyaline, border, and vacuole cells in the chick inner ear. *Journal of Comparative Neurology* **318,** 64–82.

OFSIE, M. S. & COTANCHE, D. A. (1996) Distribution of nerve fibres in the basilar papilla of normal and sound-damaged chick cochleae. *Journal of Comparative Neurology* **370,** 281–94.

POJE, C. P., SEWELL, D. A. & SAUNDERS, J. C. (1995) The effects of exposure to intense sound on the DC endocochlear potential in the chick. *Hearing Research* **82,** 197–204.

RAPHAEL, Y. (1992) Evidence for supporting cell mitosis in response to acoustic trauma in the avian inner ear. *Journal of Neurocytology* **21,** 663–71.

RAPHAEL, Y. (1993) Reorganization of the chick basilar papilla after acoustic trauma. *Journal of Comparative Neurology* **330,** 521–32.

ROSAHL, T. W., GEPPERT, M., SPILLANE, D., HERZ, J., HAMMER, R. E., MALENKA, R. C. & SUDHOF, T. C. (1993) Short-term synaptic plasticity is altered in mice lacking synapsin I. *Cell* **75,** 661–70.

RYALS, B. M., STALFORD, M. D., LAMBERT, P. R. & WESTBROOK, E. W. (1995) Recovery of noise-induced changes in the dark cells of the quail tegmentum vasculosum. *Hearing Research* **83,** 51–6.

SAUNDERS, J. C., ADLER, H. J. & PUGLIANO, F. A. (1992) The structural and functional aspects of hair cell regeneration in the chick as a result of exposure to intense rounds. *Experimental Neurology* **115,** 13–17.

SLEPECKY, N. B. (1996) Structure of the mammalian cochlea. In *The Cochlea* (edited by DALLOS, P., POPPER, A. N. & FAY R. R.) pp. 44–129. New York: Springer.

STONE, J. S. & COTANCHE, D. A. (1992) Synchronization of hair cell regeneration in the chick cochlea following noise damage. *Journal of Cell Science* **102,** 671–80.

TAKASAKA, T. & SMITH, C. A. (1971) The structure and innervation of the pigeon's basilar papilla. *Journal of Ultrastructure Research* **35,** 20–65.

TANAKA, K. & SMITH, C. A. (1978) Structure of the chick's inner ear: SEM and TEM study. *American Journal of Anatomy* **153,** 251–71.

UEDA, T., GREENGARD, P., BERZINS, K., COHEN, R. S., BLOMBERG, F., GRAB, D. J. & SIEKEVITZ, P. (1977) Subcellular distribution in cerebral cortex of two proteins phosphorylated by a cAMP-dependent protein kinase. *Journal of Cell Biology* **83,** 308–19.

WANG, Y. & RAPHAEL, Y. (1996) Re-innervation patterns of chick auditory sensory epithelium after acoustic overstimulation. *Hearing Research* **97,** 11–18.

WHITEHEAD, M. C. & MOREST, D. K. (1985) The development of innervation patterns in the avian cochlea. *Neuroscience* **14,** 255–76.

ZIDANIC, M. & FUCHS, P. A. (1996) Synapsin-like immunoreactivity in the chick cochlea: specific labeling of efferent nerve terminals. *Auditory Neuroscience* **2,** 347–62.