

# SYNAPTIC STRUCTURES IN THE LATERAL LINE CANAL ORGAN OF THE TELEOST FISH *LOTA VULGARIS*

ÅKE FLOCK, M.B., and JAN WERSÄLL, M.D.

From the Department of Otolaryngology and the Department of Histology, Karolinska Institutet, and Gustav V Research Institute, Stockholm, Sweden

## ABSTRACT

This paper is a preliminary report on some of the results of electron microscopic studies on the lateral line canal organ of the teleost fish *Lota vulgaris*. It deals with the ultrastructure of the synaptic area on the hair cells of the sensory epithelium and describes the nerve endings as well as a complicated system of foldings of the hair cell plasma membranes enclosing portions of the hair cell cytoplasm in the synaptic area. These findings are discussed in the light of present knowledge of the ultrastructure of other receptoneuronal synapses.

## INTRODUCTION

The importance of correlating the ultrastructure of the synaptic area in various synapses, including neuromuscular junction and receptoneuronal synapses, with present knowledge of neurophysiology and neuropharmacology has been stressed by several authors (2, 17, 9, 19, 12, 13).

Extensive work has been done on the structure of interneuronal synapses (4, 3, 7, 17), the neuromuscular junction (1), and on the receptoneuronal synapses in the retina (19, 20), the inner ear (11, 12, 18, 21), the taste buds (6, 10, 22), the cutaneous sense organs (15), and on the epidermal neuro-masts of the lateral line system (21, 22). So far no publication has appeared on the ultrastructure of the canal organ in the lateral line system of fish.

Since special physiological significance has been assigned to the membranous structures in the synapse, the present paper deals especially with such structures in the recepto-neuronal synapse of the fish canal organ.

The structure of the various cell types found in this epithelium will be discussed elsewhere, though

the general pattern of the sensory epithelium is indicated by Fig. 1.

## MATERIAL AND METHODS

Six fishes, weighing 0.8 to 1.6 kg, were prepared for this investigation. After decapitation of the animal an incision was made into the supraorbital canal, and the lateral canal was cut through about 8 cm behind the gillcover. A glass pipette was inserted into the supraorbital canal which was perfused caudally with fixation fluid. The skin was dissected away over the relevant area, and the semitubules of bone containing the organs were cut out and put into the fixation fluid.

Buffered 1 per cent osmium tetroxide solution and buffered 3 per cent potassium permanganate solution were used as fixation fluids. After rinsing in Ringer solution and dehydration, the specimens were embedded in butylmethacrylate, Araldite or Epon.

Specimens for phase contrast microscopy and electron microscopy were cut with glass knives on an LKB Ultratome. The sections were floated on 20 per cent acetone and collected on formvar membranes stretched across a single large hole or multiple holes of copper grids.

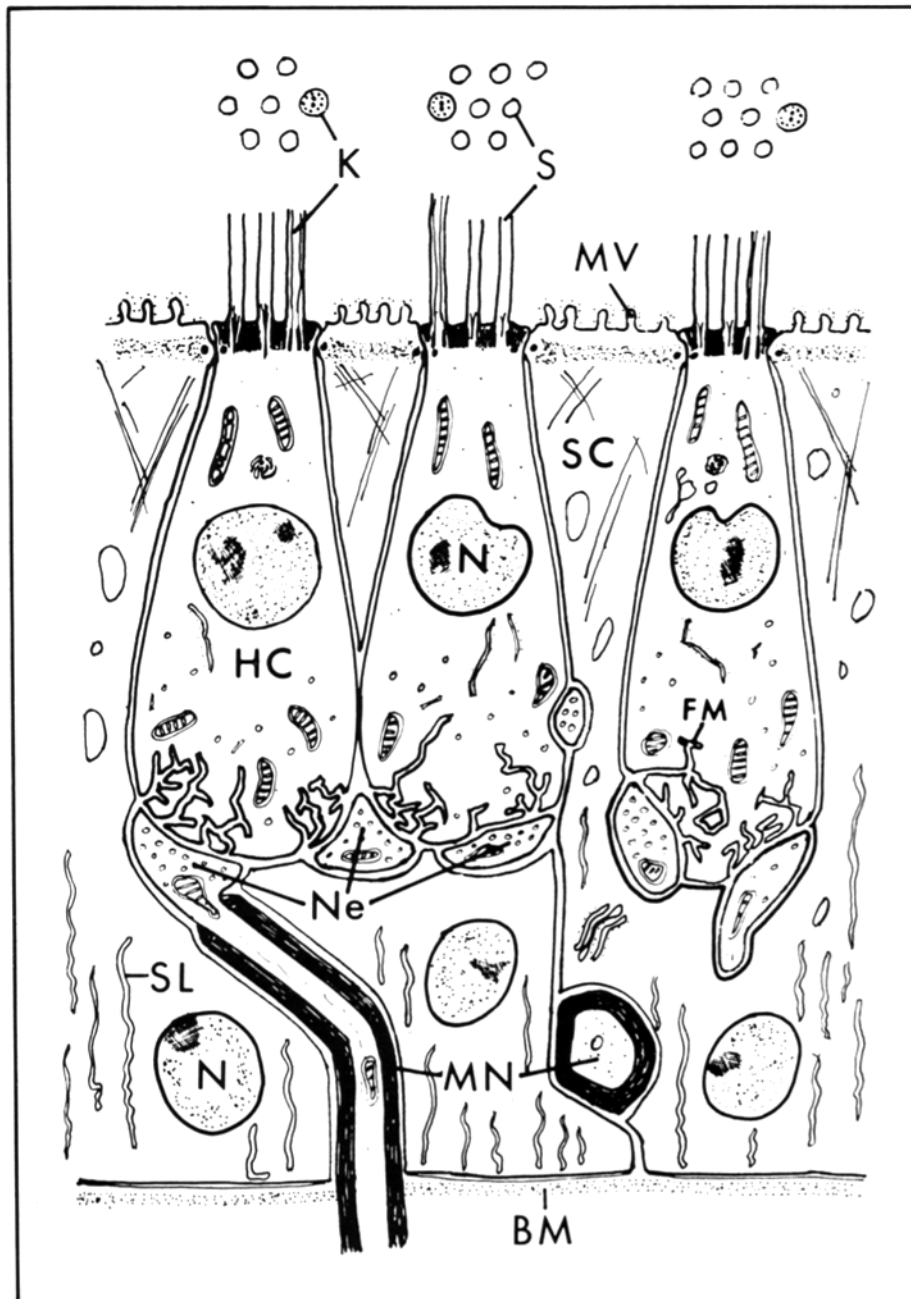


FIGURE 1

Highly schematic drawing of the sensory epithelium of the lateral line canal organ. *HC* = hair cell, *SC* = supporting cell, *MN* = myelinated nerve fiber, *Ne* = nerve ending, *K* = kinocilia, *S* = stereocilia, *MV* = microvilli, *N* = nucleus, *FM* = folding membrane system, *SL* = supporting lamellae, *BM* = basal membrane.



FIGURES 2 TO 4

Three sections taken from a series through the base of a hair cell, *HC*, showing the complicated system of folding plasma membrane, *FM*, and the relation to the nerve ending, *Ne*. On these sections the process of folding can be seen to give rise to an enclosed cytoplasmic partition, *CP*, which in the last section seems to have no connection with the cell cytoplasm. The series shows, however, the true communication with the hair cell cytoplasm, (Potassium permanganate fixation).  $\times 76,000$ .

Electron micrographs were taken at 1000 to 40,000 times magnification in a Siemens Elmiskop I with 50 or 20  $\mu$  objective apertures and subsequently photographically enlarged to the magnification desired.

#### OBSERVATIONS

The synaptic area of some of the nerve endings at the basal part of the hair cells in the sensory epi-

thelium of the lateral line canal organ is composed of (a) one or several rounded nerve endings, (b) a 100 Å wide synaptic cleft, (c) the hair cell plasma membrane, from which is derived (d) a complicated system of interdigitating folds and tubules.

The nerve endings contain a large number of ellipsoid or round vesicles 200 to 400 Å in diameter. Each vesicle is surrounded by a membrane and

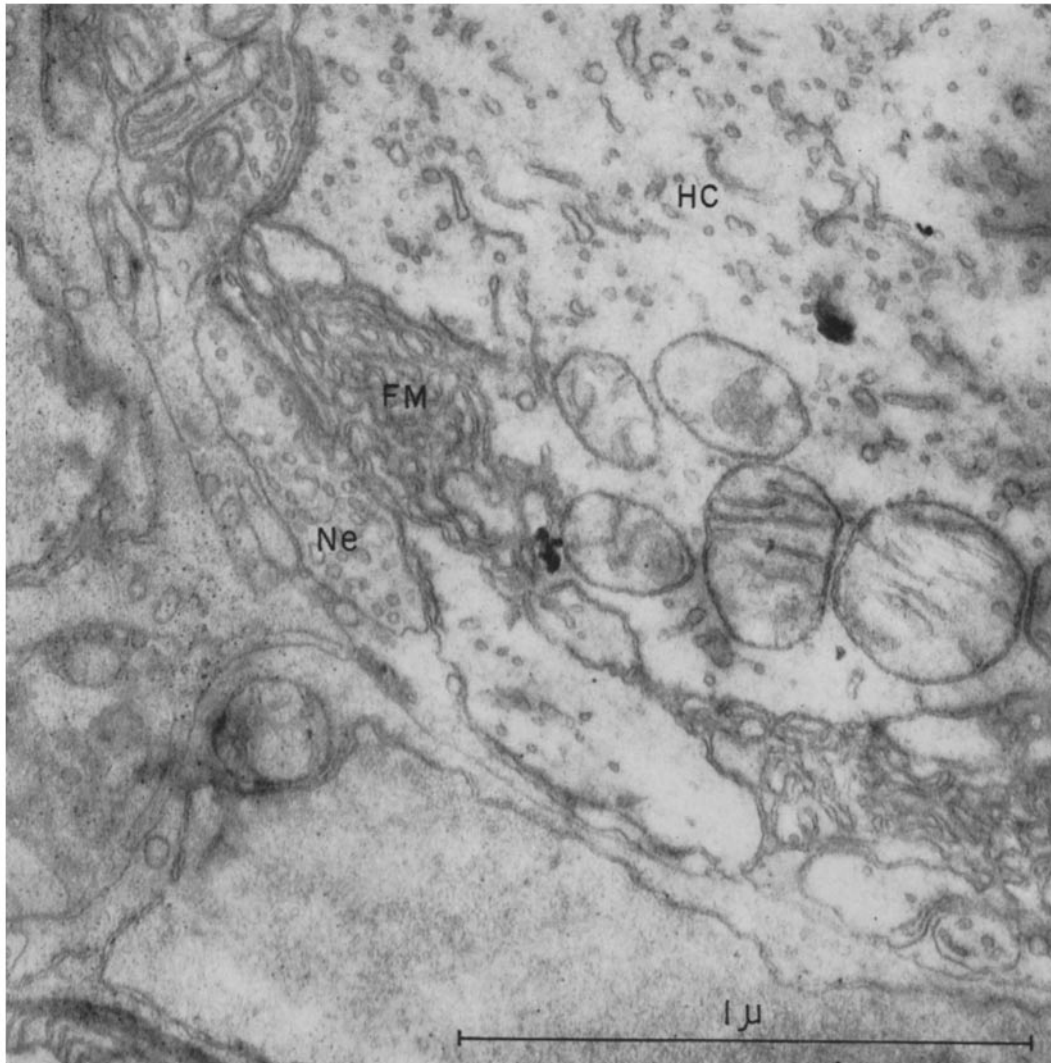


FIGURE 3

contains a rather opaque substance darker than the axoplasm. A few mitochondria limited by a double membrane and containing some irregularly shaped inner membranes and tubules are seen in each nerve ending. The ground substance between the densely packed vesicles is somewhat opaque. The vesicles are spread throughout the nerve endings and are found along all portions of the plasma membrane of the nerve ending. We have found no special concentration of vesicles close to that part of the membrane facing the hair cell cytoplasm.

The plasma membrane of the nerve ending is a single layer of uniform thickness.

A synaptic cleft, 100 A in width, separates the plasma membrane of the nerve ending from that of the hair cell. No difference in thickness is found between these two membranes. The distance between two nerve endings is larger than the width of the synaptic cleft.

In the synaptic region the plasma membrane of the hair cell forms a complicated system of interdigitating folds and tubules enclosing cytoplasmic partitions which appear to be completely

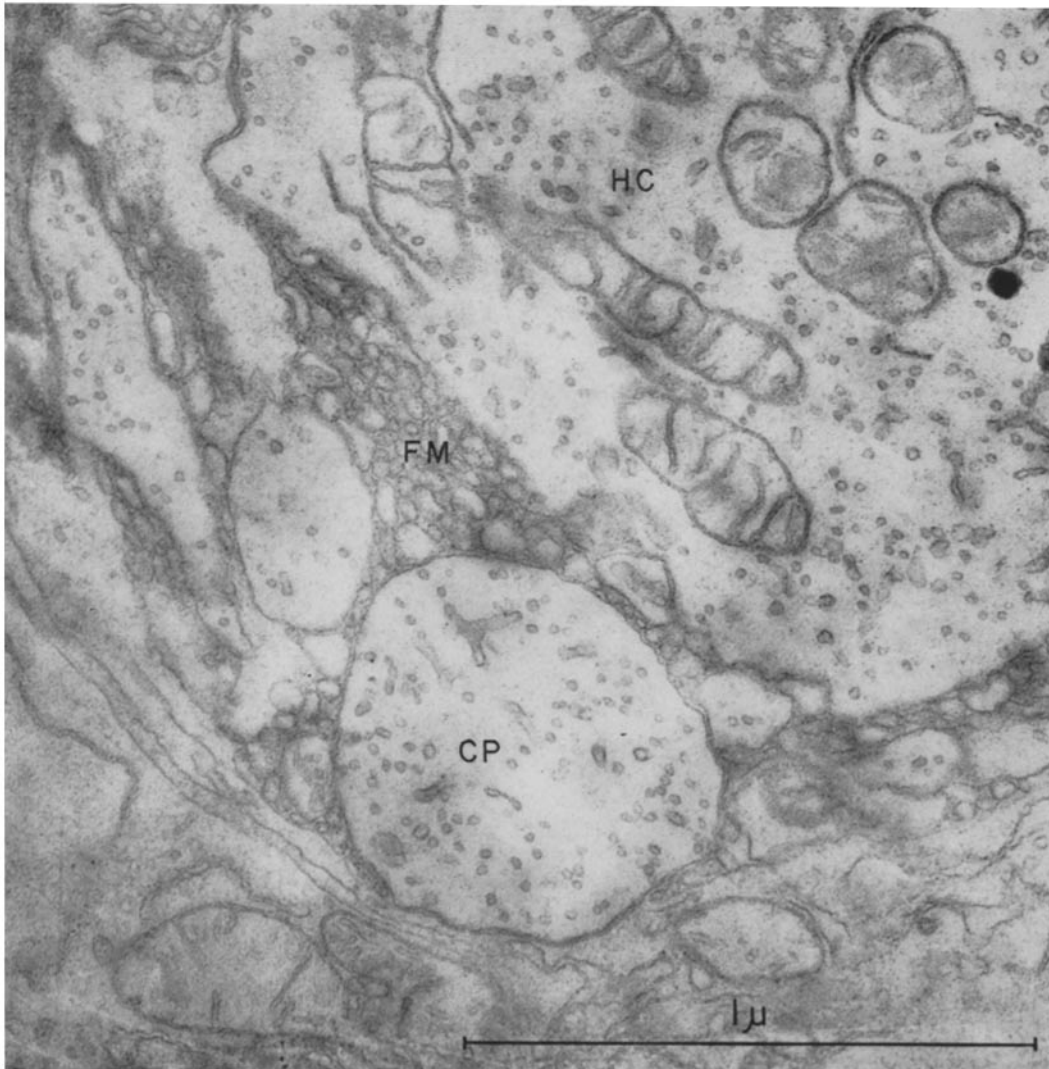


FIGURE 4

closed off from the rest of the cell when only a single section is observed. When such partitions are traced through a series of sections, it can be seen, however, that they have a true communication with the hair cell cytoplasm (Figs. 2 to 4). A complete reconstruction of the membranes of such areas is being made.

The hair cell cytoplasm also contains vesicles, short tubules and mitochondria. The vesicles are more sparsely scattered in the ground substance of the hair cell than in the nerve endings. The central parts of hair cell vesicles and tubules are pale. These formations are not particularly con-

centrated in the vicinity of the plasma membrane of the cell. As the cytoplasmic partitions in some sections appear as isolated, rounded structures lying outside the cell body, they might easily be taken for nerve endings if the difference in inner structure was not so prominent. This difference makes it easy to distinguish the cytoplasmic partitions from nerve endings in any of the figures presented.

The inner membranes of the mitochondria of the hair cell are found to be mostly parallel to each other.

## DISCUSSION

Most terminal nerve fibers end in a swelling when they make contact with the innervated cell (3, 4), though the shape, number, and localization of the swellings may vary in different tissues.

On the sensory receptor cells some nerve fibers have button-shaped endings on the cell surface (6, 10, 11, 14, 15, 17, 18, 20, 22); other endings form nerve chalices enclosing the greater part of the sensory cell surface (11), while still other endings lie within a deep groove formed by the invaginated plasma membrane (19, 22). Button-shaped endings like those described in the present work are thus found on the hair cells in the organ of Corti, on the type II cells in the vestibular sensory epithelia of guinea pigs, and on all receptor cells in the vestibular organ of fish (14). They are also found in the epidermal neuromasts (22) and in the taste buds (6, 10, 22).

Ellipsoid globules or round vesicles have been described in most nerve endings. However, the density and the diameter of these structures vary. In interneuronal synapses the most dense accumulation of these vesicles has been found on the pre-synaptic side (7, 16, 17). In sensory organs there is a considerable difference in the structure of nerve endings even on the same sensory cell. Thus, two different types of endings, one with many vesicles and the other with fewer vesicles, can be distinguished in relation to the vestibular sensory cells (23), the external hair cells of the organ of Corti in mammals (11), and the hair cells of the epidermal neuromasts (22). The significance of these synaptic vesicles has been discussed by several authors (5, 8, 9, 17). So far we have found only one type of nerve ending on the hair cells of the canal organ.

## BIBLIOGRAPHY

1. ANDERSSON-CEDERGREN, E., *J. Ultrastruct. Research*, 1959, Suppl. 1.
2. BODIAN, D., *J. Comp. Neurol.*, 1937, **68**, 117.
3. BODIAN, D., *Physiol. Rev.*, 1942, **22**, 146.
4. CAJAL, R. Y., *Trab. Lab. Inv. Biol. Univ. Madrid*, 1934, **29**, 1.
5. CASTILLO, J. B., and KATZ, B., *Progr. Biophys., Biophys. Chem.*, 1956, **6**, 137.
6. DE LORENZO, A. J., *J. Biophysic. and Biochem. Cytol.*, 1957, **4**, 143.
7. DE ROBERTIS, E., and BENNETT, H. S., *J. Biophysic. and Biochem. Cytol.*, 1955, **1**, 57.
8. DE ROBERTIS, E., PELLEGRINO DE IRALDI, A., RODRIGUEZ, G., and GOMEZ, C. J., *J. Biophysic and Biochem. Cytol.*, 1961, **9**, 1.
9. ECCLES, J., *Physiology of the Nerve Cells*, Baltimore, Johns Hopkins Press, 1957.
10. ENGSTRÖM, H., and RYTZNER, C., *Ann. Oto-rhino-laryng.*, 1956, **65**, 361.
11. ENGSTRÖM, H., and WERSÄLL, J., *Exp. Cell Research*, 1958, Suppl. **5**, 460.
12. ENGSTRÖM, H., *Acta Oto-laryng.*, 1958, **49**, 153.
13. GRAY, E. G., and WHITTAKER, V. P., *J. Physiol.*, 1960, **153**, 35.
14. LOWENSTEIN, O., and WERSÄLL, J., *Nature*, 1959, **184**, 1807.

The synaptic cleft is a non-opaque interspace of a uniform width of about 100 Å between the plasma membrane of the nerve ending and that of the sensory cell. No structure has been found in the interspace, as is the case in the motor end-plate of the muscle (1).

Folding of the plasma membrane has been described in the synaptic area of the sarcolemma of the muscle fiber. Such foldings are also found in the electric organ of the eel. In the rod spherules in the retina a number of synaptic vacuoles, having no communication with the cytoplasm of the sensory cell, are seen in an intimate relationship to the terminal processes of the dendrites (19, 20). The plasma membrane of the hair cells of the organ of Corti is doubled at the area of contact with such nerve endings that have many vesicles.

The portions of the hair cell cytoplasm enclosed by the folding of the plasma membrane, described in the present article, contain the vesicles and tubules typical of hair cell cytoplasm. The distance between the two layers of plasma membrane formed by this folding is very constant, being about 100 Å, equal to the width of the synaptic cleft.

The significance of this folding of the plasma membrane is not known. Since the foldings are regularly found at the base of the cell and are generally related to one or more nerve endings, they might be associated with the transmission of impulses from the hair cell to the nerve endings, presumably by providing an increased surface area for transport of electrolytes.

This work has been supported by grants from the Swedish Medical Research Council and from the Therese och Johan Anderssons Minne.

Received for publication, December 12, 1961.

15. MONTAGNA, W., *Advances Biology of Skin*, New York, Academic Press, Inc., 1961, **1**.
16. PALADE, G. E., and PALAY, S. L., *Anat. Rec.*, 1954, **118**, 335.
17. PALAY, S. L., *J. Biophysic. and Biochem. Cytol.*, 1956, **2**, 193.
18. SMITH, C. A., and SjöSTRAND, F. S., *J. Ultrastruct. Research*, 1961, **5**, 109.
19. SjöSTRAND, F. S., *Proceedings of the International Conference on Electron Microscopy held at London, 1954*, **428**.
20. SjöSTRAND, F. S., *J. Ultrastruct. Research*, 1958, **2**, 122.
21. TRUJILLO-CENÓZ, O., *An. Fac. Med. Montevideo*, 1959, **44**, 469.
22. TRUJILLO-CENÓZ, O., *Z. Zellforsch.*, 1961, **54**, 654.
23. WERSÄLL, J., *Acta Oto-laryng.*, 1956, Suppl. 126.