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Ultrastructural and Ultracytochemical Studies on the Pineal Organ of the Floating Goby, *Chaenogobius annularis* Gill

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Abstract

The pineal organ of the floating goby, *Chaenogobius annularis*, was investigated by electron microscopy and ultracytochemistry (argentaffin reaction) in order to localize endogenous 5-hydroxytryptamine (5-HT). The compact pineal organ of the goby was mainly composed of photoreceptor, supportive, and ganglion cells. Besides these cell types, phagocytic and degenerating cells existed in the pineal end-vesicle. Some photoreceptor cells appeared darker than others due to an abundance of free ribosomes and the development of Golgi apparatus, without showing noticeable regional differences in distribution in the pineal organ. The greater part of the cytoplasm of supportive cells was occupied by the smooth endoplasmic reticulum. Although none of the cell types showed distinct morphological features suggestive of secretory activity of the pineal organ, numerous pinocytotic vesicles were found within endothelial cells of the blood capillaries surrounding the pineal organ. Ultracytochemically, both the photoreceptor and supportive cells showed positive argentaffin reactions, with reaction products precipitated diffusely over the cytoplasmic matrix but not over any cytoplasmic organelle. Ganglion, phagocytic, and degenerating cells were exclusively negative to the test. The results are discussed in terms of the possibility that both the photoreceptor and supportive cells of the pineal organ of the floating goby could accumulate 5-HT in their cytoplasmic matrix.

Introduction

The presence of indoleamines in the pineal organ of teleost fishes has been demonstrated by fluorescence histochemistry (Hafeez and Quay, 1969; Owman and Rüdeberg, 1970; Falcon et al., 1980; van Veen et al., 1980), and by chromatographic separation (Fenwick, 1970). In addition, hydroxyindole-*O*-methyltransferase (HIOMT), an essential enzyme for melatonin biosynthesis, has also been detected in the pineal organ of several fish species (Hafeez and Quay, 1970; Smith and Weber, 1976a, b; Birks and Ewing, 1981a, b). These facts indicate that the pineal organ of teleost fishes also functions as a secretory organ similarly to that found in higher vertebrates. In teleosts, however, no information concerning ultrastructural localization of indoleamines in the pineal organ has been obtained thus far.

The present study was designed to investigate cytological localization of indoleamines, especially 5-hydroxytryptamine (5-HT), in the pineal organ of the floating goby, *Chaenogobius annularis*, by means of an electron microscopical argentaffin reaction.

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Material and Methods

A total of 26 specimens of the floating goby, *Chaenogobius annularis*, of both sexes, ranging from 82 to 125 mm in standard length, were used in the present study. Fish were collected in October 1982 from the Ryukei River in the suburbs of Hakadate. They were maintained thereafter in an outdoor pond of the laboratory under natural conditions, and were fed daily on chipped meal of Antarctic ocean krill. At sampling times, fish were anesthetized in ethyl 4-aminobenzoate, measured and sacrificed by quick decapitation between 13 : 00 and 14 : 00.

For electron microscopy, the pineal organ attached to the dorsal cranium *in situ* was carefully removed and fixed in 2% paraformaldehyde-1.5% gultaraldehyde in 0.2M cacodylate buffer (pH 7.4) for about 3 hours at room temperature. After washing in cold 0.1M cacodylate buffer the specimens were postfixed in 1% osmium tetroxide in 0.2M cacodylate buffer (pH 7.4) for 2 hours at 4°C. The fixed specimens were dehydrated in a graded alcohol series and embedded in Epon. Ultrathin sections cut with glass knives were stained with uranium acetate and lead citrate, and then examined with a Hitachi HU-12 electron microscope.

For the detection of indoleamines, the pineal organ was fixed in 2.5% glutaraldehyde in 0.2M cacodylate buffer (pH 7.4) for 4 hours at room temperature. The fixed specimens were briefly washed in the same buffer, dehydrated and embedded in Epon. Ultrathin sections mounted on nickel grids were immersed in ammoniacal silver nitrate solution, which was prepared according to the method of Lu and Lin (1979), for 2 to 3 hours at 60°C. The grids were thoroughly washed with distilled water, dried, and examined electron microscopically.

Results

A small and compact pineal organ of the floating goby, *Chaenogobius annularis*, was located medially beneath the dorsal roof of the cranium just in front of the transversal cartilage. A thick disk-shaped end-vesicle of the organ, measuring about 100 μm in length, was connected at its base to the dorsal roof of the diencephalon by a long and thin pineal stalk.

Electron microscopy

The pineal end-vesicle was mainly composed of three types of cells : photoreceptor, supportive and ganglion cells (Fig. 1). Besides these cell types, phagocytic cells and degenerating cells were occasionally found in the end-vesicle.

In general, the cytoplasm of photoreceptor cells contained oval mitochondria with tubular cristae, short cisternae of rough endoplasmic reticulum, well-developed Golgi apparatus composed of several lamellae and many clear vesicles, and abundant free ribosomes (Fig. 4). Among the photoreceptor cells, there were some which appeared darker than the others. The dark cells were rich in free ribosomes and had mitochondria with matrices that were somewhat electron-dense (Figs. 5, 6). Furthermore, the Golgi apparatus existing in these cells was more developed than in the other, light photoreceptor cells. In some cases the mitochondria formed a

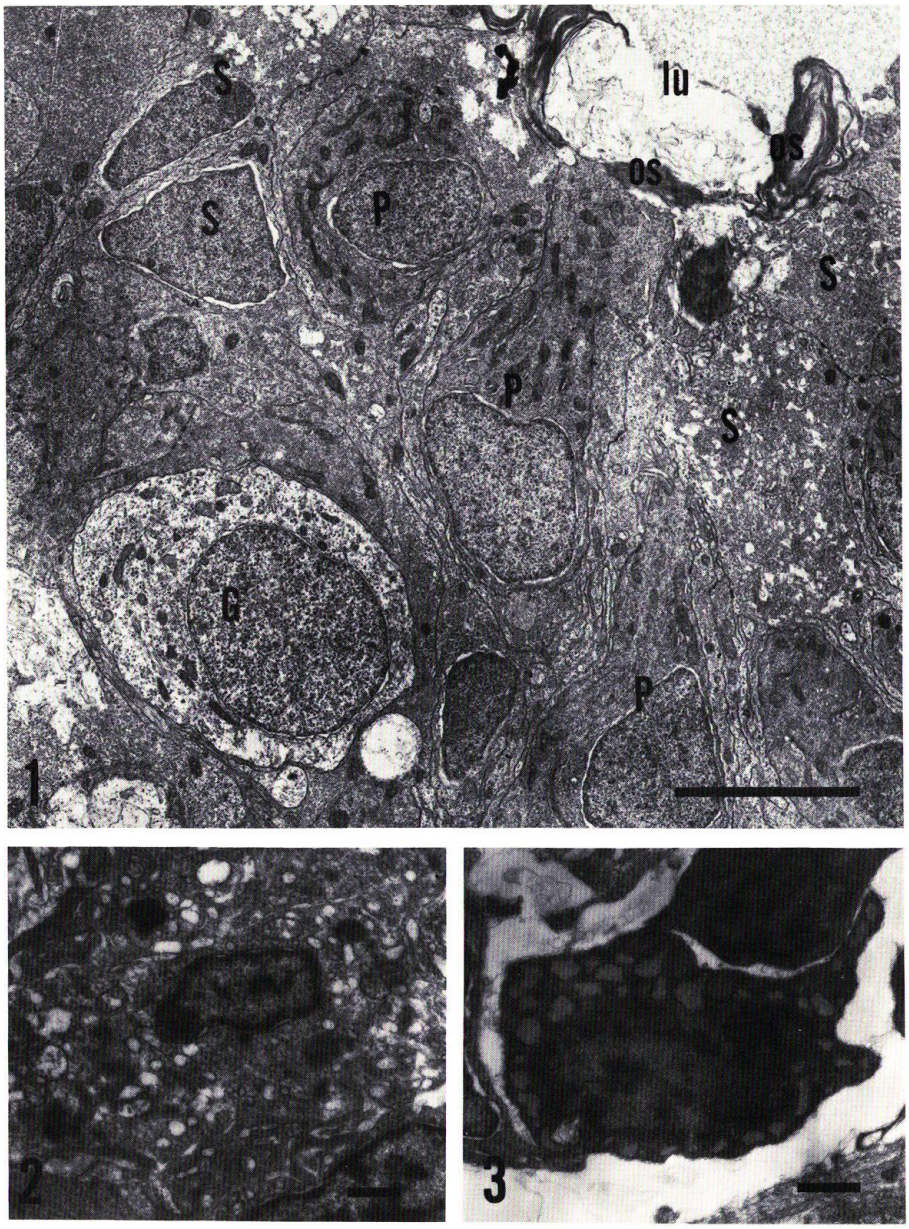
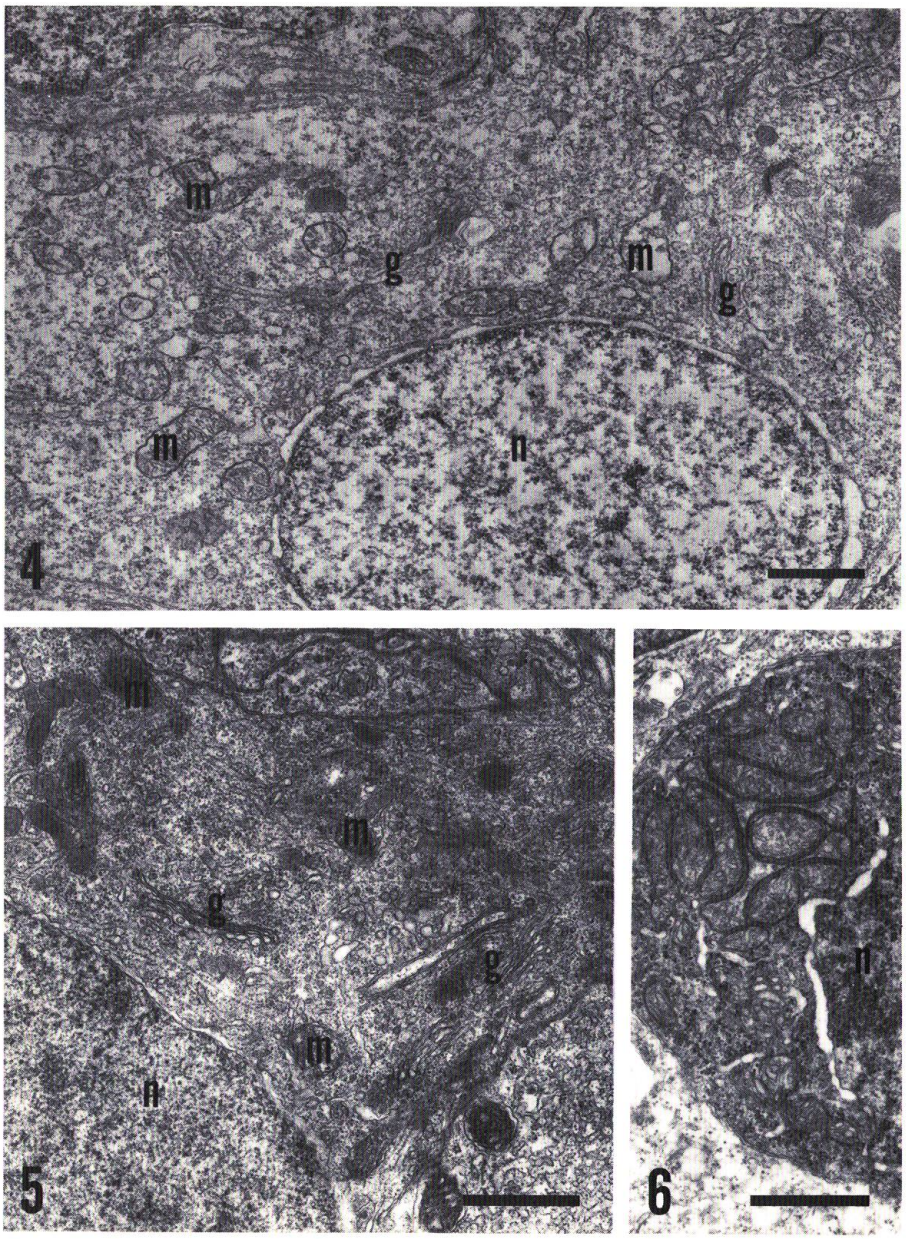


Fig. 1. Pineal parenchyma of the floating goby, showing photoreceptor cells (*P*) with the outer segment (*os*), supportive cells (*S*), and a ganglion cell (*G*). *lu*, pineal lumen ; *os*, outer segment. Bar indicates 5 μm.

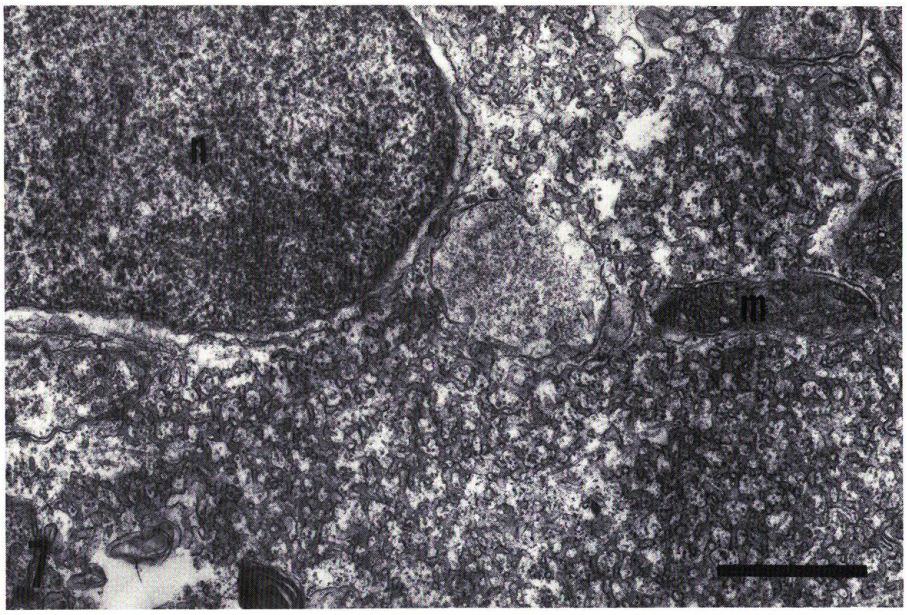
Fig. 2. A phagocytic cell appearing in the vicinity of the outer segment of photoreceptor cells. The cell contains many vesicles and dense bodies. Bar indicates 1 μm.

Fig. 3. A degenerating cell found in the pineal parenchyma. Broad intercellular space between the cell and neighbouring cells is noted. Bar indicates 1 μm.



Figs. 4–6. Basal nucleated part of photoreceptor cells. A light cell (Fig. 4) and dark cells (Figs. 5, 6) are shown. Fig. 6 shows mitochondria forming a compact cluster. *g*, Golgi apparatus ; *m*, mitochondrion ; *n*, nucleus. Bars indicate 1 μm.

compact cluster, tangled together in a complicated way in the supranuclear cytoplasm of the dark photoreceptor cells (Fig. 6). Regional differences in distribution of the light and the dark photoreceptor cells were not distinct in the pineal



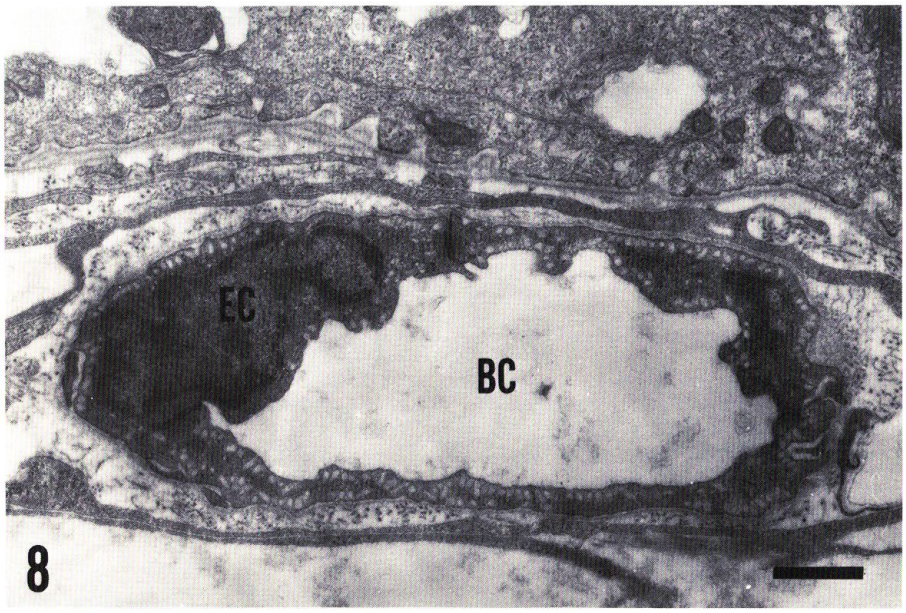


Fig. 7. Cytoplasm of a pineal supportive cell occupied by abundant tubular cisternae of the smooth endoplasmic reticulum. *m*, mitochondrion ; *n*, nucleus. Bar indicates 1 μm.

Fig. 8. Endothelial cells of a blood capillary, showing numerous pinocytotic vesicles lying along the periphery of the cytoplasm. *BC*, blood capillary ; *EC*, endothelial cell. Bar indicates 1 μm.

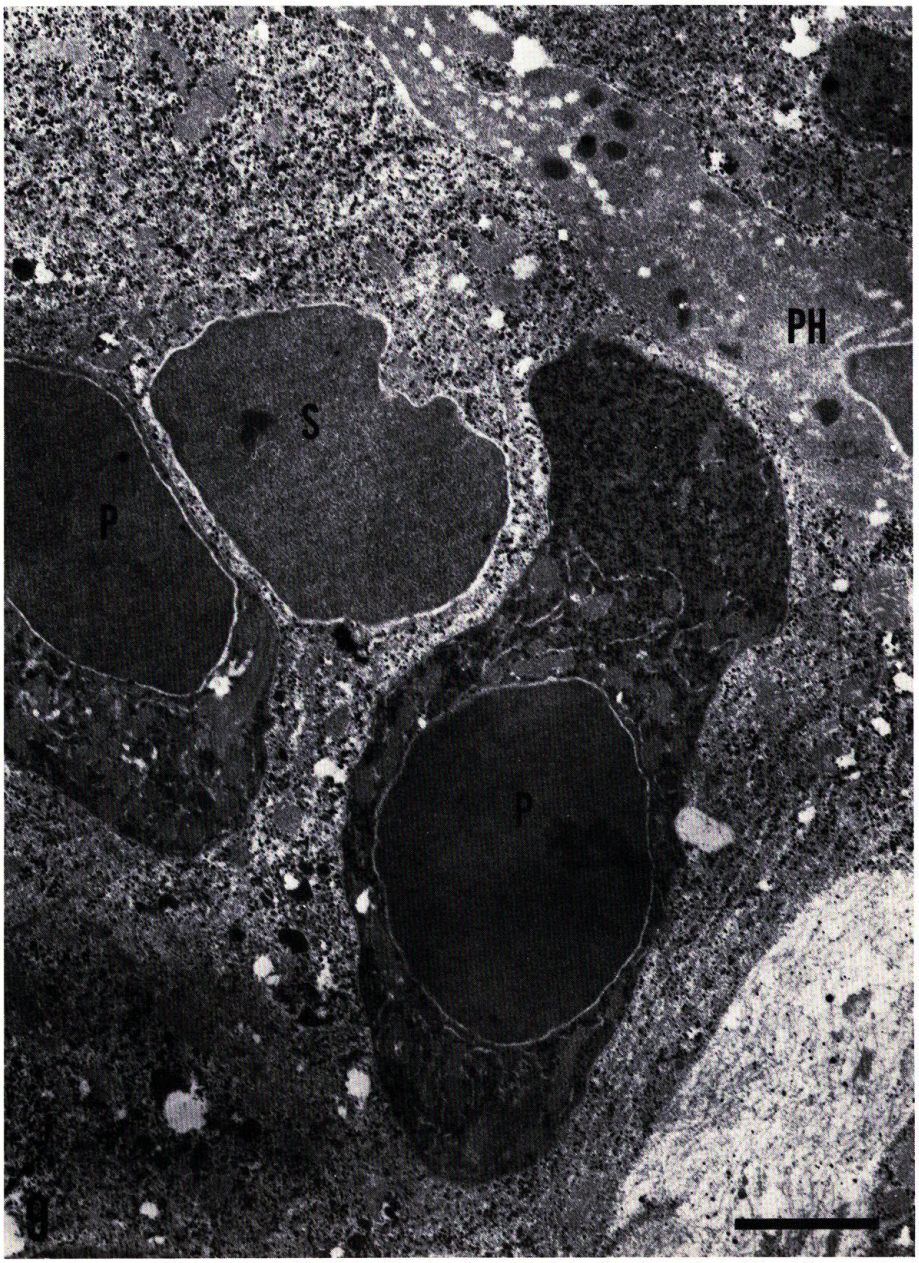


Fig. 9. Argentaffin reaction of cells in the pineal organ of the floating goby. Fine silver grains are precipitated selectively over the cytoplasmic matrix of both photoreceptor (*P*) and supportive cells (*S*). *PH*, phagocytic cell. Bar indicates 2 μm.

organ of the floating goby, although a few photoreceptor cells with similar ultrastructural characteristics were found to exist together in groups. Photoreceptor cells invariably had well-organized outer segments, and showed no notable signs of secretory activity such as the occurrence of dense-cored vesicles and particular clear vesicles in the cytoplasm.

The cytoplasm of the supportive cells was spread between the photoreceptor and ganglion cells, and the supportive cells had junctional complexes of the intermediate type with neighbouring photoreceptor cells at their neck region (Fig. 1). The basal end of the supportive cells bordered on the pericapillary space surrounding the organ, showing scarcely any signs of infolding. The greater part of the supportive cells was occupied by tubular cisternae of the smooth endoplasmic reticulum (Fig. 7). Oval mitochondria with tubular cristae, short cisternae of rough endoplasmic reticulum, and a number of lysosome-like dense bodies were also present in the cytoplasm. Golgi apparatus were rare in the cytoplasm of these cells.

Ganglion cells were generally found to be distributed along the periphery of the end-vesicle. These cells were large in size, approximately 15 μm in diameter, and had spherical nuclei. Their perinuclear cytoplasm was markedly electron-lucent when compared with that of the other cell types of the organ (Fig. 1). A number of small and oval mitochondria, short and flat cisternae of the rough endoplasmic reticulum, lysosome-like bodies with granular contents, well-developed Golgi apparatus, polysomal ribosomes, and neurofilaments were present in the perinuclear cytoplasm of the ganglion cells.

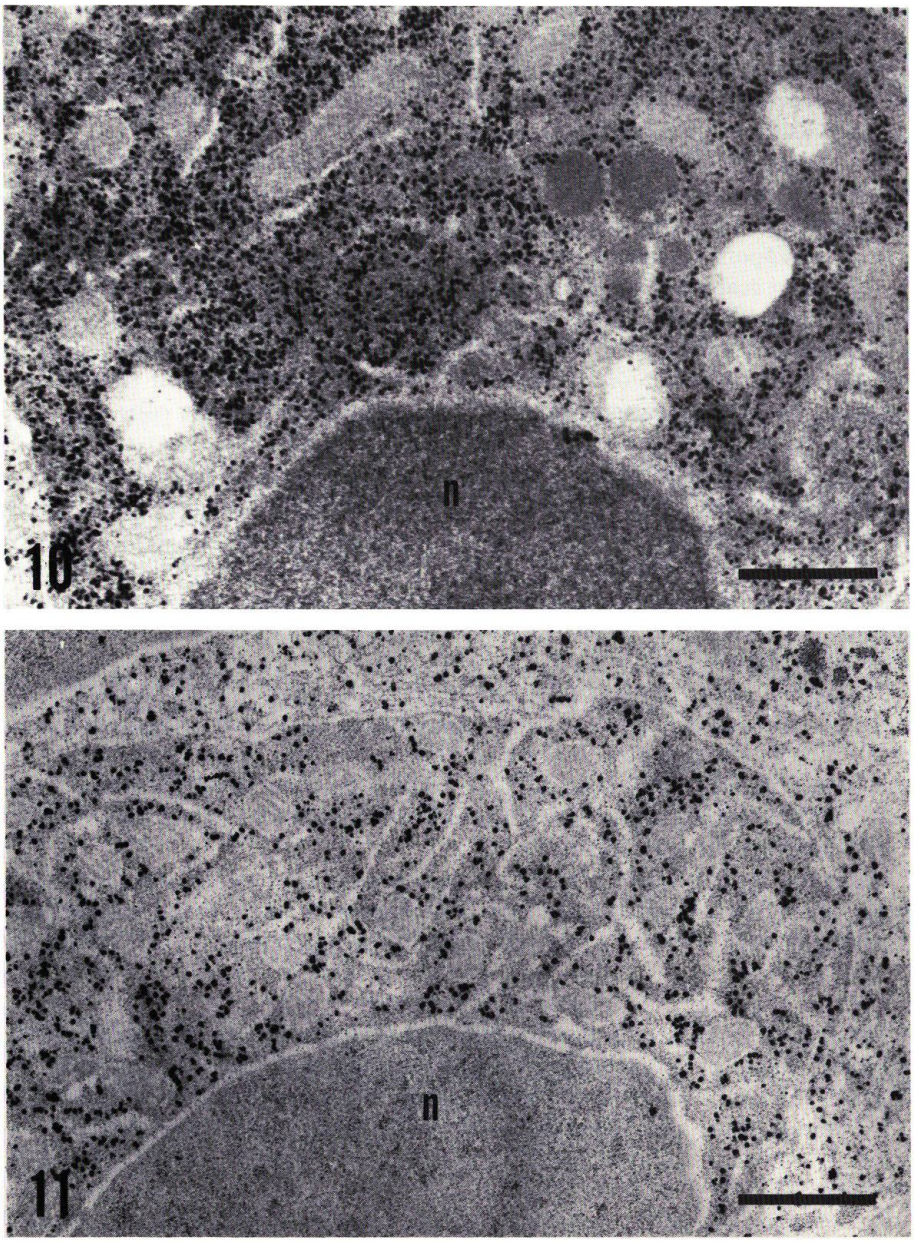
Phagocytic cells appeared adjacent to the outer segment of the photoreceptor cells in the pineal lumen or adjacent to degenerating cells in the pineal parenchyma. These cells were characterized by having many clear vesicles and dense, membrane-bound bodies in their cytoplasm (Fig. 2). The organelles showed a wide variation in size. Phagocytic cells also contained mitochondria with electron-lucent matrices and well-developed Golgi apparatus.

Degenerating cells were found to occur sporadically within the pineal parenchyma. They were irregular in shape and appeared to be much electron-dense, contrasting sharply with the other cell types of the organ (Fig. 3). Moreover, there were broad spaces between these cells and the neighbouring cells. It was difficult to determine the origin of these cells in the present study.

The pineal organ of the floating goby was surrounded by a number of blood capillaries. Endothelial cells of the capillaries were thick and were marked by the presence of numerous pinocytotic vesicles along the periphery of the cytoplasm (Fig. 8). No fenestrations were observed in the endothelium of the capillaries.

Argentaffin reaction

By treating ultrathin sections of pineal organs of the floating goby with ammoniacal silver nitrate solution, both the photoreceptor and supportive cells showed a positive argentaffin reaction, while the ganglion, phagocytic, and degenerating cells showed a negative reaction (Fig. 9). Fine silver grains were precipitated diffusely but selectively over the cytoplasmic matrix of both the photoreceptor and supportive cells. No reaction was observed in the nucleus or in cytoplasmic organelles such as the mitochondria, endoplasmic reticulum, and lysosome-like bodies. In general, photoreceptor cells were more crowded with silver grains than



Figs. 10, 11. Photoreceptor cells stained with argentaffin reaction, showing a cell with a greater number of silver grains (Fig. 10) and a cell with relatively a small number of grains (Fig. 11). *n*, nucleus. Bars indicate 1 μm.

supportive cells were. Photoreceptor cells with a greater number of silver grains (Fig. 10) and those with a relatively small number of grains (Fig. 11) in the cytoplasmic matrix were intermingled in the same pineal organ.

Discussion

Secretory functions of the pineal organ have been clearly shown for teleost fish by various methods of investigation. However, there has been no direct morphological evidence for secretory activity in the pineal cells of fishes, though some ultrastructural features of the cells have been considered to be related to synthetic or secretory activity of the pineal organ (Herwig, 1979). For instrance, some authors believe that dense-cored vesicles occurring in the pineal cells of various fish species may contain pineal secretions such as indoleamines and proteinaceous material (McNulty, 1981 ; Omura and Ali, 1981). Among teleost fishes, however, there are other species which lack the dense-cored vesicles in the cells of the pineal organ. In the floating goby, as well, no dense-cored vesicles were present in any of the cell types of the pineal organ. However, the presence of Golgi apparatus and abundant free ribosomes in pineal photoreceptor cells of the floating goby, in addition to well-developed smooth endoplasmic reticulum in pineal supportive cells, is highly suggestive of a high level of metabolic activity in these cells. McNulty (1982) reported that in the pineal organ of the goldfish, *Carassius auratus*, the size and number of Golgi apparatus existing in photoreceptor cells was altered under conditions of constant light and constant darkness. Remarkable development of the smooth endoplasmic reticulum in supportive cells was also observed in the pineal organ of the ice goby, *Leucopsarion petersi* (Ito and Takahashi, 1983). Furthermore, numerous pinocytotic vesicles occurring within endothelial cells of blood capillaries surrounding the pineal organ may indicate an active interchange of materials between the blood and the pineal cells of the floating goby.

The argentaffin reaction used in the present study has generally been applied to certain neurons and amine-producing endocrine cells to reveal the localization of reducing compounds such as catecholamines and 5-hydroxytryptamine (5-HT) ; the specificity of the reaction for these amines has been thoroughly investigated in various vertebrates (Tramezzani et al., 1964 ; Cannata et al., 1968 ; Håkånson et al., 1971). However, the argentaffin reaction cannot distinguish 5-HT from catecholamines. In the present study, fine silver grains indicating the presence of reducing compounds were found distributed selectively over the cytoplasmic matrix of photoreceptor and supportive cells of the pineal organ. This suggests that pineal cells of the floating goby may contain catecholamines or 5-HT.

The demonstration of pineal monoamines has thus far been carried out by the fluorescence histochemical method of Falck-Hillarp in several fishes such as the rainbow trout, *Salmo gairdneri* (Hafeez and Quay, 1969), the jacksmelt, *Atherinopsis californiensis* (Hafeez and Quay, 1969), the northern pike, *Esox lucius* (Owman and Rüdeberg, 1970 ; Falcon et al., 1980), and the three-spined stickleback, *Gasterosteus aculeatus* (van Veen et al., 1980). In these investigations, yellow fluorescence corresponding to indoleamines (5-HT or 5-HTP) appeared uniformly over the entire pineal organ, while green fluorescence corresponding to catecholamines occurred in the meningeal tissue close to the organ. Considering the findings of these studies,

it is highly likely that the positive argentaffin reaction of the pineal cells observed in the present study indicates the occurrence of 5-HT, but not of catecholamines, in the cells of the floating goby. The present study further suggests that the 5-HT is localized in the cytoplasmic matrix of the cells. In the pineal gland of higher vertebrates, 5-HT has been shown to be stored in dense-cored vesicles existing in rudimentary photoreceptor cells (Collin, 1979, 1981) or in pinealocytes (Lu and Lin, 1979). It is interesting to note in this context that in the three-spined stickleback, *Gasterosteus aculeatus*, yellow 5-HT/5-HTP fluorescence was shown to exist diffusely over the whole pineal parenchyma, even in photoreceptor cells where no dense-cored vesicles were detectable ultrastructurally (van Veen et al., 1980).

Recent papers frequently deal with the occurrence of two types of photoreceptor cells in the fish pineal organ (Falcon, 1979; Ito and Takahashi, 1983; Meiniel and Vivien-Roels, 1983). Meiniel and Vivien-Roels (1983) suggested that two categories of photoreceptor cells, viz. photoreceptor cells *sensu stricto* and photoneuroendocrine cells, were present in the pineal organ of the five-bearded rockling, *Ciliata mustela*. In the floating goby, some photoreceptor cells of the pineal organ were highly electron-dense while others were low in electron-density. Otherwise, however, there were no notable differences in their ultrastructure which were useful in distinguishing between the two photoreceptor cell types. Furthermore, pineal photoreceptor cells of the floating goby invariably showed a positive argentaffin reaction, although there was a slight quantitative difference in the number of reaction products. The difference in intensity of argentaffin reaction may imply that some biosynthetic activity of indoleamines is gradually altered in individual photoreceptor cells. Thus, in the pineal organ of the floating goby, the activities of the photoreceptor cells seem to be altered as a result of the actions of unidentified elements; the cells do not seem to be classifiable into different categories in terms of their function.

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