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Innervation patterns in neuromasts of the mottled sculpin, Cottus bairdi

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INNERVATION PATTERNS IN NEUROMASTS OF THE MOTTLED SCULPIN, *Cottus bairdi*

The mottled sculpin, *Cottus bairdi* has a lateral line system composed of both canal and superficial neuromasts and distributed on both the head and trunk. These neuromasts are mechanoreceptors sensitive to low frequency vibrations caused by movement of the surrounding water. Behavioral experiments have indicated a sensitivity difference between the head and trunk in which the head has a higher sensitivity than does the trunk to equivalent stimuli e.g. those produced by potential prey. Neurophysiological experiments indicate that canal rather than superficial neuromasts are most responsible for this observed behavior. My research is an attempt to test one of the possible mechanisms by which the sensitivity difference between head and trunk neuromasts might come about. It is my hypothesis that this is due, at least in part, to a greater convergence of sensory hair cells onto their afferent nerve fibers in head canal neuromasts. My study uses anatomical protocols to see if populations of neuromasts do in fact differ in this respect.

The results of my investigation show that all canal

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CHAPTER I

INTRODUCTION

Some of the earliest references to the lateral line system are found in the scientific literature of the 17th century. Early investigators noted that the skin of some fish exhibited small circular openings or pores which were latter proven to communicate with a system of subcutaneous canals (Disler, 1971). The actual nature of this system remained enigmatic through most of the 19th century, despite numerous investigations. Moreover, limited largely by the undeveloped state of microscopical techniques at the time, it was not until the middle of the 19th century that what is now called the lateral line system was considered as anything but a system which secreted the mucous that covers the surface of most fish (Dijkgraaf, 1989). It is now widely recognized that the lateral line is a mechanoreceptive system which is sensitive to low frequency vibrations caused by movement of the surrounding water (for review of lateral line systems see Coombs *et al.*, 1989). Such mechanoreceptive organs are found in a wide variety of species, including cyclostomes, elasmobranches, teleosts and larval amphibia (Hama & Yamada, 1977).

The mechanosensory lateral line system is characterized

by the morphology of its endorgans, the neuromasts, which consist of sensory receptor cells (hair cells), supporting cells and mantle cells (Jørgensen, 1989). Typical neuromasts of the lateral line system in fish, e.g. both elasmobranchs and teleosts, can be divided into two groups: superficial neuromasts, which are located on the surface of the skin, and canal neuromasts, which are embedded in the walls of lateral line canals, which lie underneath the epidermis of the head and trunk. Canal neuromasts communicate to the outside environment through an elaborate pore system. Within the canal the axis of the sensory strip, which is comprised of sensory hair cells and supporting cells, is typically, but not always, parallel to the long axis of the canal itself (Webb, 1989). Although superficial and canal neuromasts differ in their location on the body and the size and number of hair cells per neuromast, the basic mechanism of sensory transduction appears to be the same, i.e. both respond to deflection of their apical ciliary bundles by local mechanical stimuli, particularly by water movement in relation to the fish. Some studies have shown that the superficial neuromasts respond best to velocity changes while canal neuromasts respond best to acceleration (Kroese and Shellert, 1987, Denton and Gray, 1988, Coombs and Janssen, 1990). Such mechanosensitivity allows fish to detect the presence of predators or prey, to orient properly to the current, to maintain position in a school and to avoid obstacles (Bond, 1979).

The neuromasts of the aquatic anamniotic vertebrates are composed of sensory hair cells and supporting cells, all surrounded by mantle cells (Jørgensen, 1989). The neuromasts are covered by a gelatinous cupula which extends into either the surrounding water in superficial neuromasts or canal fluid in canal neuromasts. From the apical surface of each hair cell, a bundle of sensory hairs protrudes into the overlying cupula. Each sensory hair bundle consists of a single acentric kinocilium, which is a true cilium with a conventional 9+2 pattern of microtubules, and stereocilia which are in fact modified microvilli, despite their name (Flock 1967). Within each neuromast there are two populations of ciliary bundles, with opposite but parallel orientation of their mechanosensitive axes as determined by the position of the kinocilium relative to the stereocilia. Thus, each hair cell has its own axis of sensitivity (Rouse and Pickles, 1991). The sensory hair bundles act to transmit the mechanical energy of cupular displacements caused by water or canal fluid to the mechanosensitive site in the hair cells where the receptor mechanism is activated. Displacement of the stereocilia toward the kinocilium is excitatory and results in a increase in nerve impulse frequency. Displacement in the opposite direction i.e. away from the kinocilium is inhibitory and results in a decrease in nerve discharge rate (Flock, 1967).

Within the neuromast, the supporting cells, which are believed to contribute secretory materials to form the cupula

(Iwai, 1967), extend from the apical surface of neuromast to the basal lamina, and surround and separate the hair cells. The hair cells make synaptic contacts basally with nerve fibers of the lateral line nerve, which innervates the neuromast. Mantle cells, situated on the lateral margins of neuromast, surround the sensory and supporting cells and separate the neuromast from surrounding tissue (Münz, 1989).

Neuromasts are innervated by both afferent and efferent fibers (Fritzsch, 1989). Both types of nerve endings contact the basal portion of hair cells. Afferent endings, which are relatively abundant, contain few vesicles, but numerous microtubules, microfilaments and mitochondria. The cytoplasm of the hair cells innervated by these fibers contains a dense synaptic body at the area of synaptic contact between nerve ending and hair cell. Each afferent fiber branches to innervate hair cells having the same orientation of ciliary bundles (Russell, 1976). These fibers transmit sensory information to the central nervous system. The other type of nerve endings, the efferent, are smaller and not as common as the afferent endings. Efferent endings have numerous vesicles, both clear and "dense-core", inside the nerve ending (Flock, 1967) and the hair cell with which they make synaptic contact contains a flattened, membranous sac, the subsynaptic cisterna, in the adjacent cytoplasm. The action of efferent stimulation can lead to inhibition of the nerve discharge, reducing hair cells sensitivity and thus protecting hair cells

from over stimulation caused by the fish's own movements (Roberts and Meredith, 1989). In bony fish, information from neuromasts on the head is carried into the central nervous system by dorsal and ventral branches of the anterior lateral line nerve (ALLN), and information from more caudal regions is carried by the posterior lateral line nerve (PLLN). In some species, there is an additional middle lateral line nerve, e.g. the gar (Song 1991). Mechanoreceptive lateral line fibers terminate in both the medulla and cerebellum (McCormick, 1989).

Sculpins, demersal fish of the Cottidae family, are found from shallow stream water (<1M) to the depths (322M) of Lake Superior (McPhail and Lindsay, 1970). Four sculpin species are present in Lake Michigan, segregated according to depth. *Cottus bairdi* is the shallowest living species, living at <1 to 10M (Hoekstra, 1984). The lateral line system of the mottled sculpin, *Cottus bairdi*, like that of most bony fish, consists of both superficial and canal neuromasts, which are distributed on head and trunk of the fish (Janssen *et al.*, 1987) (figure 1). The superficial neuromasts, which are located directly on the surface of the skin, are much smaller and more symmetrical. The canal neuromasts, which are situated within subdermal, fluid-filled lateral line canals are larger and asymmetrical (Janssen *et al.*, 1987). These canals communicate with outside environment through a pore system in which a single neuromast

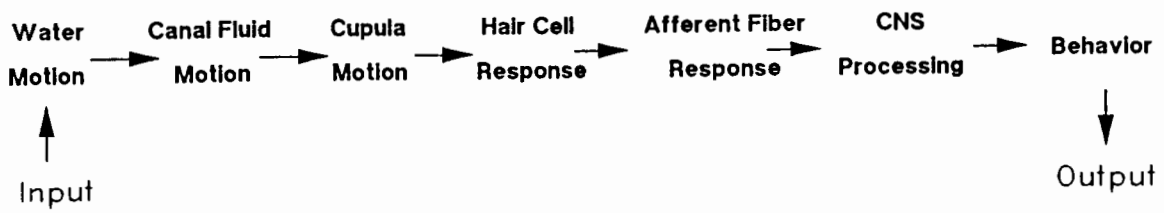
is usually situated between two adjacent pores. The neuromasts found in mandibular canal on the head (100mm standard length fish) are the largest neuromast (about 0.06mm) of the entire system and have the largest number of hair cells (about 700 hair cells per neuromast), whereas those on the trunk are smaller (0.015mm) and have the smallest number of hair cells (about 200 hair cells per neuromast) (Janssen *et al.*, 1987). The head has a higher density of neuromasts than does in trunk. Superficial neuromasts are the only mechanoreceptor found in the tail region.

The ultrastructure of neuromasts in the mottled sculpin is similar to that in other fish. The neuromast consists of sensory hair cells, supporting cells and mantle cells, and a cupula which overlies the apical surface of the neuromasts. Lateral line nerve fibers, both afferent and efferent, make synaptic contact with the basal portion of the hair cells (Jones *et al.* 1989). These investigators found that afferent and efferent ending have the same morphological characteristics as described in other fishes (see above). Some of the myelinated nerve fibers, in both superficial and canal neuromasts, terminate their myelination just before crossing the basal lamina and some keep their myelin sheath all the way to the base of the hair cells in the upper third of the neuroepithelium.

Behavioral experiments have shown that the mottled sculpin is not uniformly sensitive to hydromechanical

stimulation along its body, although the mechanoreceptors extend nearly the entire length of the fish. Behavioral results indicate that the threshold distance for detection of *Daphnia* positioned directly in front of the snout, where there are no canal neuromasts, is significantly less than for prey detected elsewhere along the head and trunk (Hoekstra and Janssen, 1986). Similar results were obtained for threshold distance for detection levels of a vibrating sphere, where the trunk was found to be significantly less sensitive than the adjacent head regions (Coombs and Janssen, 1989). Thus the lateral head has the highest sensitivity while the tail region, where there are no canal neuromasts appears the least sensitive of the whole body (Coombs and Janssen 1989).

Responsiveness to a given stimulus could be affected at several levels, as is indicated in the following diagram.



For purposes of discussion, these levels can be grouped into three broad categories: (1) gross biomechanical considerations, which includes the effects of factors such as canal geometry and cupular properties, (2) factors originating in the neuromast proper, including basic physiological

properties of hair cells *per se* as well as interactions between hair cells and their innervating nerve fibers, and (3) processing within the central nervous system. These categories are indicated on the diagram above.

Both canal width and the compliance of the material of which the canal is constructed can be shown to affect the output from a neuromast. Based on physical and mathematical modeling by Denton and Gray (1988, 1989), it has shown that at low frequencies the sensitivity of a rigid (bony) canal will increase as canal width increases. Moreover, if the canal is more compliant i.e. covered with soft tissues (as is often the case) rather than rigid, sensitivity can be further increased through resonance. Indeed, based on both measured and calculated values, Denton and Gray (1988) have estimated that *Poromitra*, a fish with wide, compliant canals would be about 8 times more sensitive to a given stimulus than would a fish with smaller, rigid canals. With respect to the cupula, van Netten (1991) has shown that the overall response of canal cupulae to fluid movement depends on a number of factors, including the stiffness of the cupula, which depend primarily on the number of hair cell bundles, and the overall mass of the cupula.

Within the neuromast itself, a variety of factors can considerably affect its response to stimulation. For example, hair cells in most vertebrate inner ear systems are markedly heterogenous with respect to their constituent hair cells.

Hair cells can be divided into two types, Type I and Type II, according to the morphology of hair cell and their innervating terminals, e.g. in the cristae of the guinea pig (Wersäll, 1956). Type I cells have a flask-shaped body with a broad, rounded basal region, a narrow neck, and a flared apical region. Their entire basal and neck region is encased in one afferent synaptic terminal (Ades and Engströ, 1965; Jørgensen and Andersen, 1973). Type II hair cells are columnar or ovoid with a relatively flat apex, are contacted by afferent and efferent nerve fibers, both of which usually terminate in small boutons that cover only part of the lateral and basal surface area of the hair cell (Wersäll and Bagger-Sjöbäck, 1973). Type II cells, which are the more common of the two types, are found in all inner ear organs. The Type I cells are widely found in mammalian and avian vestibular organs. These two types of hair cells may have different physiological properties. In the mammalian cochlea, two such distinct morphological populations of hair cells exist, the inner and out hair cells. Aside from gross morphological differences, these 2 population form qualitatively and quantitatively different associations with their innervating nerve fibers. The hair cell:afferent fiber ratio, in inner hair cells is 1:20-30 in the cat and 1:8-24 in the greater horseshoe bat ((Bruns and Schmieszek, 1980; Liberman, 1980), In outer hair cells, this ratio is 10:1 in the cat and 6-12:1 in guinea pig (Kimura 1975). Both inner and outer hair cells are also

innervated by efferent fibers. In the inner ear of teleost fish, it was long believed that there was only one kind of hair cell, the Type II hair cell (Wersäll, 1960). However, Hong *et al.* (1991) and Popper *et al.* (1993) have provided new evidence that both Type II hair cells and Type I-like hair cells exist in the inner ear of the oscar, *Astronotus ocellatus*. Such heterogeneity has not yet been reported for the hair cells of the lateral line system. Were this to be the case, however, we might speculate that the hair cell type *per se* could affect sensitivity of the lateral line system.

Sensitivity may also be affected by the number of hair cells converging onto a single afferent nerve fiber, i.e. the degree of convergence. Thus, fibers innervating neuromasts with large numbers of hair cells, such as the large mandibular canal neuromasts, may contact more hair cells than fibers contacting trunk neuromasts, the smallest canal neuromast in the system. This higher convergence of hair cells onto their innervating fibers might lead to a higher sensitivity through spatial summation of the postsynaptical potential given by individual hair cells. Thus, for a stimulus of a given amplitude, afferent fibers receiving a greater amount of convergence would be more strongly depolarized via postsynaptical spatial summation than would a nerve fiber receiving little convergence. The result in a greater sensitivity of afferent nerve fibers innervating these

neuromasts.

Finally, if we consider the ratio of hair cells to their innervating fiber as a factor of peripheral neural circuitry, central convergence of afferent fibers projecting onto central neurons may also be a factor affecting the overall sensitivity, although evidence for this hypothesis has not been demonstrated.

The behavioral response of *Cottus bairdi*, in which the head is more sensitive than the trunk, might be affected at any of these levels, individually or in combination. The purpose of this study is to test one of these possible levels, specifically that there might be higher convergence of hair cells to nerve fibers in head canal neuromasts than in the trunk.

CHAPTER II

MATERIAL AND METHODS

Twelve mottled sculpins, *Cottus bairdi*, ranging in size from 51 to 80mm, standard length (SL) were collected by SCUBA diving in Lake Michigan near Chicago. Fish were maintained in aquaria at 14-15°C on a 12:12 hour light:dark cycle, and fed live *Daphnia* and pieces of frozen squid.

Prior to fixation, fish were anesthetized with an overdose (>25mg/L) of tricaine methanesulfonate (MS-222) until respiratory movements ceased and then immersed in modified Karnovsky's fixative (2% paraformaldehyde, and 2% glutaraldehyde in 0.2M sodium cacodylate buffer at pH 7.4) for 24 hours at 4°C. Small tissue pieces containing canal and superficial neuromasts from both head (mandibular canal and the mandibular line of superficial neuromasts) and trunk (anterior parts of trunk canal and their accessory superficial neuromasts) were dissected from the surrounding tissue and left in the same fixative at 4°C until further processing. Mandibular canals, which are in part surrounded by bone, were decalcified using "D.calcifier" (Lerner Laboratories; active ingredients: hydrochloric acid, colloid stabilizer and surfactant) for 48 hours at room temperature. Alternatively,

neuromasts from both the mandibular and trunk canals from an additional four fish were decalcified using 5% saturated EDTA at 4°C for four weeks, with a change of solution every three day after primary fixation (see results). Prior to dehydration, all specimen were washed in 0.2M sodium cacodylate buffer at pH 7.4 and post-fixed for one hour in 2% osmium tetroxide (OsO4). Dehydration was carried out through increasing concentrations of acetone (30%, 50%, 75%, 95%, 100% X 3) with each step lasting five minutes. Following initial dehydration in 30% acetone, tissues were stained *en bloc* with 2% uranyl acetate in 30% acetone for one hour. Final infiltration and embedding was in EPON 812, following conventional protocols. In order to facilitate orientation, tissues were pre-embedded on flat molds, cut from these molds and then post-embedded in BEEM capsules (Ted Pella, Inc.) such that sections could be cut perpendicular to the long axis of the hair cells.

For scanning electron microscopy, selected neuromasts were fixed and dehydrated as above and dried in a solution of Peldri II (Ted Pella, Inc.), the canal were dissected open and then shadowed with 500Å of gold-palladium.

Each neuromast was serially sectioned from its apical surface to 30 to 50μm deep into the subjacent connective tissue, (i.e. in a plane parallel to the skin surface), thus generating sections not only of the hair cells but also of the fibers that innervate them. Studies in our laboratory on

sculpins as well as those on the ratfish, *Chimaera monstrosa* (von Lubitz, 1981) have indicated that most fiber branching occurs close to the neuromast i.e. within the neuromast proper or just beneath the basal lamina. In our preliminary studies, nerve fiber number decreased rapidly with distance below the basal lamina and then remained relatively constant in the range of from 30 to 50μm below it, (e.g. in a typical trunk canal neuromast, more than 70 fibers are present about 5μm below the basal lamina, 40 fibers at 25 μm below it, 30 fibers at 35μm and 30 fibers at 50μm). Therefore, counts of nerve number made in this range more accurately reflect the number of nerve fiber innervating a specific neuromast prior to any ramification. Hair cells and myelinated nerve fibers were counted using semi-thin (0.5μm) sections of material stained with 1.5% toluidine blue in sodium borate viewed under light microscope. Hair cells were distinguished from surrounding supporting and mantle cells based on (1) location within the neuromast and (2) cellular and nuclear morphology. Initially, light microscope identification of hair cell identity was confirmed using sequential semi-thin toluidine blue stained sections and thin (60-70nm) sections stained with lead citrate and uranyl acetate, and examined with a JEOL 1200 EX transmission electron microscope.

Nerve fiber diameters were measured using the Bioquant Image Analysis system and a digitizing pad (R & M Biometrics) on the same sections from which number of nerve fiber was

determined. Since not all fibers were cut in perfectly transverse section, the shortest distance within myelin sheath across the axon through the center point was measured. Data thus collected represent the minimum diameter of the fiber.

CHAPTER III

RESULTS

1. Morphology of superficial and canal neuromasts

The findings of the present study are in agreement with those of previous studies with regard to the general morphology and distribution of canal and superficial neuromasts (see review of literature for a comprehensive bibliography). A diagram showing the arrangement of the lateral line system in *Cottus bairdi* based on these studies is shown in figure 1. The most obvious difference between canal and superficial neuromasts are those of shape, size and location. Compared with superficial neuromasts, which are round when viewed from above and are located directly on the surface of the skin (figure 2), canal neuromasts are elliptical and situated on the bottom of subdermal canals (figure 3). Moreover, the number of hair cells present in the sensory epithelium of superficial neuromasts is much less than that of canal neuromasts, e.g. mandibular canal neuromasts have an average of 520 hair cells, whereas trunk canal neuromasts have about 224; for mandibular line superficial neuromasts and trunk accessary superficial neuromasts these values are 51 and 46, respectively. (details, see below). In

light microscopic observation of plastic semi-thin sections canal neuromasts have their sensory strips parallel to the long axis of the canal, whereas in superficial neuromasts, the axis of the sensory strip is less defined, at least in sectioned material. In both types of endorgan, the innervating lateral line nerve fibers travel through the connective tissue underneath the neuromast and enter the neuromast directly through the basal lamina. Generally, these nerve fibers ramify just before they penetrate the basal membrane, or do so immediately thereafter (figure 4), although individuals may vary slightly in this respect.

Light microscopic observation of serial cross sections of neuromasts (perpendicular to the long axis of hair cell) show hair cells arranged in the apical 2/3 of the endorgan. In superficial neuromasts, hair cell nuclei are located in a cluster in the center of the neuromast (figure 5.), whereas in canal neuromasts, they lie along the long axis of the neuromast to form a narrow band e.g. a sensory strip, down the middle of the neuromast paralleling the canal axis (figure 6). A single round and darkly stained nucleus is present in the basal portion of each hair cell. Within the sensory portion of both canal and superficial neuromasts, hair cells can be subdivided into two types, a "light cell" and a "dark cell", based on the staining of the cytoplasm. There are otherwise no obvious differences between these two types of cells, although in electron microscopy, the plasma membrane of dark

cells has a more irregular outline compared to that of light cells (figure 7). Hair cells can be unambiguously distinguished from the supporting cells in light microscope by their position within the neuromast and nuclear shape. Their nuclei are nearly round in cross section and situated in the central area of the hair cell. When viewed under the electron microscope, hair cell cytoplasm contains numerous vesicles, rough endoplasmic reticulum and mitochondria distributed throughout the cell (figure 8). The supporting cells extend from the basal lamina to the apical surface of the neuromast, surrounding the sensory epithelium as a whole as well as each hair cell individually. Their irregularly shaped nuclei are located on the basal region of the cell (figure 9). A Golgi apparatus is usually found in the apical non-nuclei region of the supporting cell. The rest of the cytoplasm is filled with well developed rough endoplasmic reticulum, in which the width of the cisternal cavity varied widely. Mitochondria are present throughout the cell. Thus, the neuromast as a whole appears in light microscopy to have two nuclear layers, a deep layer with irregular nuclei (supporting cells) and an overlying i.e. more superficial layer of round nuclei (hair cells). between the somatic epidermal tissues and the sensory strip are mantle cells, which have the same general appearance as the supporting cells. Two additional cell types are present in the mantle cell zone. The first type, which is only seen in superficial neuromasts, usually occur in clusters of 2-3

cells. At the light level their cytoplasm is largely unstained (figure 5). TEM studies revealed a lucent cytoplasm containing degenerating organelles (figure 10). It seems unlikely that this degeneration is artifactual since other cell types are well preserved and this cell type has been only observed in superficial neuromasts. The second cell type is found both in superficial and canal neuromast (figure 11), but is seen more frequently in canal neuromasts than in superficial neuromasts. The most striking characteristic of these cells is their size, which is much larger than all other cells in the neuromast. That, and their pale staining make them easy to distinguished in light microscopy. At the TEM level, there are numerous vesicles in cytoplasm, a full complement of organelles and a rough endoplasmic reticulum arranged in elaborate whorls and concentric spirals (figure 12).

Lateral line nerve fibers make synaptic contact with hair cells at their basal portion. In both superficial and canal neuromasts, nerve fibers either maintain their myelination all the way to the base of the hair cells or, in some instances, lose their myelin sheath before crossing the basal lamina (figure 13). This variability was also observed at the TEM level in a study of superficial neuromasts in *Cottus bairdi* (Jones *et al.* 1989).

Because this study is mainly based on light microscopy, it is impossible to distinguish between the afferent and efferent nerve fibers. However, electron microscope

observations made in the course of this study as well as those of Jones *et al.* (1989) provide evidence that both afferent and efferent endings are in fact present in superficial and canal neuromasts of the mottled sculpin (figure 14).

2. Hair cell and nerve fiber counts.

Preparations of neuromasts for both light and transmission microscopes were obtained from 12 mottled sculpins. Superficial and canal neuromasts from both head and trunk were selected from each individual. Based on type and location, neuromasts were divided into four different groups: head superficial (Lm, mandibular line), trunk superficial (Ts), head canal (MD, mandibular canal) and trunk canal (TR) neuromast. Nerve fiber counts were made deep to the basal lamina as shown in figure 15. All specimens were from the left side of the fish.

Data for three dependent variables, i.e. hair cell number, nerve fiber number, and the hair cell/nerve fiber ratio were analyzed as analysis of covariance (ANCOVA). For each ANCOVA, fish length was the covariate and location (head vs trunk) and type (canal vs superficial) were group variables. The interaction between location and type was also included in the model. Preliminary analysis indicated a strong correlation of the variance with means for all three dependent variables. This problem was corrected by using a log transformation of the data for each ANCOVA. The results are summarized in Table 1.

(A). Hair cell number: A total of 9 neuromasts from the mandibular canal was examined. The range in hair cell number was from 240 to 690 with 521 per neuromast the average value (Table 2). Twelve specimens from trunk canal neuromasts were examined. The number of hair cells ranged from 135 to 300 with an average 224 per neuromast (Table 3). Thus, in trunk canal neuromasts, the number of hair cells was less than that of mandibular canals, but still higher than that of any superficial neuromast. Superficial neuromasts from both head and trunk had the smallest number of hair cells in the entire system. Among eleven examined fish, hair cell number ranged from 30 to 70 with an average 50 per neuromast in head superficial neuromasts (Table 4)and 46 per neuromast in trunk superficial neuromasts (Table 5). The ANCOVA indicated no length effect and that the interaction term was significant ($P < 0.001$). It was therefore necessary to compare the means of location X type cell using a Newman-Keuls test. Statistical analysis indicated that canal neuromasts from different locations had statistical differences in the hair cells number, with mandibular canal neuromasts having more hair cells than trunk canal neuromasts ($p < 0.001$). Statistical analysis showed that there was no difference between head and trunk superficial neuromast in this respect ($p > 0.05$). These results are presented in Figure 16.

(B). Nerve fiber number: Lateral line nerve fibers, which innervate both canal and superficial neuromasts, travel

directly through the base of that neuromast into the sensory organ (figure 17). In mandibular canal neuromasts, there are 37 to 73 nerve fibers passing into the sensory epithelium, with an average number of 48.3 fibers. In trunk canal neuromasts, the range is from 25 to 60, with an average 39.7 nerves per neuromast. The ANCOVA indicated no length effect and that the interaction term was significant ($P < 0.005$) and again, the means of location X type cell were compared by a Newman-Keuls test. There was a statistical difference in fiber number between canal and superficial neuromasts ($P < 0.001$), but no statistical differences between mandibular canal and trunk canal ($P > 0.10$), or between mandibular line and trunk accessory superficial neuromasts ($0.05 > P > 0.01$). These results are presented in Figure 18.

(C). Relationship between number of hair cells and nerve fibers in canal neuromasts. The relationship between two variables, hair cell and nerve fiber in both mandibular and trunk canal neuromasts has been statistically analyzed using linear regression. Analysis of Variance (ANOVA) has shown a statistically significant ($P < 0.004$) relation between the number of hair cells and the number of nerve fibers in trunk canal neuromasts, but not between the number of hair cells and number of nerve fibers in mandibular canal neuromasts ($P > 0.55$). A comparison of the slopes of regression line based on the data was not statistically distinguishable (t-test, $0.5 < P < 0.10$) (Figure 19).

(D). Hair cell/nerve fiber ratio: Based on the hair cell and nerve fiber counts, the ratio of hair cell to nerve fiber was calculated. As is shown in Table 2-5 and figure 20, mandibular canal neuromasts had a higher ratio (11:1) than trunk canal neuromasts (5.85:1). Mandibular line superficial neuromasts had a ratio of 3.91 and trunk accessory superficial neuromasts had ratio of 2.70. The ANCOVA indicated no length effect and that the interaction term was not significant ($P>0.15$). Both the location and type factors were significant ($P< 0.001$ for each case) indicating that the head tends to have a higher ratio for both canal and superficial neuromasts than the trunk does and the canal neuromasts tend to have a higher ratio regardless of location. These results are shown in figure 20.

3. Distribution of fiber diameter.

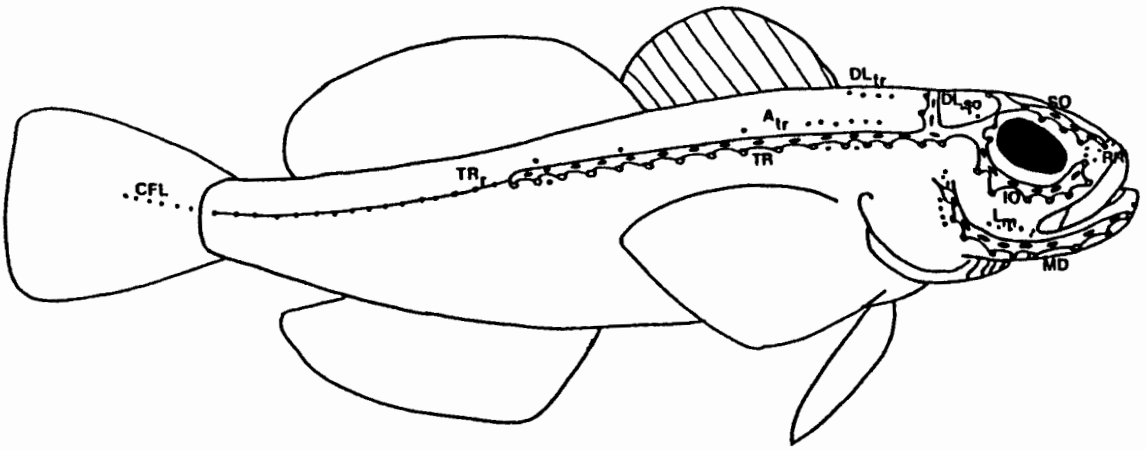
Decalcification of mandibular canal samples were necessitated by the fact that these neuromasts are partially embedded in bone, which must be decalcified prior to sectioning. Significant differences in nerve fiber morphology were observed depending on whether the tissue was decalcified or not.

Initially nerve fibers from all four groups of neuromasts from each of 12 fish were measured. Mandibular canal neuromasts were decalcified using an acid decalcifier. Nerve fiber diameter was measured under light microscope. The distribution of the fibers in the nerves innervating

mandibular canal, trunk canal, mandibular line superficial and trunk accessory superficial neuromasts are shown in figure 21. At first glance, mandibular canal fibers also appeared to have thicker myelin sheaths i.e. more layers. Accordingly, these fibers were examined with TEM to see if this is indeed the case. TEM observation suggested, however, that this increase in thickness was in fact an artifact of acid decalcification as the membranes were grossly swollen with numerous membranous blebs. Non-acid treated fibers did not show such swelling. Accordingly, we repeated a portion of this study using both mandibular and trunk canal neuromasts and a gentler decalcification procedure using EDTA (5%, pH 7.4). Specimens in this experiment were from four additional fish with lengths of 75, 81, 82 and 86mm (SL). ANCOVA statistical analysis showed no length effect on the fiber diameters ($p>0.10$). Under TEM, there was no evidence of the degeneration which occurred with acid decalcifier. The results of the fiber morphology with acid decalcifier and EDTA treatments are shown in figure 22. The distribution from two group are shown in figure 23.

FIGURE 1 Distribution of superficial (SNM) and canal (CNM) neuromasts in the adult mottled sculpin. SNMs are indicated by filled circles, CNMs by filled ellipses, canals by thin lines and their pores by open circles.

CNM	**IO**	infraorbital
	SO	supraorbital
	MD	mandibular
	PRO	preopercular
	ST	supratemporal
	TR	trunk
SNM	**RRso**	
	RFRio	rostral replacements
	CFRio	
	Lm	mandibular
	Lo	opercular
	DLso	supraoccipital
	DLtr	dorsal trunk
	Atr	trunk
	TRr	trunk replacement
	CFL	caudal fin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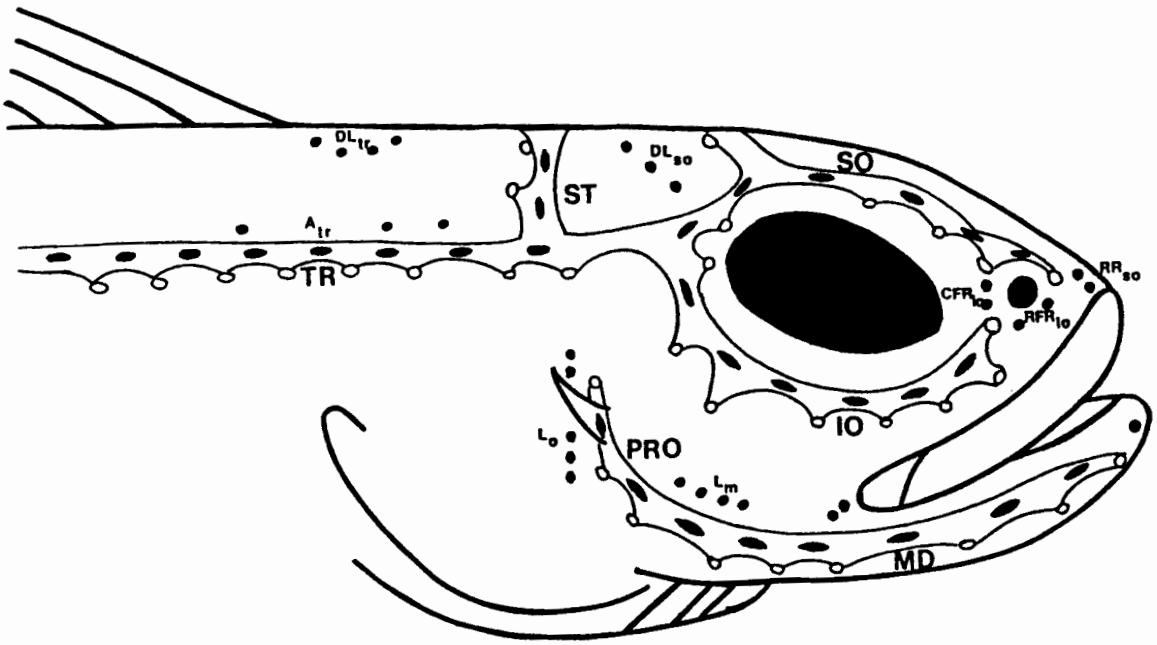
TR_r
CFL
RR
IO
L_m
MD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DL_so
SO
ST
A_tr
TR
RR_so
CFR_io
RFR_io
IO
L_o
PRO
L_m
MD

FIGURE 2 Scanning electron microscope micrograph of superficial neuromast. (a). Arrow indicates a superficial neuromast is surrounded by epithelium (E). Bar = 50μm. (b). Same neuromast as in (a). Whole superficial neuromast is surrounded by epithelium (E). On the surface of neuromast, sensory strip (SS) and zone of mantle cells (MC) can be distinguished. Bar = 10μm.

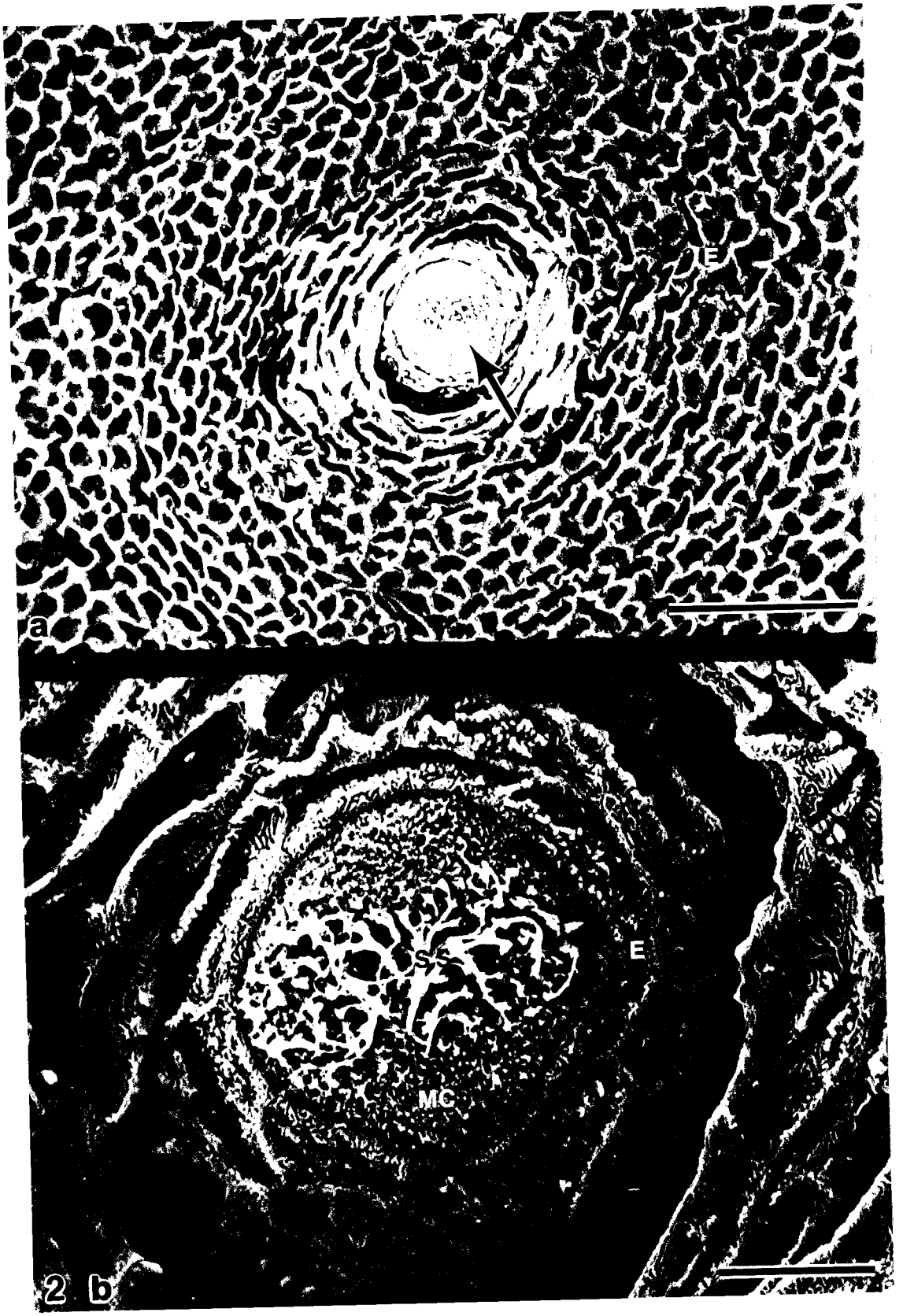

E
a
E
MC
2 b

FIGURE 3 Scanning electron microscope micrograph of a mandibular canal neuromast. (a). A canal neuromast (CNM) is situated on the bottom of the canal (C). Double headed arrow indicates the longitudinal axis of canal. Bar = 50 μm. White block area in (a) is magnified showing in (b). Sensory strip (SS) is comprised of sensory hair bundle (arrow). Mantle cell zone (MC) also can be seen. Bar = 10μm.

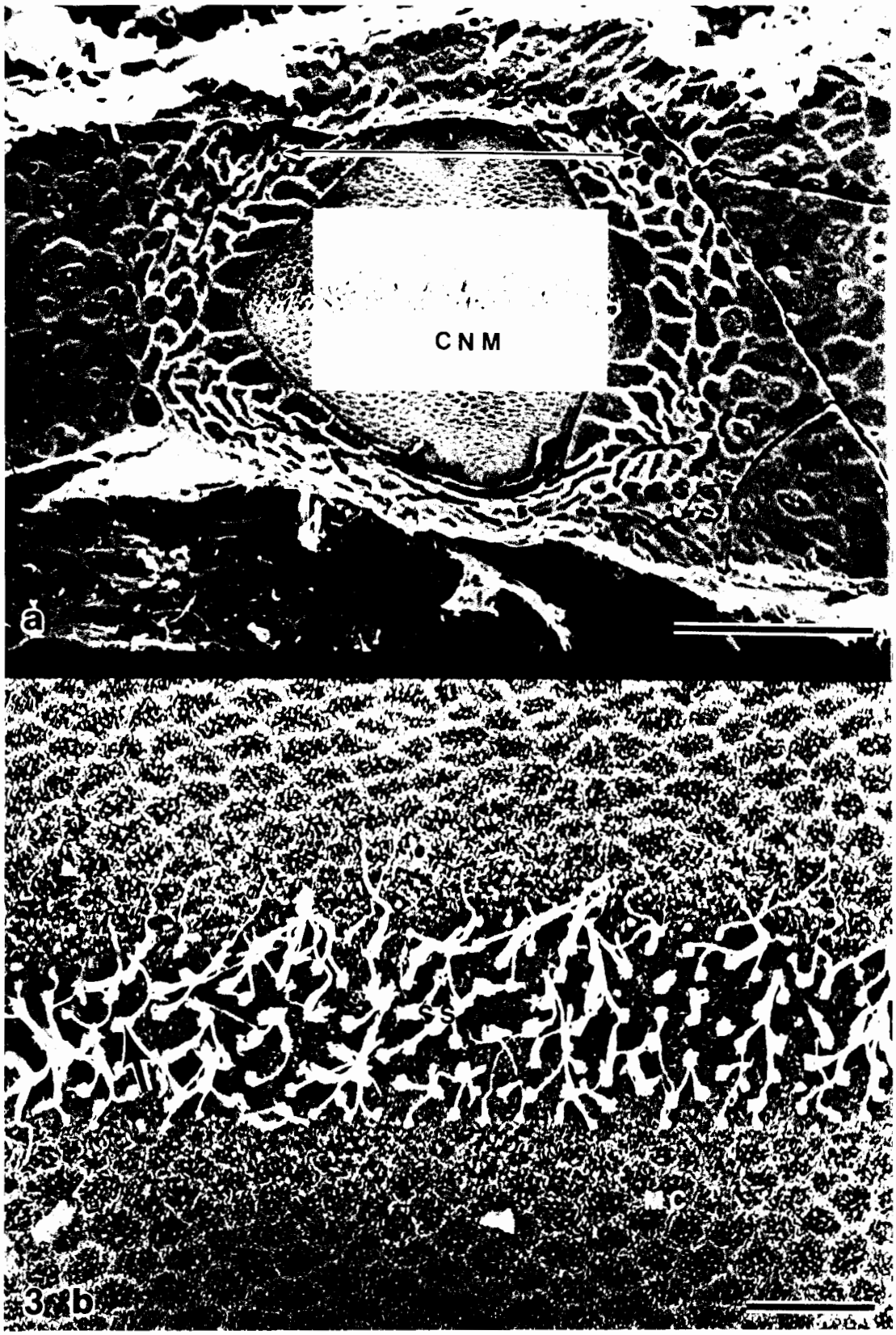

CNM
a
ss
c
3a&b

FIGURE 4 Transmission electron microscope micrograph of the basal portion of a canal neuromast. Lateral line nerve fiber (NF) enters the neuroepithelium (NE) through the basal lamina (BL). Presumptive ramifications are indicated by arrows. Bar = 2μm.

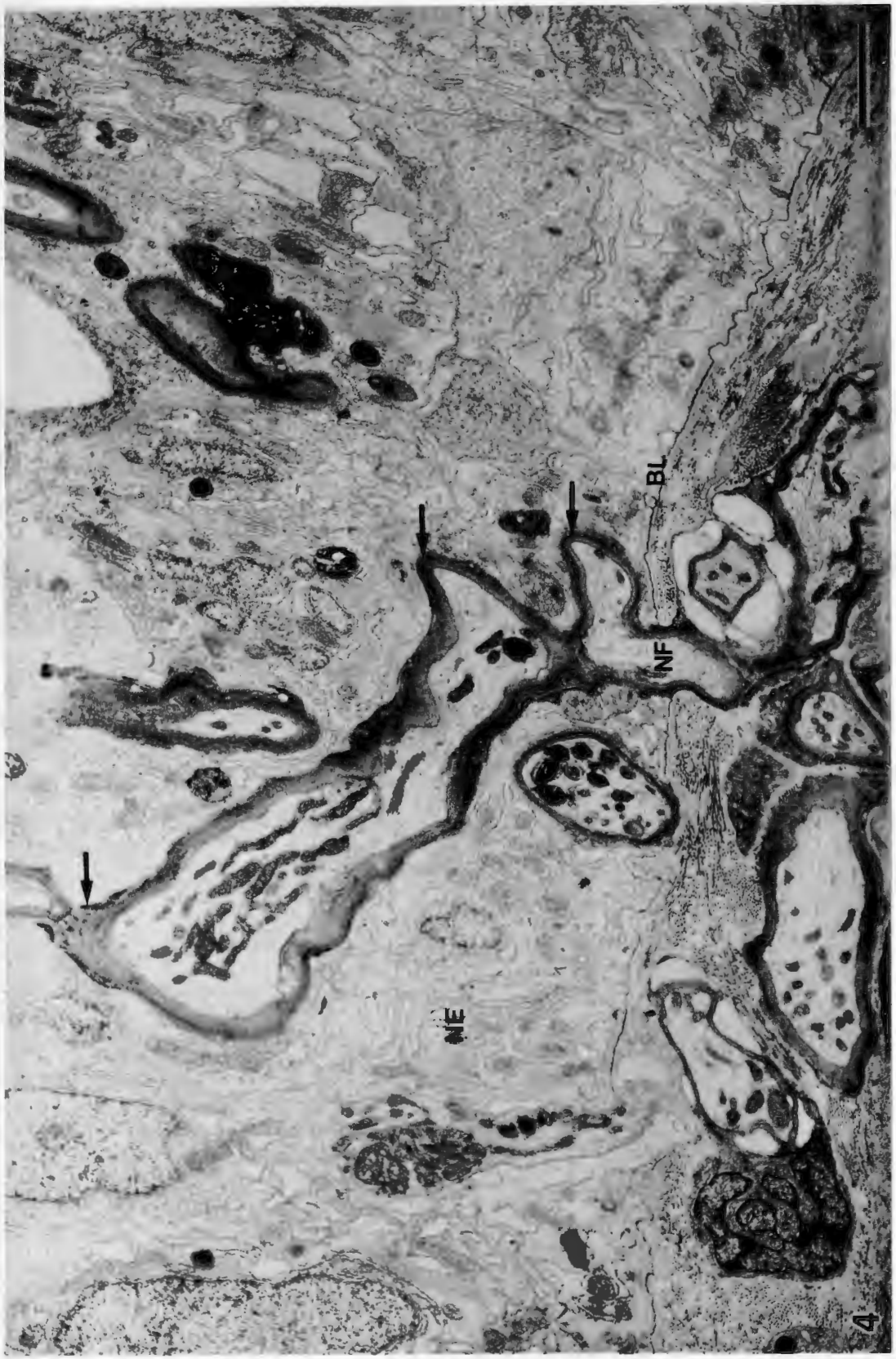
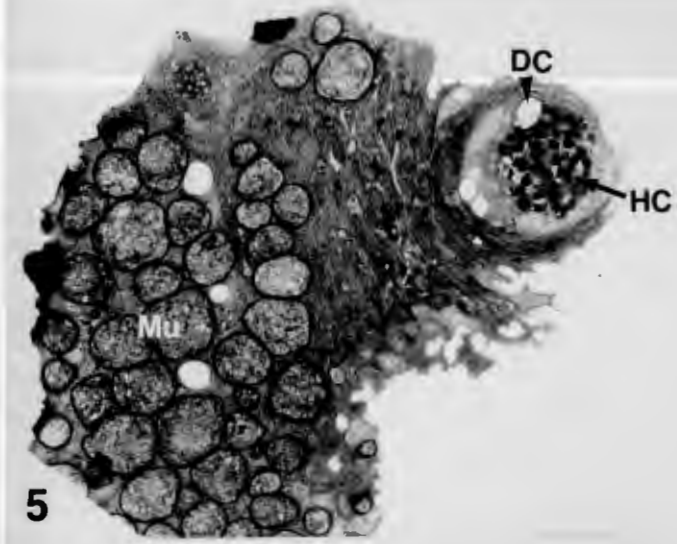
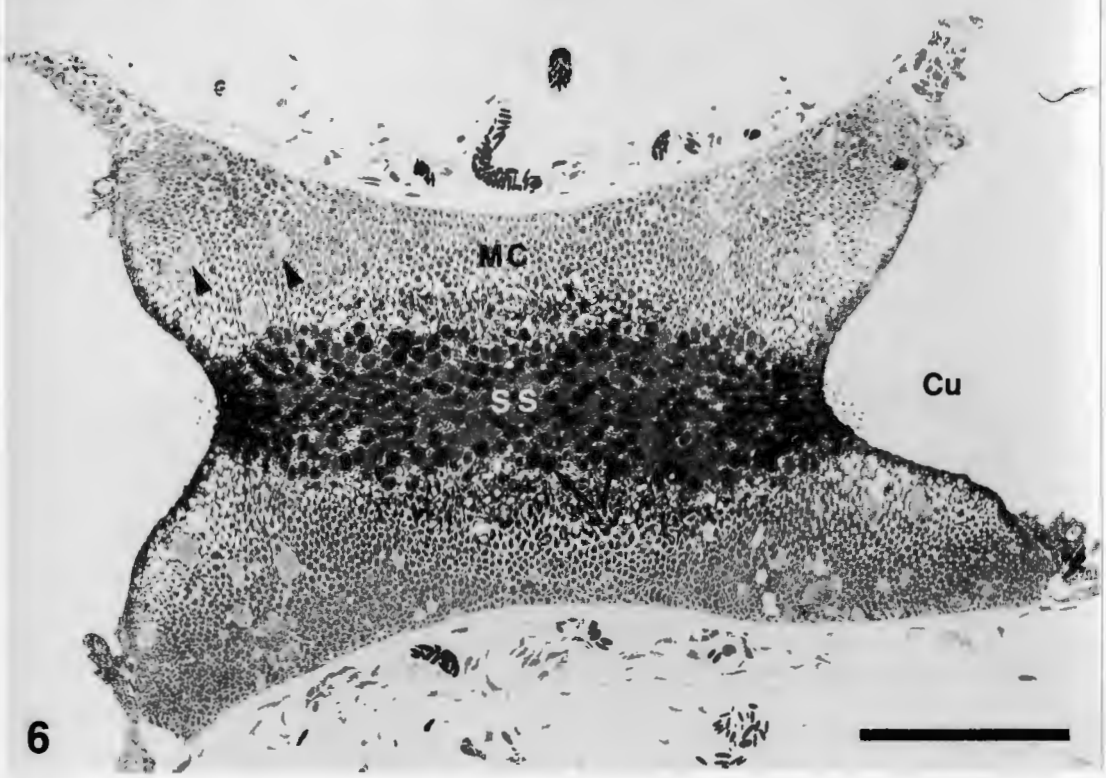

BL
NF
NF
4

FIGURE 5 Photograph of plastic section of a superficial neuromast (SNM). Hair cells (HC) are present in the sensory area. A degenerating cell (DC) can be seen on the margin of the neuromast. Mucous cells (Mu) associated with the epithelium are also shown in photograph. Bar = 100μm.

FIGURE 6 Photograph of plastic section of a mandibular canal neuromast. Hair cells (arrows) comprise the sensory strip (SS), and they are distinguished from mantle cells (MC) and big cells (Arrow heads). Cupula (Cu) can be seen clearly in this photograph. Bar = 100μm.



5



6

FIGURE 7 Transmission electron microscope micrograph of hair cells with different densities. Dark hair cells (DHC) has an relatively irregular outline as compared to light hair cells (LHC). Hair cells with a density intermediate between light and dark hair cells are also present (IHC). Between hair cells are supporting cells (SC). Bar = 2μm.

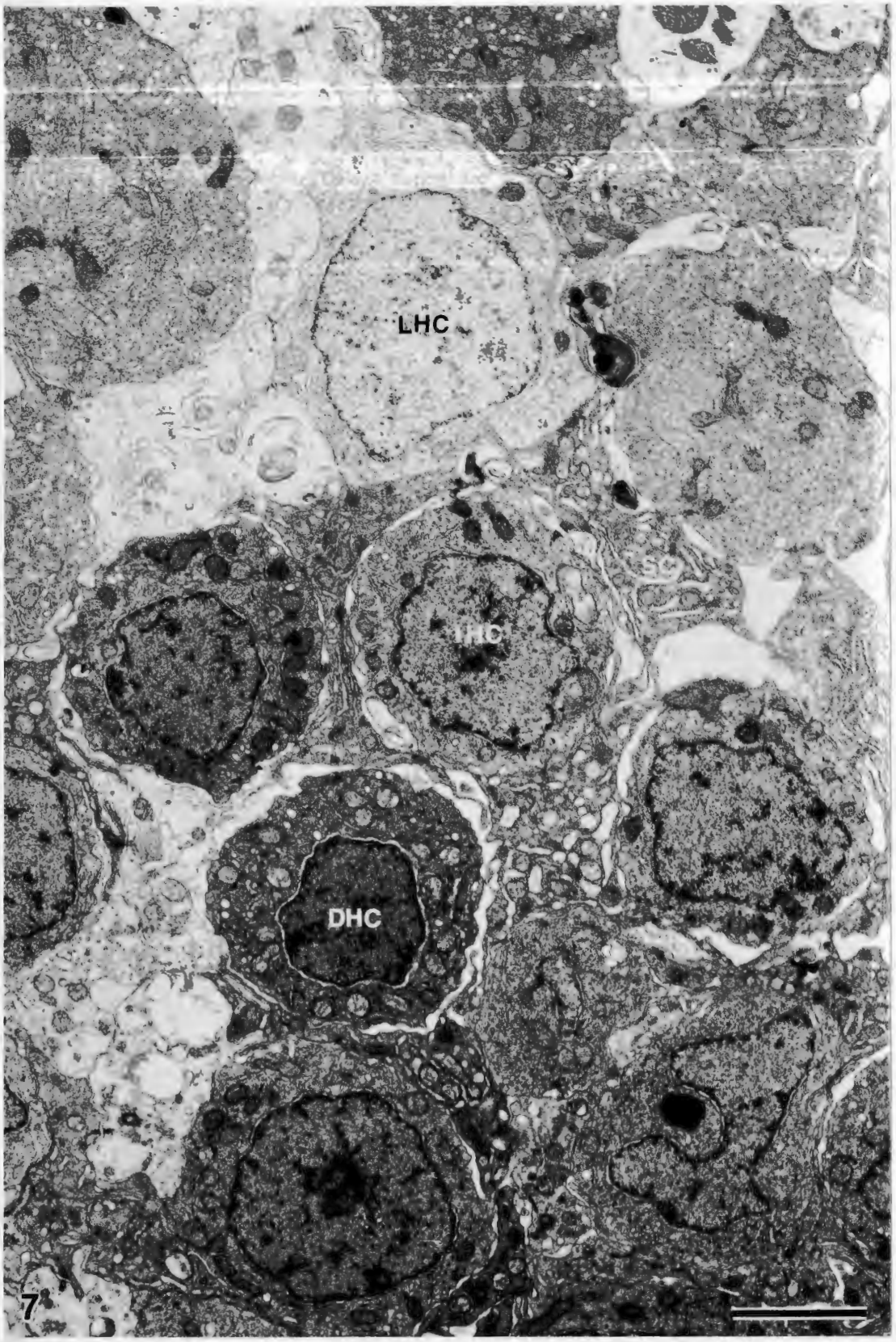
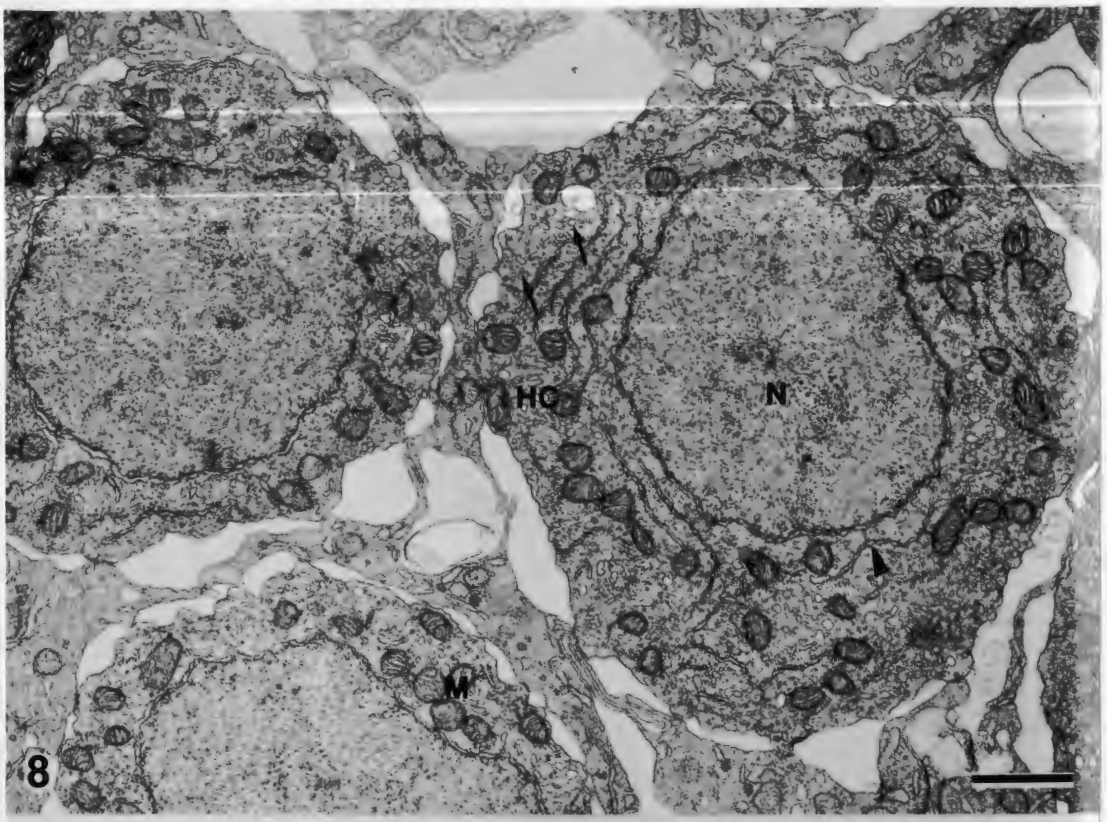
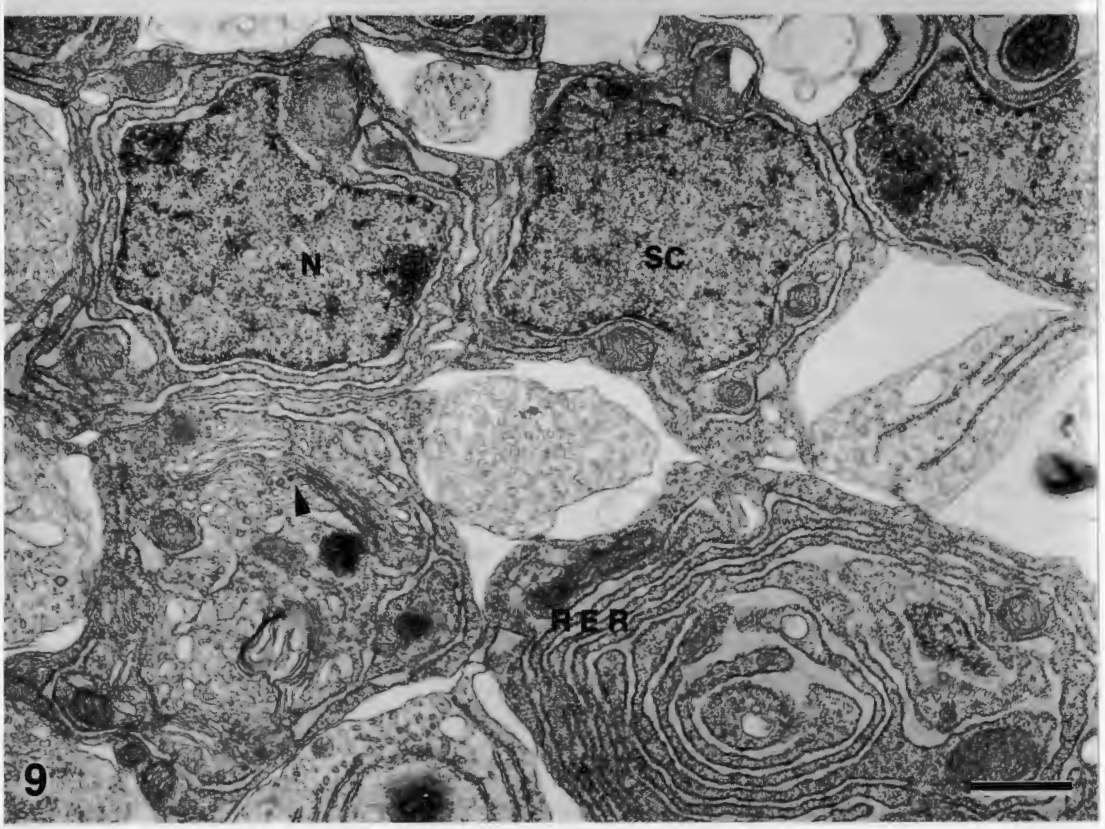

LHC
LHC
DHC
7

FIGURE 8 Transmission electron microscope micrograph of three hair cells (HC). They have round centrally located nuclei (N). Mitochondria (M) and rough endoplasmic reticulum (arrow head) are present in cytoplasm. Numerous membranous vesicles (arrows) are also present in cytoplasm of the cell. Bar = 1μm.

FIGURE 9 Transmission electron microscope micrograph of five supporting cells (SC). Supporting cell has a large irregular shape nucleus (N). Highly developed rough endoplasmic reticulum (RER) and Golgi complex (arrowhead) are present in cytoplasm. Bar = 1μm.



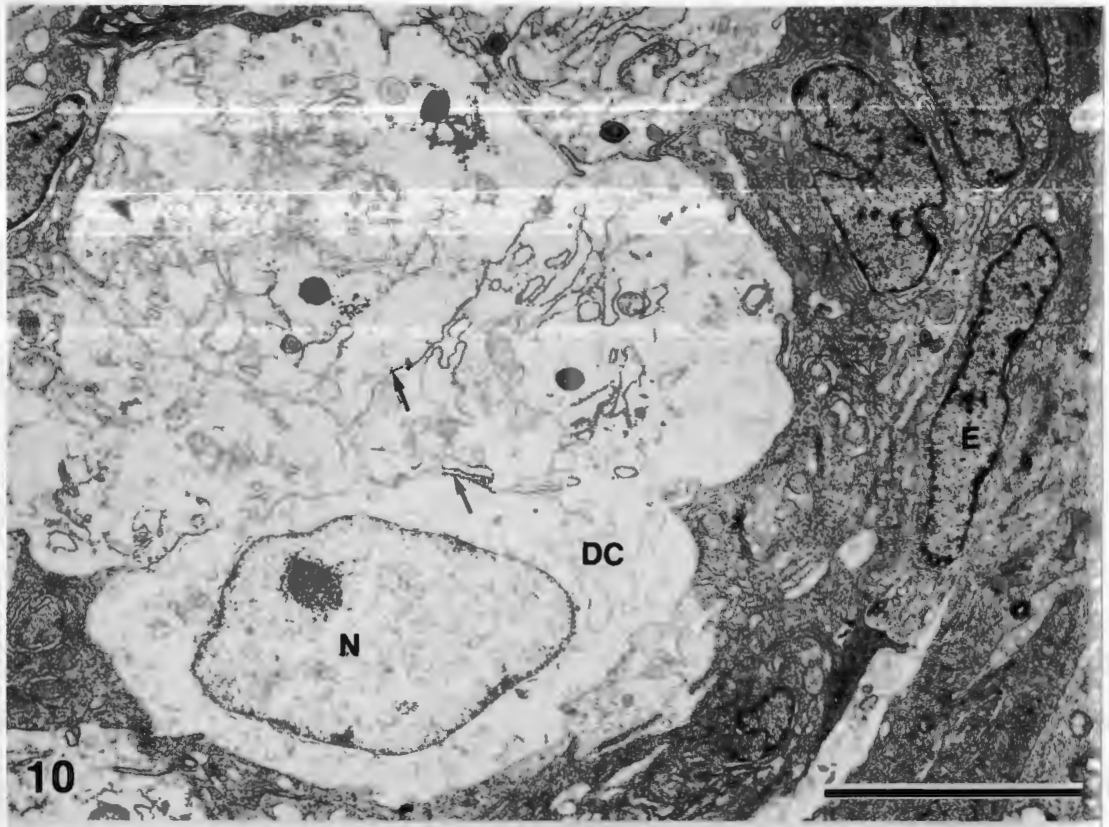
8



9

FIGURE 10 Transmission electron microscope micrograph of a cluster of degenerating cells (DC) found in the outer margins of a superficial neuromast. Four cells are clustered together and can be distinguished by incomplete membrane (arrows). Cytoplasm of cells have degenerating organelles, a nucleus (N) is present in one of these cells. Some epithelia cells (E) are in right side of micrograph. Bar = 5μm.

FIGURE 11 Transmission electron microscope micrograph of a portion of the sensory strip (under dashed line) and mantle cell (MC) zone (above dashed line). Within mantle cell zone, several big cells (BC) are present. Hair cells (HC) are within sensory strip. Bar = 5μm.


E
DC
N
10

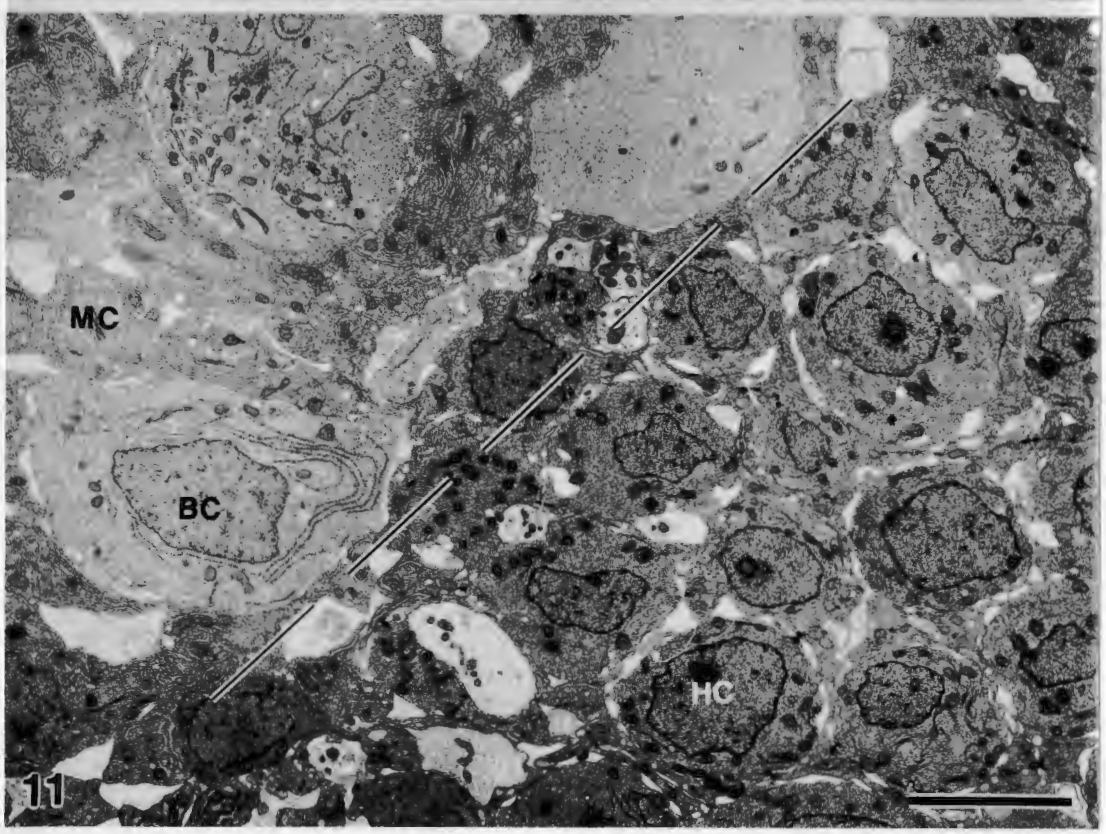

MC
BC
HC
11

FIGURE 12 (a). Transmission electron microscope micrograph of big cell (BC) with an nucleus (N) in mantle cell (MC) zone. Bar = 2μm. (b). Higher magnification of the cytoplasm of the big cell. Rough endoplasmic reticulum (RER) is arranged elaborately in parallel and concentric patterns. Arrows indicate numerous vesicles in the cytoplasm. Bar = 1μm.

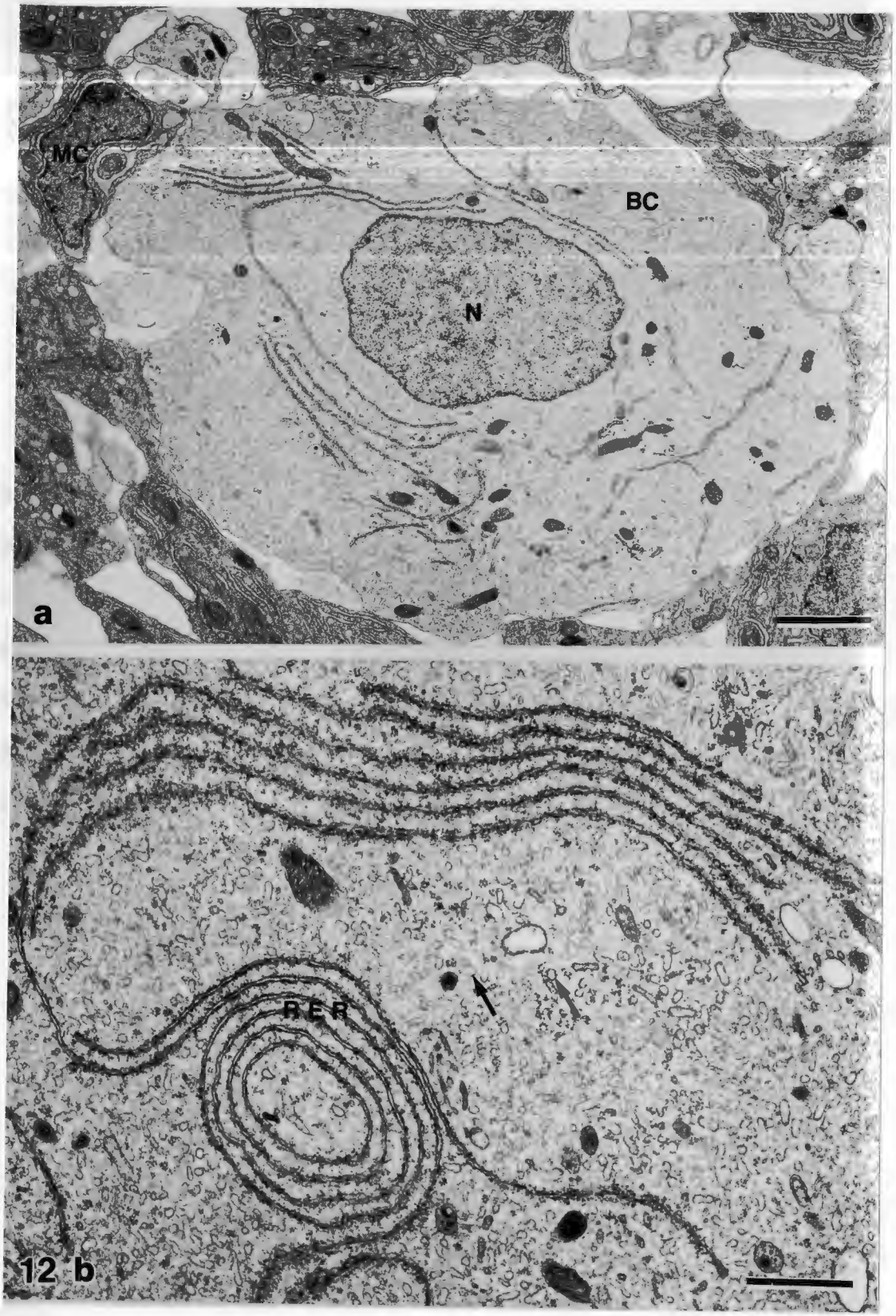

MC
BC
N
a
RER
12 b

FIGURE 13 Transmission electron microscope micrograph of basal end of a neuromast. Two myelinated nerve fibers (MF) are seen beneath the basal lamina (BL). One retains its myelination within neuroepithelium (NE), the other's myelination terminates before traversing the basal lamina at arrow and continues as an unmyelinated fiber (UF) within neuroepithelium. Bar = 500nm.

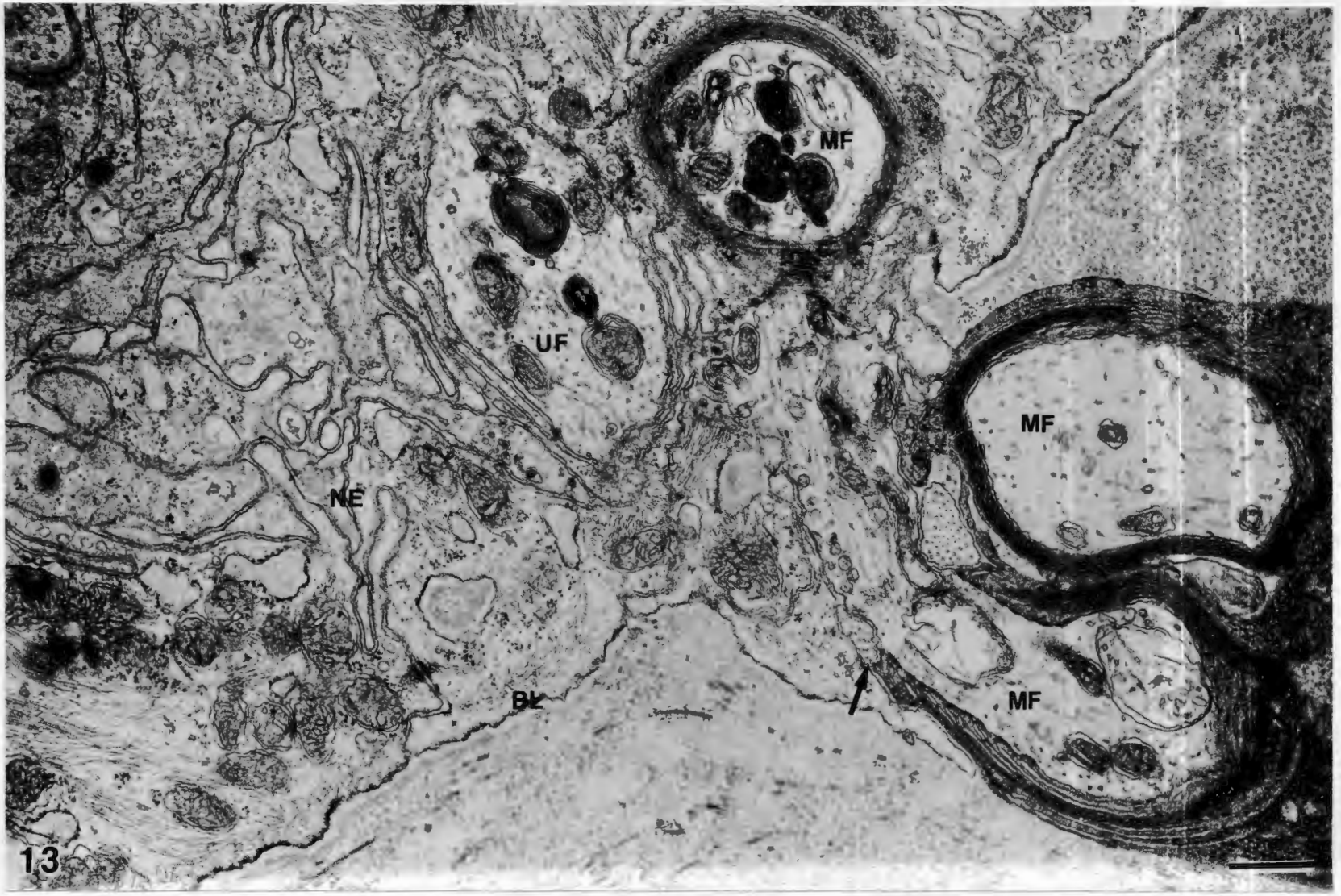
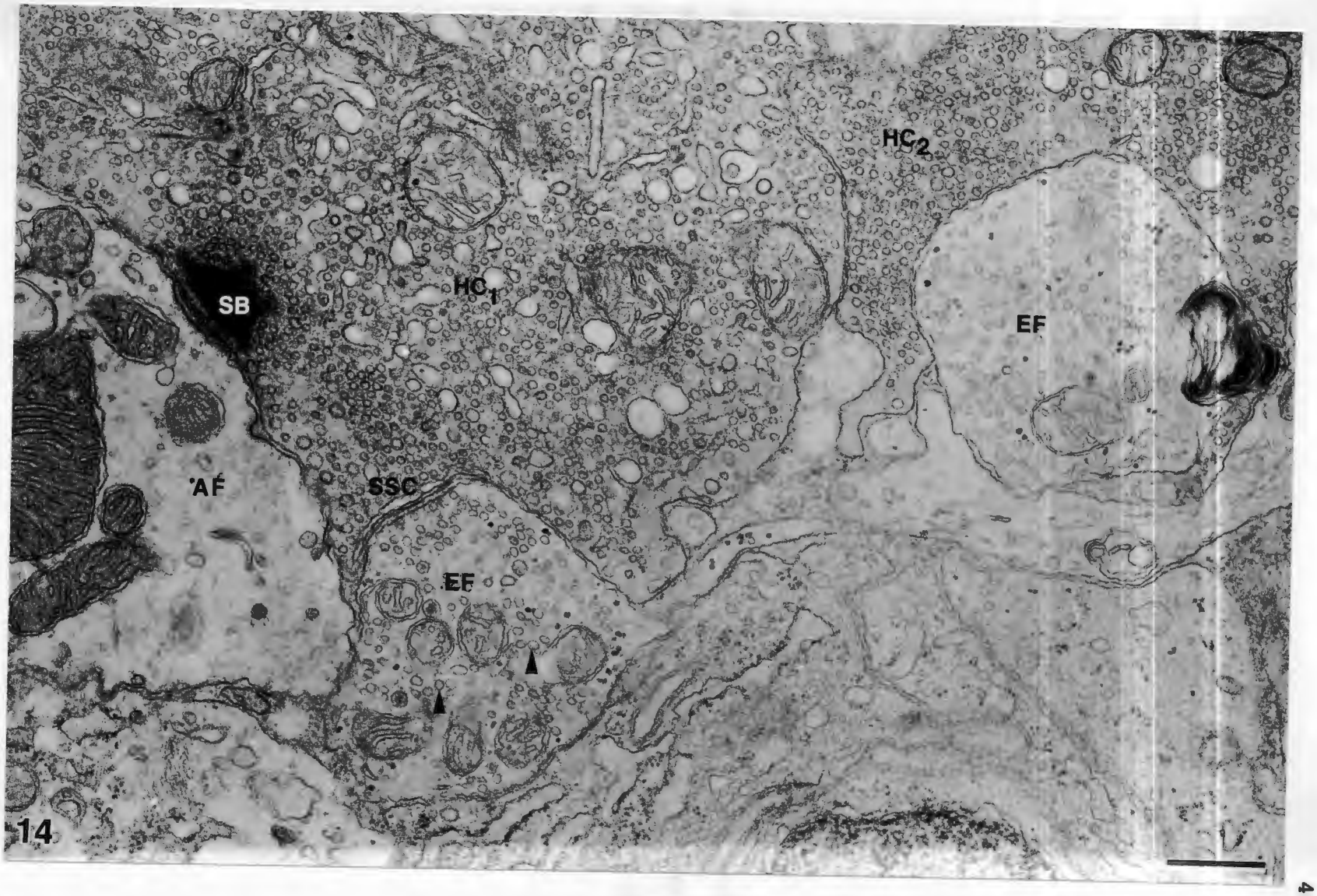

MF
UF
MF
NE
BL
MF
13

FIGURE 14 Transmission electron microscope micrograph of one hair cell (HC_1) contacting with two different types of nerve endings. Afferent ending (AF) has a light cytoplasm and few vesicles; presynaptic body (SB) within the hair cell cytoplasm is surrounded by a corona of synaptic vesicles. Efferent ending has relatively darker cytoplasm and contains numerous synaptic vesicles (arrowhead); subsynaptic cisterna (SSC) within hair cell cytoplasm. A second hair cell (HC_2) with an associated efferent is also present. Bar = 500nm.



14

FIGURE 15 Photograph of a plastic section of lateral line nerve innervating (a) superficial neuromast and (b) mandibular canal neuromast in connective tissue (CT). Arrows indicated single axons with their myelin sheath. Bar = 50 μm.

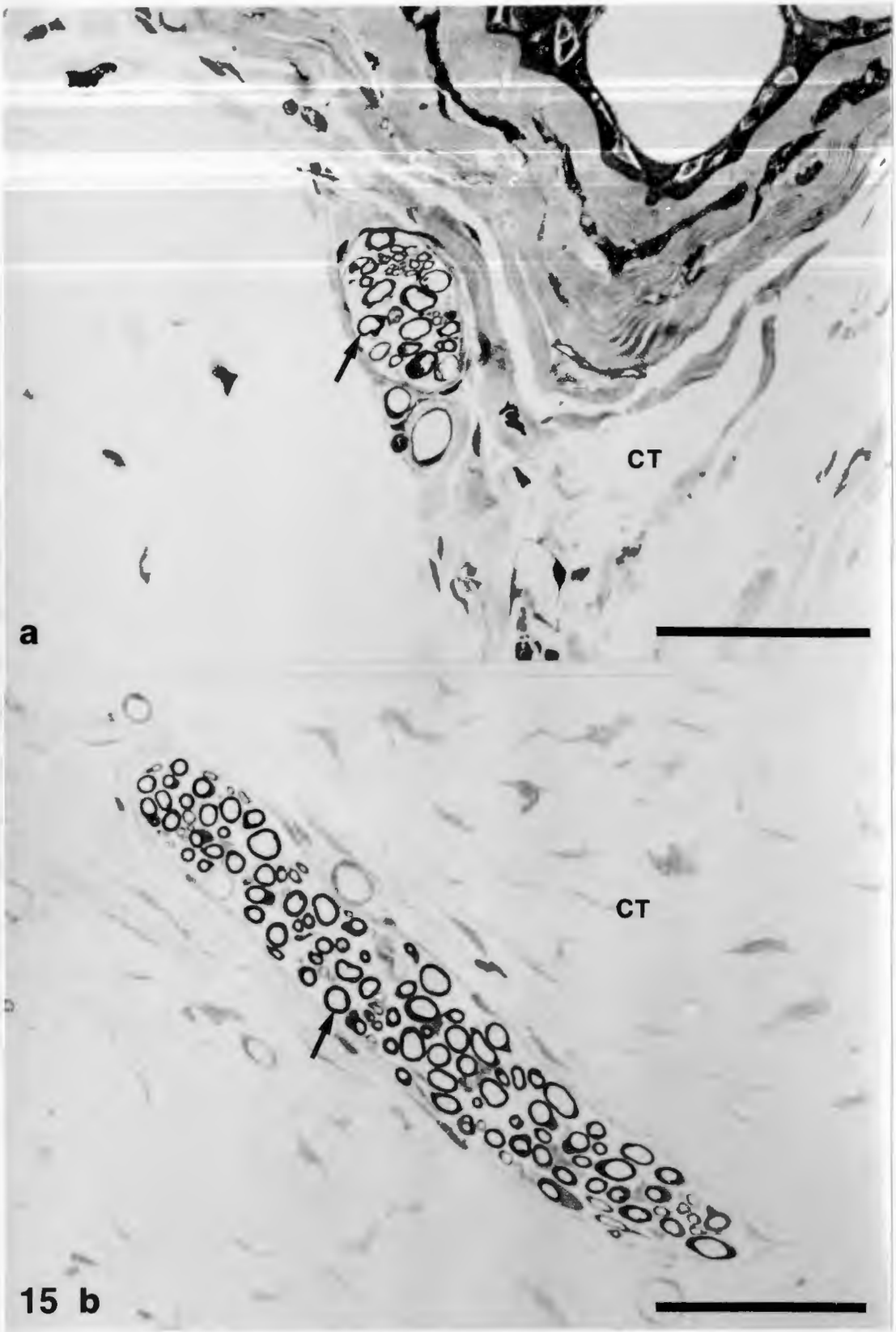

CT
a
CT
15 b

FIGURE 16 Number of hair cells (ln scaled) in four groups of neuromasts. Mandibular canal neuromasts (MD) had significantly higher number of hair cell than trunk canal neuromasts (TR). (Newman-Keuls, test $p<0.001$). Canal neuromasts had significantly higher number of hair cell than superficial neuromasts ($p<0.001$). There was no significant different number of hair cell between head (Lm) and trunk (Ts) superficial neuromast. Underline indicates groups for which there is no significant difference at $0.10>P>0.05$.

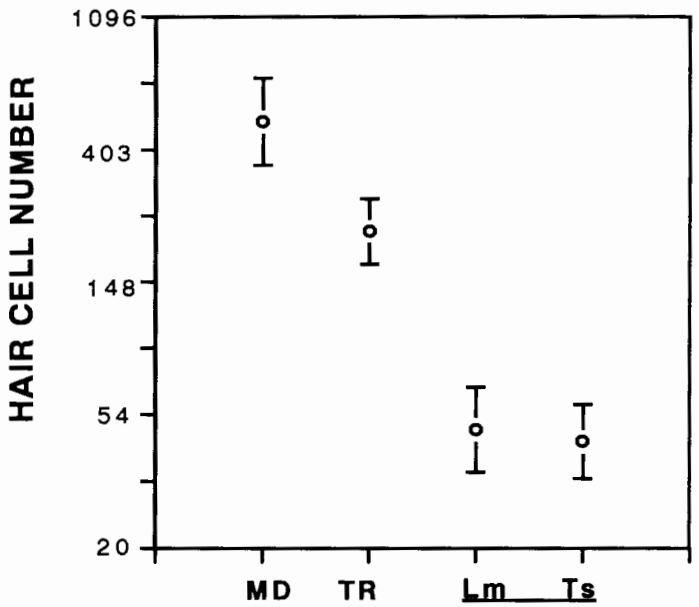

HAIR CELL NUMBER
1096
403
148
54
20
MD
TR
Lm
Ts

FIGURE 17 A lateral line nerve bundle from a canal neuromast viewed in a whole tissue block (a) and in a plastic section (b) from that block. Nerve fibers (NF) travel in connective tissue (CT) below neuromast. Portion of the canal (C) and border of canal (arrowheads) can be seen. Bar = 100 μm.

17 a
NF
c
b
NF
c

FIGURE 18 Number of nerve fiber (ln scaled) in four groups. Canal neuromasts had significantly higher number of nerve fibers than superficial neuromasts (Newman-Keuls test, $P<0.001$). There was no significant difference between mandibular and trunk canal neuromasts ($p > 0.10$) with respect to nerve fiber number. There was no significant different number between head (Lm) and trunk (Ts) superficial neuromasts in this respect ($0.05 > p > 0.01$). Underlines indicate groups for which there are non-significant differences.

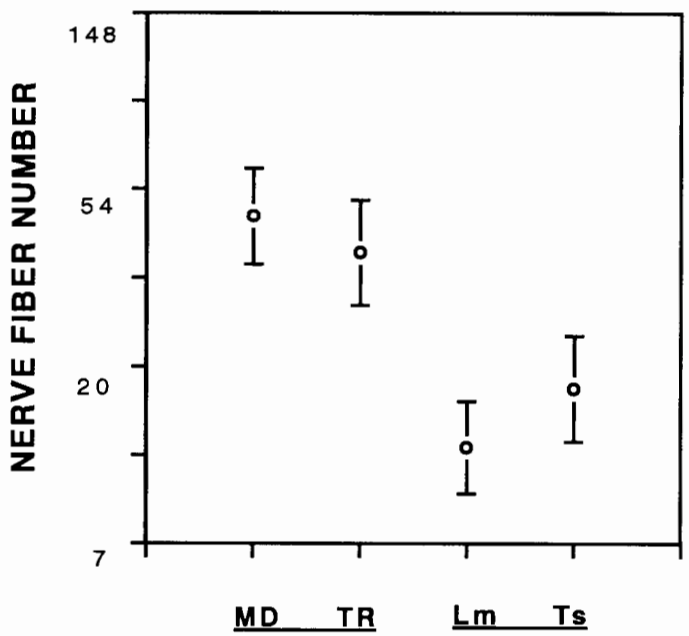

NERVE FIBER NUMBER
148
54
20
7
MD
TR
Lm
Ts

FIGURE 19 Linear regression between number of hair cells and number of nerve fiber in canal neuromasts. Trunk canal neuromasts (TR) had a statistically significant relation between these two variables (ANOVA, $P < 0.004$). Mandibular canal neuromasts (MD) did not show statistical relation between these two variables (ANOVA, $P > 0.55$). Statistical comparison of the two slopes was not statistically significant (t-test, $0.5 < P < 0.10$).

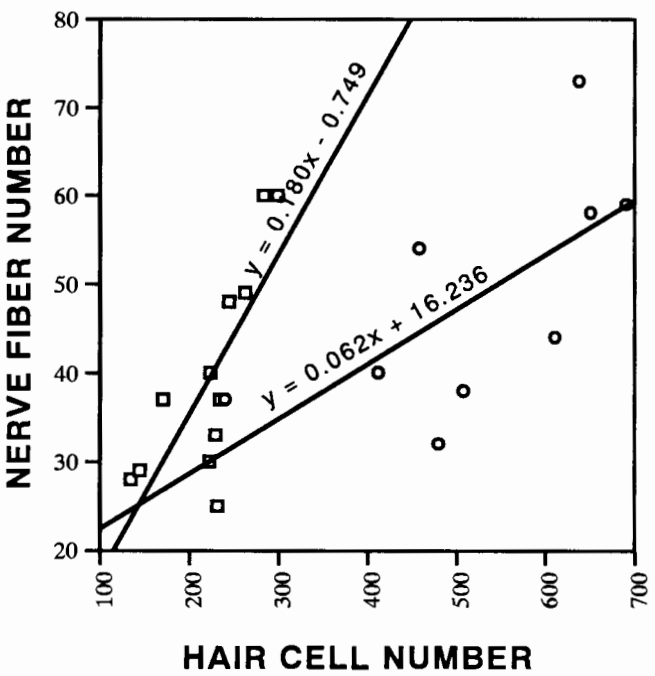

NERVE FIBER NUMBER
HAIR CELL NUMBER
y = 0.180x - 0.749
y = 0.062x + 16.236
80
70
60
50
40
30
20
100
200
300
400
500
600
700

FIGURE 20 Ratio of hair cell to nerve fiber (ln scaled). Mandibular canal neuromasts (MD) had a significantly higher ratio than trunk canal neuromasts (TR). (ANCOVA, $p<0.001$). Canal neuromasts had a significantly higher ratio than superficial neuromasts ($p<0.001$). Mandibular line superficial neuromasts (Lm) a had significant higher ratio than trunk accessary superficial neuromasts (Ts) ($P<0.001$).

CNS = canal neuromast

SNM = superficial neuromast

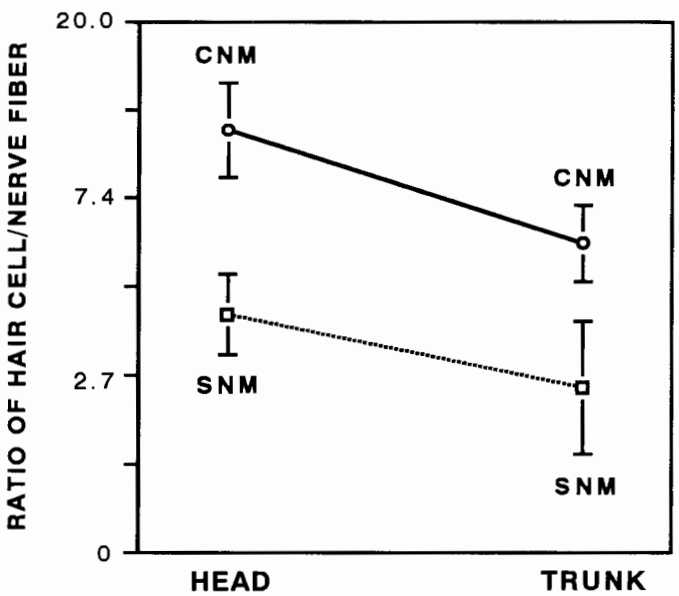

20.0
7.4
2.7
0
RATIO OF HAIR CELL/NERVE FIBER
CNM
CNM
SNM
SNM
HEAD
TRUNK

FIGURE 21 Distribution of lateral line nerve diameter. Abscissa, diameter in μm. Ordinate, frequency as percentage of total.

(a). Trunk canal neuromast (TR), mean of 11 nerves.

(b). Mandibular canal neuromast (MD), mean of 9 nerves.

(c). Mandibular line superficial neuromast (Lm), mean of 12 nerves.

(d). Trunk accessory superficial neuromast (Ts), mean of 12 nerves.

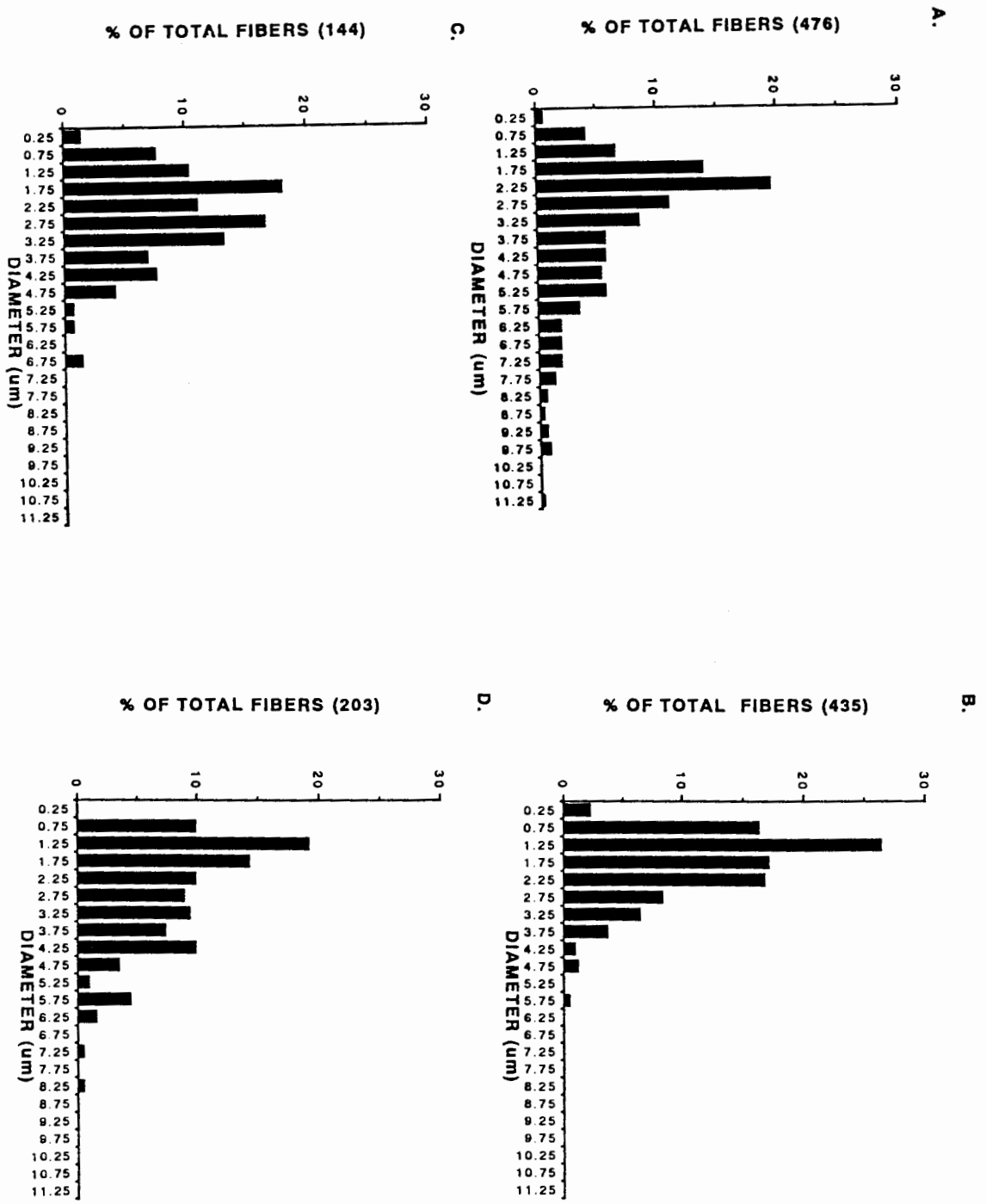

A.
% OF TOTAL FIBERS (476)
DIAMETER (um)
0.25
0.75
1.25
1.75
2.25
2.75
3.25
3.75
4.25
4.75
5.25
5.75
6.25
6.75
7.25
7.75
8.25
8.75
9.25
9.75
10.25
10.75
11.25
0
10
20
30
C.
% OF TOTAL FIBERS (144)
B.
% OF TOTAL FIBERS (435)
D.
% OF TOTAL FIBERS (203)

FIGURE 22 Transmission electron microscope micrograph of lateral line nerve fibers. (a). Nerve fiber (NF) processed without decalcifier, Myelin sheath (MS) did not show any degeneration. (b). Mandibular canal neuromast processed with acid decalcifier. Axon (Ax) has been compressed by swelling of the myelin sheath (MS) which had numerous blebs (arrow). (c). Mandibular canal neuromast decalcified with EDTA. Nerve fiber (NF) with myelin sheath did not show any degeneration or axon compression. Bar = 2 μm.

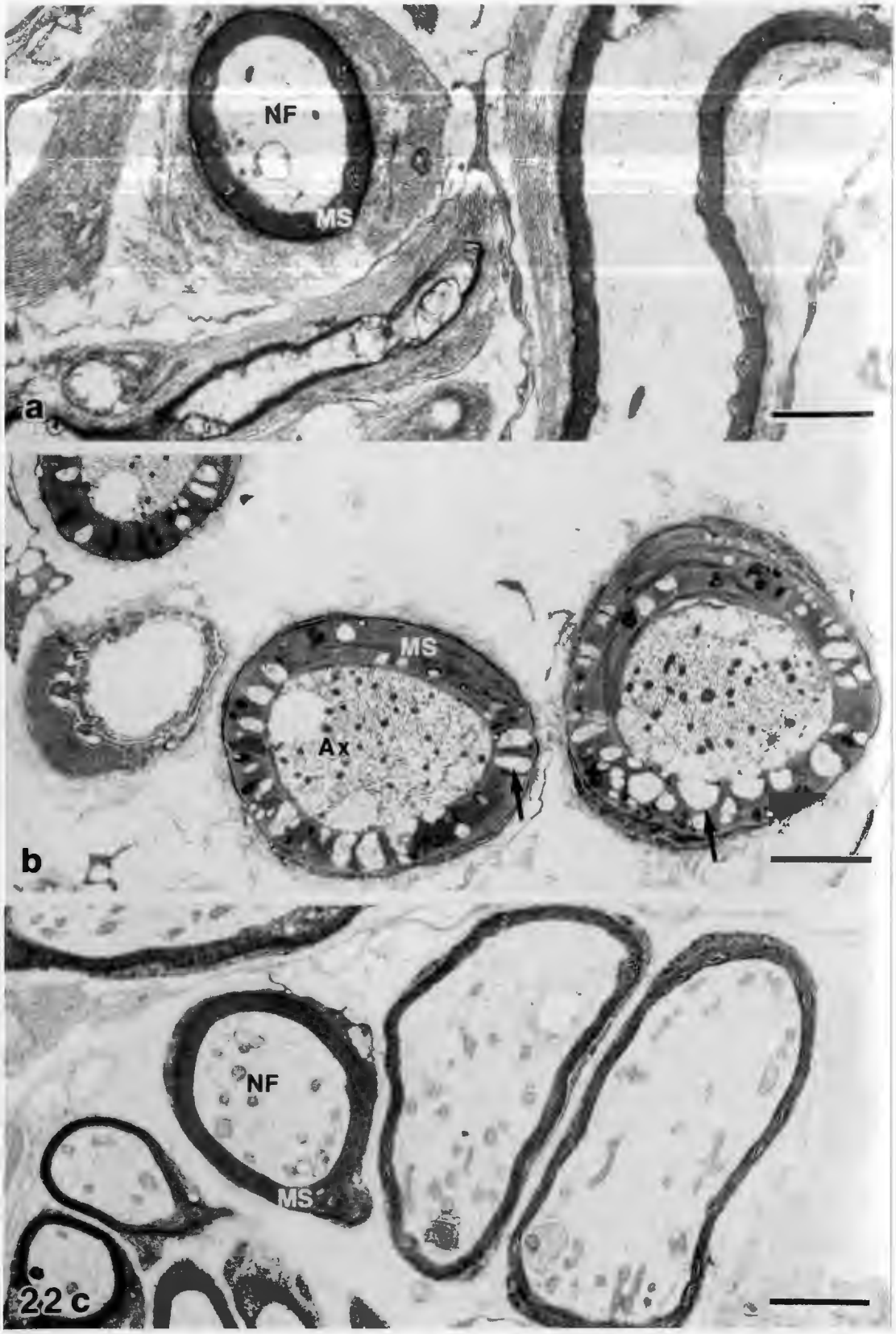
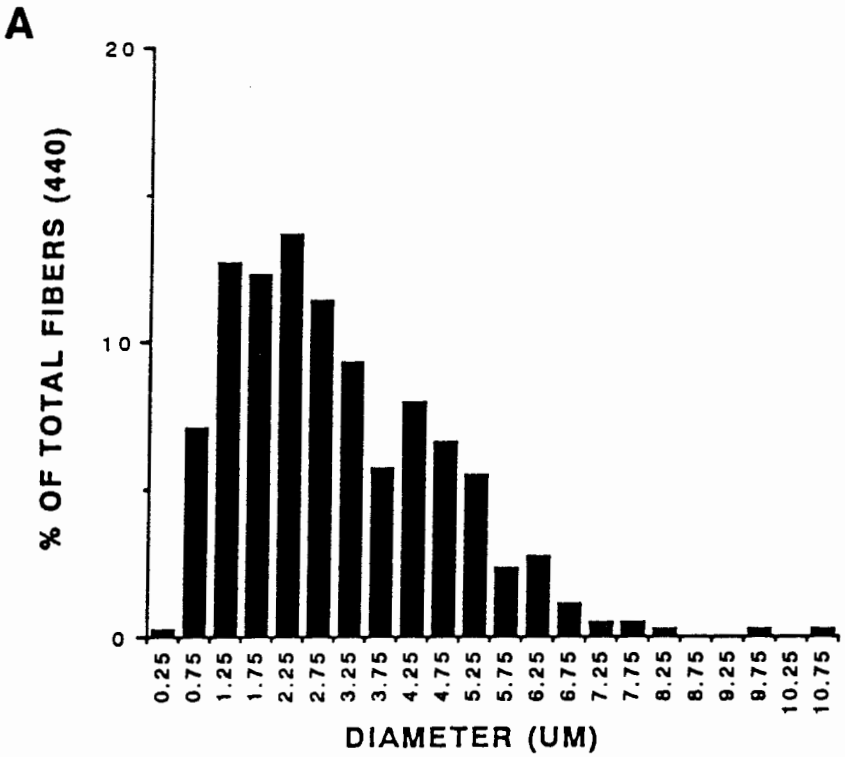

NF
MS
a
MS
Ax
b
NF
MS
2.2 c

FIGURE 23 Distribution of lateral line nerve diameter innervating canal neuromasts (treatment with 5% EDTA). Abscissa, diameter in μm. Ordinate, frequency as percentage of total.

(a). Mandibular canal neuromast, mean of 4 nerves.

(b). Trunk canal neuromast, mean of 4 nerve.


A
20
10
0
% OF TOTAL FIBERS (440)
0.25
0.75
1.25
1.75
2.25
2.75
3.25
3.75
4.25
4.75
5.25
5.75
6.25
6.75
7.25
7.75
8.25
8.75
9.25
9.75
10.25
10.75
DIAMETER (UM)

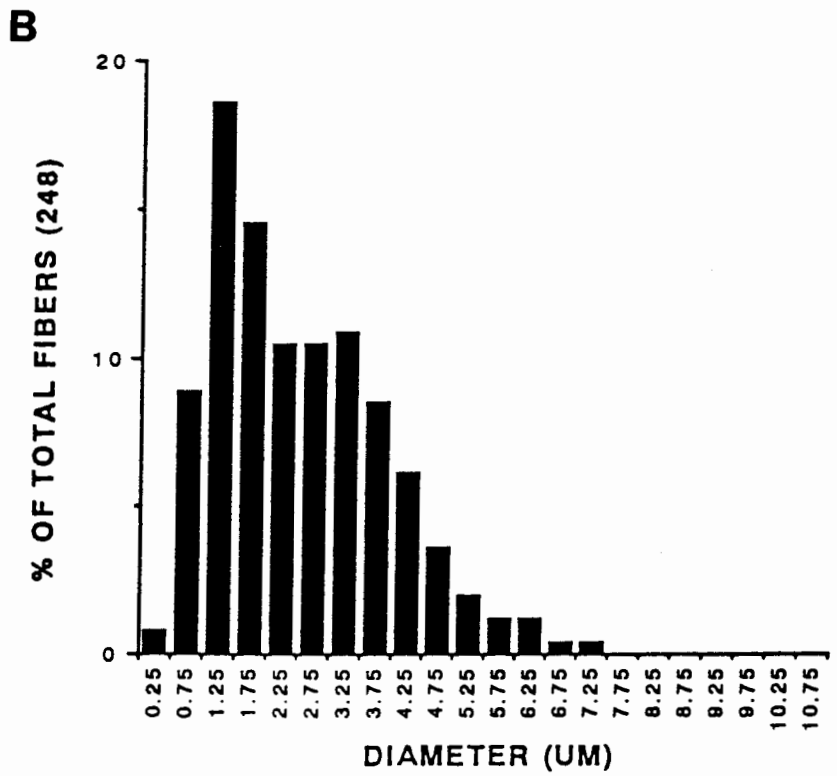

B
20
10
0
% OF TOTAL FIBERS (248)
0.25
0.75
1.25
1.75
2.25
2.75
3.25
3.75
4.25
4.75
5.25
5.75
6.25
6.75
7.25
7.75
8.25
8.75
9.25
9.75
10.25
10.75
DIAMETER (UM)

TABLE 1. Three analyses, each with a different variable of length (SL), location (head or trunk), type (superficial or canal) and interaction between location and type. Hair cell number, nerve fiber number and ratio of hair cell to nerve fiber are different sources for each analysis. Asterisk indicates statistically significant differences.

TABLE 1. ANALYSIS OF COVARIANCE

A. HAIR CELL NUMBER

Source	F	P
Length	2.461 (1,38 df)	0.125
Location	51.466 (1,38 df)	0.000*
Type	232.134 (1,38 df)	0.000*
Location*Type	48.347 (1,38 df)	0.000*

B. NERVE FIBER NUMBER

Source	F	P
Length	0.000 (1,38 df)	0.988
Location	0.320 (1,38 df)	0.575
Type	93.722 (1,38 df)	0.000*
Location*Type	5.760 (1,38 df)	0.021*

C. RATIO OF HAIR CELL/NERVE FIBER

Source	F	P
Length	2.281 (1,38 df)	0.139
Location	43.693 (1,38 df)	0.000*
Type	110.257 (1,38 df)	0.000*
Location*Type	17.257 (1,38 df)	0.000*

TABLE 2. MANDIBULAR CANAL NEUROMAST (MD)

Fish	Length of fish (mm)	Hair cells	Nerve fibers	Ratio of cells/fibers
1	80	-*	-	-
2	75	690	59	11.70
3	74	-	-	-
4	70	-	-	-
5	70	480	32	15.00
6	68	458	54	8.48
7	65	650	58	11.20
8	64	637	73	8.70
9	63	508	38	13.37
10	56	610	44	13.86
11	53	240	37	6.94
12	51	412	40	10.30
Mean	65.75	520.60	48.33	11.01

* Represent missing data.

TABLE 3. TRUNK CANAL NEUROMAST (TR)

Fish	Length of Fish (mm)	Hair cells	Nerve fibers	Ratio of cells/fibers
1	80	235	37	6.31
2	75	223	30	7.40
3	74	171	37	4.62
4	70	232	25	9.28
5	70	300	60	5.00
6	68	263	49	5.37
7	65	224	40	5.67
8	64	245	48	5.10
9	63	145	29	5.00
10	56	284	60	4.73
11	53	135	28	4.82
12	51	230	33	6.97
Mean	65.75	223.92	39.67	5.85

TABLE 4. MANDIBULAR LINE SUPERFICIAL NEUROMAST (Lm)

Fish	Length of Fish (mm)	Hair cells	Nerve fibers	Ratio of cells/fibers
1	80	67	13	5.15
2	75	30	12	2.50
3	74	-*	-	-
4	70	50	15	3.30
5	70	38	8	4.75
6	68	46	13	3.54
7	65	70	15	4.67
8	64	61	20	3.05
9	63	35	9	3.89
10	56	68	14	4.86
11	53	33	10	3.30
12	51	60	15	4.00
Mean	65.75	50.73	13.09	3.91

* Represent missing data.

TABLE 5. TRUNK ACCESSARY SUPERFICIAL NEUROMAST (Ts)

Fish	Length of fish (mm)	Hair cells	Nerve fibers	Ratio of cells/fibers
1	80	46	12	3.83
2	75	50	26	1.92
3	74	-*	-	-
4	70	53	11	4.82
5	70	30	14	2.41
6	68	31	14	2.21
7	65	70	20	3.50
8	64	40	21	1.95
9	63	46	17	2.71
10	56	65	20	3.25
11	53	35	22	1.59
12	51	40	26	1.54
Mean	65.75	46.00	18.46	2.70

* Represent missing data.

CHAPTER IV

DISCUSSION

The results of the present study are in agreement with previous studies of the lateral line in *Cottus bairdi* with respect to (1) the disposition and composition of the system as a whole and (2) the morphology of its endorgan, the neuromast (Janssen *et al.*, 1987). In this last respect we extend our knowledge of neuromast morphology in the sculpin by reporting the presence of cell types possibly involved in neuromast growth and/or repair, these include "light" and "dark" cells in the sensory strip and "big" cells in the mantle cell zone. The two major cell types, hair cells and supporting cells, were both present in the sensory strip and were delimited from the surrounding somatic epithelium by mantle cells. These types of cells were easily distinguished from each other morphologically. Two populations of hair cell were present, one lightly staining with a smooth, almost round profile in TEM section, the other more darkly staining with an irregular outline. Between the light and dark hair cells, there are cells which appear to be in transition from light to dark cell. Such dark hair cells have also been found in the shark canal neuromasts (Tester and Kendall 1969) and in the

free neuromasts of *Xenopus* (Shelton, 1971, Campantico *et al.*, 1983). Aside from staining, these cells did not show much difference in their size or complement of organelles in the present study. However Shelton (1971) found that in free neuromasts of *Xenopus*, the cytoplasm of dark and light hair cells did in fact differ. Dark cells had a higher density of cytoplasmic organelles and complex membrane whorled and contained vacuoles and large mitochondria. There is still no clear functional explanation of these light and dark cells, although it has been demonstrated that hair cells become darker after denervation and, finally disappear (Delaveuve, 1974). Thus, one possible explanation is that hair cell changes from light to dark may represent a maturation process culminating in cell death and loss, but conclusive evidence is still lacking.

The large unknown cell type present on the periphery of the sensory strip, yet still within the mantle cell zone, may represent newly differentiating hair cells. For many years, it was believed that damaged hair cells in the mammalian inner ear could not regenerate. Recently Forge *et al.* (1993) presented clear evidence demonstrating the regeneration of hair cells in the guinea pig utricle. Mechanosensory hair cells in the fish and amphibian lateral line system and ear (Corwin 1981, 83, 85. Jørgensen 1981) are produced throughout life and increase in number along with increasing animal size.

Rouse and Pickles (1982) have shown in *Apogon* and *Parapriacantus* that newly produced hair cells are found at a higher density on the edge of the sensory strip. Likewise, in the sensory epithelia of the inner ear in elasmobranchs and amphibians, developing hair cells are added at the margins of the sensory area (Lewis and Li, 1973, 1975; Corwin, 1985). The consensus of these studies suggest that this post-embryonic production of hair cells makes it possible to extend the size of the sensory epithelium with the growth of the fish. Corwin (1977a) using autoradiographic labelling observed that in shark macula neglecta, some cells present in the nonsensory zone of the marginal epithelium eventually migrate into the peripheral sensory epithelium, although no morphological description of these cells was presented. Similarly, in the present study these big cells were not present in the sensory strip *per se* but at its margin. Thus, they can be suspected to be immature hair cells.

The formation of new hair cells can also be triggered by damaging the sensory epithelium (Balak *et al.*, 1990). The origin of the regenerating hair cell in this situation is likewise not clearly understood. Two hypotheses of new hair cell production have been proposed. First, supporting cells may act as progenitors (Corwin, 1989; Balak *et al.*, 1990; Warchol *et al.*, 1993) giving rise to new hair cells. The study of Balak *et al.* (1990) suggests that the mantle cells were the source of the

new hair cells in neuromasts formed by budding from pre-existing neuromasts, whereas supporting cells acted as progenitors in the replacement of damaged or killed hair cells in the central area of the neuromast. Likewise, Forge *et al.* (1993) examined the morphology of regenerating hair cells and concluded that the replacement of damaged hair cells was indeed from supporting cells in the region where the original hair cells were lost. The second hypothesis suggests that hair cells originate from some other unknown precursor cells which give rise to two oppositely orientated hair cells at the same time (Rouse and Pickles 1991).

A major goal of the present study was to test the hypothesis that the behaviorally measured, differential sensitivity of head and trunk neuromasts could, at least in part, be explained by differing degrees of convergence between hair cells and their afferent nerve fibers. In this model, the greater sensitivity in the head region is due to a higher convergence of hair cells onto their innervating afferent fibers, which allows for greater spatial summation of receptor potentials. Thus, fibers innervating larger number of hair cells would reach their thresholds for firing at lower stimulus magnitudes than those innervating fewer hair cells. As we know, there are numbers of factors or levels, including canal structure, cupula properties, hair cells and their innervating fibers and central nervous system processing, which may all be the factors to affect the responsiveness of

the system. We only examined this last possible mechanism. In fact, it is probable that interaction between all elements of the system contribute to the final sensitivity of the system.

The ratio of hair cells to innervating fiber in different lateralis systems in vertebrates has been the object of study by many investigators. Convergence has been demonstrated in a variety of hair cell based sensory endorgans. A ratio of 57:1 for the macula neglecta of sharks in genus *Carchahinus* has been found by Corwin (1977b). Perhaps most relevant to the present study are the studies (Best and Gray, 1982) on the utriculus and lateral line of the sprat (*Sprattus sprattus* (L.), where the ratio in utriculus was 2.7:1 for the anterior macula, 7.1:1 for the middle macula, and 7.2:1 in lateral line canal. In the sprat, the higher ratio of hair cell to nerve fiber in the middle macula was due to a greater number of hair cells as compared to the utriculus. In the lateral line at another fish, *Sarotherodon niloticus,* a ratio at 5:1 for canal neuromasts and 2:1 in superficial neuromasts has been reported (Münz, 1985). In the mottled sculpin, Janssen *et al.* (1987) have found that all canal neuromasts have higher numbers of hair cells than all superficial neuromasts, as does the present study, and also found that the neuromasts of the head canal have more hair cells than those of the trunk. The number of hair cells also varies between canals (Janssen *et al.*, 1987). For example, in fish in the size range of 50-80mm (SL), the mandibular

canal has 350-550 hair cells per neuromast, the preopercular canal has 280-450, the infraorbital canal has 260-380, the supraorbital canal has 230-370 and trunk canal have 140-200 hair cells per neuromast. Our data are in good agreement with these studies. In comparably sized fish, the number of hair cells per neuromast ranges from 240-690 (X=521) for the mandibular canal and from 135-300 (X=224) for the trunk canals. It must be noted that these investigators counted hair cells on fish ranging from 20 to 90mm (SL) and were able to establish a strong correlation between SL and number of hair cells per neuromast. In our study, we do not find such a correlation, possibly because our counts were confined to fish in a relatively narrow size range (50 to 80mm, SL). Additionally, our counts are consistently higher than those of Janssen *et al.*. This difference might result from methodological differences in counting. Janssen *et al.* counted the cell number with scanning electron microscope, i.e. hair cells were counted by the number of ciliary bundles present in the surface of neuromast, whereas we counted cells directly from plastic semi-thin sections. Alternatively, the observed discrepancies might be due to exactly which neuromast in a given canal was counted, although evidence to support this positional effect is lacking, and indeed studies in both the mottled sculpin (Janssen *et al.*, 1987) and the sprat (Best and Gray, 1982) failed to demonstrate any relation between hair

cell number and position in a canal.

Statistical analysis indicates that nerve fiber numbers increase with increasing number of hair cells in both mandibular and trunk canal neuromasts. This increase is statistically significant in the latter case ($P < 0.004$) but not in the former ($P > 0.55$). The slopes of linear regressions from both mandibular and trunk canal neuromasts are not statistically distinguishable. One interpretation of these results is that it is physiologically important to maintain some optimal ratio between the number of hair cells and their afferent fibers as the neuromast increases in size as the fish grows. This seems not to be the case in the teleost macula, which adds both hair cells and nerve fibers as the fish ages (Popper *et al.*, 1984). The increase in hair cells can be 34-fold in the saccule of a cichlid fish, with the corresponding increase in nerve cells only 4.8-fold, resulting in a change in the ratio of hair cells to nerve fiber at about 30:1 in the younger fish to about 300:1 in older fish. Similarly, Corwin (1987) found that the macula neglecta in the ray has a hair cell to nerve fiber ratio of 5:1 in the juvenile and 60:1 in the adult fish due to increase in the number of hair cells along with fish growth. Since the fish used in present study were from a limited size range, it is not possible to ascertain if similar changes are occurring, and if so, whether mandibular and trunk canal neuromasts differ in this respect. Indeed, previous analysis indicates no statistically

demonstrable relation between fish standard length and any of the parameters measured in this study. A relatively larger range of fish size is needed to test this hypothesis i.e. fish ranging in length (SL) from a few mm to 100mm.

Another factor that may affect the sensitivity of the nerve fiber is its diameter. Generally, in the nervous system, the number of fibers is the main factor in determining the information carrying capacity, whereas conduction time is largely dependent on diameter and myelination (Best and Gray, 1982). A relationship between fiber diameter and different functions has been observed by Katsuki *et al.* (1951) in lateral line canal neuromasts of sea eel (*Rhyncocymba).* Their investigation demonstrated that as expected small diameter fibers had low stimulus thresholds, whereas larger fibers had high stimulus thresholds. Due to the high probability of decalcifier-induced changes in nerve fiber diameter, it is difficult to interpret our data with any degree of certainly. Further, more carefully controlled experiments are needed to resolve the role of this potentially significant factor in sensitivity.

Since this study relied almost exclusively on light microscopic determinations, it was not possible to distinguish which of the nerve fibers counted were afferent and which were efferent. Electron microscope studies have clearly demonstrated that both endings exist in superficial and canal neuromasts of the sculpin (Jones *et al.,* 1989 and the present

study). However, based on the known morphologies of afferent and efferent fiber in other system e.g. the mammalian cochlear nerve (Spoendlin, 1968), and the macula of the sprat utriculus (Best and Gary, 1982), the efferent fiber population comprises only about 1% of total innervating fibers. Somewhat higher figures were determined in the cat, where there are only 400 to 500 vestibular efferent compared with a total 11,300 to 13,200 vestibular afferent fibers (Gacek and Rasmussen, 1961, Warr, 1975), which was about 3.5% to 3.8% of the total innervating fibers. Consequently, our inability to distinguish between these two populations should at most result in only a small overestimation.

CONCLUSIONS

The general morphology of neuromast as observed in the present study is similar to that described in other water-living vertebrates: i.e. they include sensory hair cells, supporting cells, mantle cells and innervating fibers. One cell type was found in nonsensory areas of the neuromast characterized by large size and pale staining. These cells were considered to be immature hair cells.

Hair cells and nerve fibers from the lateral line system of the mottled sculpin, *Cottus bairdi*, were counted and their ratio were calculated. The ratio of hair cells to nerve fibers was higher for all neuromasts, both canal and superficial neuromasts, located on the head. This suggested the hypothesis that a higher convergence of hair cells to innervating afferent fibers could, at least in part, explain the greater responsiveness of the head to stimulation. The findings of the present investigation demonstrate anatomical support for this hypothesis.

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The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated and that the thesis is now given final approval by the Committee with reference to content and form.

The thesis is therefor accepted in partial fulfillment of the requirements for the degree of Master of Science.

6 December 93
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