THE ULTRASTRUCTURE OF THE KINOCILIUM
OF THE SENSORY CELLS IN THE
INNER EAR AND LATERAL LINE ORGANS

ÅKE FLOCK, M.B., and ARNDT J. DUVALL, 3RD, M.D.

From Gustaf V Research Institute and the Department of Otolaryngology, Karolinska Sjukhuset, and from the Department of Histology, Karolinska Institutet, Stockholm, Sweden

ABSTRACT

The bundle of sensory hairs protruding from the top of each receptor cell in the vestibular and lateral line organs in the teleost fish (burbot) *Lota vulgaris* is composed of a number of stereocilia and one kinocilium located in the periphery of the bundle. The ultrastructure of the kinocilium and its basal body is described. It is found that the kinocilium is morphologically polarized by the asymmetric arrangement of its component fibers and of the basal body by the presence of a basal foot. Peripheral fibers 5 and 6 of the kinocilium and the basal foot of the basal body are oriented away from the stereocilia; that is, in a direction coinciding with the direction of excitatory stimulation. The findings are discussed in terms of directional sensitivity.

INTRODUCTION

In the stato-acoustic and lateral line organs the sensory cells are stimulated by the shearing motion of the overlying structures, that is, the cupula, the otolithic membrane, or the membrana tectoria, in relation to the surface of the sensory epithelium. A bundle of sensory hairs protruding from the top of each receptor cell transmits the stimulus to the cell, which regulates the flow of impulses in the innervating nerve fibers. In the vestibular organs (23, 24, 37, 10, 33, 34, 11) and lateral line organs (36, 15, 8) the sensory hair bundle is composed of a number of stereocilia and one kinocilium located in the periphery of the bundle, as first described by Wersäll (37). In the organ of Corti the kinocilium is lacking (10). The hair cells in the organ of Corti do, however, possess a centriole located at the top of each cell in a position corresponding to the location of the basal body of the kinocilium of the hair cells of the vestibular and lateral line organs (16, 11, 17). It has been possible to correlate the electrophysiological response of these cells with their morphological polarization as indicated by the location of the kinocilium or the centriole (25, 15–17, 11, 8, 39, 26, 14)[1]. The presence of modified kinocilia in other sensory systems suggests a sensory function for the kinocilium or its derivatives in the inner ear and lateral line organs, a fact which has directed the special attention of this article to the microarchitecture of these structures.

MATERIAL AND METHODS

The study was carried out on the teleost fish *Lota vulgaris* (burbot). After decapitation, the appropriate organs were perfused with 1 per cent osmium tet-

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Dr. Duvall, coauthor, is a visiting National Institutes of Health Research Fellow for the University of Minnesota, Minneapolis, Minnesota.

[1] See also Flock, Å., *Acta Otolaryngol.*, 1965, Suppl. 199, 1.

FIGURE 1 Section cut at a slight angle through the surface of the sensory epithelium of the macula utriculi of the teleost fish *Lota vulgaris*. The hair cells (*HC*) are interposed between supporting cells (*SC*) which, at their apical ends, are firmly joined to each other and to the receptor cells by desmosomes (*D*). *HC 1* is cut only partly below the cell surface, whereas *HC 2* and *3* are cut at the level of the cuticular plate (*Cu*). From the top of each sensory cell a bundle of sensory hairs or stereocilia (*St*) and one kinocilium (*K*) protrude into the overlying otolithic membrane. The kinocilium emerges from a basal body (*B*) located beneath the surface of the cell in an area devoid of cuticular substance (*CD*). The sensory cells are all directed with the kinocilium facing the same direction. *C*, centriole of a supporting cell. × 11,000.
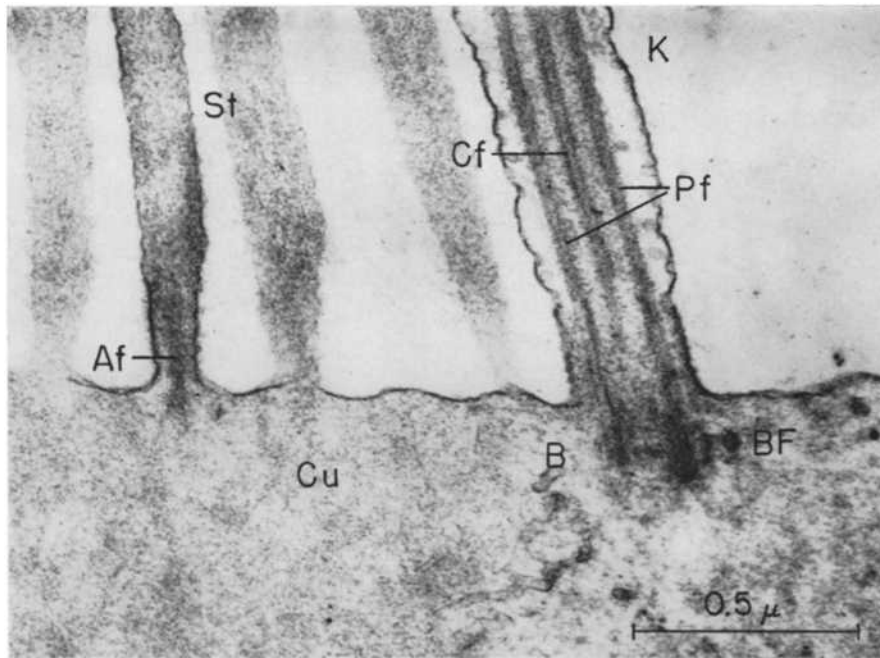
FIGURE 2   Section cut longitudinally through the kinocilium (*K*) and stereocilia (*St*) of a sensory cell in the macula utriculi. The peripheral fibers (*Pf*) of the kinocilium are continous with the wall of the basal body (*B*), while the central fibers (*Cf*) terminate above the cell surface. The lumen of the basal body contains a dense condensation. From the wall of the basal body a basal foot (*BF*) extends away from the stereocilia. Its distal end bears a globular swelling, and its base is composed of two short bars running along the wall of the basal body. Each stereocilium is composed of a plasma membrane surrounding a fibrillar core which, at its base, continues as an axial filament (*Af*), piercing the cuticle (*Cu*). × 60,000.

roxide solution buffered with veronal acetate, dehydrated in alcohol, and imbedded in Epon. Serial sections were cut with glass knives on an LKB Ultrotome, collected from 10 per cent alcohol on 100-mesh copper grids, and examined with a Siemens Elmiskop I. Most sections were stained in a 1.2 per cent solution of uranyl acetate in water or 10 per cent alcohol.

RESULTS

The sensory cells in the vestibular and lateral line organs of the teleost fish *Lota vulgaris* are flask-shaped cells about 30 to 40 μ long and with a greatest diameter of about 10 μ. Their long axis is perpendicular to the surface of the sensory epithelium. The receptor cells are interposed between the surrounding supporting cells which are planted on a basement membrane. At their apical ends, the supporting cells are firmly joined to each other and to the neighboring receptor cells by desmosomes, thus forming a rigid reticular lamina inter-

locking the hair-bearing ends of the sensory cells (Fig. 1).

From the top of each sensory cell a bundle of 40–60 stereocilia and one kinocilium protrude into the overlying cupula or otolithic membrane. The stereocilia are arranged in a regular pattern of parallel rows (Fig. 1). Their length increases stepwise towards the peripherally located kinocilium. The stereocilium is composed of an external triple-layered plasma membrane surrounding a fibrillar core, which at its base continues as an axial filament or rootlet piercing the cuticle (Figs. 1 and 2).

The kinocilium is composed of a bundle of tubular fibers emerging from a basal body located beneath the surface of the cells in an area devoid of cuticular substance (Figs. 1, 2). Fig. 3 and the schematic drawing (Fig. 8) show the composition of the peripheral part of the kinocilium, demonstrating the classical pattern of 9 peripheral double tubules or fibers surrounding a central pair of
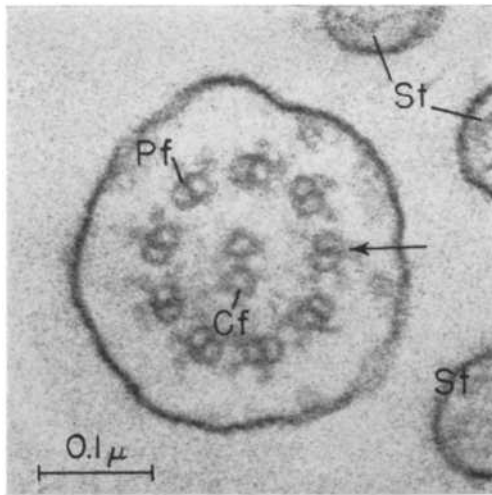
FIGURE 3 Cross-section of the kinocilium (utricular sensory cell of a fish). 9 peripheral double fibers (*Pf*) surround a central pair of fibers (*Cf*). The unpaired peripheral fiber (arrow) faces the stereocilia (*St*). × 150,000.
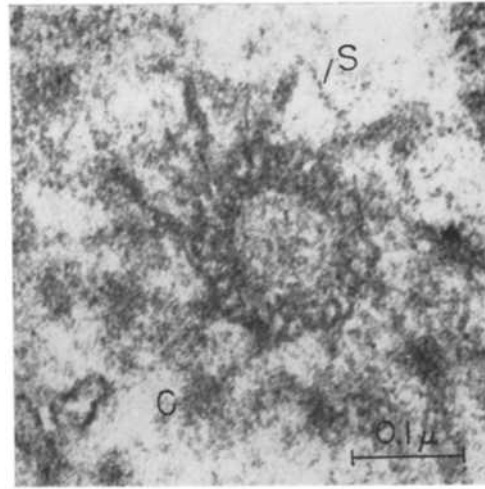

FIGURE 4 Cross-section through the upper part of the basal body, showing a system of spokes extending in a clockwise direction from the triplet tubules. A globular condensation (*C*) is found at the tip of each spoke. The spokes are interconnected by strands of osmiophilic substance (*S*). The lumen is occupied by an electron-opaque plate. × 150,000.

simple tubules. These fibers cannot be distinguished in specimens fixed in potassium permanganate, a fact suggesting that they do not contain lipids. A line connecting the two central tubules is perpendicular to the rows of stereocilia. The unpaired peripheral tubule No. 1 (numbered clockwise according to Afzelius, 1959) is oriented towards the stereocilia. Each peripheral fiber is provided with three short "arms," two of which point clockwise (when looking from inside the cell) and one of which points radially towards the surrounding triple-layered plasma membrane. Thin strands of osmiophilic substance converge towards the central fibers. In the middle of each strand a dense condensation is seen. In some kinocilia, vesicles with a diameter of about 300 A are found between the plasma membrane and the peripheral fibers (Fig. 2); in others, the plasma membrane is more or less degenerated.

Near the surface of the cell, the substructures of the kinocilium are rearranged through a complicated sequence of transformative stages leading to the formation of the basal body. These transformations, which will be described elsewhere, include the termination of the central fibers somewhat above the cell surface (Fig. 2). The ultrastructure of the basal body is schematically presented in Fig. 8. It has the shape of a short

cylinder about 0.2 μ long and 0.17 μ wide. The wall of the cylinder is formed by nine triplicate tubules which are derived from the peripheral tubules of the kinocilium. A cross-section through the upper part of the basal body (Fig. 4) shows a system of spokes protruding in a clockwise direction from the triplet tubules obliquely towards the plasma membrane at the base of the kinocilium. At the tip of each spoke, a globular condensation is found. Superficially, close to the hair cell surface, the spokes are interconnected by strands of osmiophilic substance running from each globule clockwise to the middle of the neighboring spoke. The whole structure has the appearance of a shovel-wheel. At this level the lumen of the basal body is occupied by a plate of aggregated material peripherally limited by a distinct membranous margin. At a deeper level (Fig. 5), the spokes are reduced to limited condensations outside the tubules. A dense strand runs along the inner margin of each triplicate tubule at the junction of its second and third part. Occasionally, granular or vesicular aggregates appear in the lumen of the basal body, especially at its lower end. The lumen is otherwise devoid of organized structures. Close to the bottom of the cylinder, the triplet

tubules are surrounded by a dense matrix occupying the space between them (Fig. 6). A remarkable feature of the basal body is the presence of a club-like basal foot ending with a swelling. It projects from the basal body at a location corresponding to peripheral fibers 5 and
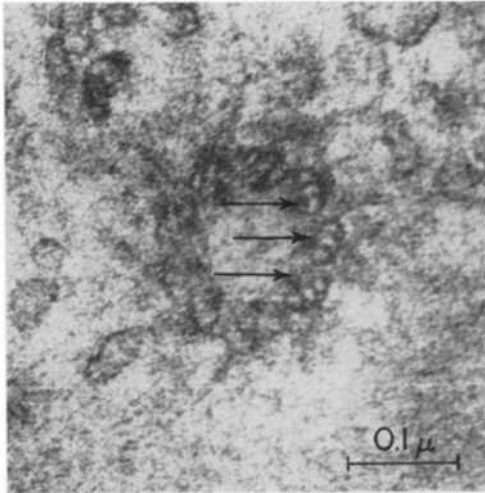

FIGURE 5 At a deeper level in the basal body, the spokes are reduced to condensations outside the tubules. A dense strand runs along the inner margin of each triplicate at the junction of its second and third part (arrows). × 150,000.

6; that is, away from the stereocilia (Figs. 2 and 8). The basal foot shows a cross-striation indicating its laminated structure (Fig. 6). and at its base is composed of two short bars running along the wall of the basal body (Fig. 2). Sometimes two basal feet project close by each other from the same basal body. In the vicinity of the basal body are found vesicles and mitochondria. The general structure of the basal body resembles that of centrioles found in other cells (Fig. 7), including the inner hair cell of the guinea pig cochlea (9).

DISCUSSION

The same arrangement of the sensory hair bundle has been observed in the vestibular organs of many species (38), as well as in the lateral line organs of fish (36, 15) and frog (8). The presence of a kinocilium in the bundle of sensory hairs of vestibular sensory cells was first described by Wersäll (37) who found that its structure was similar to that of kinocilia and flagella of other ciliated cells.

It was observed by Löwenstein and Wersäll (25) in the thornback ray that in each crista ampullaris the sensory hair bundles are all oriented in the same direction. They found that the morphological polarization of the sensory cells coincides with their directional sensitivity in such a
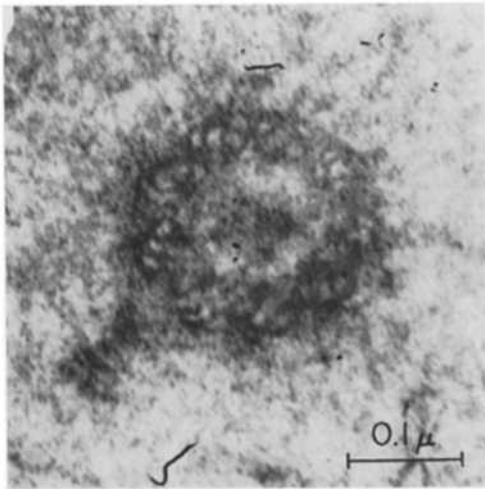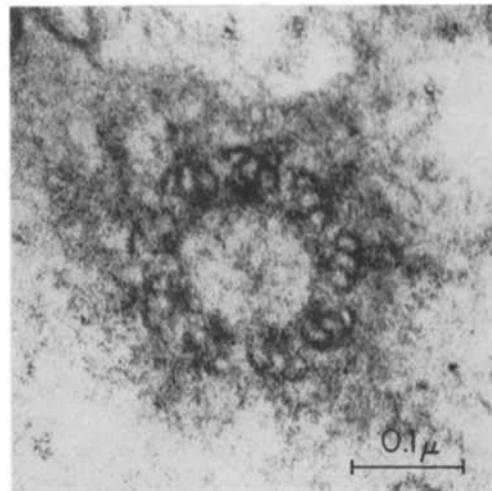

FIGURE 6 Section through the basal body showing the basal foot associated with it. The laminated structure of the basal foot is seen. A dense osmiophilic substance appears in the lumen of the basal body. × 150,000.

FIGURE 7 Cross-section through a centriole of a supporting cell in the utricular macula of a fish. Its resemblance with the basal body of the kinocilium of the vestibular hair cell is great. × 150,000.
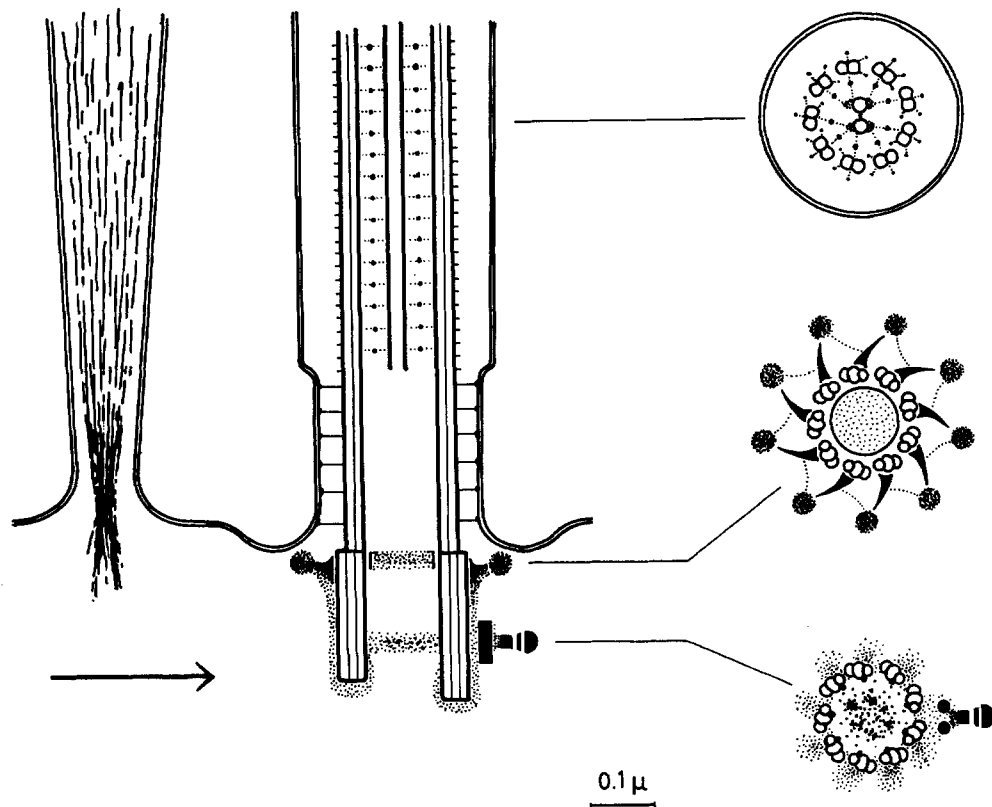
FIGURE 8 Diagrammatic reconstruction of the vestibular hair cell kinocilium and its basal body, showing transverse sections at the levels indicated. The arrow at the lower left indicates the direction of excitatory stimulation.

way that mechanical stimulation, that is, cupular displacement approaching the cells in a direction from the stereocilia towards the kinocilium is accompanied by a depolarization and an increased discharge frequency in the innervating nerve fibers, while stimulation in the opposite direction is followed by hyperpolarization and decrease of discharge rate. In the lateral line organ of the burbot, Flock and Wersäll (15) demonstrated the presence of two groups of sensory cells oriented with the kinocilia facing opposite directions. Assuming that the correlation of morphological and functional polarization of the receptor cells is the same as in the crista ampullaris, the double wave form of the microphonic effect recorded from the lateral line organ could be explained by the superposition of the antagonistic responses derived from the two oppositely oriented groups of cells.[1] In the macula utriculi of the burbot the pattern of orientation of the sensory cells, recently investigated by Flock

(14), agrees with the electrophysiology of the organ. In the organ of Corti a similar correlation is found between the location of the centriole in the outer hair cells and the directional sensitivity of these cells (17). Friedmann's idea (18) that in the organ of Corti of the fowl embryo otocyst some cells were equipped with a kinocilium, contributes toward emphasizing the relationship between the centriole and the basal body.

It is found in the present study that the directional sensitivity of the hair cells of the vestibular and lateral line organs is indicated not only by the location of the kinocilium in relation to the stereocilia, but also on an ultrastructural level by the asymmetric arrangement of the component fibers of the kinocilium and by the presence of a basal foot protruding from the basal body. Peripheral fibers 5 and 6 (numbered according to Afzelius, 1959) and the basal foot always point away from the stereocilia; that is, in the direction of excitatory stimulation (Fig. 8). A line connecting the two

central fibers is perpendicular to this direction. Similar results have been obtained independently by Löwenstein, Osborne, and Wersäll in the thornback ray (26).

This observation is interesting when one considers the relation between the ultrastructural organization and the direction of the effective stroke in actively beating cilia of metazoa and protozoa. In the ciliated epithelia of metazoa the cilia of any particular cell beat in a single plane, and the direction of the effective stroke is usually irreversible. A line connecting the central fibers is approximately perpendicular to the direction of beat (12, 2, 4). It was shown by Gibbons (20) that the basal body of the gill cilia of a lamellibranch was equipped with a basal foot and that the effective stroke was directed towards the basal foot and peripheral fibers 5 and 6. The same relationship has been observed on the tentacles of a gastropod mollusk (27) and in tracheal epithelium of rat (20). On the other hand, it is well known that many protozoa are able to change the direction of their effective stroke according to the direction in which the organism is swimming (29, 31). Electron microscopic studies on the cilia of such organisms have shown that the pair of central fibers have a random orientation (20, 28) and that the simple cylindrical basal bodies are devoid of asymmetrical structures such as a basal foot (19, 28, 30). It has been suggested that in metazoa the restricted capacity for movement of the cilia in more than one plane is related to the polarization of the basal body by means of its basal foot, while in protozoa the capacity of the cilia to beat in many directions is related to the fact that the basal body is not polarized (13).

The association of an analogous morphological polarization of the kinocilium and its basal body, in one case, to the direction of beat of motile cilia, and in the other to the directional sensitivity of vestibular and lateral line sensory cells, is remarkable and directs our attention to the presence of modified kinocilia also in other sense organs and in neural tissue (5). In the olfactory epithelium, the terminal sensory processes emerging from the apical swelling of the dendrite of the olfactory neuron closely resemble ordinary cilia (3, 6, 36). The initial stage in the morphogenesis of the outer segments of retinal rods is the development of a primitive cilium from a centriole which is destined to be the basal body of the cilium (7). In ultrastructure, the basal body of the retinal rod is surprisingly similar to the basal body of vestibular sensory cells, and it also possesses a basal foot projecting from its wall (35). In the more primitive eye of a starfish, each sensory cell is provided with kinocilium which does not appear to be modified (22). In the auditory organ of the locust, the dendrites of the sensory neurons end in cilia which are surrounded by a scolopale coupled to the tympanum (21).

A similar architecture has been observed in the plate organs of the antenna of the honeybee, for which the function of auditory or other vibratory perception has been suggested (32). In all these organs, the sensory function assigned to the modified cilia is generally agreed upon (13).

In the vestibular and lateral line organs, the sensory hairs transmit the mechanical stimulus to the sensory cells which regulate the impulse frequency in the innervating nerve fibers. A pervading principle is the directional sensitivity of the receptor units. This functional polarization coincides with a morphological polarization of the sensory cells indicated by the position of the kinocilium in the sensory hair bundle as well as by the asymmetric structure of the kinocilium and its basal body, a fact which tends to ascribe to this morphological polarization a functional significance in terms of directional sensitivity.

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