Three Types of Photoreceptors in the Pineal and Frontal Organs of Frogs: Ultrastructure and Opsin Immunoreactivity*

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Summary. The pineal complex in frogs (Rana esculenta, R. temporaria, R. tigrina, R. arvalis) was studied by conventional electron microscopy and postembedding rhodopsin immunoelectron microscopy. Three types of photoreceptor cells were found in both the pineal and frontal organs. In the pineal organ, most of the photoreceptors exhibited rhodopsin-immunoreactive outer segments and large inner segments with a large ellipsoid of densely packed mitochondria (“rod-like” photoreceptors). A small number of photoreceptors was rhodopsin-immunonegative (“cone-like” photoreceptors). In both Rana esculenta and R. temporaria, the latter were either supplied with an oil droplet and an ellipsoid in their inner segment, or they were electron-lucent with a small inner segment without an ellipsoid. In contrast, the frontal organ displayed many immunonegative “cone-like” outer segments and few rhodopsin-immunoreactive “rod-like” photoreceptors. In both organs, the basal processes of the photoreceptor cells were found to form ribbon-containing axonal pedicles which synapsed with the dendrites of secondary neurons. The latter rarely received any further afferences by conventional synapses.

The frog pineal organ is considered a predominantly “rod-type” and the frontal organ a “cone-type” photosensory organ. The presence of three kinds of pineal/frontal photoreceptors is discussed in connection with the occurrence of different photopigments (rhodopsin/porphyropsin, iodopsin, ultraviolet and/or blue pigments) enabling the animal to discriminate by the pineal complex environmental light in various ranges of the spectrum.

The pineal complex (extracranial frontal and intracranial pineal organs) of anuran amphibians is known to be photosensitive, being composed of photoreceptors, glial cells, intrinsic secondary neurons and a synaptic neuropil. The latter is connected by the pineal/frontal (organ-) nerve and by the pineal/epiphysal tract, respectively, with the habenular, pretectal and tegmental regions as well as with the hypothalamic periventricular gray (Paul et al., 1971; Zilles and Nickeleit, 1979; Eldred et al., 1980; cf. Vollrath, 1981). Ultrastructurally, the photoreceptor cells are endowed with outer segments that resemble the cones of the retina in the arrangement of their membrane disks (Hyla regilla: Eakin, 1961; Eakin et al., 1961, 1963; Rana esculenta, R. temporaria: Oksche and Von Harnack, 1962, 1963; Oksche and Vaupel-Von Harnack, 1963; Rana

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However, the antigenicity of the frog pineal photoreceptor has been found to be predominantly of the rod-type (Vigh-Teichmann et al., 1980a, 1982, 1983a, b; Vigh-Teichmann and Vigh 1983, 1985, Vigh et al., 1982, 1985, 1986). Previously, Eldred and Nolte (1981) distinguished two kinds of photoreceptors by their length, density of cytoplasm and distribution of mitochondria in the frontal organ of Rana pipiens. Further, in the pineal organ of Rana esculenta, two kinds of photoreceptors were described by either the absence or presence of an oil droplet in their inner segment (Vigh-Teichmann and Vigh, 1983; Vigh et al., 1985). The presence of an oil droplet is typical of retinal cones of this species.

Moreover, our immunocytochemical studies revealed two main classes of pineal photoreceptors differing by the antigenicity of their outer segments to antisera raised against the opsin of bovine retinal rods: rhodopsin-immunonegative and immunopositive ones. The oil droplet-containing cells belonged to the immunonegative photoreceptors (Vigh-Teichmann and Vigh, 1983; Vigh et al., 1985). These results were direct evidence that the pineal photoreceptors elaborate at least two different photopigments.

Recently double labeling with rhodopsin and photoreceptor-specific S-antigen antisera has brought to our attention further differences in the antigenicity of the photoreceptors in the amphibian pineal organ, a pattern suggesting the presence of three kinds of photoreceptors (Vigh-Teichmann and Vigh, 1986a, b; Vigh et al., 1986; Vigh-Teichmann et al., 1986).

Since we earlier found rhodopsin-immunoreactive photoreceptors in the frontal organ by light microscopy (Vigh-Teichmann et al., 1980a, b), the present study was aimed at comparing the frontal and pineal organs of various frog species (Rana esculenta, R. temporaria, R. tigrina, R. arvalis) by use of rhodopsin immunoelectron microscopy. In addition, the immunocytochemical data were correlated with ultrastructural particularities of the photoreceptor cells of the pineal complex.

MATERIAL AND METHODS

Twenty five adult frogs (Rana esculenta, R. temporaria, R. trigrina, R. arvalis) of both sexes were used in the present study. The animals were kept under lighted laboratory conditions, or in the dark at 6°C for up to several months. The specimens were anesthetized with urethane prior to fixation between 10:00 and 17:00. The pineal organ, frontal organ and retina were fixed by immersion or transcardiac perfusion with 1% or 5% glutaraldehyde dissolved in a phosphate or cacodylate buffer of varying osmolality, at pH 7.2. For conventional electron microscopy, part of the material was postfixed in 1% buffered OsO₄ for 2 hr. After washing in buffer overnight, the material was dehydrated in graded series of ethanol and embedded in Araldite.

Silver to gold-colored ultrathin sections collected on grids were used for the detection of opsin immunoreaction as described previously (Vigh et al., 1983, 1985; Vigh-Teichmann et al., 1984). Two polyclonal antibovine opsin antisera were applied, raised against the opsin band prepared by SDS gel electrophoresis from rod outer segments of bovine retinas in the sheep (antisera Nr. 2235/17-45 provided by Dr. D. S. Papermaster, New Haven, Ct, USA: Converse and Papermaster, 1975; Papermaster, 1982) and the rat (Dr. P. Röhlisch, Budapest: Szél et al., 1985). The specificity of the latter antiserum was tested by immunoblotting (Szél et al., 1986b). The primary
antisera were diluted appropriately (1:100 to 1:250,000) in phosphate-buffered saline (PBS)-0.5% human serum albumin (HSA) of pH 7.4 for up to 48 hr at 4°C. Goat anti-rat-immunoglobulin (IgG) gold-complex (GAR G15), rabbit anti-goat-IgG gold-complex (RAG G5) or protein A colloidal gold (G5 or G15) obtained from Janssen Pharmaceutica, Beerse, Belgium served as second-step reagents to visualize the antigentic sites. Protein A-gold was also applied as a third step reagent after incubation with rabbit anti-rat-IgG (Sigma, Munich, FRG) or rabbit-ant-sheep-IgG (DAKO, Immunochemicals, Copenhagen, Denmark).

Control sections incubated without primary antisera, with preimmune serum (1% egg albumin Grade III, Sigma, Munich, FRG) or with either the anti-IgG-gold or protein A-gold complexes alone, were negative. The ultrathin sections were counterstained with uranyl acetate and lead citrate and photographed with a JEM 6C electron microscope.

Semithin sections of material opsin-immunoreacted with goat peroxidase-anti-peroxidase (DAKO, Immunochemicals, Copenhagen, Denmark), rabbit anti-sheep-IgG-peroxidase (Human Olto Anyag Termelő Vállalat, Budapest, Hungary) or avidin-biotin-peroxidase (Vector Labs, Burlingame, CA, USA) (technical details, see VIGH-TEICHMANN et al., 1984) served for orientation.

RESULTS

I. Pineal organ

In conventional ultrathin sections of the pineal organ of all the species studied, we observed two main classes of photoreceptor cells. They were characterized either by the relatively electron-dense cytoplasm of the perikarya, axonal processes and dendritic inner segments, or by the electron-lucent appearance of these elements (Fig. 1). In Rana esculenta and R. temporaria, some of the electron-dense photoreceptors exhibited an oil droplet in their inner segment (Fig. 1). Our conventional and immunoelectron microscopic results are mainly concerned with these two species, since they are often used in electrophysiological and other experimental work.

1. Rhodopsin-immunoreactive photoreceptor

In all species studied, most of the photoreceptor cells were relatively electron-dense and rhodopsin-immunoreactive. They occurred as singles, pairs, or triples from the ventricular orifice of the pineal stalk to the most distal pineal. Their elongated large inner segments were often found to be connected by zonulae adherentes. The inner segments lacked an oil droplet but contained a large ellipsoid, an accumulation of closely packed mitochondria oriented longitudinally (Fig. 1, 2a). Fingerlike—calycal—processes projected from the inner segment to cover the outer segment.

The membrane lamellae of the outer segment were of medium electron-density and strongly rhodopsin-immunoreactive by means of rat and sheep antibovine rhodopsin antisera (Fig. 1, 2a, b). Usually, the immunopositive outer segments were relatively long (up to 5 μm) and large (Fig. 2b).

The perikarya of the photoreceptor cells were broad, located close to the ependymal surface. In Rana esculenta, R. temporaria and R. arvalis, the basal processes of the photoreceptors were observed to form basket-like terminal enlargements in the neuropil. These large axonal pedicles contained an electron-dense hyaloplasm, ribosomes, glycogen granules, groups of mitochondria and accumulations of synaptic vesicles (diameter
Fig. 1. Three types of photoreceptors (1–3) in the pineal organ of the frog *Rana esculenta*. 1 Oil/lipid (L) droplet-containing electron-dense photoreceptor, 2 electron-lucent photoreceptor with loosely arranged mitochondria (M), 3 electron-dense photoreceptor. E ependymal cell, MB myeloid bodies, N nucleus, V ependymal microvilli. Arrows: electron dense zones in the outer segment. ×5,900. Insets a–c. A higher magnification of the membrane lamellae of the three kinds of photoreceptors reveals a cone-like appearance. a. At some places (asterisks), the intracellular spaces of the oil droplet-containing photoreceptor contain electron-dense material. ×59,000.
50–75 nm) associated with ribbons of various length (Fig. 3). In some of the terminal enlargements of the green frog pineal, the ribbons were rather long (up to 2.2 μm in length). In a number of pedicles, the synaptic vesicles were crowded around electron-dense spherules (diameter 0.2 μm). The pedicles encircled groups of thin neuronal dendrites in a basket-like manner (Fig. 3a) and formed ribbon synapses on them. Both the pre- and the postsynaptic membranes exhibited distinct thickenings (Fig. 3b).

2. Oil droplet-containing photoreceptor

In *Rana esculenta* and *R. temporaria*, there was a considerable number of a second type of electron-dense photoreceptor: its inner segment contained an oil/lipid droplet distal to the ellipsoid (Fig. 1, 4a). Sometimes, even two oil droplets could be detected in the same inner segment. Calycal processes extended from the inner segment alongside the outer segment (Fig. 4).

The outer segment of the oil droplet-containing photoreceptor cell was rhodopsin-immunonegative (Fig. 4b) and rather long (up to 6.5 μm in length). The outer segment appeared to differ from those of the other kinds of photoreceptors by the narrow
Fig. 3. Details of terminal enlargements of the axons of the rhodopsin-immunopositive, electron-dense type of photoreceptor in the pineal organ of *Rana esculenta*. a. The large axonal pedicle (P) encircles dendrites (D) of intrinsic pineal neurons in a basket-like manner. Arrows: synaptic ribbons. ×12,000. b. Both the presynaptic membrane of the pedicle (P) and the postsynaptic membrane of the dendrite (D) exhibit a thickening (arrow). M mitochondria. ×26,500
extracellular spaces between its photoreceptor lamellae. Furthermore, an osmiophilic material could be observed in the intracellular cytoplasmic spaces of the outer segment (Fig. 1, inset). The amount of this electron-dense material varied in the assemblies of membrane lamellae, forming alternating stripes from proximal to distal sections within the same outer segment (Fig. 1).

The oil droplet-containing photoreceptors were found scattered in the whole pineal organ, from the entrance of the pineal recess to the most distal pineal. Their perikarya were located at the level of those of the rhodopsin-immunoreactive photoreceptors. The basal processes of the oil droplet-containing cells formed large axonal terminals. These pedicles contained accumulations of synaptic vesicles (diameter 60–80 nm) and slightly arched ribbons. The ribbons were sometimes thickened at their distal ends, touching the synaptic membrane. These pedicles, identified in serial sections, appeared to be rather similar to those of the rhodopsin-immunoreactive photoreceptors.
Fig. 5. Details of the electron-lucent type of photoreceptor of the pineal organ of *Rana esculenta* (a and b) and *Rana temporaria* (c). a. Overview of the photoreceptor (P), M mitochondria of the ellipsoid, PN nucleus of the photoreceptor, E ependyma with myeloid bodies and nucleus (N). ×7,300. b. Inner segment (IS) with loosely arranged mitochondria (M). O outer segment, asterisk: connecting piece of the cilium. ×22,000. c. Portion of the rhodopsin-immunonegative outer segment (O) of an electron-lucent photoreceptor. A few gold particles (black dots) represent background staining. Non-osmicated material. ×38,000
In *Rana tigrina* and *R. arealis*, oil droplets could not be detected in the inner segments of the retinal and pineal photoreceptors. In the tiger frog, rhodopsin-immunonegative pineal photoreceptors could be observed in considerable numbers. Most of the immunonegative outer segments were smaller than the immunoreactive ones. The opsin-immunonegative outer segments appeared to display membrane lamellae with narrow extracellular spaces, as is typical of the oil droplet-containing photoreceptors.
3. Rhodopsin-immunonegative electron-lucent photoreceptor

This third type of photoreceptor was characterized by a relatively electron-lucent hyaloplasm, a small inner segment with randomly distributed mitochondria, and an ellipsoid usually located in a thickening of the intraependymal dendrite (Fig. 1, 5a, b). Oil droplets were absent. Such photoreceptors occurred in small numbers, scattered in the pineal epithelium. An accumulation of cells of this type could be observed in the distal portion of the pineal organ.

Using antibovine rhodopsin antisera, we found the electron-lucent photoreceptors to display opsin-immunonegative outer segments (Fig. 5c). The latter measured up to 5.4 μm in length. The thickness of the photoreceptor lamellae was about that of the immunoreactive, electron-dense photoreceptors lacking an oil droplet (Fig. 1, 5). However, the outer segment appeared to be less electron-dense (Fig. 1).

The perikarya of the electron-lucent pineal photoreceptors were located farther away from the pineal lumen than those of the other types of photoreceptors. The nucleus of the former displayed a pronounced nucleolus and few heterochromatic areas. The perikaryon gave rise to a basal process forming a basket-like terminal enlargement. This pedicle contained some endoplasmic reticulum, ribosomes, further glycogen granules, groups of mitochondria and accumulations of synaptic vesicles (diameter 45–50 nm) around ribbons (Fig. 6a, b). The pedicles encircled either single or groups of thin dendrites and formed ribbon synapses on them (Fig. 6a). A synaptic membrane thickening could be observed on the presynaptic pedicle as well as on the postsynaptic dendrites of the intrinsic pineal neurons (Fig. 6b).

4. Intrinsic pineal neuron

Nerve cells were found scattered singly or in twos and threes. Relatively large neuronal perikarya were located more basally in the pineal tissue, near the basal lamina. Smaller neurons were observed at the level of the cell bodies of the photoreceptors. The apical portion of the nerve cells almost reached the diverticles of the pineal lumen but was covered by a thin ependymal layer (Fig. 7a). It could not be demonstrated whether such neurons would send ciliated dendritic terminals into the lumen of the pineal organ. However, the neuronal perikarya were found to give rise to solitary cilia extending into the intercellular space (Fig. 7b). Cilia were also observed to arise from basally located neurons, sometimes toward the external surface of the pineal nervous tissue.

The neuronal perikarya were characterized by elements of the endoplasmic reticulum, numerous ribosomes, mitochondria, small Golgi areas and rare granulated vesicles (90–140 nm in diameter). Such granulated vesicles could seldom be observed in dendritic profiles of the neuropil. In addition to the ribbon-containing axonal pedicles of the photoreceptor cells, there were few common axon terminals that formed axo-somatic or axo-dendritic synapses on the intrinsic pineal neurons, particularly of the proximal pineal. Their presynaptic axoplasm contained synaptic and few relatively large granulated vesicles (Fig. 7c). The latter measured 90–140 nm in diameter. The postsynaptic membrane was thicker and more electron-dense than the presynaptic one (Fig. 7c).

5. Internal and external pineal glial boundaries

Some comments seem necessary on the cytological peculiarities of the ependyma covering the pineal lumen and the external basal lamina. In general, the ependyma contained a relatively electron-lucent hyaloplasm and several lunula-shaped myeloid bodies.
Fig. 7. Details of intrinsic neurons and ependyma of the pineal organ of *Rana esculenta*. 

a. The neuronal perikaryon (plasmalemma dotted) is covered by a thin ependymal layer (arrow). B myeloid body, MV ependymal microvilli, N nucleus, Z zonula adherens. ×34,000.

b. A solitary cilium (C) arises from an intraependymal neuron into the intercellular space. ×38,000.

c. Axo-somatic synapse (arrow) formed on large intrinsic pineal neuron by an axon terminal containing synaptic and granulated vesicles. N nucleus. ×43,000.

d. Bundles of parallel running tubules (T) in an ependymal process of the dorsal wall of the pineal organ. ×37,000.
B. Vigh and I. Vigh-Teichmann:

(see also Kelly and Smith, 1964) consisting of rather thin, closely packed electron-dense membranes of the endoplasmic reticulum (Fig. 1, 5, 7). Such myeloid bodies occurred in the apical as well as more basal portions of the cells. The ependymal processes formed endfeet on the basal lamina of the pineal tissue. It was remarkable that they contained bundles of tubules (for the retina see Braevevelt, 1982) measuring 40-45 nm in diameter. They appeared to run in slight waves parallel to the longitudinal axis of the cell (Fig. 7d). The number of tubules varied from about 50-170 within single bundles (Fig. 7d). Such basal ependymal processes with tubules were numerous at the dorsal circumference of the pineal organ. Its ventral surface was covered by glial endfeet lacking tubules.

In the material studied, neurohormonal axon terminals containing synaptic vesicles with or without ribbons could not be detected on the pineal basal lamina. However, processes of unidentified origin were found, containing numerous pleomorphic granulated vesicles of varying density (diameter up to 170 nm); these penetrated the row of ependymal endfeet and directly contacted the basal lamina of the ventral pineal surface.

Furthermore, it seems noteworthy that though the pineal organ is known for its saccular structure, it displayed rather small saccules at its most distal tip. Here, we found scattered places where the pineal epithelium became rather flat and eventually disappeared. In these pore-like areas, the CSF of the pineal lumen directly touched the external basal lamina.

The data on the three kinds of photoreceptor cells of the pineal organ are summarized in a schematic drawing (Fig. 13).

II. Frontal Organ

The frontal organ was studied in Rana esculenta, R. temporaria and R. tigrina. Three types of photoreceptor cells—resembling those of the pineal organ—could be distinguished in Rana esculenta and R. temporaria (Fig. 8-10).

1. Rhodopsin-immunoreactive photoreceptor

A small population of electron-dense photoreceptors was rhodopsin-immunoreactive and characterized by relatively large inner segments with an ellipsoid of closely packed mitochondria and the absence of an oil droplet (Fig. 8a, c, d). Such photoreceptor cells were mainly found in the rostro-medial portion of the organ. Their outer segments measured up to 5.4 μm and displayed photoreceptor membrane lamellae of medium thickness (Fig. 8).

2. Oil droplet-containing photoreceptor

This second type of frontal photoreceptor resembled the corresponding type in the pineal organ (Fig. 8b, 9). It was rhodopsin-immunonegative and contained an oil droplet in its inner segment (Fig. 9b). The outer segment displayed relatively electron-dense membrane lamellae (Fig. 8b, 9c). Such photoreceptors occurred in rather small numbers, often as pairs, in the frontal organ of Rana esculenta and R. temporaria, especially in their medio-posterior portion.

With our material, it was difficult to distinguish the axonal pedicle of the rhodopsin-immunoreactive type of photoreceptor lacking an oil droplet from that of the oil droplet-containing one because of their cytological similarity.

3. Rhodopsin-immunonegative electron-lucent photoreceptor

In the frontal organ, most of the photoreceptor cells belonged to this type. It contained
an electron-lucent hyaloplasm that was somewhat denser than that of the corresponding pineal photoreceptor. Aggregations of glycogen granules and numerous loosely arranged mitochondria in the inner segment were also characteristic of this type of cell (Fig. 8b, 10a–c). It occurred in groups of two or three. The outer segment (measured length up to 4.8 μm) was composed of membrane lamellae ultrastructurally similar to those of the rhodopsin-immunopositive photoreceptors (Fig. 10a, b). The
Fig. 9. Details of the electron-dense, oil droplet-containing photoreceptor in the frontal organ of *Rana esculenta*.  

**a.** Lipid droplet (L)-containing inner segment.  
**B** basal bodies of the outer segment cilium,  
**E** ellipsoid.  
× 20,000.  

**b.** The outer segment (O) of the oil droplet containing photoreceptor is rhodopsin-immunonegative. In non-osmicated, resin-embedded material the oil droplet is represented by a vacuole (V).  
× 17,500.  

**c.** The outer segment (O) exhibits rather electron-dense membrane lamellae.  
**IS** inner segment with lipid droplet (L).  
× 35,000
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Fig. 10. Details of the electron-lucent type of photoreceptor in the frontal organ of *Rana esculenta*. 

a. Outer (O) and inner segment (IS) containing loosely arranged mitochondria (M) and glycogen granules. E ependyma, MB myeloid body. ×18,000. 

b. The extracellular space of the membrane lamellae of the outer segment (O) is relatively wide. IS inner segment. ×39,000. 

c. Rhodopsin-immunonegative outer segment (O) of the electron-lucent photoreceptor. A few gold particles correspond to background staining. ×26,000
extracellular spaces of the membrane lamellae appeared to be wider than those of the oil droplet-containing photoreceptor (Fig. 10b).

The three types of photoreceptors of all the species studied formed basal, often basket-like axonal pedicles (Fig. 11a-c) similar to those of the pineal organ. The

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Fig. 11. Details of the synaptic neuropil in the frontal organ of Rana esculenta. a. Axonal pedicle (A) of an electron-dense photoreceptor. D dendrites, arrow: synaptic spherule, G granular vesicle. ×28,000. b. The axonal pedicle (A) of an electron-lucent photoreceptor forms a ribbon synapse (arrow) on a dendrite (D). M mitochondria. ×26,000. c. The synapse formed by the axon terminal (A) of an electron-dense photoreceptor on a dendrite (D) exhibits pre- and postsynaptic membrane thickenings (arrow). V1 synaptic vesicles, V2 synaptic vesicles of the axonal ending of an electron-lucent photoreceptor. ×24,000
Pedicles of the frontal organ contained mitochondria, some glycogen granules and accumulations of synaptic vesicles around ribbons. Some pedicles displayed spherules. The synapses formed by the pedicles on the dendrites of the intrinsic nerve cells exhibited pre- and postsynaptic membrane thickenings (Fig. 11c).

4. Intrinsic neurons of the frontal organ

As in the pineal organ, these neurons were located either intraependymally or basally (Fig. 12a). The basal nerve cells assembled in a larger group near the origin of the frontal nerve. We could not detect ciliated dendrite terminals of the intraependymal neurons in the lumen of the organ, and their luminal portion was only covered by a rather thin ependymal layer (Fig. 12a). Cilia of the $9 \times 2 + 0$ type were found to arise

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**Fig. 12.** Details of neuronal elements of the frontal organ (a–c) and frontal nerve (d) of *Rana esculenta.*

- **a.** The intraependymal neuron (plasmalemma dotted) is covered by thin ependyma (arrows). $M$ ependymal microvilli, $MB$ myeloid body, $N$ nucleus. $\times 14,000$.
- **b.** The solitary cilium (asterisk) of an intrinsic neuronal perikaryon projects into the intercellular space. $\times 26,500$.
- **c.** Granulated vesicles ($G$) about 100 nm in diameter in a neuronal perikaryon. $\times 28,000$.
- **d.** Granulated vesicles ($G$) about 100 nm in diameter in unmyelinated nerve fibers of the frontal nerve. $\times 28,000$.
from both the basal and the intraependymal neuronal perikarya, extending into the intercellular space (Fig. 12b). The perikarya contained electron-lucent cytoplasm, slightly developed Golgi areas and few granulated vesicles. The latter were smaller (diameter about 100 nm) than those in the pineal neurons (Fig. 12c). Granular vesicles of similar diameter but a smaller core were sometimes observed in nerve fibers of the frontal nerve (Fig. 12d).

The distribution and quantity of the three types of photoreceptors is different in the pineal complex. Whereas the pineal organ is characterized by a predominance of

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**Fig. 13.** Schematic illustration of the three types of pineal photoreceptors in the frog pineal organ. 1 Rhodopsin-immunopositive photoreceptor with an ellipsoid in its inner segment (IS), 2 oil droplet (black spot)-containing photoreceptor, 3 rhodopsin-immune-negative photoreceptor with an ellipsoid (asterisk) in its dendrite. A axon forming terminal on nerve cell. Arrows indicate intercellular cilia; D postsynaptic dendrites of secondary neurons (N); E ependyma; IS inner segments; O outer segments; P basket-like axonal pedicles of photoreceptor cells.
DISCUSSION

Our previous light microscopic studies provided immunocytochemical evidence for the presence of photopigments in the photoreceptor cells of the pineal complex in a number of species from cyclostomes to birds (for reviews see VIGH-TEICHMANN and VIGH, 1985, 1986a, b; VIGH et al., 1986). In anurans, rhodopsin, S-antigen and vitamin A was demonstrated immunocytochemically in the pineal organ (VIGH-TEICHMANN et al., 1980a, b; VIGH et al., 1982, 1986; VIGH-TEICHMANN and VIGH, 1983, 1985, 1986a, b; VIGH and VIGH-
Immunoelectron microscopy showed that the opsin-immunoreactive sites were located on the photoreceptor membranes (Vigh and Vigh-Teichmann, 1986). Immunoelectron microscopy showed that the opsin-immunoreactive sites were located on the photoreceptor membranes (Vigh and Vigh-Teichmann, 1981; Vigh et al., 1983, 1985; Vigh-Teichmann et al., 1986), a location typical of the transmembrane protein rhodopsin (Ovchinnikov et al., 1982; Ovchinnikov, 1984; Hargrave, 1982; Hargrave et al., 1983, 1984; Pappin et al., 1984).

Since one population of the pineal photoreceptors was rhodopsin-immunonegative, as were most of the retinal cones, we suggested the existence of cone- and rod-like photoreceptors (Vigh-Teichmann and Vigh, 1983, 1985; Vigh et al., 1985, 1986). This view was strengthened by the fact that the S-antigen, which binds specifically to photolyzed and phosphorylated rhodopsin (Pfister et al., 1985), was found in the putative pineal rods but was absent from the cone-like cells (Vigh-Teichmann et al., 1986).

Our present ultrastructural and immunocytochemical results continue this question of different types of photosensory cells. Our findings reveal one rod- and two cone-like photoreceptors in both the pineal and frontal organs of the frogs _Rana esculenta_ and _R. temporaria_: 1) rhodopsin-immunoreactive, electron-dense, 2) rhodopsin-immunonegative oil droplet-containing, and 3) rhodopsin-immunonegative, electron-lucent photoreceptors (Fig. 13).

These photoreceptors differ not only by their antigenicity to the antibovine rhodopsin antisera used but also by their cytological characteristics. Since in the retina a similar pattern can be found (Vigh et al., 1983; Szel et al., 1985, 1986a), it may be suggested that the three kinds of photoreceptors of the pineal and frontal organs elaborate different photopigments. These photopigments (rhodopsin and/or porphyropsin, iodopsin, ultraviolet and/or blue pigments?) may enable the animal to detect light of differing wavelengths, and by this, adjust its behavior to changing environmental conditions.

Our findings are in accord with those earlier electrophysiological data which demonstrated neuronal responses with spectral sensitivities at different absorption maxima: in the pineal organ 500/570 nm (Dodt and Morita, 1964) and 425/525 nm (Morita, 1969) and in the frontal organ 355/515 nm and 570 nm (Dodt and Heerd, 1962; Hamasaki, 1970; Eldred and Nolte, 1978; Korf et al., 1981; Meissl and Dodt, 1981; Dodt and Meissl, 1982).

The rod-like photoreceptors demonstrated by us seem to correspond to those photosensory elements causing inhibitory achromatic responses in secondary neurons at an absorption maximum of about 500 nm—a wavelength typical of rhodopsin. The two cone-like photoreceptors lacking rhodopsin immunoreactivity are thought to be identical with structures responsible for light perception in the ultraviolet/blue and orange (iodopsin?) ranges of the spectrum. Photopigments sensitive to these wavelengths were already identified in the retina (Papernower et al., 1977; Witkovsky et al., 1981; Szel and Rohlich, 1985; Szel et al., 1986a).

It seems contradictory that ultrastructurally the rod-type pineal outer segment resembles a retinal cone in the arrangement and shape of the membrane lamellae. However, its content of rhodopsin and of rod-specific enzymes/peptides playing a role in photochemical transduction (Applebury, 1984; Kuhn, 1984; Stryer, 1984; Pfister et al., 1985; Vigh-Teichmann et al., 1986) speaks in favor of the rod-like nature of this type of photoreceptor. In our opinion, the rod- or cone-like appearance of an outer segment only indicates a higher (rod) or a lower (cone) level of its surface differentiation (=differing sensitivity for light intensity?), independently of the type of photopigment (=wave-length specificity) actually elaborated in the photosensory cell.

Our ultrastructural and immunocytochemical results support the hypothesis of Hamasaki (1970), who claimed that three different types of photoreceptors exist in the
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frontal organ; one inhibitory kind for luminance responses and two kinds causing chromatic responses. In chromatic responses, the photoreceptors were thought to act by inhibitory and excitatory transmitters (Hamasaki, 1970).

In our present investigations we could not find morphological evidence for the existence of interneurons, neither in the pineal nor in the frontal organ. Such elements were thought to function in the transfer of information between the photoreceptors (Dodt and Meissl, 1982). This question necessitates further studies dealing with the transmitters of both the photoreceptors and the secondary neurons (see Meissl and George, 1984) of the pineal complex. At the present level of knowledge, a simple hypothesis is offered in Figure 14 on possible synaptic connections resulting in chromatic and achromatic responses, respectively.

Finally, we wish to emphasize that the large number of rod-like photoreceptors found in the pineal organ indicates its predominantly rod-type light perceiving capacity (see also Vigh-Teichmann et al., 1980a; Vigh et al., 1982). In contrast, the frontal organ represents a predominantly cone-type photoreceptor structure possibly sensitive to orange and/or ultraviolet light.

Since little is known about the immunocytochemical characteristics of the supposed cone-like photoreceptors of the pineal and frontal organs, further studies are needed to identify their photopigments and specific peptides/ enzymes acting in photochemical transduction.

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