Japanese Journal of Ichthyology
Vol. 18, No. 1 June 15, 1971

魚類学雑誌
18巻1号 1971年6月15日

Fine Structure of the Large Pit Organ of the Goby, *Chaenogobius castaneus*

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(Received December 22, 1970)

Abstract The supposed similarities between the large pit organ and lateral-line canal organ were confirmed by the present investigation: the receptor cell is provided with sensory hairs which are arranged in a characteristic pattern, described in the receptor cell of the canal organ, and are innervated by two kinds of nerve endings, i. e., non-granulated and granulated, although the former appears more frequently than the latter. The supporting cells which may have sustentacular function and secretory activity are divided into two kinds, the central and peripheral, mainly on the basis of their topographic position in the large pit organ. While these two kinds of the supporting cells show no remarkable differences in their basic structure, the size and shape of secretory granules found in the apical cytoplasm are different between them. Whether such morphological differences of the granules correspond to the differences of chemical natures of substances contained in the granules or not is the subject of future research.

Introduction

The goby, *Chaenogobius castaneus* (O'Shaughnessy), has a number of large pit organs (free or naked neuromasts) on its head and trunk. The distribution and light-microscopical structure of the organ of the goby were examined by Satō (1954), but the fine structure of the organ has not yet been observed in the goby, not to speak of other adult teleosts. However, the organs of the embryos of *Cnesterodon decemmaculatus* and *Fitzroyia lineata* (Trujillo-Cenóz, 1961) and the larvae of medaka, *Oryzias latipes*, (Iwai, 1967) were investigated ultramicroscopically. According to Dijkgraaf (1963), the large pit organs are put into the group of the ordinary lateral-line organ which comprises lateral-line canal organs. Accordingly, it is possible that the fine structure of the large pit organ may be very similar to that of the canal organ, the fine structure of which was reported by Flock and Wersäll (1962a, b), Flock (1965), Flock and Duvall (1965), Hama (1965) and Petraitis (1966). The present investigation was designed to ascertain the similarities in the fine structure of these two organs.

Material and Methods

The gobies, measuring 50–55 mm in total-length, were obtained at Hirosaki and used as the material. The cheek skin containing the large pit organs was excised from the anesthetized goby and was cut into small blocks of roughly 1.5 mm^3. They were fixed for 1 hour in cold 1.25 or 2.5% glutaraldehyde buffered at pH 7.4 with phosphate buffer, and then were postfixed for 2 hours in cold 1% osmium tetroxide buffered at pH 7.4 with veronal-acetate buffer. After fixation, they were rapidly dehydrated through a series of graded concentrations of ethyl alcohol, and then embedded in Epon 812, according to Luft's method (Luft, 1961), through two changes in propylene oxide. Ultra-thin sections were cut on a Porter Blum MT-1 ultramicrotome and stained with Millonig's lead solution (Millonig, 1961) or saturated aqueous solution of uranyl acetate, and examined with a Hitachi HS-7D electron microscope.

Results

The large pit organ consists of several pear-shaped receptor cells and long slender supporting cells. Each receptor cell occupies upper half of the organ and does not reach the basement membrane of the epithelium, in which the organ is embedded. The nerve fibers enter into the organ penetrating the basement membrane raised at the organ and make the synaptic contact with the receptor cells. A jelly-like cupula extends out from the summit of the organ.

Receptor cells. On the upper surface of the receptor cell, sensory hairs project into the cupula where fine fibrous networks are visible (Fig. 1). Sensory hairs are composed of one kinocilium and 30–40 stereocilia, and are arranged in the hexagonal disposition, as described by Flock and Wersäll (1962b), Flock (1965) and Hama (1965) in the lateral-line canal organ. The kinocilium, 0.3 to 0.4 μ in diameter, shows "9+2" pattern of filaments. The stereocilia with a diameter of about 0.2 μ, slightly less at their origin, have filamentous cores which extend into the cuticle located immediately beneath the surface plasma membrane of the receptor cell (Fig. 1). As Flock and Wersäll (1962b) and Flock (1965) already stated, the length of the stereocilia increases stepwise towards the kinocilium, and each kinocilium of the adjacent receptor cells points in opposite directions, towards the head or towards the tail alternatively.

Near the free surface of the organ, a junctional complex (Farquhar and Palade, 1963) is found on the plasma membrane between the receptor cell and the adjacent supporting cell. In the supra-nuclear region, microtubules

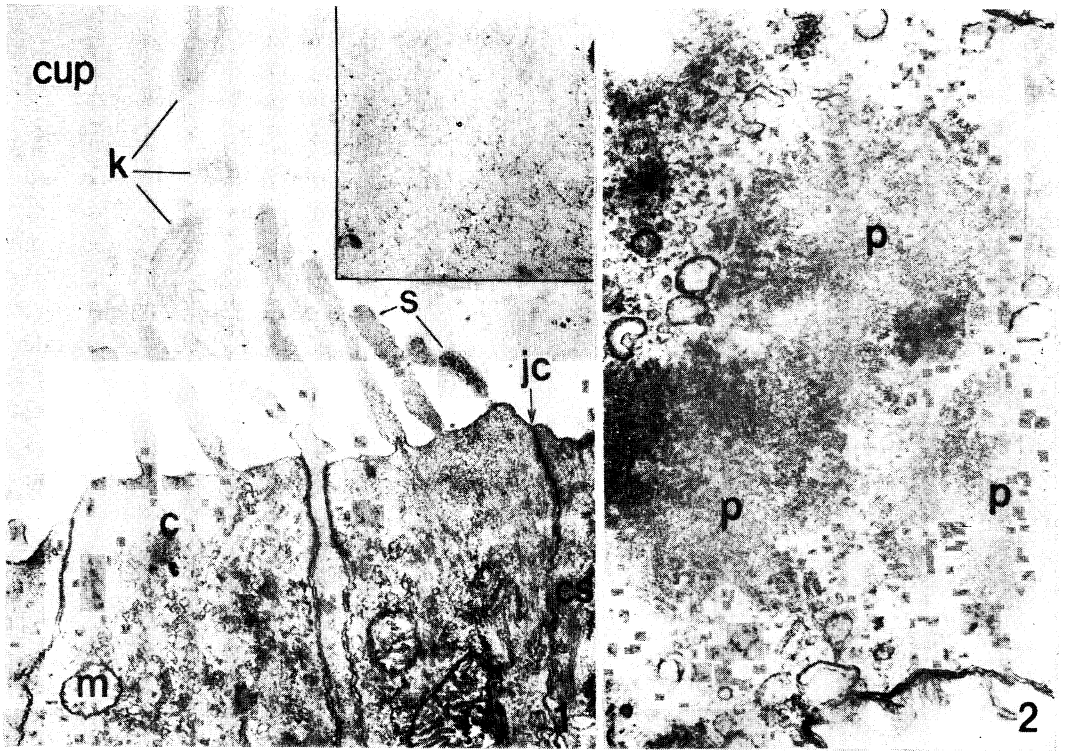

Fig. 1. The apical part of the receptor cells (r). c, cuticula; cs, central supporting cell; cup, cupula; jc, junctional complex; k, kinocilium; m, mitochondrion; s, stereocilia. ×14,000. Insertion is a high power electron micrograph of the cupula where fine fibrous networks are visible. ×54,000.

Fig. 2. Crystalloid structure of the pigmented granules (p) in the receptor cell. ×36,000.

measuring about 200Å in diameter, vesicles with a diameter of about 400Å to 1000Å, glycogen-like dense granules of about 300Å in diameter, tubule- or cistern-like structures, mitochondria, free ribosomes and a few multivesicular bodies are observed. The microtubules run along the long axis of the cell from the cuticular region to the nuclear level. Most of the tubule- or cistern-like structures mentioned above seem to be smooth-surfaced endoplasmic reticula.

The nucleus of the cell is roughly oval, and the nuclear envelope shows occasionally deep infoldings. One characteristic feature of the peri-nuclear region is the occurrence of uncapsulated bodies which were reported by Sato (1963) under the name of pigmented granules, and they appear to be crystalloid structures in their high magnification (Fig. 2). The bodies in question will be reported elsewhere. As another feature, some Golgi complexes are found in this region.

Most part of the infra-nuclear region is occupied by numerous vesicles with a diameter about 400Å (Fig. 3). These vesicles increase gradually in their numbers according to advancing from the nuclear level towards the basal part of the cell. Besides these vesicles, mitochondria, smooth-surfaced endoplasmic reticula, free ribosomes and vacuoles with a diameter ranging from 0.1 to 0.3 μ are also found in this region.

Nerve endings. Each receptor cell is innervated by several nerve endings which make contact with the basal surface of the cell and show considerable variations in size and shape. They are generally ellipsoidal, and do not locate in a deep invagination, but in a shallow depression of the receptor cell surface. In the receptor cell of the large pit organ, typical calyx type of the nerve endings is invisible. The nerve endings are divided into two different types, i. e., non-granulated and granulated, as in the receptor cell of the lateral-line canal organ (Flock, 1965). The non-granulated nerve ending corresponds to the "first type" named by Hama (1965), and has a characteristic feature containing some mitochondria and few vesicles (Fig. 3). In close association with the non-granulated nerve ending, one or more spherical homogenous dense bodies measuring 0.5 μ or so in diameter are found in the cytoplasm of the receptor cell. The dense bodies may be recognized as a kind of synaptic bar, after Flock (1965). These bodies have no limiting membrane, and are surrounded by a row of vesicles of about 400Å in diameter. At the dense bodies, the synaptic membranes of the receptor cell and the nerve ending are slightly convex towards the nerve ending and exhibit similar elaborate ultrastructure to the synaptic membranes in the receptor cell in the lateral-line canal organ of *Lota vulgaris* (Flock, 1965). The synaptic cleft is a non-opaque interspace of a slightly irregular width measuring about 150 to 200Å. However, the synaptic cleft associated with the dense body has an interspace of a uniform width of about 200Å.

As described by Flock (1965), the granulated nerve ending contains numerous vesicles with a diameter of about 400Å. This nerve ending is referable to the "second type" named by Hama (1965). The mitochondria contained in the cytoplasm of this ending show a tendency to be generally smaller than those of the non-granulated. In the receptor cell cytoplasm adjacent to the synaptic membrane of the granulated nerve ending, a flattened cistern is observed frequently (Fig. 4). This cistern may be analogous to the accessory double membrane (Flock, 1965) or to the structure described by Hama (1965). An interspace between the flattened cistern and synaptic membrane of the receptor cell is 80 to 120Å in width, and seems to be more opaque than the matrix of the cytoplasm. The synaptic cleft in this type of synapse has uniform width, measuring about 200Å, and the synaptic membranes are not wavy, but are relatively smooth. As Hama (1965) reported already, these two types of the nerve endings are sometimes found existing on the same receptor cell. On the whole, however, the

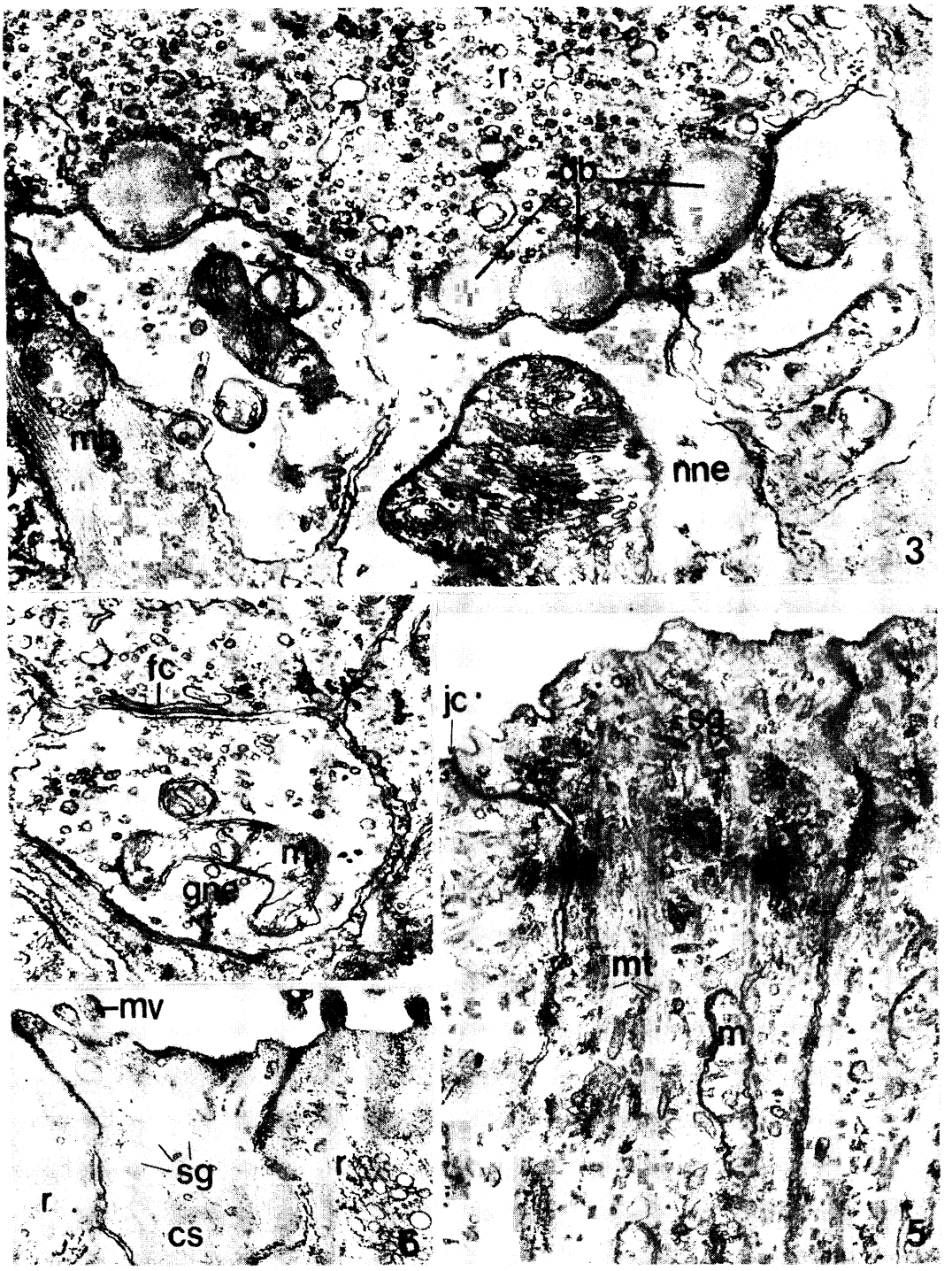

Fig. 3. The infra-nuclear region of the receptor cell (r) and non-granulated nerve endings (nne). db, dense bodies; mb, multivesicular body. ×30,000.
Fig. 4. The granulated nerve ending (gne). fc, flattened cistern. ×30,000.
Fig. 5. The apical region of the peripheral supporting cells (ps). mt, microtubules; sg, secretory granules. ×24,000.
Fig. 6. Secretory granules (sg) found in the apical region of the central supporting cell (cs). mv, microvillus. ×24,000.

non-granulated type is found more frequently than the granulated.

The large pit organ seems to be innervated by both the myelinated and the unmyelinated nerves. The former lose their myelin sheath before or after the nerves penetrate the basement membrane, and then branch off. The non-granulated nerve endings are possibly derived from the myelinated nerves. However, the relation between these two kinds of the nerves and these two types of the nerve endings could not be ascertained in the present observation.

Supporting cdlls. The supporting cell is a slender element, distinguished evidently from the receptor cell or ordinary epithelial cells surrounding the large pit organ. Most of the supporting cells reach from surface of the epithelium to the basement membrane and are provided with some microvilli with a diameter ranging from 0.1 to 0.2 μ on their free surface. Each supporting cell is equipped with an elongated ovoid nucleus which is situated always below the basal region of the receptor cell. At the apical circumferences, the adjacent cells show the junctional complex. The supporting cells are divided into the following two kinds according to their topographic position in the large pit organ, though they show no fundamental differences in their basic structural elements: one is the peripheral supporting cell which is located in the peripheral portion of the large pit organ, and the other is central supporting cell which embraces directly each receptor cell.

In the area extending from the apical surface of the peripheral supporting cell to the Golgi complexes in the middle region, numerous oval or elongated oval granules of about 0.1 to 0.4 μ in major axis and about 0.04 to 0.1 μ in minor axis are found (Fig. 5). The granules show considerable variations in density and are enclosed with an indistinct limiting membrane. They seem to be related to the secretion of the cupular component. Smaller and more slender mitochondria than those observed in the receptor cell, ill-developed endoplasmic reticula, bundles of fine filaments, microtubules running along the long axis of the cell, and free ribosomes are also observed in the apical region. The fine filaments are connected with the desmosomes. The microtubules, similar to those observed in the receptor cell, are found more numerously in this region than the middle and basal (Fig. 5). Although Petraitis (1966) observed the closely packed mitochondria in the supporting cell of the lateral-line canal organ of *Fundulus*, such a structure was not found in the present investigation.

Middle region of the peripheral supporting cell is characterized by the well-developed rough-surfaced endoplasmic reticula and well-developed Golgi complexes which are arranged parallel to the long axis of the cell (Fig. 7). The cisternae of the endoplasmic reticulum and the Golgi lamellae are filled with moderately dense substance. Some of the cisternae of the endoplasmic reticulum show a tendency to surround the mitochondria, and are frequently located closely parallel to the Golgi lamellae (Fig. 7). The width of the cisternal cavity varies considerably. In the Golgi area, secretory granules, vesicles or vacuoles with various sizes and densities are observed. The mitochondria contain sometimes a myelin-like swirl structure with various sizes (Fig. 8). Bundles of fine filaments, microtubules, free ribosomes, multivesicular bodies and capsulated dense bodies which may be possibly lysosomes are also present in this region. The dense bodies mentioned just above include occasionally a fine lamellar structure.

The basal region, i. e., peri-nuclear and infra-nuclear cytoplasm, of the cell contains similar organelles to those found in the middle region. In this region, however, the Golgi complex and the secretory granules are almost invisible. The cisternae of the endoplasmic reticulum are connected with the external nuclear membrane in several points. Most of the mitochondria are surrounded by the

Fig. 7. The middle region of the peripheral supporting cells. er, rough-surfaced endoplasmic reticulum; f, fine filaments; g, Golgi complex; i, inter-cellular space. ×20,000.

Fig. 8. The basal region of the peripheral supporting cells. bm, basement membrane; ms, myelin-like swirl structure; n. nucleus. ×15,000.

Fig. 9. The middle region of the central supporting cell. d, desmosome; nf, nerve fiber. ×36,000.

rough-surfaced endoplasmic reticulum (Fig. 8). The central supporting cell shows similar fine structures to those of the peripheral one mentioned above. However, the secretory granules found in the former can be distinguished from those of the latter in their shape and size. The granules of the former are roundish oval and are measured about 0.1 to 0.3 μ in major axis and 0.1 to 0.2 μ in minor axis (Fig. 6). Generally speaking, moreover, the central supporting cells contain more numerous fine filaments as compared with the peripheral ones. As Hama (1965) described already in the lateral-line canal organ of a congrid fish, *Rhynchocymba nystromi nystromi*, the central supporting cells are frequently found to embrace intra-epithelial nerve fibers with mesaxon-like membrane infolding beneath the receptor cell (Fig 9.).

Comment

One of characteristic intra-cellular elements of supporting cells is the secretory granules. As Petraitis (1966) suggested already, the secretory granules seem to be generated in the middle region, especially in Golgi complexes. Then they perhaps migrate to the apical region, and are probably discharged to form the cupula. The differences of shape and size between the secretory granules in the peripheral supporting cells and those in the central supporting cells require further examination, because such differences have not been reported in the lateral-line canal organ. In the middle and basal region of the supporting cells, the rough-surfaced endoplasmic reticulum often wraps around mitochondria. The profile seems to suggest the close association between mitochondria and rough-surfaced endoplasmic reticulum.

The microtubules are found in both the receptor cell and the supporting cell. The microtubules found in the receptor cell may be supposed to play some roles in transduction, as guessed by Jande (1966) in the receptor cell of the lateral-line organ of frog tadpoles, though Flock (1965) stated that their importance is obscure. On the other hand, the microtubules observed in the supporting cell of the large pit organ of the goby may probably serve as intra-cellular cytoskeletal elements with the fine filaments.

Iwai (1967) reported that the outer surface of the peripheral supporting cells of the neuromast of the larvae of medaka is covered by a thin layer of mantle cells. In the large pit organ of the adult goby, however, such cells could not be found. The mantle cells described by Görner (1963), Flock (1965) and Jande (1966) may possibly correspond to the peripheral supporting cells in the present investigation.

Two types of nerve endings, viz., non-granulated and granulated, can be also observed in the receptor cell of the large pit organ of the goby, as in the lateral-line canal organ. Taken together the results obtained by Hama (1965), Iwai (1967) and the present investigation, it is quite possible that the non-granulated nerve endings are found more frequently in the receptor cell and are formed earlier in the development of the nerve ending than the granulated ones.

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ビリンゴの large pit organ の微細構造

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large pit organ は通常側線器官に属し，微細構造においても側線管器と類似性のあることが推察されたが，今回の観察結果からその事実が確かめられた．例えば受容細胞から突出している2種類の感覚毛の配列様式，受容細胞と神経終末とのつくるシナプスに non-granulated type と granulated type の2種類があるなど，管器のそれらと全く同じである．支持細胞は large pit organ 内における位置関係から中心支持細胞と周辺支持細胞とに区別できる．これら2種の支持細胞はその基本構造において大きな相違はないが，分泌顆粒の形と大きさに関しては，これら2種の支持細胞間で相違がある．これに伴なって顆粒内容物の性質まで異なるものか否かは今後の研究に待ちたい．

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