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Structure of the Pineal Organs of *Anguilla anguilla* L. and *Lebistes reticulatus* Peters (Teleostei)*

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Summary. Physiological and electron microscopic studies have shown that in different teleosts the pineal organ has a photosensory function (see Discussion). This partly contradicts earlier light microscopic results in teleosts which associate strongly folded and highly vascular pineal organs, and their acidophilic inclusions with secretory function. Although the pineal organ of the eel is "much reduplicated and highly vascular", there is no clear evidence of secretory activity in this form (see Tilney and Warren, 1919). The pineal organ of the guppy belongs to a different structural type and consists of a stalk and an end-vesicle with a narrow lumen. Pflugfelder (1953) has concluded after epiphysectomy that the pineal organ of the guppy may have an endocrine function. In view of these inconsistencies in the interpretation of teleost pineal systems, the pineal organs of the eel (*Anguilla anguilla*) and the guppy (*Lebistes reticulatus*) were examined with the electron microscope. Although the tissue layers covering the pineal organ are very different in the two species, the pineal organs in both species have well-developed receptor cells with characteristic outer segments. In the pineal organ of the eel, synapses with synaptic rods (ribbons) are observed. The pineal organs of the eel and the guppy show all of the structural elements typical for a pineal sense organ. Evidence obtained from other teleost species (e.g., *Esox lucius*) shows that pineal organs with a sensory apparatus may also form biogenic amines (Owman and Rüdeberg, 1970); this has not yet been investigated in the eel or the guppy. The present results indicate that caution should be exercised in classifying teleost pineal organs after conventional light microscopic examination into predominantly sensory and nonsensory (secretory) types. A parapineal organ is present in the adult eel but is missing in the guppy.

Key-Words: Pineal organ — Photoreceptor cells — *Anguilla anguilla* — *Lebistes reticulatus*.

Introduction

The pineal region in teleost fishes is characterized by great morphological variation (Studnička, 1905; Tilney and Warren, 1919; Holmgren, 1959). This variation is also observed in adjacent parts of the brain, and also in the thickness and pigmentation of the overlying tissues of the head.

According to Breder and Rasquin (1950), bony fishes may be divided in three groups with respect to the organization of the tissues that cover the pineal organ. In one of these groups the tissues overlying the pineal organ are transparent and form a "pineal window", whereas in another group the tissues seem to be opaque. In the third group the entrance of light into the pineal organ is regulated

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by appropriately placed chromatophores. Breder and Rasquin have also found a correlation between the above-mentioned group characteristics and the reaction of different teleost species to light. Species with a pineal window have been found to show positive phototactic movements in most cases, while those with opaque pineal covering layers have generally responded with negative phototactic movements; species with variable pineal cover are not clearly differentiated in this respect.

As a consequence of the variation in the organization of the pineal covering layers, different amounts of light must be presumed to reach the pineal organs in different species. The question now arises whether the pineal organ that receives much light is ultrastructurally different from that which receives little or no light. Recent electron microscopic research has shown that the pineal organs of at least ten species of teleosts have well-developed photoreceptor cells (*Salmo gairdneri*, Breucker and Horstmann, 1965; *Phoxinus laevis*, Oksche and Kirschstein, 1966, 1967, 1971; *Mugil auratus*, *Uranoscopus scaber*, *Sardina pilchardus*, Rüdeberg, 1966, 1968; *Plecoglossus altivelus*, Omura *et al.*, 1969; *Carassius auratus*, Takahashi, 1969; *Esox lucius*, Owman and Rüdeberg, 1970; *Thunnus thynnus*, Murphy, 1971; *Pterophyllum scalare*, Bergmann, 1971). Some of these fishes have a pineal window, e.g. *Sardina*, *Plecoglossus* and *Thunnus*, while others have not been classified in regard to the pineal covering layers. Only one species, *Uranoscopus scaber* (Rüdeberg, 1966, unpublished results), with certainty belongs to the group having seemingly opaque pineal covering layers, but in this context it must be stressed that light penetrates through the skull into the brain even in rats (Ganong *et al.*, 1963). Therefore it seems almost unavoidable that at least small amounts of light penetrate into the pineal organ also of teleosts lacking pineal windows (see also Morita and Bergmann, 1971). Nevertheless, the amount of light received by the pineal organ of *Uranoscopus* must be very much smaller than that received by the pineal of e.g. *Thunnus* or *Sardina*. It is not yet fully known, if fishes belonging to the group with a seemingly opaque pineal covering layers have ultrastructural characteristics indicating the same photosensory function that has been electrophysiologically proved in several teleost species (*Salmo gairdneri*, Dodt, 1963, Morita, 1966; *Plecoglossus altivelis*, *Pelteobagris undiceps*, Hanyu *et al.*, 1969; *Mugil cephalus*, Hanyu and Niwa, 1970; *Pterophyllum scalare*, Morita and Bergmann, 1971). The electron microscopic results obtained on *Uranoscopus* indicate that no basic difference exists, but of course other species must be investigated before a generalization can be made.

The eel, *Anguilla anguilla*, L., is a teleost species lacking a pineal window and having a thick, tough skin covering the pineal region. Even after removal of this skin the pineal organ cannot be discerned through the braincase and the overlying connective tissue. This species was therefore chosen for the present investigation (cf. Oksche and Vaupel-von Harnack, 1965) as a representative of the teleost group provided with an opaque pineal cover.

Light-dependent spontaneous (swimming and snapping movements) and conditioned (light-food-training) responses in the blinded eel have been reported by de la Motte (1964) after illumination of the pineal region of the head. However, in the eel the success of the training was not as distinct as in other fishes, e.g. *Phoxinus*. In the eel, also the trunk and tail regions were found to be light-sensitive. A light sensitivity of the head was observed in *Lebistes* shortly after

blinding; unfortunately a training program has not been performed with this species (de la Motte, 1964).

In the paper of Omura and Oguri (1969) the pineal organ of the guppy, *Lebistes reticulatus* Peters, has been classified as a special "small space type" of organ which is characterized by having only remnants of a pineal lumen. This morphological feature might indicate a transformation of the sensory pineal organ with its related functions (cf. Fenwick, 1970 a, b, c; Hafeez, 1970; Hafeez and Quay, 1970b) into a gland-like structure with more pronounced endocrine effects, as suggested by Pflugfelder (1953, 1954, 1956). It should be noted, however, that the results of Pflugfelder have been disputed by Peter (1968). The guppy was included in the present investigation to give the structural basis for the discussion of the mentioned questions.

The guppy belongs to the group of teleosts which have variable pineal covering layers, as evidenced by the fact that the pineal organ can be observed through the skull roof after removal of only the scale that covers the pineal area (cf. Rasquin, 1958).

Material and Methods

Animals. Eight adult eels, *Anguilla anguilla*, L., weighing 150 to 405 g were caught in the Sound between Malmö and Copenhagen on the 20th of October 1970 and killed by decapitation on the same day.

Series of histological sections of seven brains of the guppy, *Lebistes reticulatus* Peters, from the collection of Professor Oksche were at my disposal. These brains had been taken from guppies of six different stages between newborn and adult. Further six adult guppies, of body length 30–50 mm, were obtained from commercial sources and killed by decapitation before fixation.

Light Microscopy. After fixation for five days in Bouin's fluid, five brains of the eel were dehydrated in alcohol, embedded in paraffin, sectioned at 8 μm, and impregnated with silver protargol according to Bodian and Ziesmer.

The seven guppy specimens from the collection of Professor Oksche had been sectioned at 10 μm and stained with chrome alum-haematoxylin-phloxin according to Gomori and Bargmann (5 series) or with iron haematoxylin according to Heidenhain. Three other guppy brains with surrounding bony braincases were fixed in Bouin's fluid for 48 hours, decalcified in 7% HNO_3, dehydrated, embedded in paraffin and sectioned at 7 μm. All three specimens were impregnated in silver protargol according to Bodian and Ziesmer. One sagittally sectioned complete series was used to trace the pineal tract.

Electron Microscopy. The pineal organs of three eels and three guppies were rapidly dissected under a binocular microscope and immersed in 1% osmium tetroxide in 0.13 M phosphate buffer according to Millonig. After fixation for 60–90 min, dehydration was performed in ethyl alcohol. During the dehydration the specimens were stained *en bloc* for 30 min in a solution of 0.5% uranyl acetate and 1% phosphotungstic acid in 100% ethyl alcohol. Embedding was performed in Vestopal W (Jaeger) and Durcupan (Fluka). Sections of a thickness between 500 and 1000 Å were produced with the Porter-Blum MT2 and Reichert OMU-11 ultramicrotomes. The contrast was further improved with lead citrate (Venable and Coggeshall) and the ultramicrographs were obtained with a Siemens Elmiskop I at 60 kV.

Results

Light Microscopy

The dorsal side of the head of *eel* is not provided with any specialization to permit the entrance of light into the pineal organ. Not even after removal of the tough skin is it possible to observe the pineal organ which in many other species (e.g. the guppy, see below) can be seen through the bony braincase.

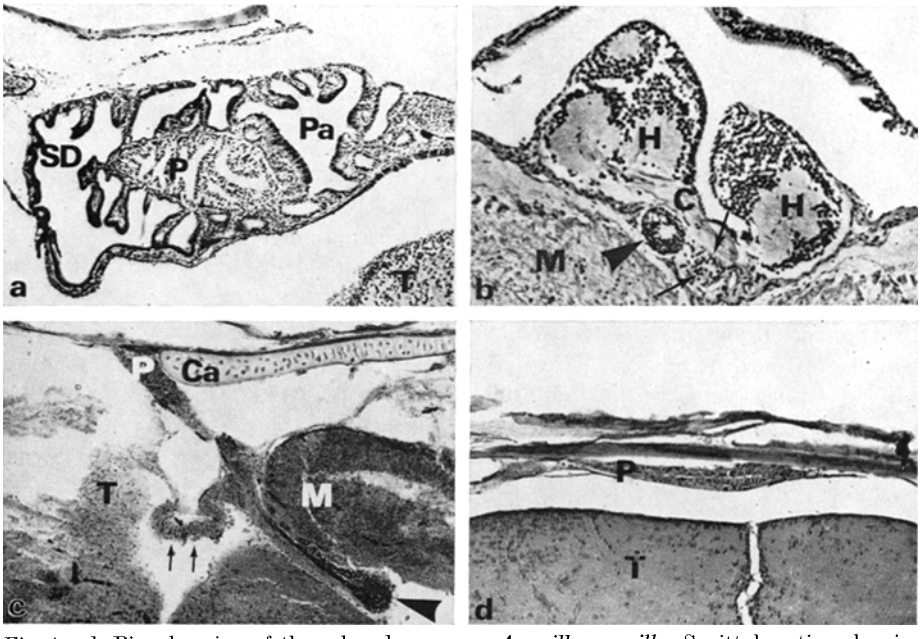

Fig. 1a–d. Pineal region of the eel and guppy. a *Anguilla anguilla*. Sagittal section showing a lateral part of the pineal organ, *P*, surrounded by the dorsal sac, *SD*, posteriorly, and the paraphysis, *Pa*, anteriorly. *T* telencephalon. Bouin. Silver protargol. 8 μm. × 60. b *Anguilla anguilla*. Horizontal section through the habenular ganglia, *H*, the proximal part of the pineal stalk, *small arrows*, the parapineal organ, *large arrow*, and the habenular commissure, *C*. *M* mesencephalon. Bouin. Silver protargol. 8 μm. × 96. c *Lebistes reticulatus* of body length 7 mm. Paramedian section through the pineal region showing the pineal organ, *P*, resting against the skull roof anterior to a cartilage rod, *Ca*. The stalk of the pineal organ joins the brain posterior to the choroid plexus of the third ventricle, *small arrows*, and anterior to the mesencephalon, *M*. *T* telencephalon, *large arrow* posterior limit of commissura posterior. Bouin. Iron haematoxylin. 10 μm. × 140. d *Lebistes reticulatus*, body length around 15 mm. Transverse section showing the pineal end-vesicle, *P*, lying close to the skull roof. A narrow lumen can be seen in the pineal organ, but this lumen is still more obscured in older animals. *T* telencephalon. Bouin. Chrome alum-haematoxylin-phloxin. 10 μm. × 130

The pineal stalk of the eel is situated in a deep furrow formed by the dorsal sac, the lateral folds of which meet on the dorsal side of the stalk and of the proximal part of the end-vesicle. The distal part of the end-vesicle rests on the paraphysis, the lateral folds of which also meet on the dorsal side of the pineal. In this way, the pineal organ is surrounded by the dorsal sac (posteriorly) and the paraphysis (anteriorly) except in a small central area of the dorsal wall of the end-vesicle which is resting against the roof of the braincase. Probably, the walls of the paraphysis and the dorsal sac do not prevent light from reaching the pineal organ, however, consisting only of simple, columnar epithelia devoid of pigments (Fig. 1a).

The thickness of the pineal epithelium varies very much, due to conspicuous epithelial ridges which project into the pineal lumen (Fig. 1a). The area of the inner epithelial surface is thus greatly increased. The pineal lumen is obviously

somewhat reduced in volume by the epithelial ridges, but nevertheless remains as a distinct cavity. The lumen is connected to the third ventricle via a narrow canal in the stalk.

A conspicuous pineal tract runs in the dorsal wall of the stalk, from the pineal end-vesicle to the region of the posterior commissure. In semi-thin sections (1 μm) of Vestopal W- and Durcupan-embedded tissue, it was possible to observe three types of cells in the pineal organ of the eel: receptor cells, supporting cells, and a few larger cells with nerve cell characteristics.

A parapineal organ was found in all light microscopically investigated specimens of the eel. This organ is a small, globular cell mass measuring 50—100 μm in diameter and situated to the left of the pineal stalk and connected to the left habenular nucleus through a small parapineal tract (Fig. 1 b).

The pineal region of the braincase of the *guppy* is covered only by a thin skin containing a relatively large mid-dorsal scale. After removal of the scale the pineal organ can be seen shining through the braincase. The amount of light entering the pineal organ is clearly dependent on the state of expansion of the chromatophores. In the brain of the guppy there is neither a paraphysis nor a dorsal sac. Consequently the pineal organ is not coated by these structures as in the eel, but runs freely from the brain to the roof of the braincase. The end-vesicle is dorsally flattened and enters in close contact with the overlying bony roof (Fig. 1 c, d). The pineal lumen is reduced in volume, and the guppy pineal organ belongs to the "small space type" of Omura and Oguri (1969). Sections of 1 μm taken from embeddings for electron microscopy show that the pineal lumen consists of an extremely narrow and winding slit. According to Omura and Oguri (1969) the lumen in the guppy has no connection with the third ventricle. It should be noted, however, that such a connection may be so narrow that only an electron microscopic investigation of the stalk could reveal its existence.

Due to the very small size of the pineal organ of the guppy, it is very difficult to study it in any detail with the light microscope. A silver impregnated specimen, however, shows a very inconspicuous pineal tract running in the dorsal wall of the pineal stalk.

No parapineal organ was observed in the guppy.

Electron Microscopy

The ultrastructural organization of the pineal tissue of the eel and of the guppy was found to agree very well with that of the pineal organs of those teleosts which have already been investigated by means of electron microscopy (Breucker and Horstmann, 1965; Rüdeberg, 1966, 1968; Oksche and Kirschstein, 1967, 1971; Omura *et al.*, 1969; Takahashi, 1969; Owman and Rüdeberg, 1970; Murphy, 1971; Bergmann, 1971). Therefore, the general features of the receptor (sensory) cells and of the supporting cells will not be described in the present paper. Instead, attention will be drawn to a few special points which are thought to be relevant for the discussion of pineal functions or which have not previously been described.

a) The Eel

The Outer Segment. The outer segment of the pineal receptor of the eel is very similar to that of the pike (Owman and Rüdeberg, 1970), consisting of

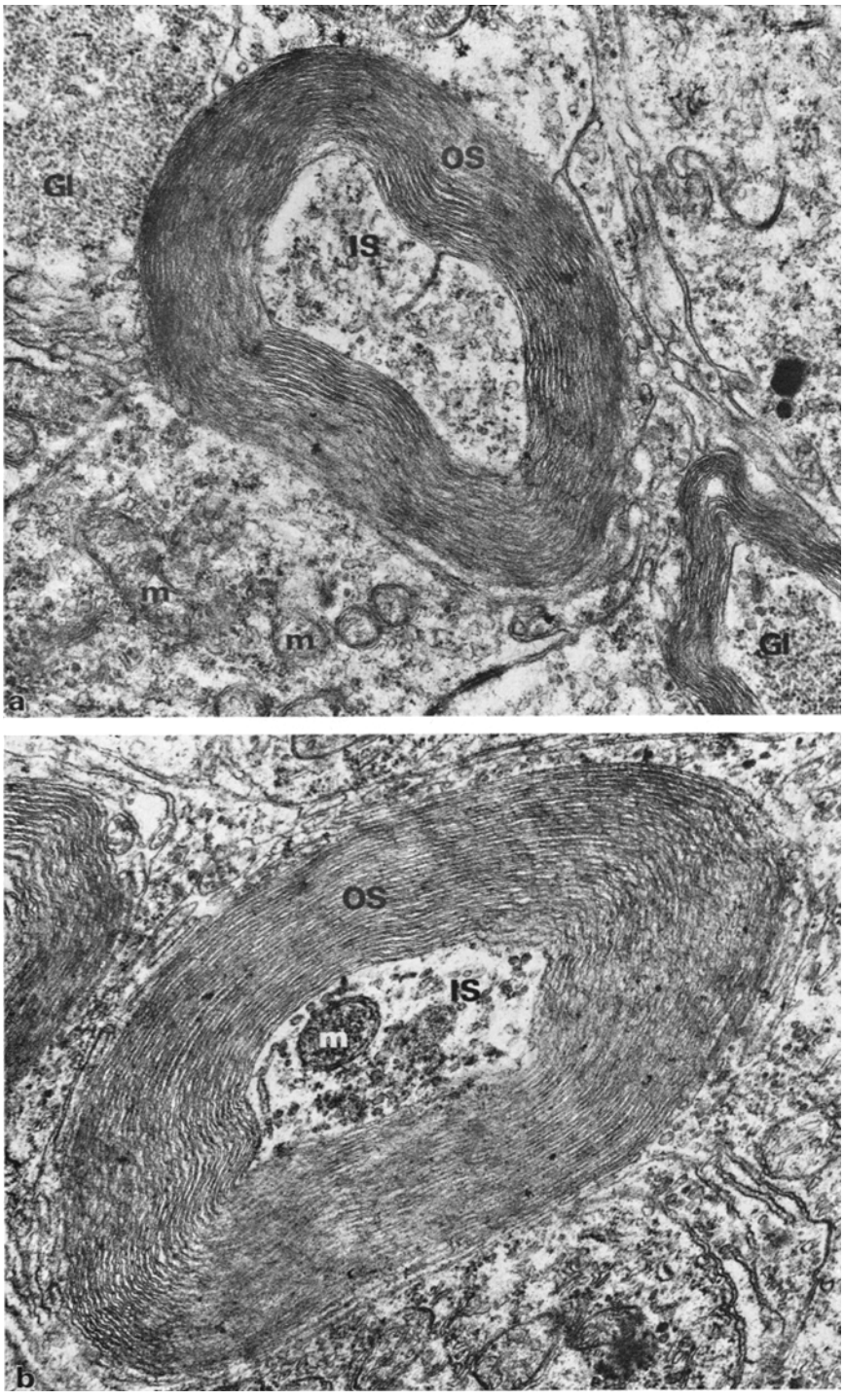

Fig. 2a and b. Pineal ultrastructure of *Anguilla anguilla*. Transverse section of two typically cup-shaped outer segments. *OS*, covering the distal parts of the inner segments. *IS*, Some neighbouring receptor cells contain glycogen. *Gl*, at least partly localized in inner segments. *m* mitochondrion. a $\times$ 18400. b $\times$ 27000

Fig. 3. Pineal ultrastructure of *Anguilla anguilla*. Longitudinal section through the distal parts of a receptor cell. The inner segment, *IS*, contains much glycogen, *Gl*, and an aggregation of mitochondria, *m*, which form the so-called 'ellipsoid'. The ciliary connecting piece, *Cc*, originates in a diplosome, *arrow*, and represents the common connection with the inner segment of the outer segment lamellae. *OS*, Instead of forming a cup around the inner segment the outer segment lamellae are directed upwards in a peculiar way. The structure *Ma* which is situated close to the outer segment, is thought to belong to a macrophage. *cy* cytosome, *G* Golgi complex, *S* supporting cell. × 13800

20—30 (as few as 10 and as many as 50 saccules are sometimes observed in single outer segments) flat saccules stacked on the inner segment. Each saccule has a thickness of 200—300 Å. The stack of saccules covers the outer surface of the inner segment and thus generally acquires the form of a bowl or cup (Fig. 2a, b). Occasionally, outer segments differ considerably from the common bowl type, but in all cases the cytoplasmic lamellae between the saccules are found to be continuous with the cytoplasm of the ciliary connecting piece which in turn is joined to the inner segment. The 9 fibrils of the ciliary connecting piece originate in a centriole belonging to a diplosome situated in the apical part of the inner segment (Fig. 3).

The Inner Segment. Apart from the usual cytoplasmic organelles (rough and smooth endoplasmic reticulum, ribosomes, cytosomes, an 'ellipsoid' consisting of mitochondria), the pineal inner segments of the eel often contain concentrations of electron-dense granules. These granules (diameter 200—400 Å) are judged to be glycogen granules on the basis of earlier studies on the pineal organ, although this has not been verified by histochemical methods in the present case (Fig. 3).

In the receptor cells of the eel the glycogen granules are also found in the perinuclear cytoplasm of the cell body and its processes. The latter observations agree with those concerning the pike pineal organ (Owman and Rüdeberg, 1970), but glycogen situated in the inner segment has not previously been electron microscopically observed in any teleost.

Synaptic Rods (Synaptic Ribbons). Like in other teleosts the pineal receptor cells of the eel extend towards the basal lamina with cell processes containing synaptic vesicles (diameter 400–600 Å) and synaptic rods and presumably making synaptic contacts with dendrites of ganglion cells. In most teleosts the synaptic rods of the pineal organ are relatively scarce and it is therefore difficult to observe any particular characteristics. The pineal of the eel, however, shows so many synaptic rods that it was possible to observe some new details. Among fishes only the pineal organs of the goldfish and of the European minnow are known to have a similar high frequency of synaptic rods (Takahashi, 1969; Oksche and Kirschstein, 1971).

In the pineal organ of the eel up to four synaptic rods have been observed in one single receptor cell ending. This indicates that the actual number of rods in each ending may be quite large, since the section contains only a small part of the total volume of the ending.

The length of the synaptic rods varies between 1.8 and 0.5 μm. Very often the rods seem shorter, but in such cases they have probably been cut obliquely or transversally. Usually the rods are straight (Fig. 4b, d), but more or less curved ones are not rare (Fig. 4a, c). The thickness of the rods is 400–600 Å and their largest breadth is about 2000 Å. It is not clear if all synaptic rods have more or less the same form and size, or if they vary in these respects. In the former case their different appearances in the electron micrographs are explained by different section planes alone. In the latter case different rod types further complicate the picture.

The synaptic rods are invariably surrounded by synaptic vesicles which generally do not adhere to the rod surface. Further, all synaptic rods probably rest with one end on the cell membrane of the receptor cell processes (Fig. 4a–d), although sometimes this does not appear to be true due to the plane of the section.

The point were the synaptic rod approaches the cell membrane displays some of the characteristics of the typical vertebrate synapse (Gray, 1966; Colonnier, 1968). The pre- and postsynaptic membranes are thickened by electron-dense material which is a little thicker on the presynaptic than on the postsynaptic side, contrary to the condition in the normal synapse. Electron-dense material is seen to be accumulated also in the synaptic cleft in the form of a thin layer (Fig. 4a). Between the end of the synaptic rod and the presynaptic membrane thickening there is a very narrow cleft of not more than 100 Å in width. This

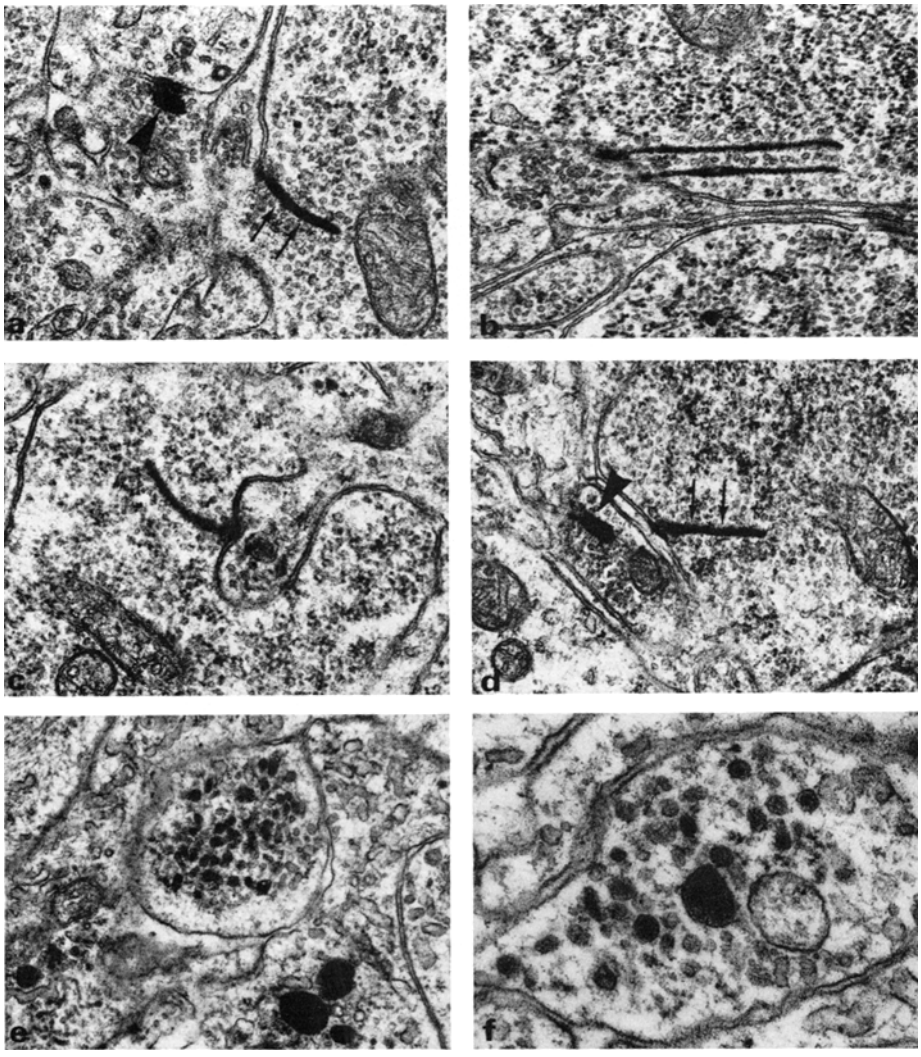

Fig. 4a–f. Pineal ultrastructure of *Anguilla anguilla*. a Longitudinally sectioned synaptic rod, *small arows*, and probably obliquely sectioned end of synaptic rod, *large arrow*. The latter is provided with pre- and postsynaptic membrane specializations. Note the synaptic vesicles in the cytoplasm around the synaptic rods. × 25000. b Two longitudinally sectioned synaptic rods. × 25000. c A curved synaptic rod. Note invaginated postsynaptic structure. × 20000. d A longitudinally sectioned synaptic rod, *small arrows*, and a probably transversely sectioned one, *large arrow*. × 20000. e and f Densecore vesicles, diameter 600–1000 Å, rarely up to 2000 Å. e × 18000; f × 36000

cleft is often obscured by electron-dense material of unknown origin, but there are no visible synaptic vesicles (Fig. 4a, c).

Only rarely the points of contact between the synaptic rods and the cell membrane are located in connection with an invagination of the postsynaptic

Fig. 5. Pineal ultrastructure of *Anguilla anguilla*. A macrophage, *Ma*, situated between several outer segments, *OS*. Some of the cytosomes, *cy* contain membranous structures. × 13800

element into the receptor cell cytoplasm (Fig. 4c) in a way similar to that of the synapses with synaptic rods in the photoreceptor cells of the retina (Pedler, 1969; Dowling, 1970). In most pineal synapses containing synaptic rods the post-synaptic element is situated adjacent to the receptor ending without being invaginated into it.

Dense-core Vesicles. In a few cases small profiles, possibly nerve fibers, have been found to contain dense-core vesicles of 600–1000 Å diameter mixed with a few larger ones up to 2000 Å in diameter (Fig. 4e, f).

Macrophages. Occasionally, macrophages were observed in or near the pineal lumen. Within the cytoplasm of these cells numerous cytosomes contain what may be rests of the outer segment membranes (Fig. 5).

No unequivocal signs of *secretion* were observed in the pineal organ of the eel. There were no secretion granules, filled vesicles or cisternae.

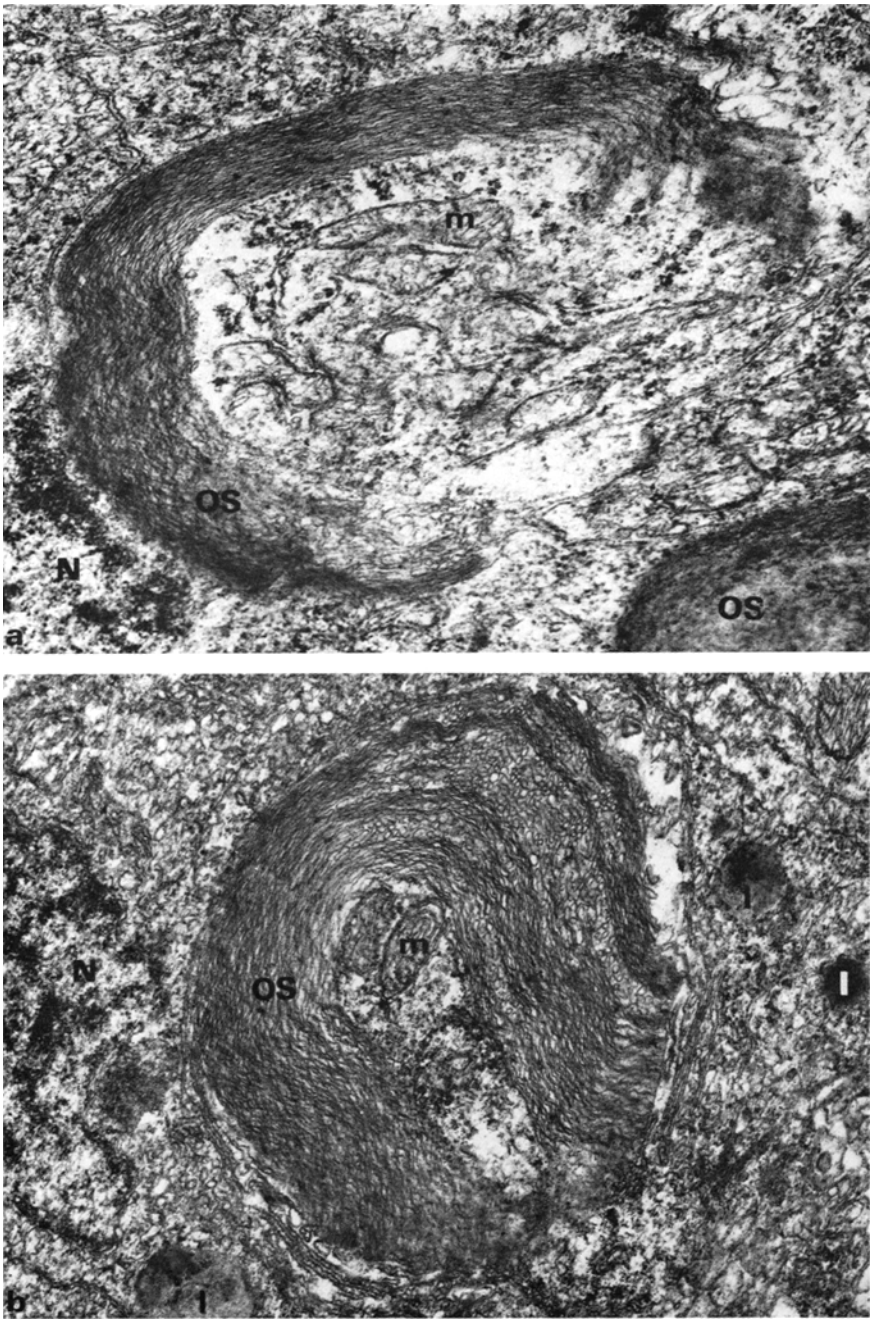

Fig. 6a and b. Pineal ultrastructure of *Lebistes reticulatus*. Typical outer segments. *OS*, covering inner segments. *l* probably a lipid droplet, *m* mitochondrion, *N* nucleus of supporting cell. a × 23000. b × 23000

b) The Guppy

Although the same methods were used for processing the pineal organs of the eel and of the guppy for electron microscopy, the results were different in the two species. While the tissue of the eel was ideally prepared for fine structural studies, that of the guppy was good for a general survey but lacked the clearness necessary for the study of small details. In spite of this lack of clearness the guppy tissue was well preserved and presented no artifacts. A detailed study was postponed, and only the following structures were studied:

The Outer Segment. The outer segment of the guppy receptor cell is very similar to that of the eel pineal receptor (Fig. 6). It consists of 10–50 saccules of 120–200 Å thickness and has same general shape as the outer segment of the eel. Like in the eel, the pineal organ of the guppy is provided with some outer segments of irregular shape.

The Inner Segment. No glycogen has been observed in the inner segment or in any other part of the receptor cell of other cells in the pineal organ of the guppy.

The Basal Process of the Receptor Cell. In receptor cell processes large numbers of synaptic vesicles (diameter 400–600 Å) are found. The synaptic rods are rare.

No unequivocal signs of *secretion* were found in the pineal organ of the guppy. As in the eel, no secretion granules, filled vesicles or cisternae were noted.

Discussion

The results presented above indicate that the pineal organs both in the eel and the guppy are provided with structures of photoreceptor type. Well-developed photoreceptor outer segments are the most important prerequisite for photosensitivity, since the membrane of the outer segment lamellae, or saccules, represent the site of the photochemical reaction of the photosensory mechanism (see e.g. Wald, 1961; Brown *et al.*, 1963). It has been electrophysiologically demonstrated that already the existence of very few outer segment saccules permit the developing photoreceptor cell of the tadpole retina to react specifically to light (Nilsson and Crescitelli, 1970). Further, to my knowledge all pineal organs provided with well-developed outer segments respond electrically to direct illumination. This statement is now valid for lampreys (Morita and Dodt, 1971), sharks (Hamasaki and Streck, 1971), teleosts (see p. 228), frogs (e.g. Dodt, 1964; Morita, 1965) and lizards (e.g. Dodt and Scherer, 1968; Hamasaki and Dodt, 1969).

One might expect that also a well-developed impulse transmission system between receptor cells and ganglion cells is a necessary prerequisite for the photosensory function of the pineal organ. This is certainly true, but it does not necessarily mean that the electron microscopist always can expect to find the morphological equivalent of such a transmission system in the form of synapses. The rod or ribbon synapses of the eel beautifully fill the needs of a theoretical model, but it must not be forgotten that such synapses are very rare at least in two of those teleosts species, the pineal organs of which have been electrophysiologically shown to be light sensitive! These are *Salmo irideus* (electrophysiology: Dodt, 1963; Morita, 1966; electron microscopy: Breucker and Horstmann, 1965; own unpublished results) and *Pterophyllum scalare* (electrophysiology: Morita; see Morita and Bergmann, 1971; electron microscopy: Bergmann, 1971).

It seems possible that the synapses between the pineal receptor cells and the pineal ganglion cells at least in certain teleost species are lacking special morphological characteristics.

The finding of *glycogen* in some of the receptor cells of the eel agrees with the earlier described localization of glycogen in the pineal organ of the selachian *Scyliorhinus* (Rüdeberg, 1969b) and in the pineal of the teleost *Esox* (Owman and Rüdeberg, 1970), although the glycogen has not before been observed in the inner segment. The possible significance of pineal glycogen has been discussed in some detail for *Scyliorhinus* (Rüdeberg, 1969b). It is worth noting that Murphy (1971) found glycogen in the pineal supporting cells of the tuna. This is more in accordance with the conditions in the lateral eye, where the glycogen is stored in the Müller cells (Magalhães and Coimbra, 1970). It is not known why the pineal glycogen sometimes is stored in receptor cells, sometimes in supporting cells, and in many cases seems to be entirely absent, but diurnal and seasonal variations should be investigated prior to further discussion of the matter.

The *dense-core vesicles* (cf. p. 236) found in a few cases in the pineal organ of the eel, morphologically resemble those found in the pineal organ of anurans (Ueck, 1968) and in the pineal organ of the dogfish (Rüdeberg, 1968b) and may also be comparable to the ones found by Oksche and Kirschstein (1971) in the pineal organ of the teleost *Phoxinus*. The contents of these vesicles are not known, but catecholamines have been suggested.

With the finding of the *parapineal organ* in the eel (see p. 231), the known number of teleost species provided with a parapineal organ as *adults* increases to three: *Salmo gairdneri, Esox lucius* and *Anguilla anguilla* (Rüdeberg, 1969a).

The morphology of the roof of the braincase covering the pineal organ does not seem to be reflected in the ultrastructure of the pineal organ (see also Morita and Bergmann, 1971). When the scale covering the pineal area of the guppy is taken away, the pineal organ is freely accessible to light, whereas the rest of the brain is protected from light by meningeal melanophores. On the contrary, in the eel the entrance of light into the pineal organ does not seem easier than into the rest of the brain. Still, both species have well-developed pineal sensory structures.

In a recent paper Hafeez (1971) has studied the pineal morphology of 15 different teleost species. On the basis of his results, obtained by light microscopy, Hafeez suggests that the pineal organ of certain teleosts may be predominantly sensory, whereas the same organ in the other teleosts may be specialized for a predominantly nonsensory function.

The electron microscopic findings of the present paper call for caution in the interpretation of light microscopical results. In the light microscope it is hardly possible to discern the well-developed outer segments in the pineal organ of the eel or the guppy and both organs have been suspected to be more glandular than sensory in nature (Pflugfelder, 1953, 1954, 1956; Oksche and Vaupel-von Harnack, 1965). Nevertheless, the present electron microscopic results make it possible to allege that both species maintain the sensory function of the pineal organ. No sign has been observed which indicates secretion or other nonsensory functions, if one excludes those general features which are found also in other teleost pineals (see Owman and Rüdeberg, 1970). It must be stressed, however, that the negative observations in no way exclude neuroendocrine functions. On the contrary, evidence is accumulating regarding the presence in teleost pineal

organs of melatonin (*Oncorhynchus*, Fenwick, 1970a), acetylserotonin transferase (*Hesperoleucus* and *Salmo*, Hafeez and Quay, 1970a), and serotonin (*Salmo* and *Atherinopsis*, Hafeez and Quay, 1969; *Esox*, Owman and Rüdeberg, 1970). These substances are very closely connected to each other, acetylserotonin transferase (a new name for HIOMT, hydroxyindole-O-methyltransferase) being the enzyme responsible for the final step in the conversion of serotonin (5-hydroxytryptamine) into melatonin (N-acetyl-5-methoxytryptamine) (Hafeez and Quay, 1970a).

Several physiological roles for melatonin in teleosts have already been proposed. Mira (1962) and Reed (1968) found melatonin to be a melanophore-contracting agent in *Scardinius* and *Nannostomus* (see also Reed *et al.*, 1969; Ruffin *et al.*, 1969). According to Fenwick (1970b) melatonin is an inhibitor of gonad functions in the goldfish. Hafeez (1970) found that melatonin decreased swimming activity, whereas Byrne (1970) discovered that this hormone decreased locomotor activity only during the light phase of the photoperiodic cycle and that serotonin increased the same activity during the dark period. For an exhaustive discussion on teleost melatonin the reader is further referred to Hafeez (1970). One possible role of pineal serotonin as a concomitant to a protein or polypeptide hormone is discussed by Owman and Rüdeberg (1970).

The hormone melatonin may well be imagined to function in all or in some of the mentioned roles, and, in fact, some earlier experiments point to the teleost pineal organ as a light-sensitive organ mediating colour change responses (von Frisch, 1911a, b; Scharrer, 1928; Schäfer, 1964). It is certainly more difficult to understand the role of a hormone in the rapid positive or negative phototactic responses exhibited by several teleosts (Breder and Rasquin, 1950; Hoar, 1955; Pang, 1965) or the role of a hormone in mediating conditioned reflexes like those demonstrated by Scharrer (1928) and de la Motte (1964). In such rapid reactions hormones probably act too slowly and nervous interactions must be presupposed. Stimuli of light reach the brain through the pineal tract, and this tract must be supposed to be connected to motor centres of the brain where the reflexes are triggered. Unfortunately, relevant information regarding the destination of the fish pineal tract is still lacking, but the interesting findings on the anuran pineal tract which were recently published by Paul *et al.* (1971), possibly will be shown to be valid also in fishes. In *Rana* the pineal nerve fibres run to parts of the brain where optical impulses and reflectory body movements are integrated (Paul *et al.*, 1971).

In the above general discussion mostly positive findings regarding pineal functions have been treated. The possibility exists, of course, that some of the alleged functions are mere exceptions or even depend on falsely interpreted facts. The negative findings of e.g. Fenwick (1970c) regarding phototactic and conditioned responses of the goldfish and of Hafeez and Quay (1970b) regarding phototactic responses of the rainbow trout may depend on species differences, but this will probably turn out to be an incomplete explanation (cf. discussion of Oksche and Kirschstein, 1971).

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