A STUDY OF THE ORIENTATION OF THE SENSORY HAIRS OF THE RECEPTOR CELLS IN THE LATERAL LINE ORGAN OF FISH, WITH SPECIAL REFERENCE TO THE FUNCTION OF THE RECEPTORS

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ABSTRACT

The morphology of the hair bundles on top of the receptor cells in the lateral line organ of the teleost fish *Lota vulgaris* is described. Each receptor cell shows a distinct morphological polarization. Two groups of receptor cells can be distingiushed, one consisting of cells polarized towards the head, the other consisting of cells polarized towards the tail. In the crista ampullaris all cells are polarized in the same direction. An hypothesis is proposed for the function of the receptor cells in the lateral line organ and the labyrinth based on a correlation of morphological and functional polarization.

INTRODUCTION

The sense organs in the labyrinth and in the lateral line system have the same origin, the auditory placode (3, 6, 8, 11). The basic structure is the same in all these organs, although they have been differentiated in different directions, each of them being especially adapted to respond to certain adequate mechanical stimuli.

The lateral line organs are found in canals on the head and along the sides of the body of fishes. The organ is built up by a group of sensory cells embraced by supporting cells forming a sensory epithelium which is innervated by nerve fibres branching under the hair cells. From the top of each receptor cell a bundle of sensory hairs protrudes into canals of the gelatinous cupula resting on the epithelium. This structure also applies to the vestibular sensory epithelia (Fig. 1).

Movements of the fluid in the canal cause a displacement of the cupula which acts upon the hair cells through the sensory hairs. The response in the hair cell is transferred to the nerve endings thereby regulating the flow of impulses in the innervating nerve fibre.

The present paper deals with the organization and structure of the sensory hairs of the receptor cells of the lateral line organ and submits a functional interpretation of the findings.

MATERIAL AND METHODS

Healthy fishes were decapitated. The lateral canal on the side of the fish was perfused with cold 1 per cent osmium tetroxide solution buffered with Veronal acetate. The sensory areas along the lateral line were dissected out and embedded in Epon after dehydration in alcohol. The specimen was sectioned with glass knives on an LKB Ultrotome and the sections were photographed in a Siemens Elmiskop I.

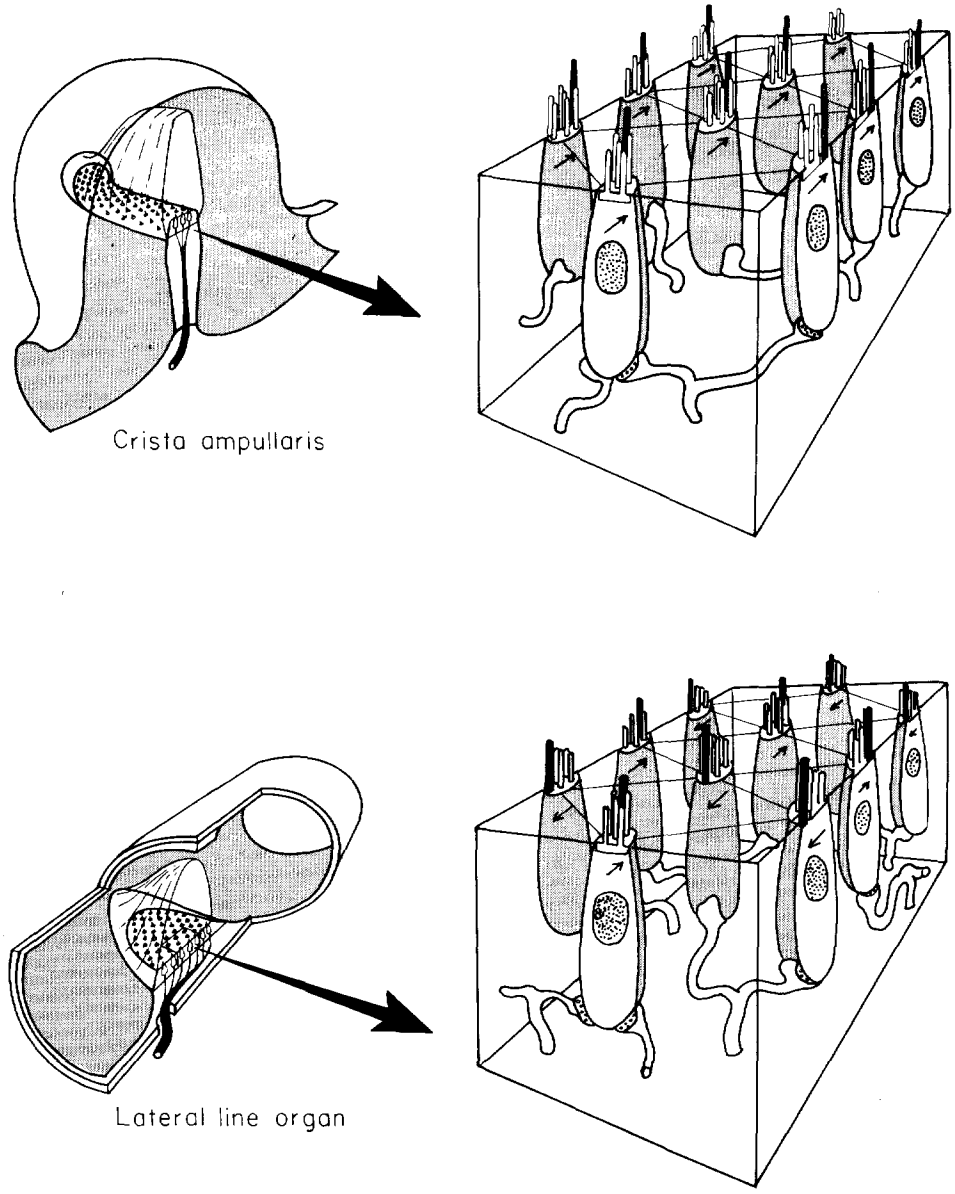


FIGURE 1

Diagram of the crista ampullaris and the lateral line organ with enlarged areas of the sensory epithelia demonstrating schematically the arrangement of the receptor cells and the orientation of the sensory hair bundles. The kinocilium is painted black. In the crista ampullaris the kinocilium is always found at the same side of the bundle, while in the lateral line organ adjacent hair cells are polarized with the kinocilia pointing in opposite directions.

The pattern of innervation of the sensory cells is imaginary. The cupula overlying the epithelium is omitted in the drawings at higher magnification.

RESULTS

The sensory hairs on each receptor cell of the lateral line organ form a bundle with a characteristic organization. Each bundle contains about fifty sensory hairs, or stereocilia, and one kinocilium (Fig. 2). The kinocilium is composed of nine peripheral double filaments arranged around a central pair of axial filaments emerging from a basal body which is presumably derived from the centriole of the cell (Fig. 3).

The kinocilium is delimited by a membrane forming a tube which, at the base of the cilium, is continuous with the plasma membrane of the cell. The diameter of the cilium is about 0.33 μ. The axial filaments are about 200 A in diameter, the space between them about 100 A. This space is occupied by an opaque substance. The axial filaments end somewhat above the cell surface. The peripheral filaments, on continuing their course down into the cell, are reorganized into the triplet filaments of the basal body. The structure of the basal body is very similar to that of the centriole and will be further described in a separate article.

The stereocilia are built up by a central core of protoplasm surrounded by a plasma membrane forming a tube, 0.15 μ in diameter. The protoplasmic core is composed of several fibrils about 30 to 40 A in diameter. In the basal part of the cilium these fibres unite to form a dense axial fibre which passes into the cuticular plate of the hair cell

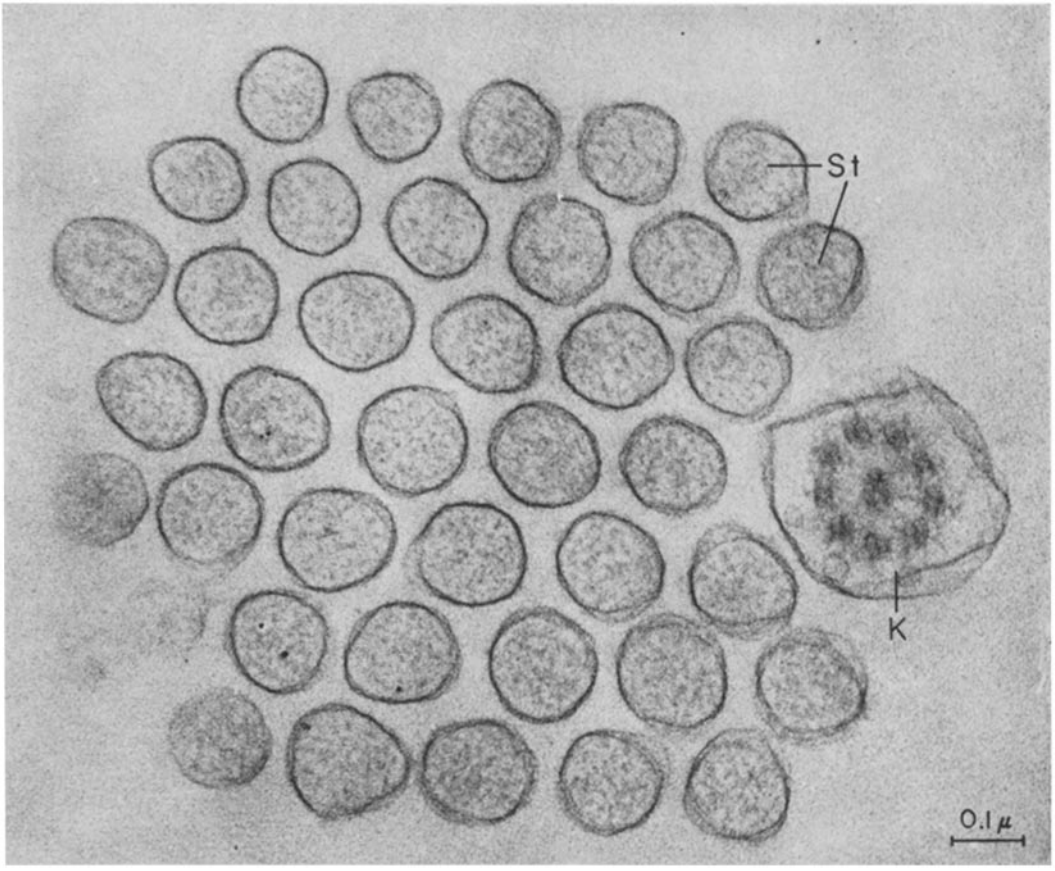


FIGURE 2

Cross-section of a sensory hair showing the organisation of the bundle. The stereocilia, *St*, are composed of a protoplasmic core containing several fine fibrils surrounded by a plasma membrane. The kinocilium, *K*, is located in a V-shaped indentation formed by the advanced peripheral rows of stereocilia. Osmium tetroxide fixation. × 100,000.

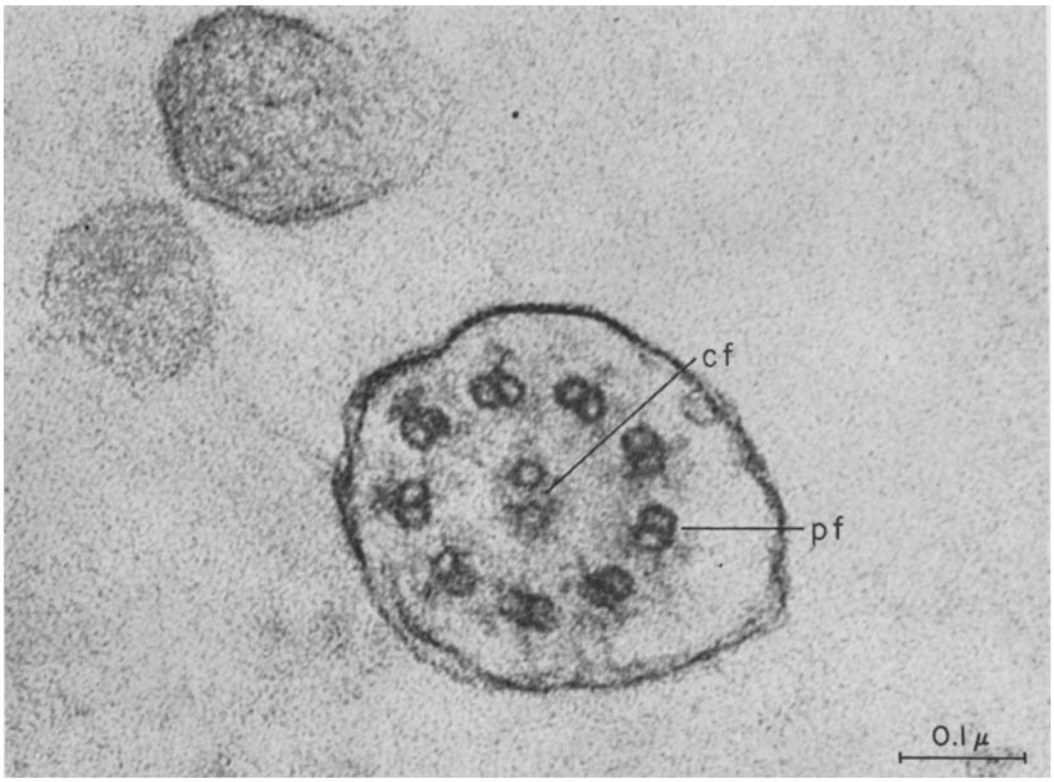


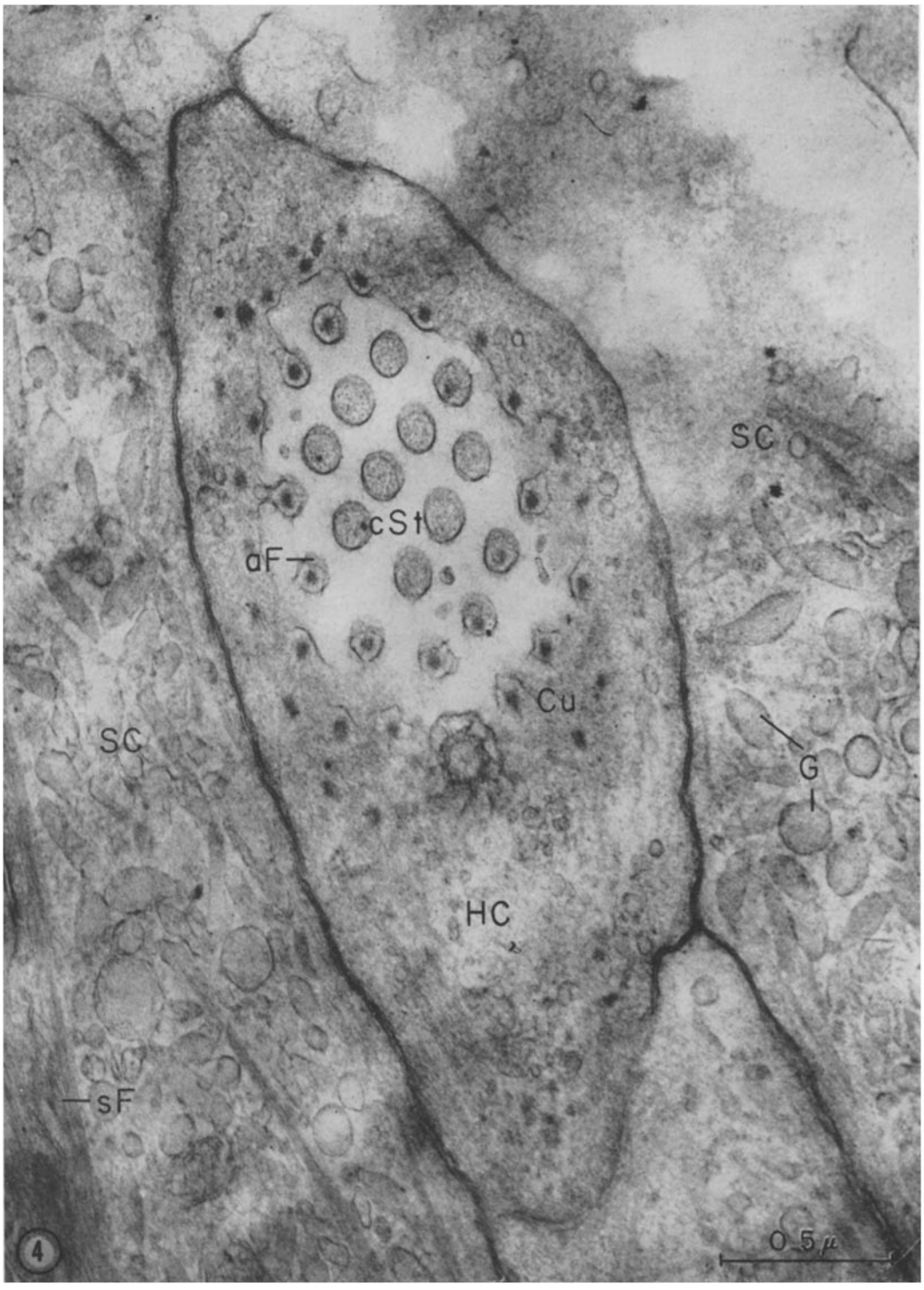
FIGURE 3

Cross-section of a kinocilium showing the nine peripheral double filaments, *pf*, and the central pair of single filaments, *cf*. Osmium tetroxide fixation. × 170,000.

(Fig. 4). The cuticular plate is granular in appearance and occupies the whole top surface of the cell apart from an area around the basal body of the kinocilium and canals, formed around the rootlets of the stereocilia. The sensory hairs are strictly organized into a definite, characteristic pattern (Fig. 2). The stereocilia are oriented along straight lines parallel to the axis of lateral line canal. The central row points towards the kinocilium, the direction of this row being almost perpendicular to a line through the axial filaments of the kinocilium. There are three rows of stereocilia on each side of the central row. The peripheral rows are advanced towards the kinocilium in relation to the

FIGURE 4

A section cut close to the surface of the epithelium showing the sensory hair bundle emerging from the top of a hair cell, *HC*. In the stereocilia located in the centre of the bundle, *cSt*, the fibrils are scattered in the protoplasm. The stereocilia located peripherally in the bundle are cut closer to the surface of the cell where the fibrils unite forming a dense axial fibre, *aF*, passing into the granular cuticula, *Cu*. The kinocilium is seen passing into the cell. The nine peripheral filaments have joined into a continuous ring sending out nine dense arms in a spiral course towards the membrane of the cilium on one side and out into the cuticula on the other side. The adjoining supporting cells, *SC*, contain large opaque granules, *G*, of various shapes and supporting fibrils *sF*. Osmium tetroxide fixation. × 50,000.


SC
cSt
aF
Cu
SC
G
HC
sF
4
0.5 μ

central row, thus forming a number of V-shaped lines one behind the other at an angle of 110 to 115° opening towards the kinocilium. In the two outermost rows the first cilium is generally lacking. Close to the hair cell short stereocilia may add further rows to the bundle, but these do not extend far from the surface of the epithelium. At a distance from the cell the number of stereocilia decreases, those lying farther away from the kinocilium ending first. At the distal end of the bundle the kinocilium is only accompanied by a single V of stereocilia and is finally travelling alone for quite a distance in the cupula (Fig. 1).

The sensory hair bundles are organized into two equally large groups distinguished by the orientation of the bundles in two directions opposite to each other but parallel to the axis of the canal (Figs. 1 and 5).

DISCUSSION

As described above, the hair bundle and the top of the hair cell in the lateral line organ are structurally distinctly polarized. The hair cells in the lateral line organ can thus be divided into two groups according to their different polarization. Adjacent hair cells are polarized with the kinocilia pointing in opposite directions, so that there is a regular alternation of orientation from cell to cell: cranial, caudal, cranial, caudal, etc. (Figs. 1 and 5). The rows of stereocilia are parallel to the axis of the canal in all bundles. This means that for both directions of cupular displacement there is an equal number of hair cells "facing" the movement of the cupula. In the crista, however, the polarization of these bundles has only one direction, which is in agreement with the findings of Lowenstein and Wersäll (5) on the ray crista and with those of Trujillo-Cenoz (10) on the epidermal neuromasts and in the crista of fish.

Considering the difference in electric response to cupular deflection in these two organs, we find the morphological polarization to be of importance for the understanding of the function of the vestibular as well as the lateral line organ receptor cells.

Different potentials have been recorded in relation to the mechanical receptors in the labyrinth and the lateral line organ, which are considered to be of importance for the function of these organs. Two of these are related to the hair cell itself, namely, the potential difference between the sensory epithelium and the endolymph, the DC potential, and the microphone potential which is an AC potential correlated with the movements of the cupula. The action potential in the nerve is initiated by depolarization of the nerve ending through the action of the sensory cell.

The microphone potential was first demonstrated in the organ of Corti by Wever and Bray (15) and has been described in the crista ampullaris by de Vries and Bleeker (12) and by Trinker (9). In the lateral line organ the microphone potential was found by de Vries *et al.* (12) and further studied by de Vries, Jielof, and Spoor (13) and by Kuijper (2).

In the crista and the organ of Corti the frequency of the microphone potential follows that of the applied stimulus (1) while the lateral line organ shows the remarkable property of responding with a microphone frequency twice that of the stimulus.

De Vries and coworkers (13) found that the characteristic double wave form of the microphone potential recorded from the lateral line organ should be interpreted as two negative peaks, one corresponding to a forward, the other to a tailward, displacement of the cupula. This was supported by Kuijper's experiments (2) in which he found a potential decrease at cranial as well as caudal cupular displacement. De Vries (11) suggested the following model for the stimulation of the hair cell in the lateral line organ. Stretching of the hair should initiate a current flow through the hair cell. If the cupula oscillates he proposed that the hair would be pulled once during each half-period, each pull producing a current flow in the same direction through the cell. This would give an AC current through the cell, which could be recorded as a corresponding microphone potential. Trinker (9) showed, however, that utriculopetal displacement of the cupula of the crista ampullaris of the horizontal canal always produced a potential decrease whereas an utriculofugal displacement produced a potential increase. The potential decrease was often greater than the increase.

This difference in characteristics of microphone potential in the organs referred to seems to imply a discrepancy in function of the hair cells in the crista and in the lateral line organ. This discrepancy has been pointed out by de Vries (11) and by Kuijper (2). De Vries suggested that the one-way stimulating effect in the crista could be explained by a difference in inclination of the hairs in relation to the cell surface and a different distribution of the hair cells on the two sides of the crista.

Knowing that in the horizontal canal the kinocilia are all directed towards the utriculus and in the vertical canals in the opposite direction (5),

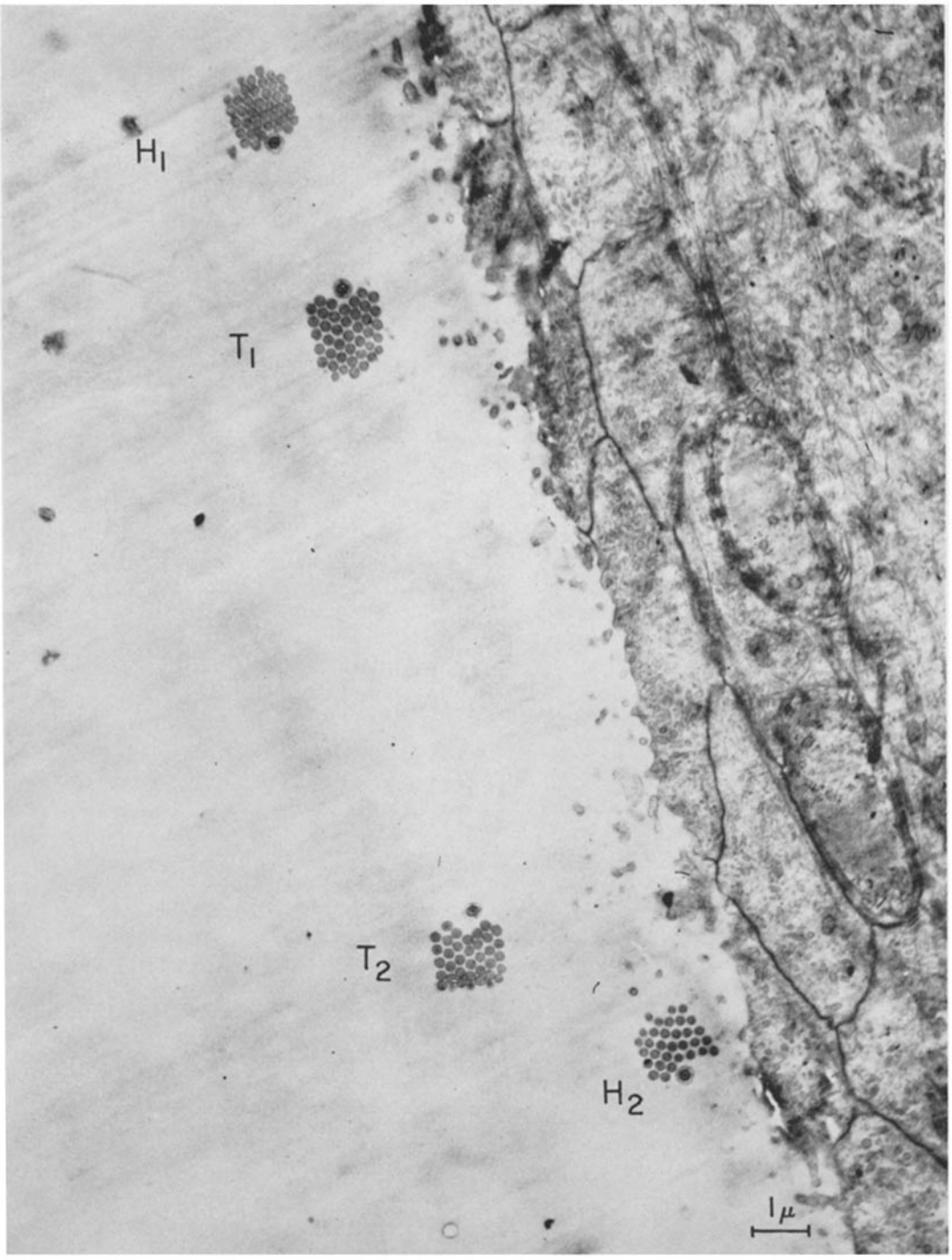


FIGURE 5

Survey picture showing the orientation of the sensory hair bundles. Two of these bundles, H_1 and H_2 are polarized towards the head of the fish, while the other two bundles, T_1 and T_2, are polarized in the opposite direction, that is towards the tail of the fish. Osmium tetroxide fixation. × 8,000.

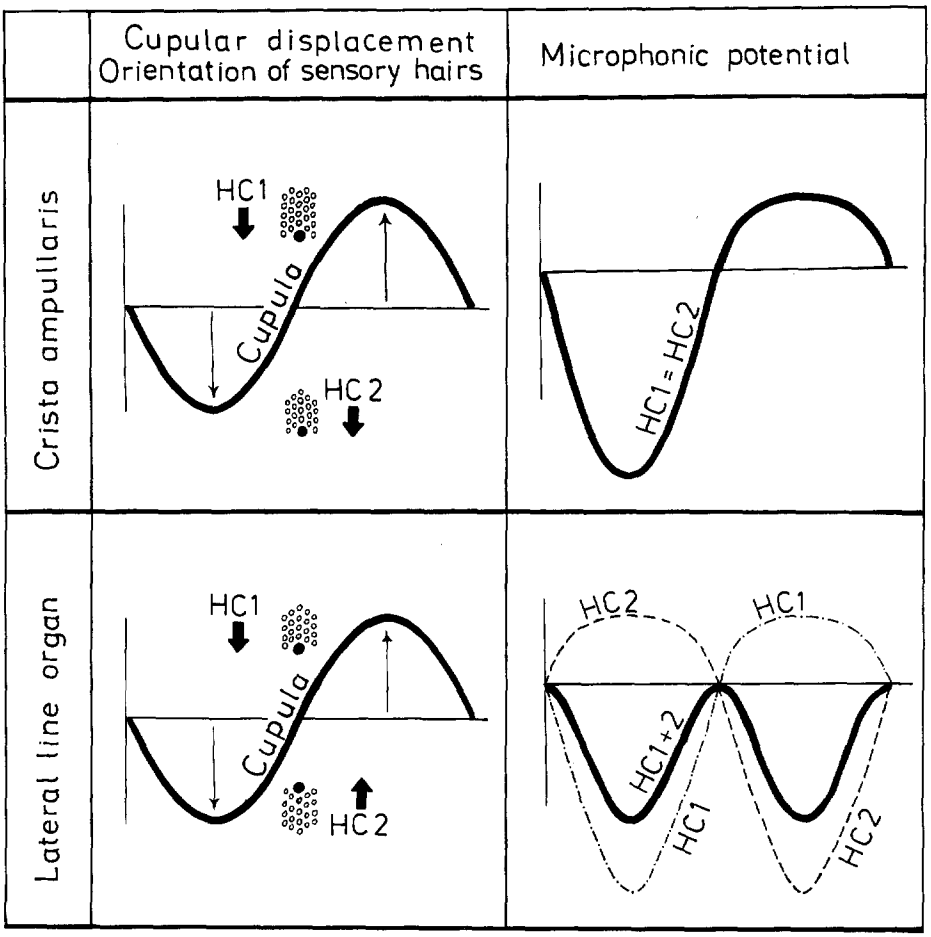


FIGURE 6

Illustration of the presented hypotheses of hair cell response to cupular displacement in two opposite directions, applied to the crista ampullaris and to the lateral line organ. In the crista, all hair cells are polarized in the same direction as is indicated by *HC1* and *HC2*. Cupular displacement in this direction is excitatory for both cells and is followed by a decrease in potential, while displacement in the other direction is accompanied by a decrease in potential as is shown by the right curve representing the sum of responses from *HC1* and *HC2*.

In the lateral line organ, *HC1* and *HC2* are polarized in opposite directions. The potential changes induced by cupular displacement through *HC1* will follow the course indicated by the dotted curve marked *HC1* in the right figure, while potential changes evoked by *HC2* will follow the course indicated by curve *HC2*. The recorded microphonic potential represents the sum of these two opposed responses, curve *HC1* + *HC2*, and will consequently show a frequency double that of the cupular displacement.

we see that stimulation in the direction of the kinocilium produces a drop in potential, away from it a rise in potential (9). Since we believe that the labyrinth receptor cells all use the same basic system for recording and transformation of mechanical stimulus, we propose that the single receptor cell of the lateral line organ shows the same functional two-way modulation as do the hair cells of the crista. Then the microphone effect of the single hair cell in the lateral line organ has to follow the frequency of the stimulus, rising one way, falling the other. The recorded microphone potential in the lateral line organ should then, according to our hypothesis, represent the superimposed responses derived from the two groups of cells which are structurally and functionally polarized in opposite directions. In the crista ampullaris the receptor cells are all arranged in the same di-

rection. Consequently the microphone potential recorded from this organ expresses the activity of the single hair cell (Fig. 6).

This concept of functional polarization can also be applied to the directional sensitivity of the impulse frequency in the single nerve fibre. This directional sensitivity was proposed by Lowenstein and Wersäll (5).

From recordings of potentials from isolated nerve fibres from lateral line organs of ray, Sand found, (7) that the frequency of the spontaneous activity in some nerve fibres increased when the canals were perfused in the head-tailward direction, and decreased in the other direction. In other fibres the reverse effects were observed. We know from the experiments of Lowenstein and Sand (4) on isolated ray labyrinths and of Zotterman on *Lota vulgaris* (16) that the impulse traffic in the nerve from the horizontal canal is increased by an utriculopetal flow and decreased by an utriculofugal flow. The vertical canals are stimulated by opposite directions of flow.

Sand's (7) observations could be explained by the existence of receptor cells, some of which stimulated the nerve endings at a displacement towards the head, others at a displacement towards the tail.

We notice that in the semicircular canals an increase in discharge frequency in the nerve fibres occurs at a displacement of the cupula towards the kinocilium, which is also the direction accompanied by a drop in microphonic effect, and that a decreased discharge rate is obtained in the direction which gave a raised potential, that is to say away from the kinociliar pole.

We know that in the lateral line organ one way displacement gives an increase in impulse traffic in some nerve fibres and a decrease in others. If we apply to these facts our knowledge of the morphological double set-up of opposing receptor cells in this organ, we see that these two groups at such one-way displacement are stimulated from opposite directions. This fact is easily explained if each receptor cell is capable of decreasing or increasing the spontaneous discharge when stimulated in the opposite direction. This also seems to be the case in the crista ampullaris, a fact which strongly implies a functional polarization of the hair cells in both organs. The morphological polarization in the crista ampullaris and in the lateral line organ and the functional polarization of the microphonic effect and of the afferent nerve fibre response lead us to the following hypothesis of hair cell stimulation, which refers to both the crista ampullaris and the lateral line organ.

The response of the hair cell is determined by the direction from which the stimulus approaches the hair bundle.

The morphological polarization of the sensory hair bundle and of the top of the cell is an indicator of this discrimination system.

A cupular displacement in the direction towards the kinociliar pole of the cell is accompanied by a fall in potential and a raised rate of discharge in the afferent nerve fibre. A displacement in the opposite direction releases a potential increase, which is accompanied by a lower afferent discharge rate. The amplitude of the displacement of the cupula determines the size of the potential change. In the lateral line organ the decrease in potential is always larger than the increase caused by the same amplitude of cupular displacement.

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BIBLIOGRAPHY

1. Békésy, G. von, *J. Acoust. Soc. Amer.*, 1952, **24**, 399.
2. Kuijper, J. W., The Microphonic Effect of the Lateral Line Organ, thesis, Groningen (Nether lands), 1956.
3. Lowenstein, O., *Proc. Roy. Soc. (B)*, 1960, **152**, 1.
4. Lowenstein, O., and Sand, A., *Proc. Roy. Soc. (B)*, 1940, **129**, 256.
5. Lowenstein, O., and Wersäll, J., *Nature*, 1959, **184**, 1807.
6. Pumphrey, R. J., *Symp. Soc. Exp. Biol.*, 1950, **4**, 3.
7. Sand, A., *Proc. Roy. Soc. (B)*, 1937, **123**, 477.
8. Stone, L. S., *J. Exp. Zool.*, **35**, 421.
9. Trinker, D., *Arch. ges. Physiol.*, 1957, **264**, 351.
10. Trujillo-Cenoz, O., *Z. f. Zellforsch.*, 1961, **54**, 654.
11. de Vries, H., *Progr. Biophysics*, 1956, **6**, 207.
12. de Vries, H., and Bleeker, J. D. J. W., *Acta oto-laryng.*, 1949, **37**, 289.
13. de Vries, H., Jielof, R., and Spoor, A., *J. Physiol.*, 1952, **116**, 137.
14. Wersäll, J., *Acta oto-laryng.*, 1960, suppl., **163**, 25.
15. Wever, G. S., and Bray, C. W., *J. Exp. Psych.*, 1930, **13**, 373.
16. Zotterman, Y., *J. Physiol.*, 1943, **102**, 313.