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Damage and recovery of hair cells in fish canal (but not superficial) neuromasts after gentamicin exposure

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Damage and recovery of hair cells in fish canal (but not superficial) neuromasts after gentamicin exposure

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Abstract

Recent evidence demonstrating the presence of two types of sensory hair cell in the ear of a teleost fish (*Astronotus ocellatus*, the oscar) indicates that hair cell heterogeneity may exist not only in amniotic vertebrates but also in anamniotes. Here we report that a similar heterogeneity between hair cell types may also occur in the other mechanosensory organ of the oscar, the lateral line. We exposed oscars to the aminoglycoside (ototoxic) antibiotic gentamicin sulfate and found damaged sensory hair cells in one class of the lateral line receptors, the canal neuromasts, but not in the other class, the superficial neuromasts. This effect was not due to the canal environment. Moreover, new ciliary bundles on hair cells of the canal neuromasts were found after, and during, gentamicin exposure. The pattern of hair cell destruction and recovery in canal neuromasts is similar to that of type I-like hair cells found in the striolar region of the utricle and lagena of the oscar after gentamicin treatment. These results suggest that the hair cells in the canal and superficial neuromasts may be similar to type I-like and type II hair cells, respectively, in the fish ear.

Keywords: Hair cell; Lateral line; Canal neuromast; Superficial neuromast; Fish; Gentamicin

1. Introduction

Early studies of the vertebrate inner ear suggested that whereas amniote vestibular endorgans have two types of hair cells (types I and II), anamniotes have only type II hair cells (Wersäll, 1956). As a consequence, diversity of hair cell types in amniotes has generally been considered to be a structurally and functionally advanced characteristic in the evolution of the vertebrate ear.

Although there have been some reports of morphological and structural diversity of hair cells in the ear of anamniotes (Hama, 1969; Corwin, 1977; Popper and Hoxter, 1981; Wegner, 1982; Jensen, 1984), there was no clear evidence of a possible homology between the anamniote and amniote hair cell types until the recent discovery of two distinct types of sensory hair cells in the ear of the teleost fish, *Astronotus ocellatus*, the oscar (Saidel et al., 1990a, b; Yan et al., 1991; Chang et al., 1992). The two types of hair cell in the oscar ear, one in the striolar region and the other in the extrastriolar region of the utricle and the lagena, may be similar to amniote type I and type II hair cells, respectively. Thus, heterogeneity of sensory hair cells may not be an advanced amniote trait, as previously proposed. In fact, hair cell diversity may extend much earlier in the evolution of the vertebrate ear than the origin of amniotes. It is still not known, however, when such diversity first occurred in the evolution of vertebrate mechanosensory systems.

The mechanosensory organs of vertebrates consist of the lateral line neuromasts and the inner ear. The neuromasts are only found in aquatic anamniotes, whereas the inner ear occurs in all the vertebrates. Ontogenetically, the sensory epithelium of both organs are closely related because the lateral line placodes differentiate from the primary otic placode (Platt, 1896; Landacre and Conger, 1913; Northcutt, 1989). The sensory epithelia in both organs contain sensory hair cells and supporting cells for detecting motion of surrounding water or fluid. Because of these similarities, the lateral line and the inner ear are collectively referred to as the octavolateralis system (Northcutt, 1980; McCormick, 1982; Popper et al., 1992). However, the inner ear has been considered to be struc-

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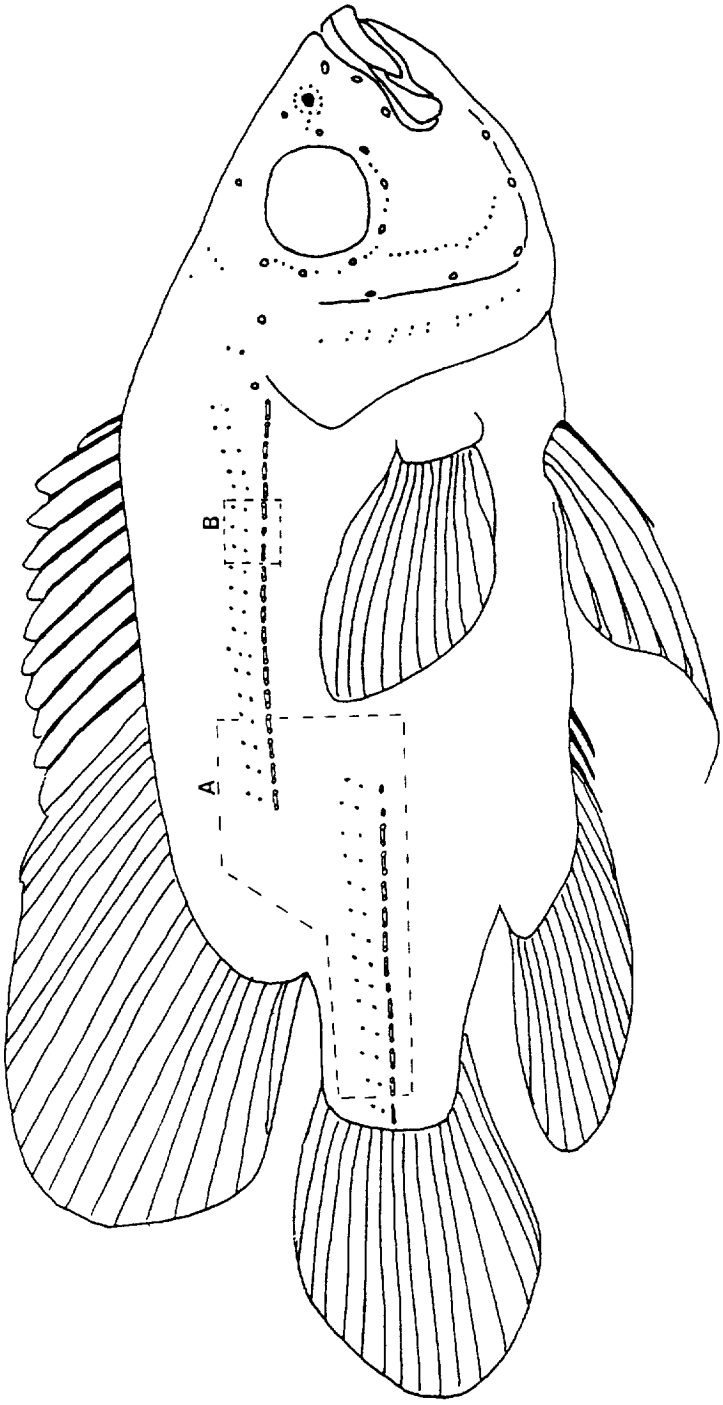

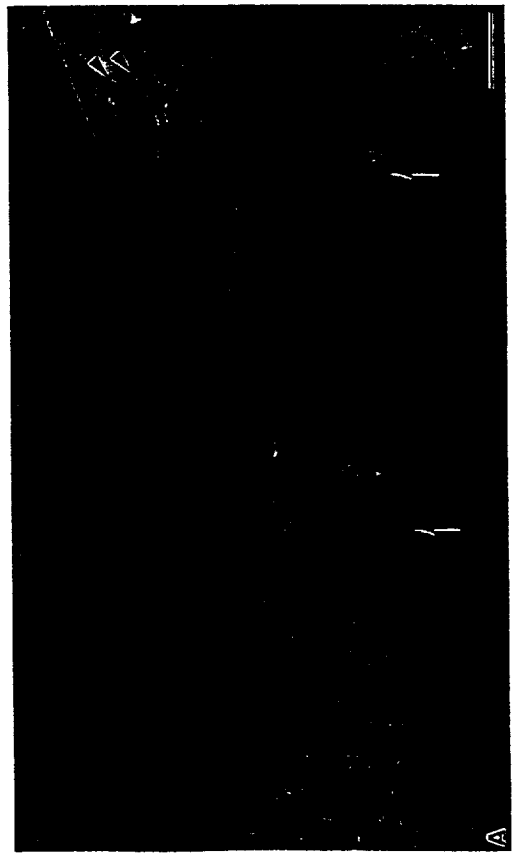

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Despite some morphological variation among species, the sensory hair cells of the lateral line neuromasts had been considered to be of only a single type (Jørgensen, 1989; Kroese and van Netten, 1989). It becomes important, however, to reexamine the hair cell types in the neuromasts in the light of the recent evidence showing the presence of two types of hair cells in the inner ear of the oscar to determine whether hair cell heterogeneity is present in the lateral line system. If heterogeneity is found in the lateral line, it could mean that this arose even earlier in the evolution of the vertebrate mechanosensory organs than suggested by the current evidence from the fish ear.

In the oscar ear, the aminoglycoside antibiotic gentamicin sulfate selectively damages ciliary bundles of hair cells in the striolar regions of the utricle without damaging hair cells in the extrastriolar regions (Yan et al., 1991; Lombarte et al., 1993). The pattern of damage in the striolar region appears similar to that observed for mammalian type I hair cells under various aminoglycoside antibiotic assaults (Wersäll and Hawkins, 1962; Wersäll et al., 1971). Thus, the types of hair cell in the fish striolar and extrastriolar regions of the utricle and lagena may be related to mammalian types I and II hair cells, respectively. The purpose of this study was to determine if the lateral line receptors of the oscar also have two types of hair cells as in the oscar inner ear. We hypothesized that if two types of hair cells occur in the lateral line neuromasts, one group would be more sensitive to gentamicin than the other. Therefore, we exposed the fish to a solution of gentamicin sulfate and examined the response of ciliary bundles of the hair cells in the neuromasts.

2. Materials and methods

Thirty oscars were divided into three experimental groups. One experimental group contained 6 fish (52–58 mm total length (TL)) while the other two groups had 12 fish each (42–48 mm TL). An additional 3 animals (44, 46, and 54 mm TL) were used as controls. The experimental fish were kept in aerated water containing 0.002% gentamicin sulfate. The water was completely changed each day to refresh the gentamicin. All experiments were done according to the policies of the University of Maryland Animal Care and Use Committee.

Fish were killed after being deeply anesthetized with tricaine methane sulfonate (MS222; Sigma). Two fish each (from the group of large fish) were killed after 4, 8, and 12 days of gentamicin exposure, and 2 fish each (from a group of small fish) were killed after 1, 2, 3, 4, 8, and 12 days exposure. The third experimental group (small fish) received gentamicin treatment until day 4, followed by 4 or 8 additional days survival during which time the water was changed daily but without gentamicin. Two fish each from this group were killed on the same schedule as the other small fish group. Control animals were kept in similarly sized tanks as the experimental animals, and their water was changed daily. The fish were killed after 4 days.

Tissue from experimental and control fish was prepared for scanning electron microscopic (SEM) examination following procedures described previously (Song and Northcutt, 1991a, b). Briefly, fish were immersed in 4% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4) and fixed for a period of time ranging from overnight to 2 days. After decalcification in 0.1% HCl for about 30 min, the fish were washed with distilled water and cut mid-sagittally. The lateral line canals were opened with microneedles. Each half of the fish was cut transversely to separate the head, trunk, and tail. After dehydration in a graded series of ethanol and critical-point drying with liquid CO_2, samples containing lateral line neuromasts were mounted on aluminum stubs, sputter-coated with gold–palladium, and viewed and photographed with an Amray 1820 SEM.

3. Results

3.1. Normal organization

The mechanosensory lateral line receptors in the oscar, as in most fishes, consist of canal and superficial neuromasts that are distributed over the surface of the head and the body in a species-specific pattern (Fig. 1). The morphological differences between the canal (or superficial) neuromasts on the head and the body are minimal. The trunk of the oscar has a dorsorostral and a mediocaudal lateral line. Each of the trunk scales in the lateral line has a single canal neuromast that is covered by a bony canal with an opening on either end of the scale. Only a few trunk canal neuromasts in each fish are not covered by bony canals (Fig. 1A). There are two or three superficial neuromasts found dorsally to each canal neuromast on the trunk of fish in the size range of our experimental groups (42–58 mm TL).

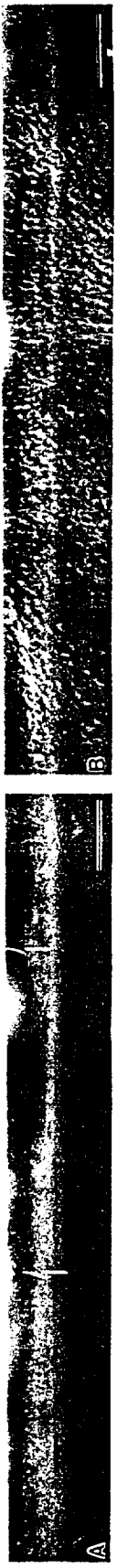

Fig. 1. Peripheral distribution of the lateral line canal and superficial neuromasts in an oscar (56 mm TL). Large dot, nares; small circles, lateral line canal openings; small dots, superficial neuromasts; small oval dots, uncovered canal neuromasts. A: SEM overview of the skin surface (with caudal portion of the trunk lateral lines) on the body. Arrowheads (upper right) point to a pair of superficial neuromasts dorsal to a lateral line scale (canal is surgically opened) from the dorso-rostral section of the trunk lateral line. Arrows point to the uncovered canal neuromast Bar = 1 mm. B: high-power view of a superficial neuromast (arrowhead in upper left). An adjacent canal neuromast sits in a surgically opened canal (long arrow in center). Both neuromasts are covered by a gelatinous cupula. Bar = 100 μm. Orientation: head to the right, dorsal to the top.

Although the canal and the superficial neuromasts in the oscar share the same basic organization, they also differ in several ways. In our experimental fish, the canal neuromasts are larger and possess over 120 hair cells, whereas the superficial neuromasts are smaller and each has about 40 hair cells (Fig. 2A,C). The density of hair cells and the length of their kinocilia are greater in superficial neuromasts than in canal neuromasts (compare Fig. 2B,D).

3.2. Gentamicin exposure

After 4 days of gentamicin exposure, superficial neuromasts in experimental animals did not show a loss of the ciliary bundles (Fig. 2C,D). However, canal neuromasts, both covered and not covered by scales, had a substantial loss of ciliary bundles with as little as 1 day of exposure (Fig. 3A,B). Control animals had no loss of ciliary bundles in either canal (Fig. 2A) or superficial neuromasts.

New small ciliary bundles on hair cells were found among the support cell microvilli in the canal neuromasts by the 2nd or 3rd day of treatment in the smaller fish and by the 4th day in the larger fish. These new bundles, however, did not attain the normal hair cell density even after 8 and 12 days of treatment (compare Fig. 4A–C and 2A).

In contrast, there were far more ciliary bundles present in the canal neuromasts of the animals that had 4 days of gentamicin exposure followed by 4 days (Fig. 4) or 8 days (Fig. 4E,F) recovery in plain water than in animals that had 8 days (Fig. 4A) or 12 days (Fig. 4B,C) of gentamicin exposure. The area covered by ciliary bundles in the canal neuromasts of the animals exposed to gentamicin for 4 days followed by 8 days of recovery (Fig. 4E,F) approached the pattern found in control animals (compare to Fig. 2A,B). The new hair cell bundles, however, appeared to have longer kinocilia than those in the canal neuromasts of the control animals (compare Figs. 4E,F and 2A,B), a characteristic similar to that of normal superficial neuromast hair cells (Fig. 2C,D).

4. Discussion

The results of our experiments demonstrate that the sensory hair cells in the canal and the superficial neuromasts respond differently to a gentamicin sulfate solution at the concentration used in our study. Moreover, the different responses to gentamicin parallel, respectively, the responses of the types I and II hair cells in the utricle and

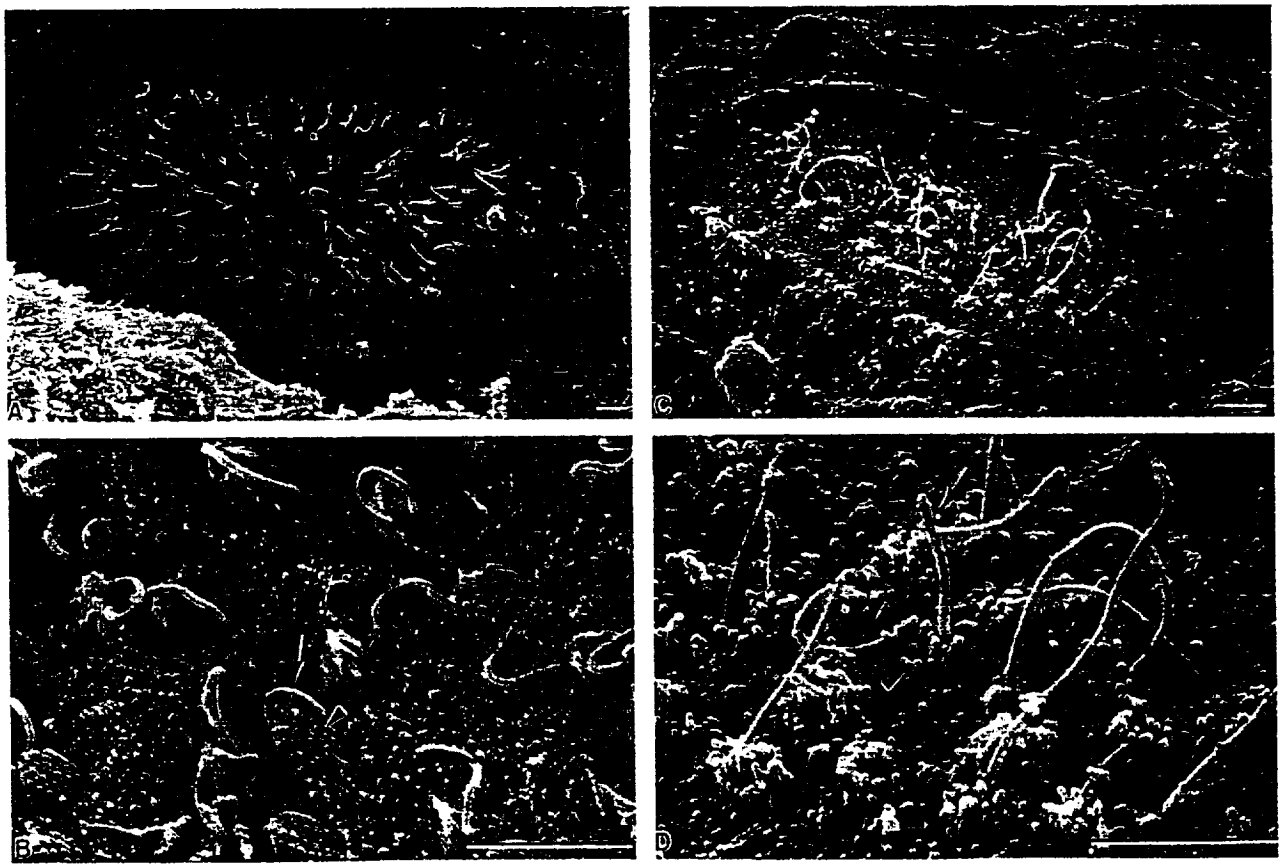

Fig. 2. Low (A) and high (B) magnification SEMs of a canal neuromast from a trunk canal of a control specimen. The bony canal was surgically opened during SEM preparation. Low (C) and high (D) magnification SEMs of a superficial neuromast from the epidermal surface dorsal to the trunk canal neuromasts from an animal continuously exposed to gentamicin sulfate for 4 days. No damage to the ciliary bundles is found. Long arrows point to a kinocilium; arrowhead points to a set of stereocilia. Bar = 5 μm.

lagena of the oscar and in amniote vestibular endorgans. These data may suggest that the heterogeneity of hair cell types occurs in both of the vertebrate mechanosensory organs, the inner ear and the lateral line neuromasts. Our experiments also demonstrate that ciliary bundles are regenerated in the canal neuromasts after exposure to, and even under the continuing presence of, gentamicin.

4.1. Classification of lateral line neuromasts

Neuromasts have been classified as canal or superficial, depending on their epidermal position (reviewed by Coombs et al., 1988; Münz, 1989). In most fishes, including the oscar, the canal neuromasts are enclosed within open grooves, bony canals, or scales, whereas the superficial neuromasts are found on the surface of the epidermis (Fig. 1). Although superficial and canal neuromasts exhibit the same basic organization, they differ from one another in a number of ways, including innervation pattern and morphology of hair cell arrangement (Hama, 1965, 1978; Münz, 1989; Song and Northcutt, 1991a, b). The canal neuromasts also appear to be more sensitive to higher frequencies of vibration than are the superficial neuromasts (Münz, 1985; Kroese and van Netten, 1989). Despite these findings, however, our knowledge of the biological significance of the morphological, physiological, and functional differences between superficial and canal neuromasts are rudimentary.

Even though there is morphological, and some functional, variation between neuromasts and their position on the epidermis, the sensory hair cells in the lateral line receptors have been considered to be only a single type (Jørgensen, 1989; Kroese and van Netten, 1989). Therefore, our results showing differences in the response to gentamicin by hair cells in the oscar indicate, for the first time, the existence of a biochemical heterogeneity of hair cells between the canal and superficial neuromasts. This, added to the known physiological differences between canal and superficial neuromasts, suggests that the two lateral line receptors may be functionally quite distinct from one another.

While the oscar, as most fishes, has two types of neuromasts, there are a number of fish species, such as the gobies (Song, 1993), that have reduced the lateral line canals in certain regions of the fish but not the neuromasts. It will be interesting and important to determine whether these neuromasts maintain distinct types of hair cells from the normal canal instead of superficial neuromasts even though both neuromasts are located on the surface of epidermis. If so, the classification of the neuromasts will be based on more meaningful criteria other than their position on the epidermis.

4.2. Types of hair cells in the lateral line

The pattern of destruction of ciliary bundles of the canal neuromast by gentamicin sulfate observed in this study is reminiscent of the damage caused by gentamicin to striolar hair cells of the oscar (Yan et al., 1991; Lombarte et al., 1993) and goldfish ears (Platt and Yan, 1993) and to mammalian vestibular type I hair cells (Wersäll, 1960; Lindeman, 1969). In contrast, the ciliary bundles of the hair cells in the superficial neuromasts were not damaged by the same dosage of gentamicin sulfate treatment. This is similar to the findings for extrastriolar hair cells of the oscar ear (Yan et al., 1991; Lombarte et al., 1993) and mammalian type II hair cells (Wersäll, 1960; Lindeman, 1969).

One question that arose in our study was whether the different response to gentamicin damage in canal versus superficial neuromasts was associated with the environment within the canal. Accumulation of gentamicin in the canal could, theoretically, have produced a much higher concentration of the drug than that presented to superficial neuromasts. To test this, we took advantage of the fact that the oscar, like most teleost fish, has a number of trunk canal lateral line scales that fail to form canals over their neuromasts (Fig. 1). If the differential damage to the canal organs was due to the concentration of drug in the canal environment, we would expect no ciliary bundle damage in

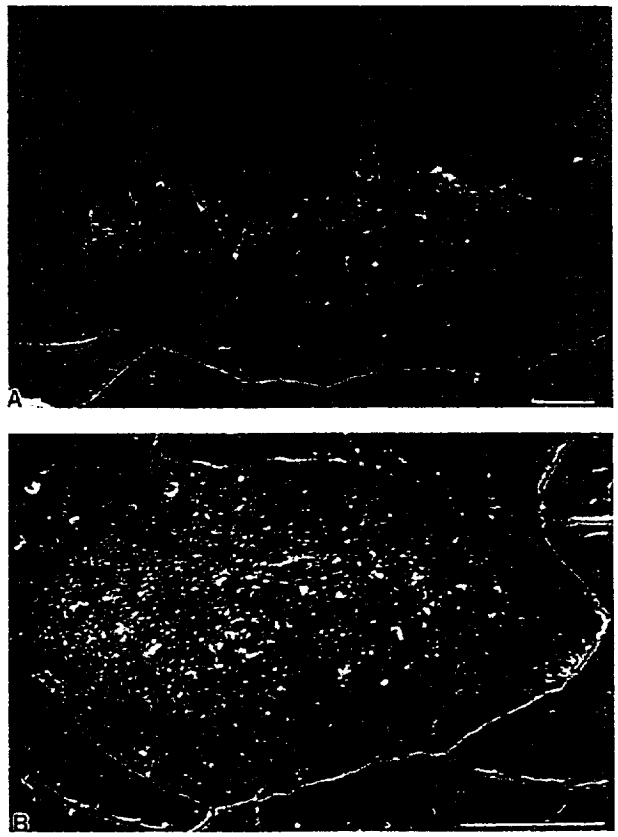

Fig. 3. SEMs of canal neuromasts after 1 day in gentamicin sulfate showing the loss of ciliary bundles on the hair cells. A: Canal neuromast where the canal cover was surgically removed during SEM preparation. B: Canal neuromast that was not normally covered by a bony canal. This is the next neighbor to the neuromast in A. Bar = 10 μm.

the neuromasts of uncovered canals. However, we found that the canal neuromasts on the uncovered scales (Fig. 3A) had damage that was similar to the damage on the neighboring covered canal neuromasts (Fig. 3B) in the same fish.

The other question concerned whether the different response to gentamicin of the hair cells in canal versus superficial neuromasts was related to the state of development of the hair cells, since the hair cells in the superficial neuromasts and the immature hair cells in the canal neuromasts share a morphological feature of having a long kinocilia (Fig. 2C–D vs. Fig. 4). It has been known that immature hair cells are less susceptible to aminoglycoside toxicity than are mature hair cells (Richardson and Russell, 1991). Our new observation of the differences in ultrastructure, innervation, and postembryonic growth pattern between the hair cells in the two classes of neuromasts, however, suggests that it is not likely the case (Song et al., 1995).

Although further study of the gentamicine susceptibility

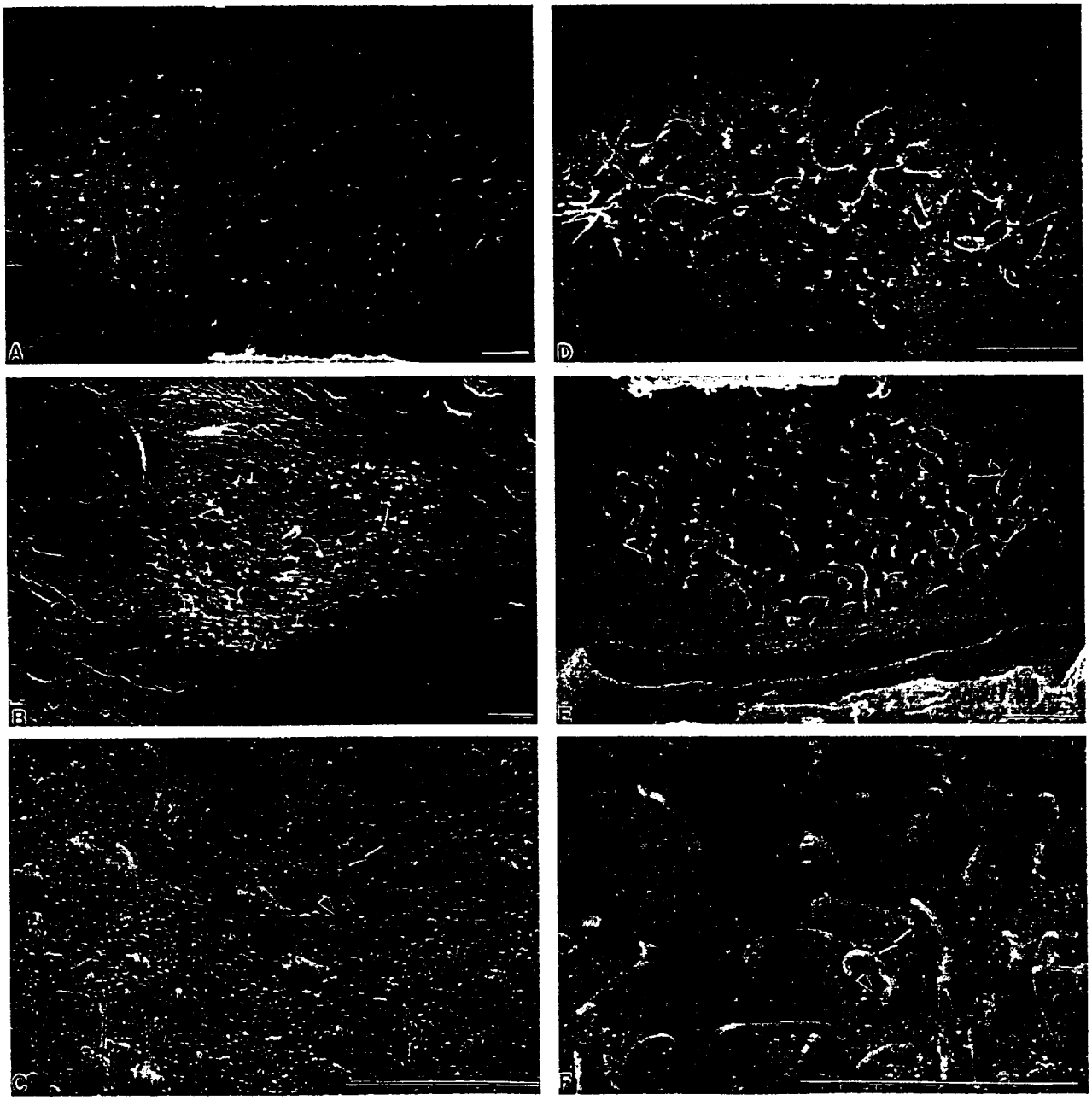

Fig. 4. SEMs of canal neuromasts after 8 (A) and 12 days (B) in gentamicin sulfate. C: Higher magnification view of (B). D: Canal neuromast after 4 days in gentamicin sulfate followed by 4 days of recovery in water with no drug (total 8 days). Low (E) and high (F) magnification view of a canal neuromast after 4 days in gentamicin sulfate and 8 days recovery (total 12 days). Long arrow points to a kinocilium. Short arrow head points to a set of stereocilia. Bar = 10 μm.

of the superficial neuromast hair cells in different length of exposure time and in different dosages of the gentamicine is required in order to understand the mechanism of the susceptibility. The results from this study, at least, suggest that the hair cells of the lateral line canal and the superficial neuromasts in the oscar may share the similar biochemical heterogeneity in response to gentamicin sulfate as the two distinct types of hair cells in the vertebrate inner ear. Because the sensory epithelia of both the lateral line receptors and the inner ear are from closely related epidermal placodes embryonically (Landacre and Conger, 1913; Metcalfe, 1985; Northcutt, 1989), it is possible that the heterogeneity between the two types of hair cells in both organs are homologous. This hypothesis should be further studied.

4.3. Regeneration of lateral line hair cells

Our results not only demonstrate that intact lateral line neuromasts of the oscar are capable of replacing hair cell ciliary bundles after aminoglycoside treatment but also that the replacement can occur in the presence of gentamicin (Fig. 3A–C) that is refreshed daily. Still, since we did not examine the hair cells with transmission electron microscopy in this study, we do not have histological evidence that there was actual hair cell destruction and regeneration. Our SEM examination of gentamicin-treated neuromasts, however, indicated that the damaged hair cells in the canal neuromasts appear to be replaced by supporting cells before the new ciliary bundles arise (Fig. 4). This suggests to us that the hair cells were completely destroyed and that new ciliary bundles must arise from new hair cells. To test this hypothesis, it will be necessary to examine the hair cell bodies of the canal neuromasts after gentamicin treatment with transmission electron microscopy.

Regeneration in the presence of an ototoxic drug has not previously been encountered in lateral line neuromasts or fish ears. The only comparable data comes from studies in the chick basilar papilla where it has been demonstrated that hair cell regeneration and differentiation can occur in the presence of ototoxic levels of gentamicin (Girod et al., 1991) and that the regenerated hair cells are not easily damaged by additional kanamycin treatment (Hashino et al., 1994). Hashino et al. (1994) suggested that kanamycin may not be incorporated into the cytoplasm of regenerated hair cells after cumulative administration of the drug and that inhibition of aminoglycoside uptake may be responsible for the drug resistance in the regenerated hair cells (Hashino et al., 1995). How this hypothesis might apply to our results, however, is not clear. In particular, we do not know if hair cell uptake of kanamycin and gentamicin is by similar mechanisms. In addition, the ototoxic drugs were administered to the hair cells by different methods (intramuscular in chicks and by immersion in fish), and this may affect the way that hair cells respond to the drugs.

Even though the suggestion of Hashino et al. (1994) may be correct, there are still other possible explanations for our results that need further study. First, it is possible that gentamicin did damage the hair cell but did not destroy the cell body, as it does in the amniote vestibular system, and that the cilia were regenerated by otherwise intact cells. Second, the response to the ototoxic effect of gentamicin may only occur late in the development of sensory hair cells and before they reach full maturity. Thus, the cells we encountered may only get to a certain stage of development and then they again are 'killed' (or damaged) by the gentamicin. The third possibility is that the regenerated cells are more like the hair cells of the superficial neuromast than those found in untreated canal neuromasts.

It is difficult to differentiate the second and third possibilities without more extended survival time after gentamicin treatment. Our results show that the regenerated ciliary bundles in the canal neuromasts have longer kinocilia than those in the control animals (compare Figs. 4E,F and 2A,B), a characteristic similar to that of normal superficial neuromast hair cells (Fig. 2C,D). The presence of longer kinocilia has been reported in regenerated hair cells in the inner ear of fishes and amphibians (Corwin, 1985; Baird et al., 1993; Lombarte et al., 1993). It also has been reported that, after aminoglycoside ototoxic drug treatment, type I hair cells are replaced by hair cells that are morphologically similar to the type II hair cells in the avian vestibular system (Weisleder and Rubel, 1992) and in the mammalian inner ear (Forge et al., 1993). Even though little is known about the regeneration or developmental stages of sensory hair cells, it is possible that vestibular type I cells develop by going through a type II stage. Therefore, the ciliary bundles we observed in the regenerated canal neuromast hair cells may be in an early stage of development in which they resemble the superficial neuromast hair cells (or the 'type II'). Thus, at the early stages, the hair cells may not be affected by ototoxic drugs because they are more type II-like. As the cells start to become more like mature canal neuromast hair cells (or striolar hair cells in the utricle), however, they may become more susceptible to the gentamicin sulfate. It is possible that, after drug damage, regenerating type II vestibular hair cells are observed first and the more normal type I hair cells would show up several weeks (or more than 60 days) late as in the cristae of birds (Weisleder and Rubel, 1992).

The third possibility, that the regenerated canal hair cells take on the characteristics of superficial neuromast hair cells and stay that way, can only be tested by observing regenerated canal hair cells for extended periods of time after gentamicin exposure.

5. Conclusions

Although further ontogenetic and phylogenetic studies to test the homology of hair cell types of the lateral lin-

receptors to the mammalian inner ear are necessary, the results of this study and new observations of the ultrastructural differences between the hair cells in the canal versus superficial neuromasts (Song et al., 1995) suggest that the lateral line receptors have considerable potential for studies on the heterogeneity of mechanosensory hair cells and their susceptibility to ototoxic drugs. The simplicity, versatility, and efficiency of ototoxic drug exposure, and the greater anatomical accessibility of the lateral line system, provide an optimal system for the study of damage and regeneration of sensory hair cells. Even though further investigations are needed to determine whether ototoxic drugs cause functional damage to the lateral line receptors, we do expect this is the case because behavioral studies have indicated that increasing doses of both neomycin and streptomycin produce increasing loss of an animal's ability to localize a wave source (the lateral line reaction) (Kaus, 1987, 1992; Blaxter and Fuiman, 1990).

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