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STRUCTURE OF THE MACULA UTRICULI WITH SPECIAL REFERENCE TO DIRECTIONAL INTERPLAY OF SENSORY RESPONSES AS REVEALED BY MORPHOLOGICAL POLARIZATION

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ABSTRACT

The anatomy of the labyrinth and the structure of the macula utriculi of the teleost fish (burbot) *Lota vulgaris* was studied by dissection, phase contrast, and electron microscopy. The innervating nerve fibers end at the bottom of the sensory cells where two types of nerve endings are found, granulated and non-granulated. The ultrastructure and organization of the sensory hair bundles are described, and the finding that the receptor cells are morphologically polarized by the presence of an asymmetrically located kinocilium in the sensory hair bundle is discussed in terms of directional sensitivity. The pattern of orientation of the hair cells in the macula utriculi was determined, revealing a complicated morphological polarization of the sensory epithelium. The findings suggest that the interplay of sensory responses is intimately related to the directional sensitivity of the receptor cells as revealed by their morphological polarization. The problem of efferent innervation is discussed, and it is concluded that the positional information signaled by the nerve fibers innervating the vestibular organs comprises an intricate pattern of interacting afferent and efferent impulses.

INTRODUCTION

Since the days of pioneering electron microscope investigation of the inner ear sensory epithelia (72, 61, 63, 13), the interpretation of structural findings has aimed at correlating morphological features with the sensory functions of the receptors. Electrophysiological studies on the function of the labyrinthine sensory organs meet with exceedingly difficult technical problems because the membranous labyrinth is more or less completely enclosed by bone. It is known, however, that in the sense organs of the acoustico-lateralis system a pervading electrophysiological principle governing the response of the receptors is the bidirectional sensitivity of the sensory units to stimulation approaching from opposite directions (15, 38, 5, 66, 68, 54). The sensory cells are thus functionally polarized.

Electron microscope studies on the sensory hairs of the receptor cells have revealed a morphological polarization of the sensory cells indicating their directional sensitivity (42, 19, 20, 21, 14, 9, 75, 43). Since the morphological and functional polarizations of the hair cells coincide, it is possible to examine the pattern of directional sensitivity of a whole organ by mapping, with the electron microscope, the orientation of the sensory cells over the sensory epithelium. This paper deals with the

results of such a study on the utricular macula, and also submits an ultrastructural description of the sensory epithelium and its innervation.

MATERIAL AND METHODS

This investigation was carried out on the teleost fish (burbot) *Lota vulgaris*. The anatomy of the labyrinth was studied in the unfixed state as well as after fixation with 10 per cent buffered formaldehyde or 1 per cent osmium tetroxide buffered with veronal acetate (52). Some specimens were stained with Sudan Black B. The surrounding bone and cartilage was carefully dissected away until the whole labyrinth could be loosened from its bony cradle and photographed in a Zeiss operation microscope (Fig. 1).

For phase contrast and electron microscopy, fishes were decapitated and, after preliminary dissection, the membranous labyrinth was perfused with cold 1 per cent osmium tetroxide buffered with veronal acetate and left for fixation for 3 hours (52). After rinsing in Ringer solution, final dissection was carried out in 70 per cent alcohol. The utricular otolith was removed by a jet of alcohol from a pipette. Dehydration was continued in alcohol and the specimens embedded in Epon (44). For mapping the polarization pattern of the utricular macula, the sensory epithelium thus embedded was divided into 50 or 20 cubes about 0.2 x 0.2 x 0.3 mm in size, the sides of which were carefully marked with respect to their topographical relationship. The area of sensory epithelium corresponding to each cube was marked on a photograph or a drawing of the macula utriculi. The cubes were mounted on Epon blanks. For phase contrast microscopy, 1-μ-thick sections were cut parallel to the surface of the epithelium with glass knives on an LKB Ultrotome. From behind the knife edge, floating on 10 per cent alcohol in a metal tray, the sections were collected on formvar membranes on single hole copper grids, transferred to objective glasses, and mounted in Epon. The sections were examined and photographed in a Zeiss photo microscope, using chiefly the 100 × phase contrast objective (Fig. 15). For electron microscopy, grey or white ultrathin sections were cut with glass knives on an LKB Ultrotome and collected from 10 per cent

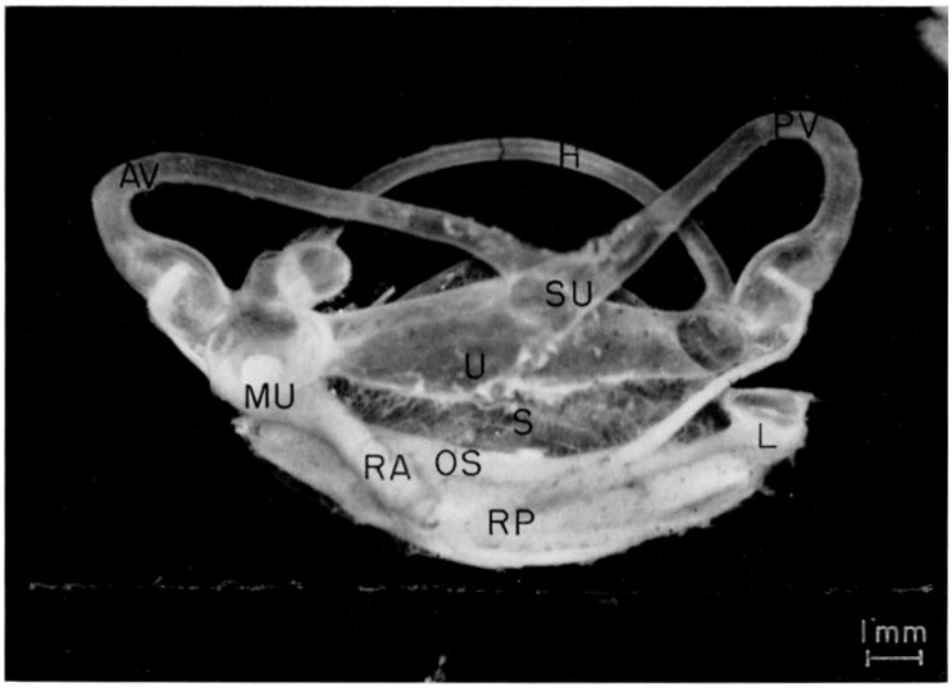

FIGURE 1 Right membranous labyrinth of *Lota vulgaris* viewed from the medial side. The utricular otolith is seen resting over the macula utriculi. The cristae ampullares are seen within the semicircular canals. *U*, utriculus; *S*, sacculus; *H*, horizontal canal; *AV*, anterior vertical canal; *PV*, posterior vertical canal; *SU*, sinus utriculi; *MU*, macula utriculi, *L*, lagena; *OS*, otolith of sacculus; *RA*, ramus anterior, *RP*, ramus posterior. Formaldehyde fixation. × 6.5.

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alcohol on 100-mesh copper grids. Most sections were stained with 1.2 per cent uranyl acetate in 10 per cent alcohol for 1 hour at 60°C (6). Electron micrographs were taken at 1,000 to 80,000 times magnification in a Siemens Elmiskop I, with 100-, 50-, 20-, or 15-μ objective apertures, and photographically enlarged to the magnification desired.

With these techniques, the entire surface of the sensory epithelium of two utricular maculae from different specimens were examined. The results were confirmed by checking various parts of the sensory epithelium of a number of utricular maculae from other specimens.

Attempts were made to study the polarization of the sensory hair bundles of whole utricular maculae, fresh or slightly stained with osmium tetroxide, laid out flat in phase contrast embedding media as described by Engström *et al.* (14). This attempt was unsuccessful because in this animal myelinated nerve fibers are found also within the sensory epithelium and they obscure the view.

RESULTS

Anatomy of the Labyrinth

The membranous labyrinth containing the sensory areas is composed of two major membranous compartments, the sacculus, onto the posterior end of which the lagena is attached, and the utriculus, with which the three semicircular canals communicate and which forms in its anterior end the recessus utriculi (Fig. 1). The semicircular canals are oriented in the three dimensions of space, one horizontal, one anterior vertical, and one posterior vertical. The two vertical canals meet at the apex of the sinus utriculi, which opens into the middle of the utriculus.

The labyrinth has a total length of about 1.5 cm and is located close to the brain stem at the level of the departure of the stato-acoustic nerve. It is supported and partly surrounded by bone, and bridges of cartilage hook the horizontal and the posterior vertical canals. The medial aspect of the labyrinth is separated from the cranial cavity only by a membranous tissue sheath.

The sensory areas are innervated by N. statoacousticus, which is divided into a ramus anterior and a ramus posterior (Fig. 1). The ramus anterior innervates the pars anterior of the labyrinth, that is, the macula utriculi, which is located in the recessus utriculi, and the cristae ampullares of the horizontal and the anterior vertical semicircular canals. It also contributes a minor branch to the innervation of the macula sacculi. The ramus posterior innervates the major part of the macula sacculi and the crista ampullaris of the posterior semicular canal and the lagena. The macula utriculi, sacculi, and lagena are covered by compact otolithic stones, while the cupula covering the sensory epithelium of each crista is of a gelatinous structure. The utricular macula has a horizontal orientation whereas the saccular macula is vertically oriented.

The lateral line organs of fishes belong to the acoustico-lateralis system, having the same origin as the labyrinth in the auditory placode (64). The lateral line organs are contained in canals on the head and along the sides of the body of the fish. They are covered by a cupula and have the same basic structure as the vestibular organs.

General Structure of the Macula Utriculi

The general structure of the pars anterior of the membranous labyrinth is seen in Fig. 2.

The sensory epithelium of the macula utriculi forms a shallow bowl occupying the floor of the recessus utriculi. Its shape is ovoid, measuring, in a full-sized animal, about 2.5 x 1.5 mm. The long axis of the macula makes an angle of about 90° with the utricular nerve stem, which, in turn, intersects the midline at an anteriorly open angle of about 70° (see also Fig. 17). The posterior part of the sensory epithelium is extended into a tongue tapering up the wall of the recessus and is called the lacinia process. The otolith measures about 1 x 1 mm and rests over the sensory epithelium. It covers only the central medial part of the epithelium and is separated from the epithelium by a gelatinous otolithic membrane which covers the whole macula and has over the lacinia, rather the appearance of a cupula.

Fine Structure and Innervation of the Sensory Epithelium

THE SENSORY CELLS: The sensory epithelium is composed of sensory cells 30 μ long and 10 μ wide reaching half-way down the epithelium, interposed between supporting cells (Fig. 3). At their apical ends, the supporting cells are firmly joined to each other and to the neighboring receptor cells by desmosomes, thus interlocking the sensory cells in a rigid reticular lamina. The apical end of each receptor cell is provided with a bundle of sensory hairs, one of which is a kinocilium. The sensory hairs are planted in a cuticular plate occupying the top of the sensory cell.

The ovoid nucleus is located in the middle of the cell and has a remarkably pale karyoplasm compared to that of mammals (76, 60, 13). A nucleolus is frequently seen close to the nuclear membrane, which is highly wrinkled and often forms deep invaginations into the apical end of the nucleus (Fig. 3).

The cytoplasm contains an abundance of mitochondria 0.5 to 3 μ long which are especially numerous in the supranuclear part and in the synaptic area. The endoplasmic reticulum is represented throughout the cell by a system of irregular double α-cytomembranes (59) or cisternae (46) lined on the cytoplasmic side with dark granules having a diameter of 100 to 150 A. Such granules are also found in irregular clusters, independent of membranes, particularly in the supranuclear part of the cell. No regularly arranged infranuclear membrane system, as described in mammalian vestibular hair cells (72), is seen.

A system of branching tubules 300 to 700 A in diameter surrounded by a single layer of small granules about 50 A in diameter is found throughout the cytoplasm (Figs. 6, 7).

The cytoplasm contains numerous vesicles 200 to 400 A in diameter concentrated in the basal part of the cell (Figs. 4, 6). Bundles of supporting fibrils run through the cytoplasm, and above the nucleus a Golgi apparatus and multivesicular bodies are often seen.

THE SUPPORTING CELLS: The sensory cells are embraced by supporting cells which generally extend from the surface of the epithelium to the basement membrane. The supporting cells are joined to each other and to the sensory cells by desmosomes, especially at their apical ends, and each cell is traversed by bundles of supporting fibrils emanating mainly from the desmosomes and running along the apex of the cell parallel to the surface of the epithelium. At the upper surface of the cell, the plasma membrane forms a number of microvilli 0.1 to 0.2 μ long. In each supporting cell two centrioles are found close to one another, one being just beneath the apical plasma membrane

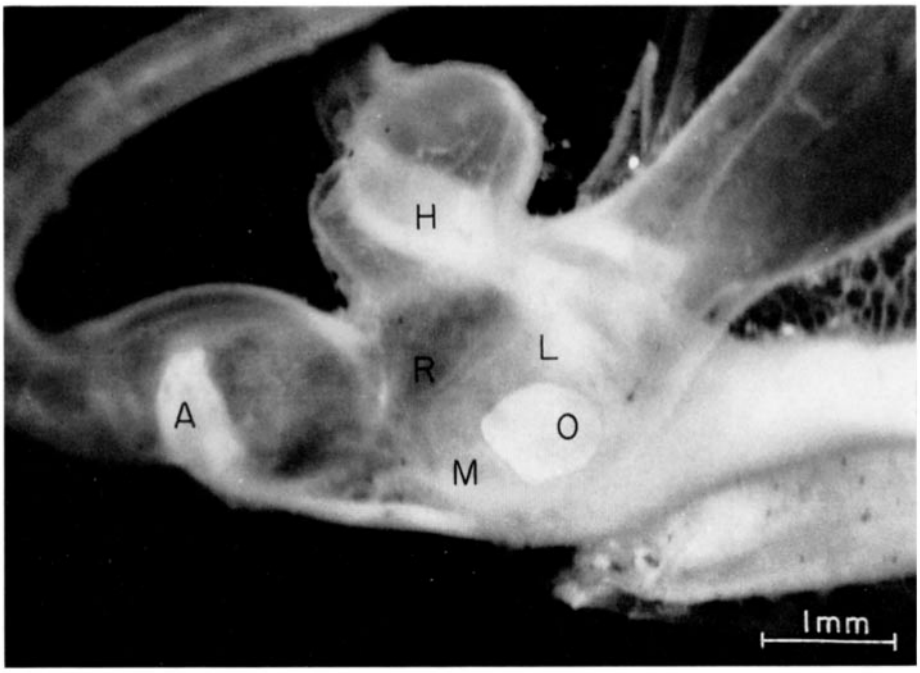

FIGURE 2 Pars anterior of the membranous labyrinth showing the general structure of the macula utriculi (*M*) occupying the floor of the recessus utriculi (*R*) and its relation to the horizontal (*H*) and anterior (*A*) cristae. The otolith (*O*) occupies the center of the macula. *L*, lacinia process of utricular macula. Formaldehyde fixation. × 17.

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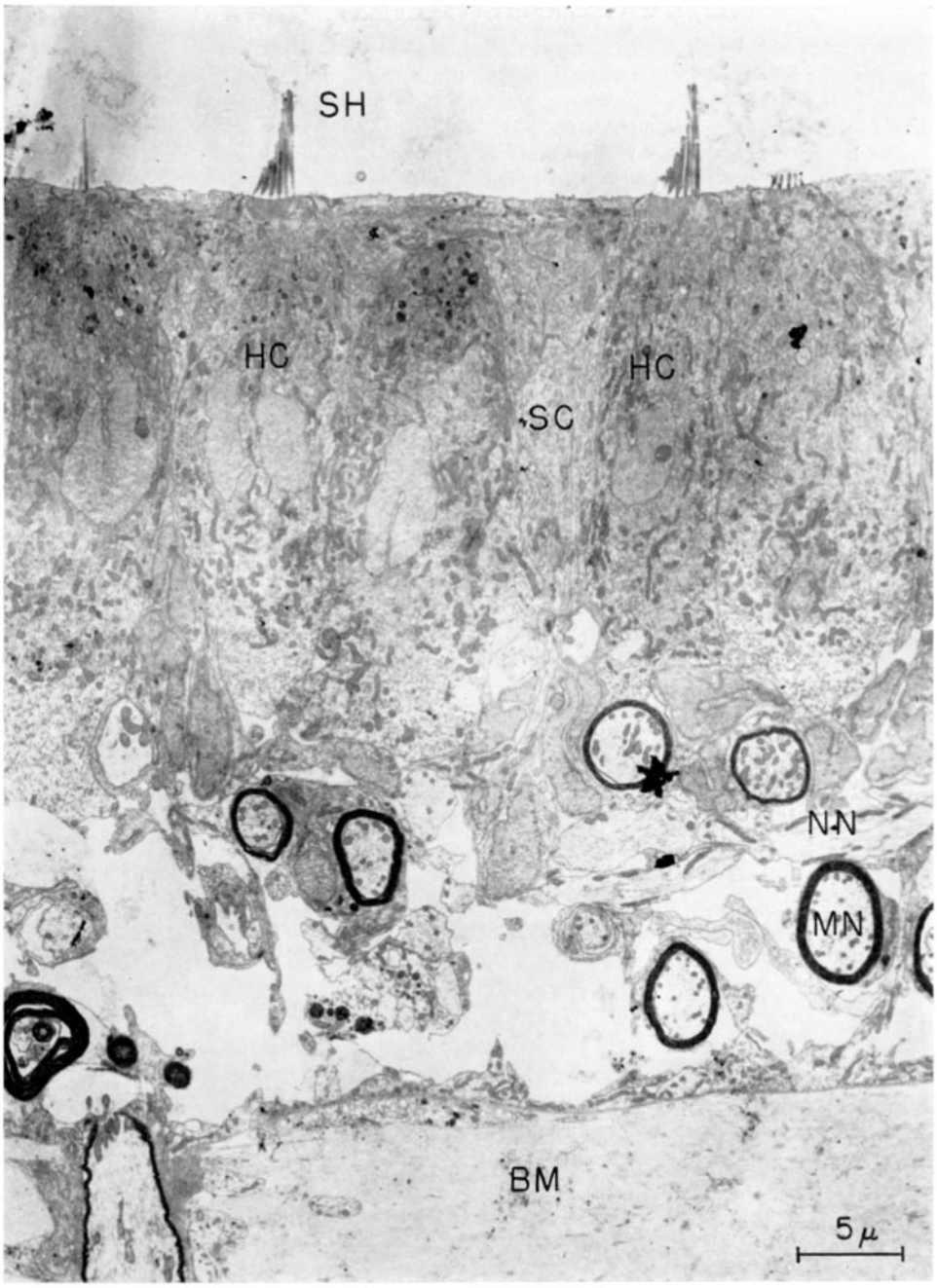

FIGURE 3 Survey picture of the sensory epithelium of the macula utriculi. The hair cells (*HC*), which are interposed between supporting cells (*SC*), are innervated by myelinated (*MN*) and non-myelinated (*NN*) nerve fibers running within the sensory epithelium, which is separated from the surrounding tissues by a basement membrane (*BM*). The stepwise increasing length of the sensory hairs (*SH*) is seen. × 2,800.

and oriented perpendicularly to it, the other having an oblique orientation. Usually the plasma membrane bulges outward above the former centriole forming a short rod or even a rudimentary cilium.

The cytoplasm contains mitochondria and an extensive endoplasmic reticulum similar to that of the hair cell but surrounding larger cleft-like spaces. Throughout the cytoplasm are found opaque granules which increase in size towards the apex and reach a final diameter of 0.2 to 0.3 μ. The presence of similar granules, although more abundant, in the crista ampullaris of guinea pig (72) was interpreted as a sign of secretory function. The nuclei are found at various levels below the middle of the epithelium and stain more heavily than those of the sensory cells. A Golgi apparatus and multivesicular bodies are also present. Toward the basement membrane the intercellular spaces often widen considerably (Fig. 3). The supporting cells guide the myelinated nerve fibers on their course within the sensory epithelium, thereby taking over inside the epithelium the function of the Schwann cells outside it.

The sensory epithelium rests on a basement membrane, which is composed of a finely fibrillar matrix in which slender cells are embedded. Beneath the basement membrane blood capillaries form an extensive network.

Innervation

Below the macula utriculi the nerve fibers of the utricular nerve spread in a fan-like fashion before entering the sensory epithelium (Fig. 2). Approaching the macula, the fibers destined for the lacinia process travel in a separate bundle.

In teleost fishes the nerve fibers generally retain their myelin sheath after penetrating the basement membrane, whereas in other animals the myelin sheath is lost at that point (50). This particular feature is observed also in the lateral line organ of this fish (18).

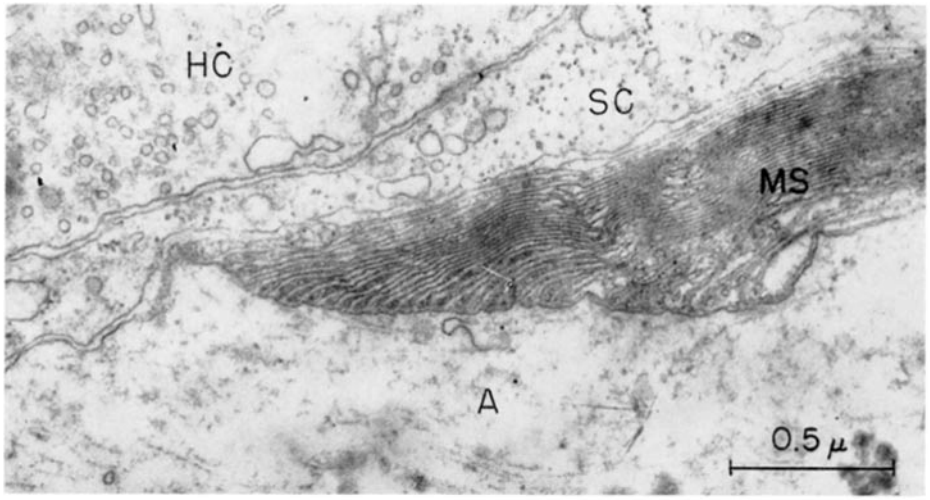

FIGURE 4 The shedding of the myelin sheath (*MS*) surrounding the axoplasm (*A*) of a nerve fiber is accomplished by the successive unfolding of the lamellae of the myelin sheath which is separated from a neighboring hair cell (*HC*) by a supporting cell (*SC*). × 43,000.

Each nerve fiber of the utricular nerve innervates many sensory cells. For example, one utricular nerve containing 2,700 fibers innervated a utricular macula calculated to contain approximately 25,000 sensory cells, the convergence in this case being about 10 to 1. Myelinated nerve fibers travel for quite a distance within the epithelium and give off branches which finally lose their myelin sheath by the successive unfolding of the lamellae of the myelin sheath (Fig. 4). At those points at which the nerve fibers branch, both outside and within the epithelium, typical nodes of Ranvier are recognized. However, unmyelinated axons containing slender mitochondria and neurofibrillae are also seen traveling within the epithelium (Fig. 3). The terminal nerve branches ramify

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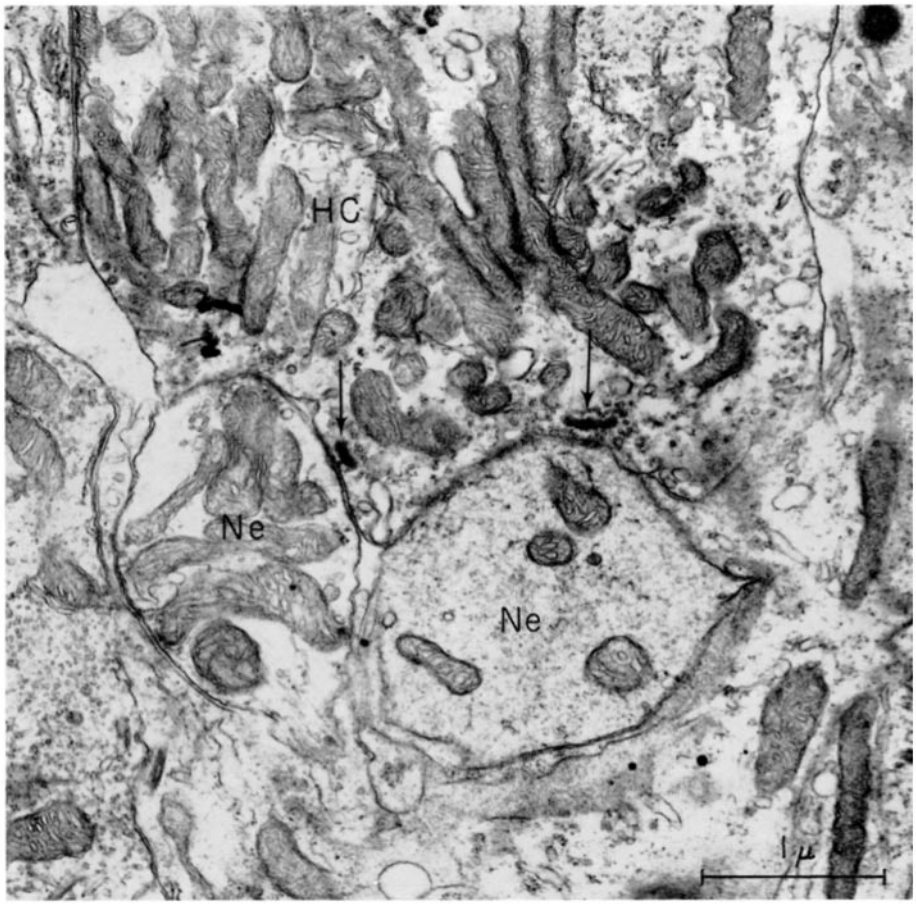

FIGURE 5 Two non-granulated nerve endings (*Ne*) at the base of a hair cell (*HC*). One ending is of the dark dotted type. Adjacent to each of them a synaptic bar is seen inside the hair cell (arrows). Many slender mitochondria are seen within the cell. × 24,000.

beneath the hair cells which are all innervated by many nerve endings.

The button-shaped nerve endings of the terminal branches innervating the sensory cells are of two different types, granulated and non-granulated (Figs. 5, 6). The non-granulated endings contain mitochondria, neurofibrillae, and some vesicles or short tubules. According to the appearance of the cytoplasm, two types of non-granulated endings can be distinguished, one type having an almost clear cytoplasm similar to that of the axon, the other having more densely staining granular cytoplasm (Fig. 5). Inside the hair cell at the area of contact with a non-granulated nerve ending, a densely staining synaptic bar surrounded by a cluster of vesicles 300 to 400 A in diameter is often found oriented along the synaptic membrane (Figs. 5, 7). The synaptic bar is separated from the subsynaptic membrane by a space 500 to 1,000 A wide which is usually occupied by vesicles.

The granulated nerve endings contain mitochondria, short tubules, and an abundance of vesicles 200 to 400 A in diameter (Fig. 6). An accessory double membrane is often found inside the hair cell along the plasma membrane associated with a granulated nerve ending.

A synaptic space 100 to 200 A wide separates the plasma membrane of both types of nerve endings from that of the hair cell.

The Sensory Hair Bundle

FINE STRUCTURE: From the top of each receptor cell, a bundle of sensory hairs projects into a canal in the overlying otolithic membrane. Each

bundle consists of a number of regularly arranged stereocilia and one peripherally located kinocilium (Figs. 8, 10).

Each stereocilium is composed of a protoplasmic core surrounded by a plasma membrane which is continuous with the cell membrane. Toward the base of the stereocilium the diameter progressively decreases from 0.2 to 0.15 μ and reaches a minimum of about 0.1 μ just above the cell surface (Fig. 8). Cross-cut bundles will, accordingly, exhibit stereocilia of different diameters, depending on the level of the section. However, a difference in actual diameter, within the limits 0.2 to 0.15 μ, also exists irregularly, as it seems, over the entire macula. The protoplasmic core has a fibrillar structure, each fibril measuring 20 to 40 A in diameter. Before reaching the cell surface, the fibrils of the core gather into a dense axial fiber. Below the surface of the cell, the fiber again resolves into fibrils penetrating in a conical fashion into the cuticular plate. Surrounding these rootlets the cuticular substance stains less densely and forms small canals lodging the rootlets.

The structure of the kinocilium is similar to that of the cilia of other cells (16). It is composed of nine peripheral double fibers surrounding a central pair of simple fibers (Fig. 9). The kinocilium is ensheathed by a plasma membrane forming a tube 0.3 μ in diameter which is continuous with the cell membrane. The central fibers end above the cell surface whereas the peripheral fibers on passing into the cell are transformed into triplet fibers making up the wall of the basal body (Fig. 8) which is located in a less densely staining area in the cuticular plate. On the side of the basal body which faces away from the stereocilia a club-shaped basal foot is found. The ultrastructure of the kinocilium and its basal body has been more thoroughly described elsewhere (17). The presence of a basal foot in the vestibular hair cells has independently been observed by Löwenstein Osborne, and Wersäll in the thornback ray (43).

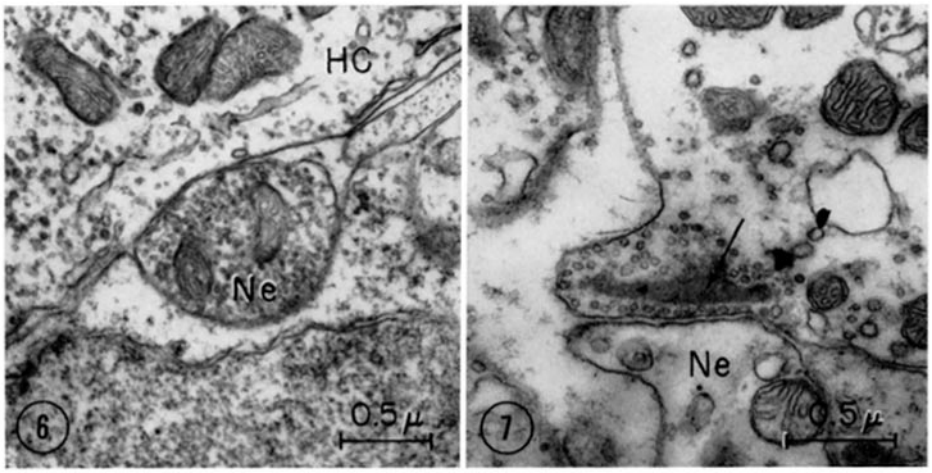

FIGURE 6 A granulated nerve ending (*Ne*) making contact with a hair cell (*HC*). × 24,000.

FIGURE 7 A densely staining synaptic bar (arrow) surrounded by a cluster of vesicles is often seen adjacent to the non-granulated nerve endings (*Ne*). × 29,000.

ORGANIZATION OF THE SENSORY HAIR BUNDLE: In the common type of bundle, 40 to 60 stereocilia are lined up behind the kinocilium in a regular pattern of 7 to 9 parallel rows (Fig. 10). The distance between two rows is greater than the distance between two neighboring stereocilia in the same row. The kinocilium is located in an indentation at the end of the bundle. This indentation is formed by the successive advancement of each of the three rows of stereocilia found on each side of the middle row, the advancing distance being half the distance between two neighboring stereocilia in the same row. An angle of about 115° opening toward the kinocilium is thus formed. The two outer rows as a rule lack the first stereocilium. A line connecting the two central fibers of

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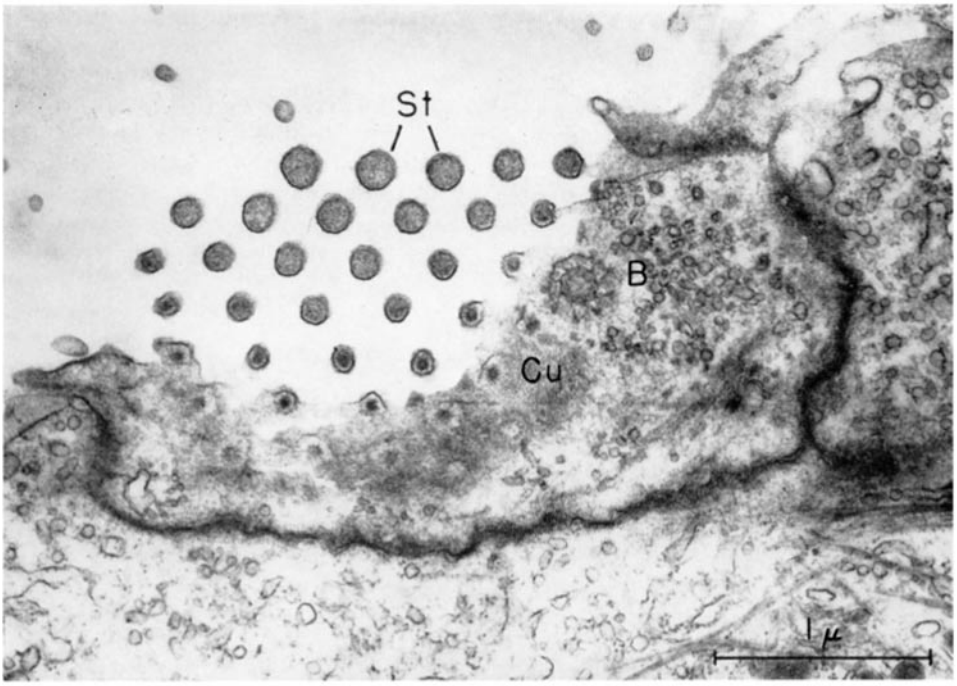

FIGURE 8 Slightly oblique cross-section through the top of a hair cell showing the stereocilia (*St*) cross-cut at successively deeper levels, finally penetrating into the cuticle (*Cu*). In one side of the cell, the basal body (*B*) of the kinocilium is seen. × 29,000.

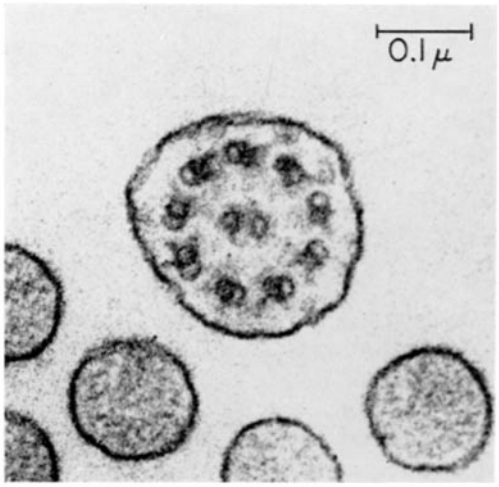

FIGURE 9 Cross-section of a kinocilium showing the arrangement of its component fibers. × 125,000.

the kinocilium is approximately perpendicular to the direction of the rows; the fibers that face away from the stereocilia are the peripheral fibers 5 and 6 (numbered according to Afzelius, 1959). Within each bundle the length of the stereocilia increases stepwise toward the kinocilium, their length ranging from 0.5 to 5 μ (Fig. 3). The kinocilium is longer than the tallest stereocilium, particularly in those parts of the macula which are not covered by the otolith but only by the otolithic membrane.

OTHER TYPES OF BUNDLES: Although the above-described arrangement of the sensory hairs is by far the most common one, other types of bundles exist. Some bundles are similar to the common type but lack the kinocilium. The horseshoe shape described by the bundle seen in Fig. 11 is encountered in perhaps one bundle out of fifteen to twenty. The arrangement of the stereocilia in this bundle might be described as two systems of rows, intersecting at an angle of about 60°, which extend into two wings half-embracing the kinocilium. Within each system the distances between the stereocilia and between the rows are as in the common bundle, each system aiming with one row at the kinocilium and opening toward it at an angle of approximately 115°. A line connecting the two central fibers of the kinocilium is approximately perpendicular to the direction of one of

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these systems, and the peripheral fibers 5 and 6 face away from the stereocilia. The kinocilium is thus rotated in relation to the axis of symmetry of the bundle. It has not been possible in these bundles to determine in what direction the basal foot is oriented, or even to ascertain the existence of one.

A less common type of bundle is provided with two kinocilia located beside each other or sometimes at slightly different levels in one end of the bundle (Figs. 12, 13). The stereocilia are arranged in parallel rows as in the common bundle, but each kinocilium is usually located in an indentation of its own. In Fig. 12, the lines connecting the central fibers in the two kinocilia meet at an angle of 90°; in other cases, they seem to be parallel to each other and perpendicular to the rows of stereocilia.

A fourth type of bundle, very rarely encountered, is seen in Fig. 14. It is a bipolar bundle provided with one kinocilium in each end. In both kinocilia, the plane connecting the central fibers is perpendicular to the orientation of the rows.

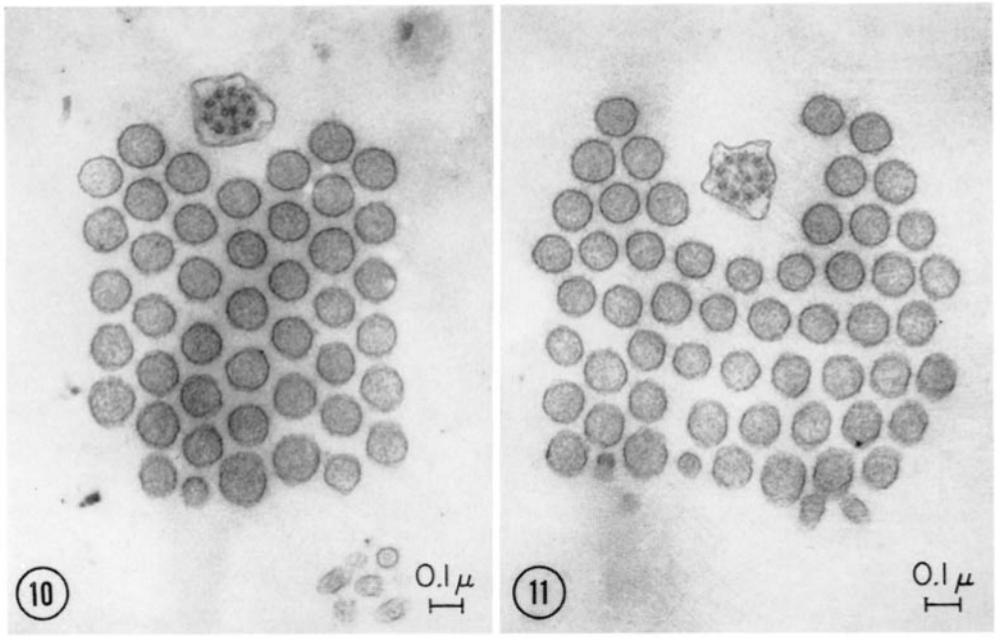

FIGURE 10 Common type of sensory hair bundle. × 40,000.

FIGURE 11 "Horseshoe" type of sensory hair bundle. × 40,000.

Orientation of the Sensory Cells

As described above, the sensory cells are morphologically polarized by the presence of a kinocilium asymmetrically placed in one end of the sensory hair bundle. The orientation of the sensory cells can consequently be determined either by phase contrast microscopy (Fig. 15) or preferably with the electron microscope (Fig. 16). By mapping the orientation of the sensory cells over the entire surface of the macula utriculi, the pattern of orientation presented in Fig. 17 is revealed.

From a point in the medial part of the macula, the sensory cells are oriented in a curved radiating pattern, the kinocilia pointing in gradually altering directions from anterior, passing lateral to posterior, covering approximately a semicircle. As seen in Fig. 17, the directions of orientation of the hair cells do not strictly emanate from one point but from along a line which is laterally oriented. This line makes an angle of 20° with the utricular nerve. Since the utricular nerve is, in its turn, anteriorly tilted at an angle of 70° from

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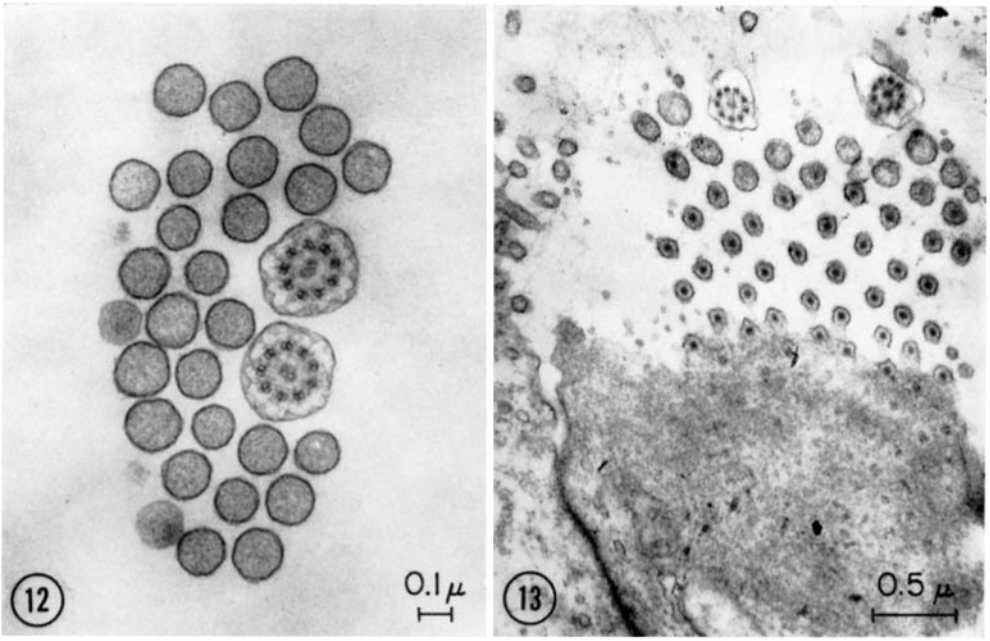

FIGURE 12 Sensory hair bundle provided with two kinocilia side by side. × 45,000.

FIGURE 13 Two kinocilia located somewhat apart in one side of the sensory hair bundle. × 22,000.

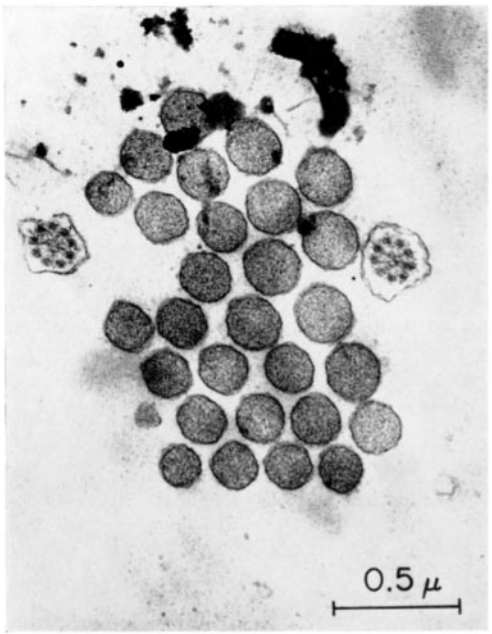

FIGURE 14 Uncommon type of bipolar sensory hair bundle having one kinocilium in each end. × 34,000.

the midline, the direction of the emanating line intersects the midline at a 90° angle. In a zone 0.15 mm wide along the anterior, lateral, and posterior margins of the macula, the hair cells are oriented with their kinocilia pointing medially in directions exactly opposite to the hair cells of the medial part. The otolith does not reach this peripheral inverted zone. Within any area the orientation between any two neighboring bundles is fairly constant, deviating maximally 10°–15° in either direction (Fig. 16). However, toward the emanating line, greater aberrations are found (Fig. 15). Close to this line the sensory hair bundles are more scarce, and many degenerated cells containing inclusion bodies are seen.

DISCUSSION

Structure and Innervation of the Sensory Epithelium

The anatomy of the labyrinth in the teleost fish *Lota vulgaris* is similar to that described for other fishes by Retzius (50), and is basically similar also to that of reptiles, birds, and mammals (50, 51),

and in these animals a conspicuous effect of evolution is the gradual development of the lagena into the organ of Corti (24).

The utricular macula in teleost fishes forms a shallow bowl containing in its center a compact otolithic stone (55) resting on a gelatinous membrane into which the sensory hairs project (50). In most other animals the sensory epithelium is covered by a lime-encrusted, gelatinous otolithic membrane containing crystals of calcite (7).

The composition of the sensory epithelium in teleosts was histologically investigated by Retzius and contributed to by Smith (60), Engström and Wersäll (13), and others (11, 73, 74, 3, 47). Two different types of hair cells could be distinguished, Type I and Type II cells, similar to those first described in the crista ampullaris of the guinea pig by Wersäll (72). The bottle-shaped Type I cells innervated by a nerve chalice enclosing the body of the cell are supposed to be more highly differentiated, while the cylindrical Type II cells innervated by a number of small nerve endings making contact with the bottom of the cell resemble the primitive hair cells found in the fish

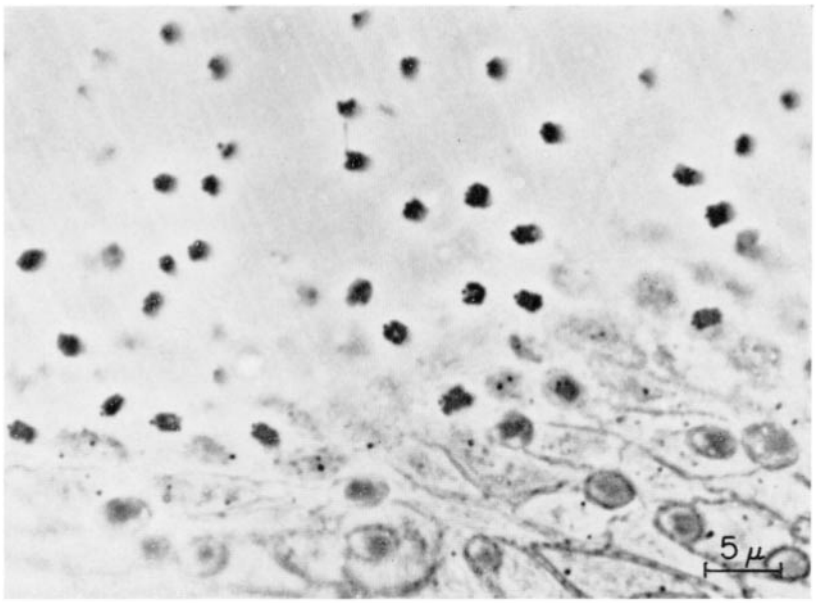

FIGURE 15 Phase contrast micrograph of a section cut tangentially to the sensory epithelium close to the emanating line in the utricular macula. The orientation of the sensory cells can be seen from the location of the kinocilium in the periphery of the bundle. × 1,900.

(50), who accurately described, within the limits of the light microscope the structure of the sensory cells and the supporting cells and the pattern of innervation. Further light microscope studies were carried out on other animals by Held (30), Kolmer (37), and others, but it was not until the electron microscope was introduced in morphological research that it became possible to determine the intricate fine-structure of the labyrinthine receptor organs.

Wersäll *et al.* (76) examined, with the electron microscope, the structure of the macula utriculi of the guinea pig. Their findings were later confirmed vestibular and lateral line organs (73, 74, 70, 75) and in the fowl embryo otocyst (23). In these primitive sensory epithelia no Type I hair cells are encountered. The utricular sensory cells described in this article resemble the Type II hair cells but in some respects differ from mammalian cells, chiefly by the vesicular content of their cytoplasm. In aquatic animals such vesicles are also seen in the sensory cells of other organs belonging to the acoustico-lateralis system (70, 18, 4).

As described above, each nerve fiber innervates many sensory cells. This was observed to be due not only to ramification of the terminal branches

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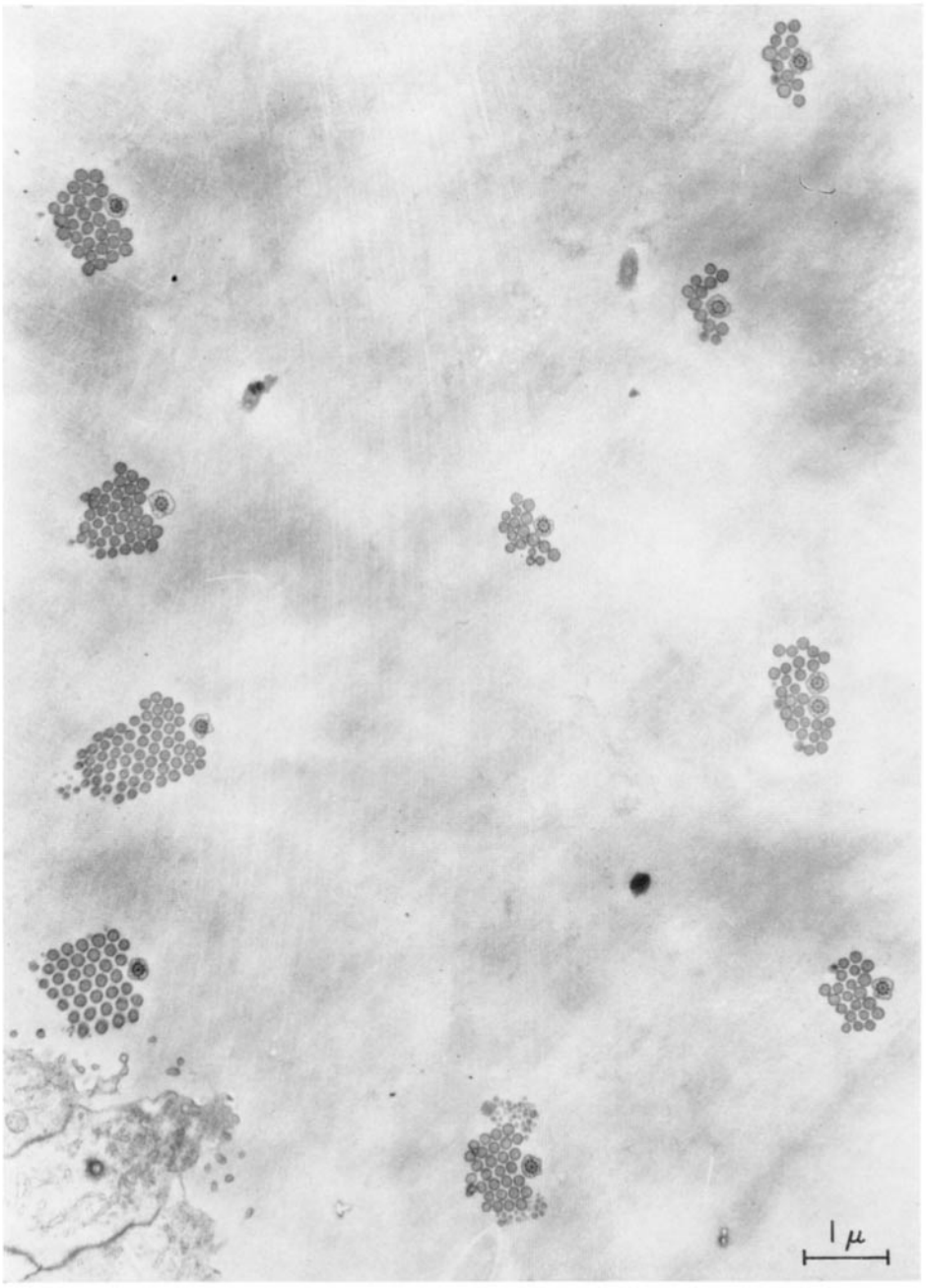

FIGURE 16 A cross-section through the sensory hair bundles reveals the consistent direction of orientation of neighboring receptor cells. × 10,000.

of the fiber but also to the fact that the utricular macula contains about 10 times as many receptor cells as nerve fibers innervating it, at least in one macula carefully examined. This convergence increases the possibility of spatial summation of sensory responses and so is apt to raise the sensitivity of the nerve fibers to simultaneous subliminal stimulation. The functional significance of an

analogous anatomical arrangement in receptor organs was recently discussed in a review by Davis (8).

The presence of two different kinds of nerve endings was first demonstrated in the organ of Corti in the guinea pig by Engström and Wersäll (12). Such a double innervation was shown also in the crista ampullaris by Wersäll (72), in the other vestibular organs by these authors (10, 13), and in the lateral line organ of the eel by Hama (28).

The synaptic bars which Smith and Sjöstrand (62) and Wersäll *et al.* (77) observed to be related to the non-granulated nerve endings on the outer and inner hair cells in the organ of Corti are similar to those described in this article. These synaptic bars resemble the synaptic ribbon found in the retinal rods by Sjsötrand (58) and in the sensory cells in the ampullae of Lorenzini by Barets and Szabo (4). These findings inply an afferent function of the non-granulated nerve endings. The presence of an identical synaptic structure in sensory cells responding to so different initial stimuli was suggested by Barets and Szabo (4) as implying a common process of transmission in these sensory synapses.

Engström (10) and Engström and Wersäll (13) suggested, on the basis of comparative morphological data, that the granulated nerve endings have an efferent activity while the non-granulated nerve endings are afferent sensory terminals. The presence of centrifugal efferent nerve fibers that reach the labyrinthine sensory areas has been shown by the work of Rasmussen (49) and Gacek (25). The efferent nature of the granulated nerve endings has recently been experimentally demonstrated by Iurato (32), Kimura and Wersäll (35), and others.[1] Hilding and Wersäll (31), and Wersäll

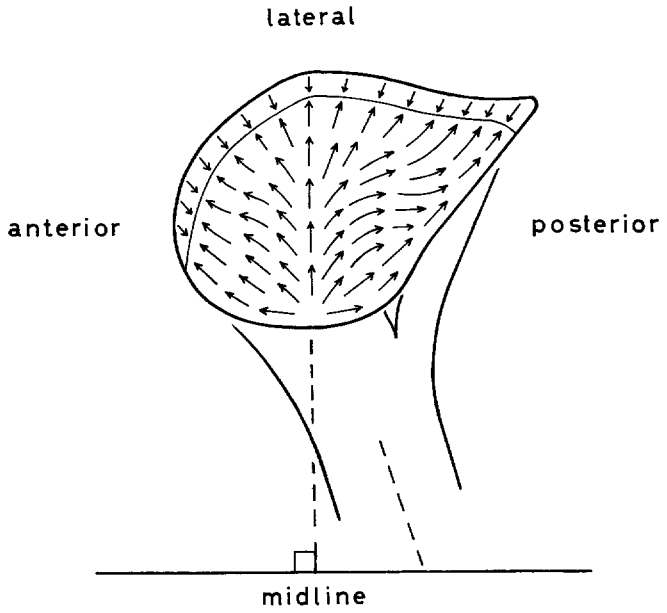

FIGURE 17 Schematic illustration of the pattern of morphological polarization of the sensory cells in the macula utriculi in *Lota vulgaris*, tentatively revealing the functional polarization of the sensory epithelium. The arrows indicate the orientation of the sensory cells in such a way that within any area the kinocilium of each sensory hair bundle leads in the direction of the arrow. According to the presented theory, the morphological polarization of the sensory cells coincides with their directional sensitivity in such a way that stimulation in a direction away from the stereocilia toward the kinocilium, represented in the figure by the direction of the arrows, gives excitation of the sensory unit, that is, depolarization and increase of discharge frequency, while stimulation in the opposite direction is inhibitory, causing hyperpolarization and decrease of resting discharge frequency.

[1] Similar findings have recently been reported by Smith, C., and Rasmussen, G., *Ann. Otol., Rhino.*

et al. (77) demonstrated the localization of specific acetylcholinesterase to the granulated nerve endings but found no esterase in the nerve chalices.

Retzius (50) observed in teleost fishes the splitting up of the sensory hairs of each receptor cell into a bundle of finer fibrils in specimens fixed in osmium tetroxide. Held (30) and Kolmer (37) observed the presence of a cilum in the periphery of the bundle in the vestibular cells, but erroneously claimed its presence also in the organ of Corti.

The fine structure of the components of the sensory hair bundle has been investigated with the electron microscope (72, 13, 19, 14), and Wersäll (73) has shown a similar arrangement of the sensory hairs of the vestibular organs in many different animals. This arrangement applies also to the sensory hair bundles in the lateral line organ (70, 19, 9). Other types of bundles such as those described in this paper have not been described before.

Morphological Basis of Functional Polarization in the Macula Utriculi

The adequate stimulus for the sensory cells in the macula utriculi is the sliding motion of the otolith produced by changes of the position of the head in the gravitational field, including linear acceleration and centrifugal forces (for references see Trincker, 1962). The sensory cells will subsequently be exposed to a variety of stimulation directions according to the direction of the positional change. In this organ, the ability of the receptor cells to signal to the central nervous system the direction from which the stimulus approaches is of great importance for the fulfillment of the equilibratory function of the organ.

In the crista ampullaris (53, 39), the macula utriculi (40), and the lateral line organ (54, 27), the discrimination of stimulating direction is electrophysiologically achieved by a two-way modulation of the resting discharge of action potentials in the innervating nerve fibers. The sensory units are thus functionally polarized. In the crista ampullaris of the horizontal canal, centripetal stimulation, that is, cupular deflection toward the utricle, is followed by an increase of discharge frequency, while stimulation away from the utricle causes a decrease of discharge (53, 39). In the vertical canals the opposite effects are encountered. Löwenstein and Wersäll (42) have shown that in the crista ampullaris of the thornback ray the sensory hair bundles are all oriented in the same direction, with the kinocilia facing toward the utricle in the crista of the horizontal canal, while in the vertical canals the kinocilia face away from the utricle. Their findings imply a functional significance of the polarization of the receptor cells.

On the basis of the morphological demonstration of two oppositely oriented groups of sensory cells in the lateral line organ of *Lota vulgaris*, Flock and Wersäll (19) proposed a theory dealing also with the microphonic effect in terms of directional sensitivity. This theory suggests that the double wave form of the microphonic effect recorded from the lateral line organ (33, 29) does not represent the function of the single sensory cell, as has hitherto been believed (8), but the sum of the superimposed anatagonistic responses derived from the two groups of cells which are structurally and functionally polarized in opposite directions. It was concluded that a bidirectional sensitivity coinciding with the morphological polarization of the sensory hair bundle seems to govern the function of the hair cells in both the crista ampullaris and the lateral line organ in such a way that stimulation of the sensory cell in a direction away from the stereocilia toward the kinocilium gives excitation of the sensory unit, that is, depolarization accompanied by increased impulse frequency in the nerve fiber, whereas stimulation in the opposite direction is inhibitory, giving hyperpolarization and decrease of resting discharge frequency (19). Recent findings of Dijkgraaf agree (9).

A correlation of functional and morphological polarizations has been observed also in the organ of Corti (20, 21, 14) and seems to be represented on an ultrastructural level by the asymmetric structure of the basal body of the kinocilium (17, 43).

It is suggested that in the utricle the same bidirectional modulation of sensory information that is seen in the crista ampullaris and in the lateral line organ is indicated by the morphological polarization of the sensory hair bundle. The pattern of morphological orientation of the sensory cells in the utricle of *Lota vulgaris* is shown in Fig. 17, tentatively revealing also the functional polarization of the sensory epithelium. This investigation has been announced and the preliminary findings discussed in earlier articles (20,

Laryngol., 1963, **72**, 489; and by Spoendlin, H., and Gacek, R., *Ann. Otol., Rhino. Laryngol.*, 1963, **72**, 660.

75). Whether a similar pattern of orientation is consistent throughout the animal series remains to be revealed. However, Engström *et al.* (14) have stated that in guinea pig and squirrel monkey the hair cells over wide areas of the macula utriculi and sacculi are oriented in the same direction while, along a certain line, this pattern reverses itself. Löwenstein *et al.* (43) have recently carried out a study on the morphological polarization of the vestibular organs which is now in press.

Löwenstein and Roberts (40) studied the influence of positional changes on the action potential frequency in single fiber preparations from utricular nerve twigs in the thornback ray. Most units showed a steady resting discharge which was increased or decreased by tilting in opposite directions, faithfully signaling by the frequency level the extent of deviation from the normal position. These units responded to both lateral and fore-and-aft tilting. Two main groups of units were found, one having its maximum of discharge activity in the side-up and nose-up position, the other in the side-up and nose-down position. It was suggested that the side-up and nose-up units are localized in the anterolateral part of the utricular macula, and the side-up and nose-down units in the posterolateral part of it. In Fig. 17, it is seen that in the burbot such responses would be yielded by the sensory cells located in the peripheral zone of the macula. In the experiments of Löwenstein and Roberts (41), the nerve twigs investigated were derived from the utricular nerve fan cut at its proximal end and peeled up towards the lateral margin of the macula. The units examined in the ray consequently corresponded only to the outer part of the macula, as stated by Löwenstein (38). It is seen, however, that in the burbot (Fig. 17) the major part of the macula, *i.e.* the central part, contains sensory cells which have their frequency maxima in exactly the opposite position. The major part of the macula would rather furnish units such as were found by Adrian (1) who, recording from the vestibular nucleus in the brain stem of cat, described gravity controlled responses to lateral and fore-and-aft tilting, showing increased impulse discharge on side-down tilting of the recording side. In a similar study on a teleost fish, Schoen (57) recorded units which were excited by side-down tilting as well as units which were excited by side-up tilting. These and many other seemingly contradictory experiments in different animals (for references see Löwenstein, 1950) contribute support to the suggested functional interpretation of the morphological polarization pattern of the utricular macula shown in Fig. 17.

Microphonic potentials have been recorded from the utricle of fishes (48). Trincker (67) measured the potential shifts in the guinea pig utricular macula, observing a two-way modulation of potentials following stimulation in opposite directions. Similar recordings were obtained in fish by Katsuki *et al.* (34). Since the added potential changes produced by all hair cells comprising the utricular macula will be simultaneously recorded, it is realized that little information about the function of the macula on a cellular level can be gained by such recordings, at least in *Lota vulgaris*. However, from Fig. 17 it is possible to predict the potential changes for any area of the utricular macula.

Sensory units of types other than the positional receptors hitherto discussed were recorded in the macula utriculi of the ray by Löwenstein and Roberts (40). Some units placed the position of their maximal discharge frequency at somewhat different positions depending on the direction of the tilt. Other units, termed "out-of-position" receptors, responded to a change in position in one and the same manner irrespective of the direction of the change. Whether the different types of bundles described in this paper correspond to these sensory units or just represent a functionally insignificant morphological variation is a matter of speculation.

It has also been shown by Löwenstein and Roberts (41) that receptors in the utricular macula that are outside the otolith-covered part are sensitive to vibration as could be expected from the exposure of these parts, especially the lacinia process, to vibratory stimuli by the coupling of the sensory cells to the gelatinous otolithic membrane (41, 38).

Functional Aspect of the Polarization of the Utricular Macula

The directional sensitivity of each utricular macula in the burbot covers a half circle with its center in the medial part of the sensory epithelium (Fig. 17). The half circle of directional sensitivity is divided into two halves by a line of cells which and laterally oriented in a direction which is perpendicular to the midline of the body of the animal, in spite of the fact that the utricular nerve makes an angle of approximately 70° with the

midline. There is consequently no anterior or posterior overlapping of sensitivity between the two maculae.

As an example to illustrate the interaction of the different parts of the maculae, the effect of tilting around a midline axis toward right-side-down will be discussed. In the right utricle, tilting in this direction will cause a laterally directed sliding force, exerted by the otolith, which will be excitatory for the cells in the central part of the macula, and maximally so for those cells which have their kinocilia pointing in this direction, but inhibitory for the cells in the peripheral zone which have their kinocilia pointing in the opposite direction (Fig. 17). Within each macula, the responses from the central part and peripheral zone consequently oppose each other. In the left utricle, tilting in the same direction will produce a medially directed sliding force evoking in the sensory units a response which is the mirror image of that of the right utricle. Thus the responses of the peripheral zones in the two maculae will oppose each other, but cooperate with the responses from the central part of the opposite macula. The central parts in the two maculae will oppose each other, but cooperate with the peripheral zone of the opposite macula.

Considering now the effect of fore-and-aft tilting around a horizontal axis connecting the two maculae, it is seen that a tilt towards nose-down will be followed, in each macula, by excitation of the sensory units in the anterior central part and the posterior peripheral zone, but by inhibition of units in the posterior central part and the anterior peripheral zone. In this case, the anterior and posterior halves within each macula will give opposing responses but both maculae will signal the positional change by identical response patterns.

It is seen that the pattern of directional sensitivity could furnish an exceedingly complicated pattern of afferent information consisting of antagonizing and cooperating responses interacting homolaterally as well as contralaterally. Therefore, it is not surprising that very conflicting results have been obtained in many studies on the peripheral equilibratory function using artificial mechanical, centrifugal, or other stimuli, as well as partial denervation or labyrinthectomy (for references see Löwenstein, 1950).

A well known effect of total unilateral labyrinthectomy is the tonus asymmetry found in all vertebrates, consisting of a preponderance of the antigravity muscles of the intact side. The experiments of Versteegh (71) indicated that the positional labyrinthine reflexes arise from the macula utriculi, and the observations of Tait and McNally (65) on frogs, in which all sensory organs of the labyrinth except one utriculus were eliminated by nerve sectioning, suggested homolateral preponderance of the antigravity muscles under the influence of the intact utricle. Much confusion has, therefore, been brought about by the observation of Lowenstein and Roberts (41) that all utricular sensory units recorded from the peripheral organ behaved as if they were linked to the antigravity muscles of the contralateral side, while the responses recorded by Adrian (1), showing increased discharge rate on side-down tilting of the recorded side, act in agreement with the expected situation. These contradictory findings can easily be brought into accord by correlating each response with the relevant part of the utricular macula, according to the pattern of directional sensitivity presented in Fig. 17.

In frogs with only one utricle intact, Tait and McNally (65) found diametrically opposed compensatory and anticompensatory reactions resulting from a slow or a quick tilt in the same direction around a horizontal axis. These authors suggested that this could only mean that the utricular macula, unlike the semicircular canals, furnishes two opposed modes of action. The anticompensatory response was seen only in the absence of vertical canal control.

It is interesting to notice that Mygind (45) made a distinction between opposing dominant and subordinate parts within the same sensory epithelium, postulating the dominant part to control the homolateral antigravity muscles and to collaborate, during lateral displacement, with the subordinate part in the opposite labyrinth, by crossed innervation.

Much interest has lately been focused on the problem of efferent innervation of the vestibular sensory epithelia. Morphological evidence suggests the presence of efferent nerve fibers ending on the sensory cells both within the vestibular sensory epithelia in mammals (25, 10, 11, 74) and in the fish utricular macula as presented in this study. It is hard to determine whether those endings represent only centripetal efferent fibers, such as described by Gacek (25), or, in addition, a homolateral efferent feed-back system connecting, on

the same side, such functionally distinct parts as described in this article.

Iurato (32) and Kimura and Wersäll (35) have shown that cutting of the crossed olivo-cochlear tract causes a degeneration of the granulated nerve endings on the outer hair cells in the organ of Corti.[1] Similar attempts by Kimura and Wersäll (36) to cut the centripetal efferent nerve fibers to the vestibular system have, so far, not been successful, inasmuch as no significant difference in degeneration of efferent and afferent endings has been observed. However, the presence of centripetal fibers has recently been experimentally confirmed. In a preliminary report, Fluur and Mendel (22) present evidence suggesting vestibular interplay in terms of contralateral efferent activity. Schmidt (56) and Gleisner and Henriksson (26) have recorded efferent impulses from the free ends of nerves detached from the vestibular organs of frog. Schmidt (56) found that stimulation of the contralateral labyrinth evoked efferent impulses reaching all vestibular sensory areas, and he also observed efferent feed-back from one ampulla to itself.

It is concluded that the positional information signaled by the nerve fibers innervating the vestibular organs comprises an intricate pattern of interacting afferent and efferent impulses. The findings presented in this paper suggest that the interplay of sensory responses is intimately related to the directional sensitivity of the receptor cells as revealed by their morphological polarization.

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