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細胞の構造についてのメダカの脳下垂体内の研究

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FINE STRUCTURE OF THE PINEAL ORGAN OF THE MEDAKA, 
ORYZIAS LATIPES

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In the medaka, Oryzias latipes, the reproductive activities of the female during the breeding period are displayed in regular association with natural or experimental photoperiodicity (Egami, 1954; Yoshioka, 1962, 1963). It does not necessarily depend on the presence of the eyes of the fish as a photoreceptor (Egami, 1959). While the photosensory function of the pineal organ has been confirmed in some teleost fishes (Dodt, 1963; Morita, 1966; Hanyu et al., 1969), no such obvious evidence has so far been obtained in the pineal organ of the medaka.

Electron microscopic studies of teleost pineal organs have also contributed to signify the possible photosensory nature of the organ (Breucker and Horstmann, 1965; Rüdeberg, 1966, 1968; Oksche and Kirschstein, 1967; Omura et al., 1969; Takahashi, 1969). Moreover, some of these studies have implied a certain secretory function of the organ (Breucker and Horstmann, 1965; Oksche and Vaupil-von Harnack, 1965; Rüdeberg, 1968). In the light of the facts that serotonin or its derivatives may be contained in teleost pineal organs (Hafeez and Quay, 1969; Owman and Rüdeberg, 1970) and that the pineal organ may have a functional concern with the gonad (Pang, 1967; Fenwick, 1970), much more information about pineal ultrastructure is required to be accumulated.

As to the physiology of reproduction of the medaka, we have demonstrated the significance of the hypothalamo-hypophysial neurosecretory system in previous papers (Kasuga and Takahashi, 1970, 1971). In this paper, the fine structural characteristics of the pineal organ of the medaka will be described.

Before going further we wish to express our cordial thanks to Professor Kiichiro Yamamoto, Hokkaido University, for his invaluable criticism and reading of the manuscript.

Material and Methods

The medaka, Oryzias latipes, used in this study were of a wild type which were collected in Yunokawa in the suburbs of Hakodate and subsequently raised in an outdoor stock pond of our laboratory. Mature females of about 30 mm in body length were sampled mainly in July for the present study. The pineal organs

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of these fish were carefully exposed as they lay on the brain, and fixed in situ in 1% 
OsO₄ in Millonig's phosphate buffer for about 2 hours. Following dehydration 
with graded ethanol series, the specimens were embedded in Epon. Ultrathin 
sections were cut with a Porter-Blum microtome, doubly stained with uranyl 
acetate and lead citrate, and observed with the Hitachi HS-7 electron microscope.

Besides, the heads of some of the fish were fixed with Bouin's fluid for the 
light microscopy on the pineal organ. Serial longitudinal sections of the head were 
stained with Delafield's hematoxylin and eosin or with Mallory's triple stain. 
Thick sections of Epon-embedded specimens were also examined light microscopi-
cally after being stained with the procedure offered by Richardson et al. (1960).

Results

Gross structure

The distal vesicle of the pineal organ of the medaka is a flattened ovoidal disc 
of 250–300 μ long, 350 μ wide and 35–40 μ thick, leaning over the posterior margin 
of the telencephalon along the median line (Fig. 1). It is connected at its ventro-
posterior region with the diencephalic roof by a columnar stalk of 30–45 μ in 
thickness. No special structures such as pineal fossa are encountered in the cranial 
roof covering the organ. The pineal vesicle is furnished with irregular, narrow 
lumina surrounded by a folded, pseudostratified wall, whereas the stalk is lacking 
in distinct lumen as described already by Omura and Oguri (1969), though quite 
narrow remnants of the lumen appear dispersely in the center of the stalk.

At least two types of cells, one with a large roundish nucleus and another with 
a smaller, rather elongated and hyperchromatic one, are recognizable light 
microscopically both in the wall of the pineal vesicle and in the stalk (Fig. 2). By 
staining 1 μ sections of Epon-embedded specimens with azur II-methylene blue 
(Richardson et al., 1960), the outer segments of sensory cells, which correspond 
to the former of the two types of pineal cells, are found to be present along the 
periphery of the pineal lumen and, though much fewer in number, in the central 
region of the stalk (Fig. 3).

In contrast to the pineal organ of the goldfish (Takahashi, 1969), the zones 
of nerve plexus, or neuropiles, are not conspicuous and the ganglion cells are 
indetectable in the organ of the medaka, so far as the present observation is 
concerned.

Sensory cell

Perhaps due to a complicated folding of the pineal cell layer, sensory cells of 
varying length and contour are encountered in the pineal organ of the medaka 
by electron microscopy. In general, the cell is a gourd-shaped one constructed
from a nucleated part with a basal process, an inner segment and an outer segment (Fig. 4). The nucleated part is the largest in size among the three parts, and is surrounded by compact cytoplasmic sheets of supporting cells. The nucleus of the cell is spherical in shape but is generally concaved to a conspicuous degree toward the distal direction of the cell. In the perinuclear region, most characteristic is a huge mass of mitochondria which lie mostly on the concaved side of the nucleus (Fig. 5). The mitochondria comprizing the mass are mostly of ovoidal shape, but some free mitochondria of markedly elongated form are also seen on many occasions. Well-developed Golgi complexes are also present in the perinuclear cytoplasm, often appearing near the mitochondrial mass (Fig. 6). They are composed of slightly expanded tubules or lamellae accompanied with small vesicles ranging from 450 to 1,100 Å in size. Large amounts of free ribosomes and a sparse endoplasmic reticulum of a rough-surfaced type are distributed over the cytoplasm of the nucleated part.

The nucleated part is provided with a slender process which extends proximally deep into the pineal parenchyma. Bundles of filaments with straggling mitochondria and small vesicles run from the perinuclear region through the process. Abutting on the outer nuclear envelope facing the process, several flattened sacs are piled up in close contact with one another (Fig. 7). They appear to have some morphological association with the endoplasmic reticulum existing in their proximity.

The inner segment of the pineal sensory cell is demarcated from the nucleated part by a blunt constriction at which the cell keeps junctions with supporting cells (Fig. 4). The inner segment is quite slender in shape in some cases but is stunted in others according to the depth of the cells in the folded wall. It contains mitochondria, lamellar cisternae of smooth- and rough-surfaced endoplasmic reticulum, free ribosomes and minute vesicles of unknown origin, all of them scarcely showing any characteristic pattern of distribution in the inner segment. On rare occasions, some lamellar structures of various appearances are included in the inner segment.

The inner segment is joined to the outer segment by a connecting cilium which is lacking in central filaments. Ciliary filaments are derived from the distal one of the two centrioles which exist in the lateral margin of the apex of the inner segment, and pass through the short connecting piece into the outer segment vertically along its lateral wall (Fig. 8).

The outer segment displays quite a wide range of variation in its shape and feature (Fig. 9). As a rule, it consists of closely piled lamellae capping the inner segment. However, a typical dome-like configuration of the lamellar stack such as that seen in the pineal of the goldfish (Takahashi, 1969) is hardly noticed in that of the medaka. The outer segments are generally stumpy, and the piled lamellae...
are extensively replaced with tubules and vesicles in most cases. By thorough observations of electron microscopic pictures, however, it is conceivable that the outer segments of pineal sensory cells bear a corresponding structure to those of retinal cone cells inasmuch as the lowermost membrane of the lamellae is contiguous to the plasma membrane of the connecting cilium and no membrane covers the margin of the lamellar piles.

Supporting cell

As recognized in Fig. 4, the supporting cells of the pineal of the medaka are readily distinguishable by higher electron density of their cytoplasmic matrix as compared with sensory cells. The main part of the cell, where a somewhat elongated and longitudinally infolded nucleus is present, generally lies deep in the pineal wall, reaching out a complicatedly ramified cytoplasm toward the inner and outer directions among sensory cells (Fig. 10). On the apical edge bordering on the pineal lumen, the cytoplasm forms many thin sheets which are often seen to surround compactly the inner and outer segments of sensory cells (Fig. 9). In the outer region of the pineal parenchyma adjoining the blood capillaries across the pericapillary space of 700–1,000 μ in width, proximal ramifications of the supporting cells are complicatedly intertwined with processes of sensory cells and, perhaps, with dendrites of ganglion cells of undetermined site, though the latter two do not come to face directly the pericapillary space (Fig. 11). The outer surface of the supporting cells with a basement membrane shows indentation of various features, displaying quite intricate structures in some places. The endothelial wall of the blood capillary frequently bears fenestrations (Fig. 11).

The supporting cell contains mitochondria, vesicles and tubules regarded as endoplasmic reticulum, a large amount of free ribosomes, and several well-developed Golgi complexes of similar configuration to those of the sensory cell. Besides these, many lysosomal dense bodies, sometimes with dense spindle-shaped cores, and oil droplets of various sizes are also noticed in various regions of the cytoplasm (Fig. 12). A ciliary process is often seen to have originated from the supporting cell. Moreover, lamellar structures or membrane scars appear on many occasions mostly in the apical region of the cytoplasm near the pineal lumen.

Other structures

No ganglion cell body is detected in the pineal organ of the medaka so far as the present observation is concerned. However, the basal process of the pineal sensory cell keeps a synaptic contact with presumed dendrites of a certain nerve cell. The basal process accumulates at its end dispersedly distributed, small vesicles of 380–450 Å in size, which are defined as synaptic vesicles (Figs. 13 and 14). The synapse is characterized by the existence of synaptic ribbons enclosed
by the synaptic vesicles. Not rarely the sensory cell process is found to embrace thin projections of the dendrite and makes the synaptic contact with them (Fig. 14). In some pictures the synapse appears to be established between the sensory cell process and the sensory cell body (Fig. 5), though further studies should be needed to draw final conclusion.

In one case in which the pineal stalk was transversally sectioned as serial as possible, it was remarked that, together with unmyelinated nerve fibers, a few myelinated ones ran along the periphery of the pineal stalk (Fig. 15). Transections of the myelinated nerve fibers were counted to only three in the lowest part of the sectioned stalk, and they amounted to more than ten in number and eventually disappeared as they got nearer to the pineal vesicle where they were indetectable in any region. These aspects seem to point out the possibility that some centrifugal or efferent innervation which has originated from the myelinated nerves may be present in the pineal organ of the medaka, though their termination on pineal cells is quite uncertain at present. Another interest, although only in two instances, is in that a structure containing a cluster of vesicles of approximately 900 Å in size with electron dense cores appeared in the neighbourhood of the myelinated nerve fibers in the upper region of the stalk (Fig. 16). The cored vesicles have some resemblance in feature to the cored granules of an adrenergic nerve element. By lack of electron microscopic pictures sufficient to make thorough study, however, the origin of this structure remains unknown in this study.

Discussion

Females of the medaka are famous for their photoperiod-dependent spawning activity during the breeding period. In the presence of males, they spawn almost daily in early morning. Being kept under some artificial conditions of photoperiodicity which is shifted to a certain extent from the natural one, they soon start to accomplish regular spawning shortly after lighting, in fair accord with the new photoperiodic cycle (Egami, 1954; Yoshioka, 1962). From the results of serial studies on the reproduction of the medaka in our laboratory, it has been ascertained that not only ovulation and spawning but also maturational progress of ovarian oocytes are responsive to natural or artificial photoperiodicity. Furthermore, it has also been confirmed that the removal of the eyes of female medaka paired with males does scarcely influence the regular reproductive cyclicity of the females, as reported by Egami (1959), and that continuous darkness suppresses the reproductive activities to lead to eventual regression of the ovary. These pieces of information have led us to expect the photosensitivity of the pineal organ which might in turn exert a certain effect on the gonad possibly through the mediation of the pituitary gland.
From the results obtained in this study, it is admitted that the pineal organ of
the medaka presents a fine structure essentially similar to that of other teleosts
examined so far (Breucker and Horstmann, 1965; Oksche and Kirschstein, 1967;
Rüdeberg, 1966, 1968; Omura et al., 1969; Takahashi, 1969). The existence of
the outer segment of a retinal cone type in sensory cells and the appearance of
synaptic structures in the basal process of these cells may point to the photosensory
nature of the pineal organ of the medaka. The accumulation of mitochondria,
which may correspond to the so-called "Ersatzellipsoid", in the juxtanuclear
region of the sensory cell seems to signify this possibility. An extensive devia­
tion of the structure from the retinal photoreceptor is, however, readily noticeable in
the lamellar structure of the outer segment: the lamellar piles are stumpy and
irregular in contour and arrangement, and the constituent lamellae are widely
replaced by tubules and vesicles. By reference to the literature cited above, these
features of the outer segment are considered to be rather common to photosensory
cells of the pineal organ of teleosts, admitting that some of the features are
fixation artefacts as stressed by several authors (van de Kamer, 1965; Oksche and
Kirschstein, 1967; Rüdeberg, 1968). A similar structural irregularity of the
outer segment of pineal sensory cells has been demonstrated by electron microscopy
in Salmo gairdnerii (Breucker and Horstmann, 1965), Phoxinus laevis (Oksche and
Kirschstein, 1967), and Plecoglossus altivelis (Omura et al., 1969), nevertheless the
photosensitivity of the pineal organ of these fishes has been confirmed electro­
physiologically (Dodt, 1963; Morita, 1966; Hanyu et al., 1969).

There were no distinct ultrastructural indications of secretion in the pineal
cells of the medaka. However, the presence of well-developed Golgi complexes
associated with many small vesicles in the sensory cell and supporting cell might
have some concern with an expected secretory function, as suggested by Rüdeberg
(1968) in his observations on the pineal organ of Sardina pilchardus sardina. It
is of further interest that the Golgi area is generally present in close proximity to
the juxtanuclear cluster of mitochondria in the sensory cell while it is distributed
rather in the apical cytoplasm in the supporting cell. Furthermore, a complicated
infolding of the outer border of the pineal parenchyma facing the blood capillaries, a
rather wide pericapillary space, and a fenestrated endothelium of the capillary
are suggestive of an active interchange of materials between the blood and the
pineal cells, which may remind us of the characteristic features of the endocrine
tissue.

Hafeez and Ford (1967) with light microscopy described an efferent innervation
in the pineal organ of Oncorhynchus nerka, suggesting the nervous regulation of
presumed secretion from the cells into the pineal lumen. In the medaka, too, the
appearance of a few myelinated nerve fibers in the pineal stalk may imply a
possibility of an efferent innervation into the pineal organ, though whether or
not these nerves are truly secretomotor in nature remain to be clarified by future studies.

Quite recently, Fenwick (1970) reported that the gonad of male and female goldfish, *Carassius auratus*, was increased in weight in proportion to the length of experimental photoperiod provided that the experiment was done during the breeding period, and that pinealectomy of the goldfish could significantly augment the gonadal response only when the operation was made in that light-sensitive phase. Although the pineal organ of the goldfish revealed little evidence of secretion in its ultrastructure (Takahashi, 1969), the organ was thus proved to exert an inhibitive control directly or indirectly over the gonad. Oguri *et al.* (1968) by radiobiological method and Hafeez and Quay (1969) by fluorescence microscopy suggested the existence of indoleamines such as serotonin and its derivatives in the pineal organ of *Salmo gairdnerii* in which an ultrastructural proof of secretion was found to be lacking (Breucker and Horstmann, 1965).

Such information as cited above seems to emphasize the need of investigating the pineal organ with a special view to elicit a seasonal variation and experimental alteration of its histochemical as well as cytological characteristics. We are now carrying out some series of studies on the pineal organ of the medaka along this line of consideration.

**Summary**

The pineal organ of mature females of the medaka, *Oryzias latipes*, sampled in July, was examined electron microscopically. The pineal vesicle of flattened ovoidal shape has narrow lumina enclosed by irregularly infolded parenchyma consisting of pseudostratified sensory cells and supporting cells. The pineal stalk shows similar structure but the lumina are only vestigial.

The sensory cells are furnished with a stumpy outer segment in which a lamellar structure of a retinal cone type shows extensive variations in its arrangement and vesicular or tubular transfigurations. The cells are characterized by an accumulation of mitochondria and well-developed Golgi complexes in the juxtanuclear cytoplasm. The basal processes of the cells are provided with synaptic ribbons and synaptic vesicles characteristic of the synaptic contact with a nervous element. The supporting cells show a complicated ramification of the cytoplasm which surrounds the sensory cells. These supporting cells are characterized by the presence of well-developed Golgi complexes associated with many small vesicles and by the dense distribution of free ribosomes in the cytoplasm. No ganglion cells are detected in the organ, but an efferent innervation of a few myelinated nerve fibers is noticed along the periphery of the pineal stalk. These characteristics of the pineal organ of the medaka were discussed in the light of photosensory nature and possible secretory function of the pineal organ of teleost fishes.
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Explanation of Plates

PLATE I

Fig. 1. A median sagittal section through the head of the medaka, revealing an organization of the pineal organ and associated structures. III, third ventricle; CP, choroid plexus; D, diencephalon; HC, habenular commissure; OL, telencephalon; ON, optic nerve; OX, optic chiasam; PS, pineal stalk; PV, pineal vesicle; SO, subcommisural organ. Hematoxylin-eosin. ×75

Fig. 2. A part of the pineal vesicle with pseudostratified sensory cells (Se) and supporting cells (Su). BC, blood capillary; L, pineal lumen. Hematoxylin-eosin. ×1,300

Fig. 3. A transverse section through the pineal stalk of an Epon-embedded specimen. Outer segments of sensory cells are seen as darkly stained structures existing in the center of the stalk. Note remnants of pineal lumina near the outer segments. Azur II-methylene blue. ×550

Fig. 4. An electron micrograph of a part of pineal parenchyma in the pineal vesicle. IS, inner segment of sensory cell; L, pineal lumen; OS, outer segment of sensory cell; Se, sensory cell; Su, supporting cell. ×8,000
H. TAKAHASHI & S. KASUGA: The pineal organ of *Oryzias latipes*
PLATE II

Figs. 5 and 6. Electron micrographs showing a fine structure of the nucleated part of pineal sensory cell. Arrows in Fig. 5 indicate the synapses of a basal process of a sensory cell with nervous element and with sensory cell body. ER, endoplasmic reticulum; F, bundle of filaments passing through the basal process of sensory cell; G, Golgi complex; L, pineal lumen; M, mitochondrion; N, nucleus of sensory cell; Se, sensory cell; Su, supporting cell. Fig. 5, ×9,200; Fig. 6, ×14,400
H. TAKAHASHI & S. KASUGA: The pineal organ of Oryzias latipes
PLATE III

Fig. 7. Perinuclear region facing the basal process in a sensory cell, showing a pile of sacs (arrow) abutting on the outer nuclear envelope. ER, rough-surfaced endoplasmic reticulum; G, Golgi complex; N, nucleus of sensory cell. \( \times 14,400 \)

Fig. 8. An outer segment of a sensory cell. A connecting cilium with basal centrioles (C) is seen connecting the outer segment (OS) with the inner segment (IS). \( \times 15,000 \)

Fig. 9. Pineal parenchyma surrounding pineal lumina (L) in the pineal vesicle, showing various features of the outer segment (OS) of sensory cells and complicated ramification of apical cytoplasm of supporting cells (Su). Arrow indicates a transection of a cilium. \( \times 8,000 \)
H. TAKAHASHI & S. KASUGA: The pineal organ of Oryzias latipes
PLATE IV

Fig. 10. A supporting cell with ramified cytoplasm. C, centriole; G, Golgi complex; M, mitochondrion; Se, sensory cell; Su, supporting cell. ×24,000

Fig. 11. Pineal parenchyma bordering on a blood capillary in the pineal vesicle. Note intricate cellular elements in the parenchyma and evaginated margin facing the pericapillary space (PS). Arrows indicate fenestrations in the endothelial wall of the capillary (BC). ×6,000

Fig. 12. Supporting cells (Su) with inclusions of varying types. Se, nucleated part of sensory cell. ×8,000
H. TAKAHASHI & S. KASUGA: The pineal organ of *Oryzias latipes*
Figs. 13 and 14. Synaptic structures in the terminal region of the basal processes of sensory cells. Synaptic ribbons and associated synaptic vesicles are demonstrated in the figures. $\times 24,000$

Fig. 15. Pineal parenchyma in the lower region of the pineal stalk. Myelinated nerve fibers are seen in the lower right corner of the figure. OS, outer segment of sensory cell; $S_s$, sensory cell; $S_u$, supporting cell. $\times 6,000$

Fig. 16. The upper region of the pineal stalk, revealing the presence of a cluster of vesicles with electron dense cores (arrow) in the neighbourhood of myelinated nerve fibers. $\times 15,000$
H. TAKAHASHI & S. KASUGA: The pineal organ of *Oryzias latipes*