Cell Tissue Res (1981) 216:113–130

Cell and Tissue Research

Pineal Complex of the Clawed Toad, *Xenopus laevis* Daud.: Structure and Function* **

H.-W. Korf, R. Liesner

Department of Anatomy and Cytobiology, Justus Liebig University of Giessen, Giessen, Federal Republic of Germany;

H. Meissl, A. Kirk

Max Planck Institute for Physiological and Clinical Research (William G. Kerckhoff Institute), Bad Nauheim, Federal Republic of Germany

Summary. The morphological and physiological properties of the pineal complex of *Xenopus laevis* were investigated in larval, juvenile and adult animals.

In a representative majority of adult *X. laevis*, the frontal organ does not display signs of degeneration. Fully differentiated frontal organs contain photoreceptors typical of the pineal complex of lower vertebrates. By means of the acetylcholinesterase (AChE)-reaction approximately 30 neurons of two different types were demonstrated in the frontal organ. The frontal-organ nerve is composed of approximately 10 myelinated and 40 unmyelinated nerve fibers. The neuropil areas of the frontal organ are generally similar to the corresponding structures of the intracranial epiphysis.

The neuronal apparatus of the epiphysis cerebri of *X. laevis* consists of (i) photoreceptor cells, (ii) ~100 AChE-positive neurons, (iii) complex neuropil areas, and (iv) a pineal tract formed by ~10 myelinated and ~100 unmyelinated nerve fibers. Some of them exhibit granular inclusions indicating that pinealopetal elements may enter the pineal complex of *X. laevis* via this pathway. The topography of the pineal tract of *X. laevis* differs considerably from that in ranid species. The most conspicuous element of the plexiform zones is the ribbon synapse. The basal processes of the photoreceptor cells may be presynaptic elements of simple, tangential, dyad or triad synaptic contacts. Conventional synapses were observed only occasionally.

Electrophysiological recordings revealed that the pineal complex of *Xenopus laevis* is directly sensitive to light. In response to light stimuli, two types of responses, achromatic and chromatic, were recorded from the nerve of the

Send offprint requests to: Dr. H.-W. Korf, Institut für Anatomie und Zytobiologie (Anatomie I: Prof. A. Oksche), Aulweg 123, D-6300 Giessen, or to Dr. H. Meissl, Max-Planck-Institut für Physiologische und Klinische Forschung (II. Physiol. Abt.: Prof. E. Dodt), Parkstr. 1, D-6350 Bad Nauheim, Federal Republic of Germany

* The authors are indebted to Professors E. Dodt and A. Oksche for initiating the study and critical reading of the manuscript

** Supported by the Deutsche Forschungsgemeinschaft

0302-766X/81/0216/0113/$03.60

frontal organ. In contrast, the epiphysis exhibited only achromatic units. The opposed color mechanism of the chromatic response showed a maximum sensitivity at approximately 360 nm for the inhibitory and at 520 nm for the excitatory event. The action spectrum of the achromatic response of the epiphysis and the frontal organ peaked between 500 and 520 nm and showed no Purkinje-shift during dark adaptation. The functional significance of these phenomena is discussed.

Key words: Pineal complex – AChE-positive neurons – Plexiform areas – Photosensory function – *Xenopus laevis*

The pineal system in several species of anurans, e.g., *Rana esculenta, R. temporaria* and *R. catesbeiana,* is sensitive to light (Dodt and Heerd 1962; Dodt and Jacobson 1963). This function is maintained by the intrapineal neuronal apparatus consisting of photoreceptor cells endowed with lamellar outer segments (Eakin 1961; Oksche and von Harnack 1963), different types of nerve cells (Paul et al. 1971; Wake et al. 1974), and nerve tracts between the pineal complex and the brain (pineal nerve or frontal-organ nerve; pineal tract or epiphyseal tract; cf. Oksche and Vaupel-von Harnack 1965). By means of microspectrophotometry, photopigments were successfully demonstrated within the pineal photoreceptor cells of the frog (Hartwig and Baumann 1974).

Considerably less information is available concerning the neurophysiological properties and the related structures of the pineal complex of *Xenopus laevis.* Myelinated fibers were observed in the epiphysis of this species by Kreht (1940). Oksche (1955) was able to demonstrate an indistinct pineal tract by means of silver impregnation. In addition, well-developed photoreceptor cells have been shown in the frontal organ of larvae (Bagnara 1965) and the epiphysis of adult animals (Ueck 1968). According to von Haffner (1950), the frontal organ of *X. laevis* shows signs of beginning degeneration immediately after metamorphosis, which may result in a complete loss of this organ in adult animals.

Considering that, in addition to the lateral eyes (cf. Denton and Pirenne 1954), the pineal system of *X. laevis* is involved in color change mechanisms, i.e., the regulation of body blanching (Bagnara 1964; for older literature see Hogben and Slome 1931; Parker 1948), the present study was undertaken to investigate its neuronal organization and the related electrophysiological properties.

Materials and Methods

A. Morphology

Six juvenile and nine adult clawed toads, *Xenopus laevis* Daud., obtained from a commercial supplier were used for the morphological part of the present study. Three juvenile and three adult animals were processed for the histochemical demonstration of acetylcholinesterase (Karnovsky and Roots 1964), and three juvenile and six adult animals for electron microscopy. The animals were sacrificed by decapitation at different times of the day (8.00 a.m., 1.00 p.m. and 6.00 p.m.). The brain and the skin region, presumably containing the frontal organ, were dissected out quickly and fixed in ice-cold phosphate-buffered glutaraldehyde (pH 7.4) for 2 h.

The specimens used for the acetylcholinesterase reaction were washed overnight in phosphate buffer containing 0.44 M sucrose at 4° C. Sagittal or horizontal frozen sections, 20 μm thick, were prepared and

treated according to Karnovsky and Roots (1964) (cf. Wake et al. 1974). Control slides were incubated in a medium free of substrate (i.e., acetylthiocholine iodide).

For electron microscopy, the frontal organ and the isolated epithalamus were washed overnight with 0.1 M phosphate buffer, postfixed with 1 % OsO_4 for 1 h and dehydrated in a graded ethanol series. Via epoxypropan, the specimens were embedded in Epon. As demonstrated in Bodian preparations (Oksche 1955) and in the AChE-material of the present study, the pineal tract of *X. laevis* extends toward the posterior commissure in a dorso-ventral direction. Thus, horizontal sections were prepared with the aim to reveal cross sections of the pineal tract. When the tract had been localized in semithin sections, ultrathin sections were prepared, stained with uranyl acetate and lead citrate. The electron micrographs were taken with a Siemens Elmiskop I at 80 kV. The fibers of the frontal-organ nerve and the pineal tract were counted at the level where one could expect the majority of these fibers to occur, i.e., the frontal-organ nerve in close proximity to the frontal organ and the pineal tract at the caudal pole of the epiphysis.

B. Physiology

The physiological experiments were performed on 103 *Xenopus laevis* of different developmental stages. Adult specimens of *X. laevis* were anesthetized with 250 mg/kg MS 222 solution (Sandoz, Basel) injected into the ventral lymph sac. Juvenile animals of stage 65/66 (Nieuwkoop and Faber 1956) were anesthetized by dissolving 2 mg/100 ml MS 222 in the surrounding water.

Electrical recordings from the frontal organ were performed in situ. To expose the nerve of the frontal organ, a piece of skin was dissected out near the inner margin of the lateral eyes leaving the region of the frontal organ untouched (Dodt and Heerd 1962). Subsequently, the nerve was cut and the portion of the skin containing the frontal organ lifted upward. Silver-silver chloride eletrodes with cotton wicks were used for recordings from the frontal-organ nerve.

For intracranial recordings from the pineal organ proper (= epiphysis) the brain was exposed and the meninges adjacent to the pineal organ removed. Glass-insulated platinium-iridium electrodes with a tip diameter of 1 to 5 µm were guided by means of a micromanipulator, its position being moved until impulse responses to light flashes were observed. The potentials were amplified and displayed on an oscilloscope. An audioamplifier served for an acoustic control. The original responses were stored on magnetic tape for subsequent analysis. For threshold measurements a window discriminator together with a leaky integrator and a Nicolet 1072 computer served as a threshold detector.

Stimuli were provided by a 150 W Xenon-arc lamp. The stimulus consisted of a light spot of 10 mm in diameter centered on the pineal area. Wavelengths were controlled by Schott interference band filters (half bandwidth ranging from 16–22 nm) and calibrated by use of a Zeiss spectrophotometer; attenuation of the light beam was achieved using neutral density absorption filters. The radiation intensity was measured my means of a Hewlett Packard radiometer.

Results

A. Morphology

In the specimens of *Xenopus laevis* examined, an intact frontal organ could be demonstrated in all juveniles and six adult animals (Fig. 1 a), whereas in three adult animals this organ showed signs of degeneration (Fig. 1 b) or was completely absent. Providing the frontal organ of *X. laevis* has not undergone degenerative changes, it contains all structural features necessary for the perception of light. Regularly lamellated outer segments (50 to 100 lamellar disks) originating from the inner segment of the photoreceptor cells protrude into the narrow remnants of the lumen of the frontal organ (Fig. 2 a). These photoreceptor cells are connected to nerve cells located within scattered, complex neuropil areas of the frontal organ. The basal processes of the pineal photoreceptor cells of *X. laevis* contain synaptic ribbons accompanied by clear synaptic vesicles (Fig. 2 b–e). Occasionally, dense-cored granules were located in the basal processes of the receptor cells (cf. Ueck

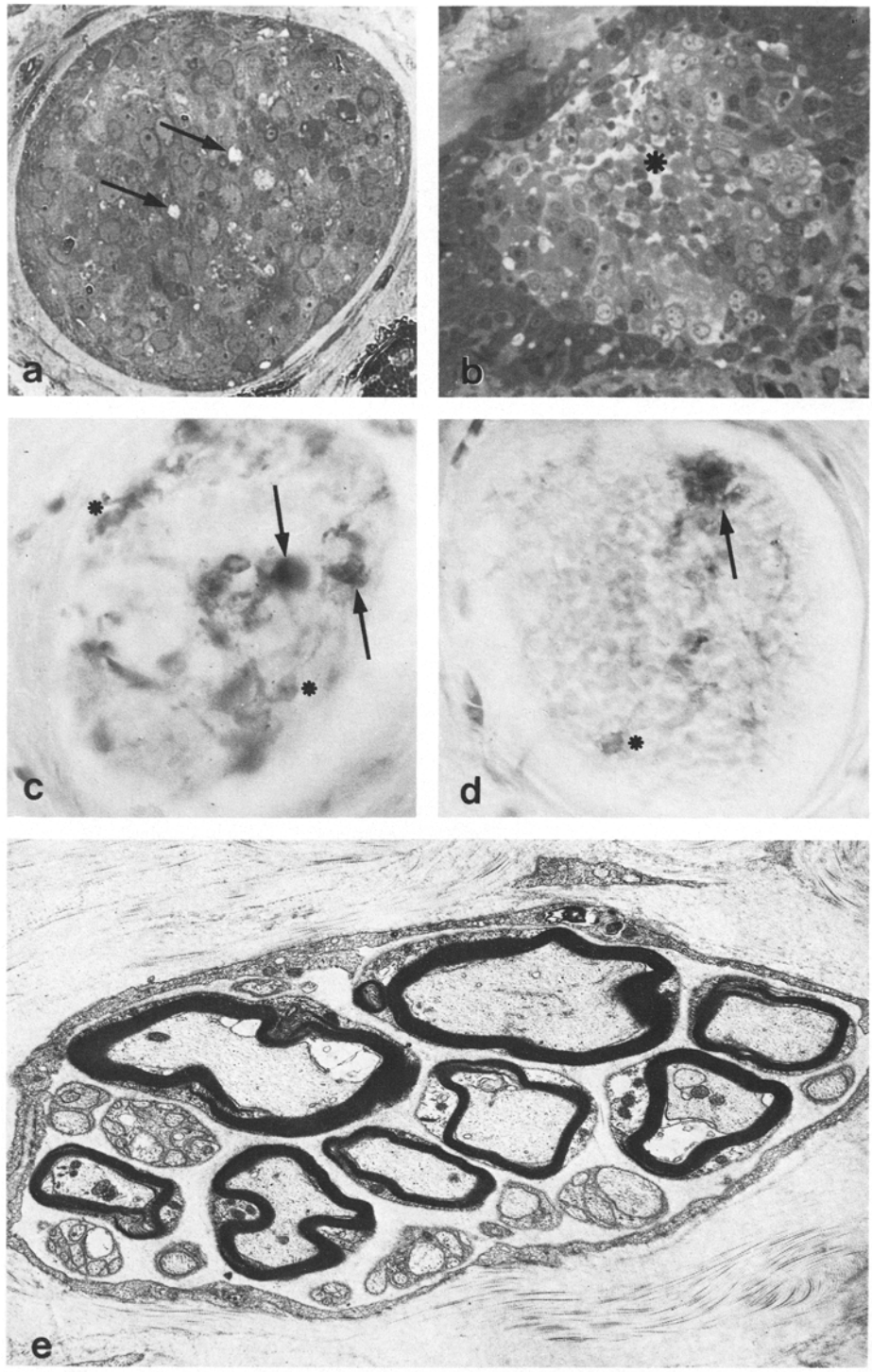


Fig. 1 a–e. Frontal organ of adult *Xenopus laevis*. **a, b** Thionin staining. Horizontal semithin sections. **a** Well-developed frontal organ devoid of signs of degeneration; note remnants of the lumen (*arrows*). **b** Partially regressed frontal organ; note karyorhexis and karyolysis (*asterisk*) in the center. **a** ×400, **b** ×400. **c, d** Acetylcholinesterase reaction. Horizontal sections. **c** Fully differentiated frontal organ displaying two different types of AChE-positive neurons: large-sized, heavily reactive perikarya (*arrows*); small, weakly stained neurons (*asterisks*). ×310. **d** Partially regressed frontal organ showing considerable loss of AChE-positive neurons: large neuron (*arrow*), small neuron (*asterisk*). ×290. **e** Electron micrograph of a cross-sectioned frontal-organ nerve consisting of eight myelinated and 42 unmvelinated nerve fibers. ×4,300

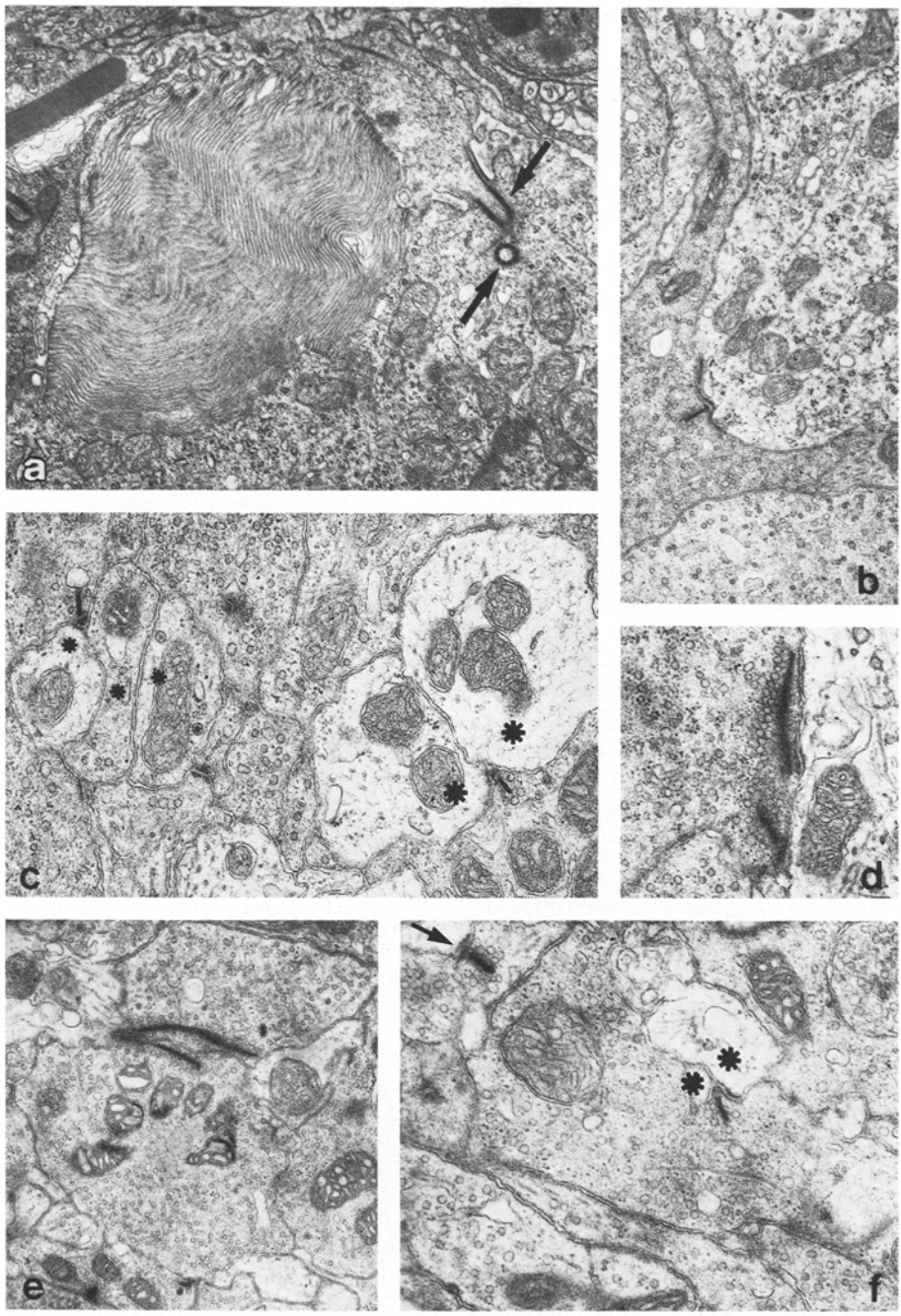


Fig. 2 a–f. Electron micrographs of the frontal organ in adult *Xenopus laevis*. **a** Photoreceptor cell with regularly lamellar outer segment; connecting piece (*arrows*) displaying a cilium of the 9+0 type. ×12,000. **b** Simple synaptic contact containing one synaptic ribbon. ×10,500. **c** Plexiform area formed by several basal processes of photoreceptor cells and dendrites; postsynaptic dendrites (*small asterisks*) of the triad synapse, one containing a dense-cored vesicle; postsynaptic dendrites of the dyad synapse (*large asterisks*). ×16,000. **d** Tangential synapse. ×19,200. **e** Two basal processes of photoreceptor cells converging to a single postsynaptic dendrite. ×12,000. **f** Plexiform area of the frontal organ; simple synaptic contact (*arrow*); dyad synaptic contact (*asterisk*). ×18,000

1968) (Fig. 2c, e). In the frontal organ of *X. laevis,* simple synaptic contacts consisting of one presynaptic and only one postsynaptic process were most frequently observed (Fig. 2b, e, f). This type of synaptic contact involves smooth-surfaced contacts or, less frequently, invaginated dendritic spines. Additionally, dyad or triad synaptic formations (Fig. 2c, f) are present in the frontal organ of *X. laevis.* Only a few conventional synapses were found, composed of a presynaptic terminal containing granular inclusions and a postsynaptic dendrite resembling the postsynaptic element of the ribbon synapse. Generally, no striking differences were found to exist between the neuropil areas of the frontal organ and the epiphysis cerebri of *X. laevis.*

By means of the acetylcholinesterase (AChE)-reaction, approximately 30 neurons were demonstrated in the frontal organ of *X. laevis,* which can be divided into two types. One types is characterized by a large-sized perikaryon, heavily stained by the AChE-reaction, whereas the other type exhibits a smaller, less stained perikaryon (Fig. 1c, d). The ratio of the neurons of these two types is approximately 1 : 1. The method applied does not indicate which type of neuron contributes to the frontal-organ nerve, although the nerve itself was successfully demonstrated by use of the AChE-reaction. Axons belonging to this nerve can be observed in semithin sections when they possess a myelin sheath. In the vicinity of the nerve, a large capillary is regularly found. In two out of nine animals investigated, the frontal-organ nerve is established by divided fiber bundles encompassing this blood vessel. Close to the frontal organ, electron micrographs of the nerve display only a few (3–10) myelinated axons. The major portion of the nerve, however, is comprised of unmyelinated elements (19 to 52) (Fig. 1e). In one case, the total number of myelinated nerve fibers exceeded that of the unmyelinated elements: 24 myelinated versus only 18 unmyelinated elements.

The frontal organs of three adult *X. laevis* showed signs of degeneration, i.e., the remnants of the neuroepithelial components exhibited karyopyknosis karyolysis or karyorhexis (Fig. 1b). Totally degenerated frontal organs completely lack AChE-positive perikarya and, correspondingly, a frontal-organ nerve.

The epiphysis of *X. laevis* is located topographically between the habenular and posterior commissures in a dorsal position, as is typical for anuran species. However, the topography of the pineal tract of *X. laevis* differs from the course of the pineal tract in Ranidae. In *X. laevis,* the tract emerges from the caudal pole of the vertically oriented epiphysis and runs toward the posterior commissure following a rather dorso-ventral course; it is deeply embedded in the pineal parenchyma (Figs. 3a; 5), whereas in ranid species the tract forms an isolated bundle before penetrating the posterior commissure in a horizontal plane.

The epiphyseal wall is mainly composed of a thin layer of neuroepithelial elements (Fig. 3a). Pineal photoreceptor cells are located within these walls, their outer segments protruding into the narrow pineal lumen. The fine structure of these cells in the epiphysis of *X. laevis* is similar to that demonstrated in other lower vertebrates. Regularly lamellar outer segments contain 50 to 100 lamellar disks. The supranuclear cytoplasm is organized into an inner and an outer segment, which are joined via a connecting piece bearing a cilium of the 9+0 type (Fig. 4a; cf. Fig. 2a). In some outer segments the lamellar disks were elongated and covered the cytoplasm of the connecting piece in the form of a cowl (Fig. 4b) or established membrane whorls at their distal tips (Figs. 4a). These whorls may be phagocytosed

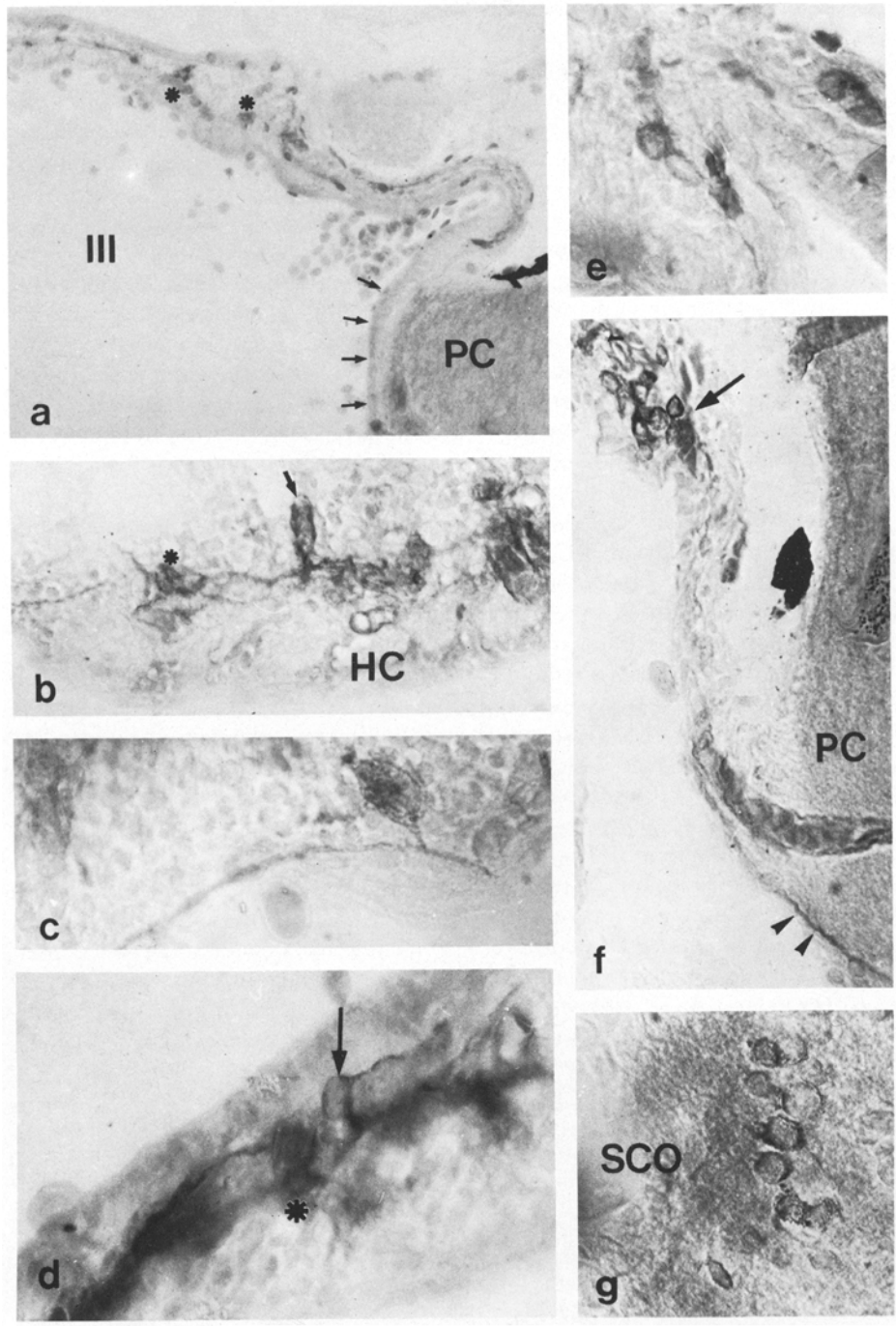


Fig. 3a–g. Acetylcholinesterase reaction in the epiphysis of *Xenopus laevis*. Sagittal sections. **a** General topography; AChE-positive neurons (*asterisks*) in the thin walls of the pineal organ proper; portion of the pineal stalk (*arrows*) tapering into the posterior commissure (*PC*); third ventricle (*III*). ×120. **b** Different types of neurons near the habenular commissure (*HC*); unipolar neuron protruding into the pineal lumen and endowed with a process branching dichotomously near the basal lamina (*arrow*); multipolar neuron located near the basal lamina (*asterisk*). ×650. **c** Unipolar neuron displaying a bipartite process near the basal lamina. ×850. **d** Unipolar (*asterisk*) and bipolar (*arrow*) neurons. ×700. **e** Multipolar neuron. ×550. **f** Accumulation of AChE-positive neurons (*arrow*) in the caudal portion of the epiphysis; pineal tract (*arrowheads*); posterior commissure (*PC*). ×200. **g** AChE-positive neurons in the region of the subcommissural organ (*SCO*). ×550

by macrophages inhabiting the pocket-like compartments of the pineal lumen. In this connection, it should be emphasized that membrane whorls observed in the pineal lumen do not principally indicate the existence of rudimentary photoreceptor cells, but may represent different stages of local degenerative events followed by regenerative processes.

The basal processes of the epiphyseal photoreceptor cells of *X. laevis* contain synaptic ribbons that are accompanied by clear synaptic vesicles (Fig. 4c, d, e). Occasionally, dense-cored granules were located in the basal processes of these cells. Compared to other amphibian species, the amount of synaptic vesicles within the receptor cells of *X. laevis* appears to be reduced. With respect to the amount of synaptic vesicles, no striking differences were found between juvenile and adult animals or between animals sacrificed at different times of the day. In *X. laevis,* the pattern and arrangement of the neuropil areas interposed between the receptor cells and the intrinsic pineal neurons do not differ fundamentally from those demonstrated in other amphibian species (Bayrhuber 1972; Flight 1973; Korf 1976) (Fig. 4a–f, h). Occasionally, the basal processes of two pineal photoreceptors appear to contact each other (Fig. 4c, g).

Certain neuronal perikarya located within the epiphysis of *X. laevis* were selectively labeled by use of the AChE-reaction. Some of these neurons could be classified as multipolar, bipolar, or unipolar elements (Fig. 3b–e); however, the majority of the stained neurons did not exhibit stained processes. The total amount of AChE-positive neurons was counted in five animals, averaging about 100 neurons. The number of AChE-positive neurons found in juvenile animals parallels the counts in adult specimens. In three animals, an accumulation of AChE-positive neurons was observed in the caudal part of the pineal organ proper (Fig. 3f).

The epiphysis of *X. laevis* is connected to the brain by a pineal tract, poorly stained in AChE-preparations (Fig. 3f).

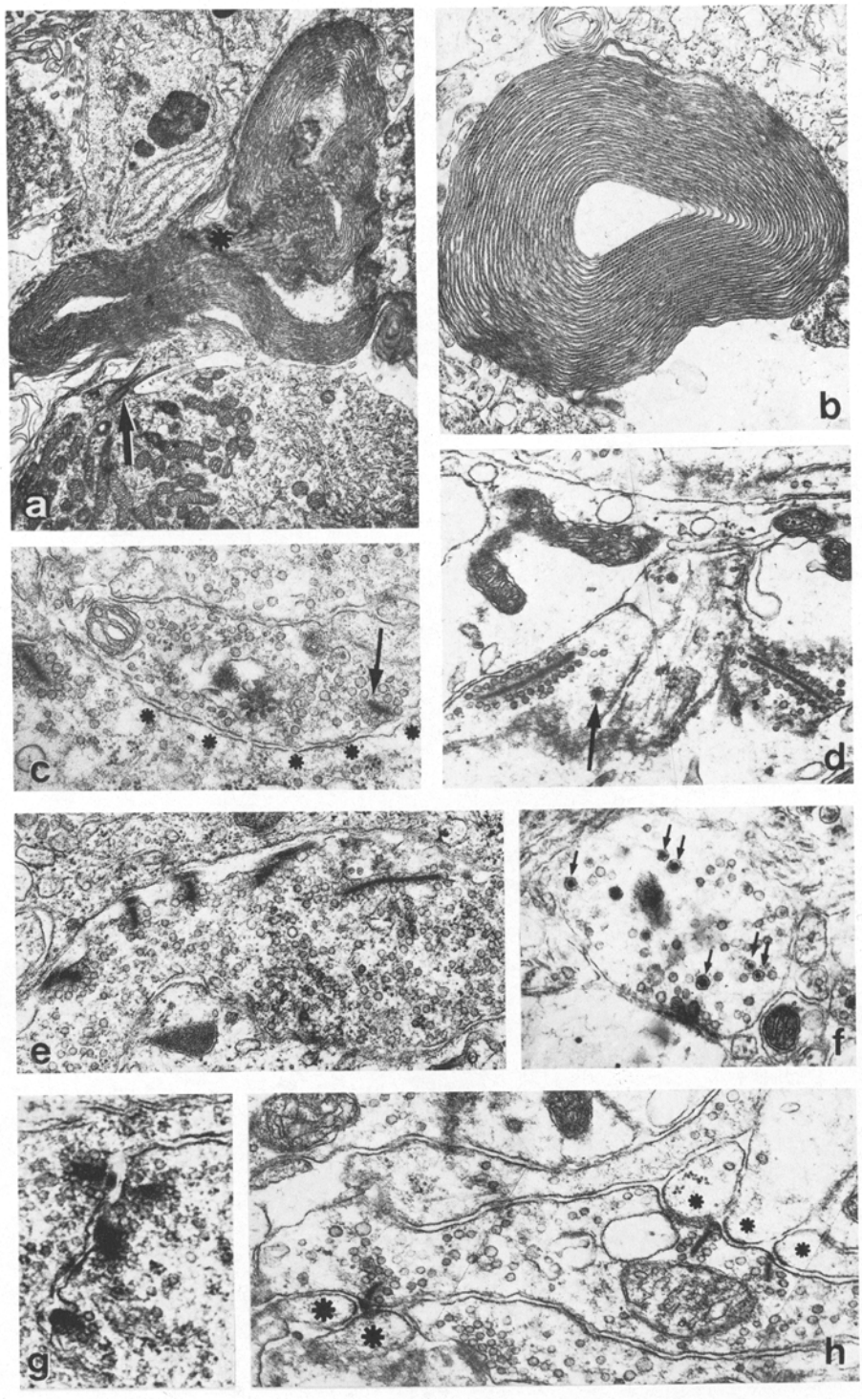
As electron microscopic investigations have shown, the pineal tract of *X. laevis* is composed of only a few myelinated nerve fibers (10–15); its major portion is formed by unmyelinated elements (95–110). Nerve fibers containing some dense-cored granules may intermingle with the unmyelinated axons (Fig. 5).

In the region of the subcommissural organ a small number of nerve cells accompanying the pineal tract displays a positive AChE-reaction (Fig. 3g).

B. Physiology

Direct evidence for the light sensitivity of the *frontal organ* of *X. laevis* was obtained by recordings of the impulse activity of nerve fibers emerging from the frontal

Fig. 4a–h. Electron micrographs of the epiphysis of adult *Xenopus laevis.* **a** Outer segment of a photoreceptor cell; lamellar disks at the distal tip forming membrane whorls (*asterisk*); connecting piece displaying a cilium of the 9+0 type (*arrow*). ×4,400. **b** Concentric arrangement of lamellar disks. ×10,200. **c** Basal processes of photoreceptor cells. Synaptic ribbon (*arrow*) directed toward the membrane of a second basal process (*asterisk*) containing a synaptic ribbon. ×22,800. **d** Basal processes of photoreceptor cells displaying synaptic ribbons, accompanied by a few clear synaptic vesicles and dense-cored granules (*arrow*). ×22,100. **e** Tangential type of synapse. ×10,320. **f** Conventional synapse. Presynaptic process containing granular inclusions (*arrows*). ×21,450. **g** Basal processes of two photoreceptor cells. Synaptic ribbons of one process point toward the membrane of the other process. Synaptic contact between two photoreceptor cells. ×22,200. **h** Plexiform areas consisting of a dyad (*large asterisks*) and a triad synapse (*small asterisks*). ×21,800


a
b
c
d
e
f
g
h

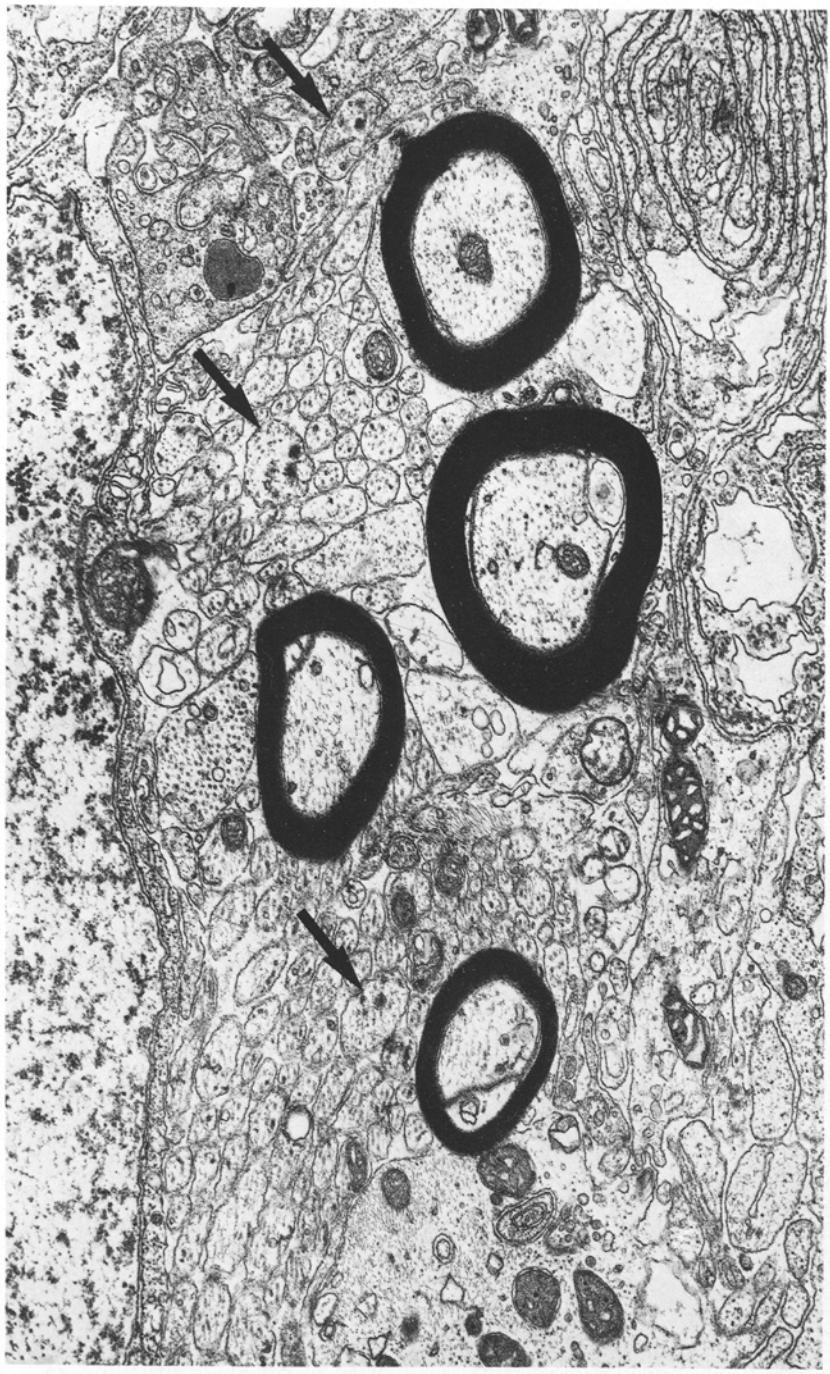


Fig. 5. Part of the pineal tract of *Xenopus laevis* embedded in the pineal parenchyma. Axons containing granular inclusions (*arrows*). ×14,000

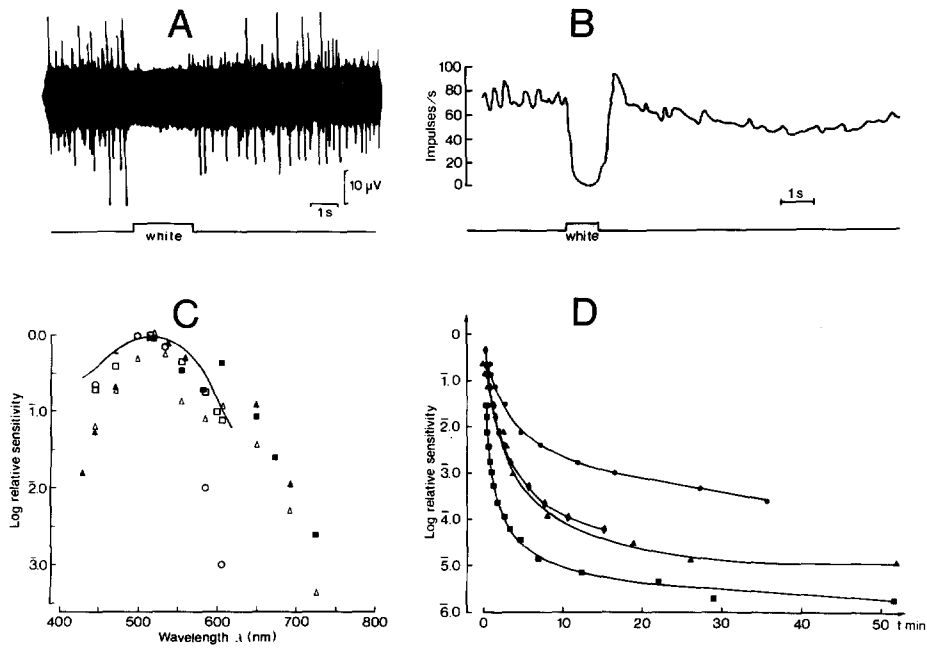


Fig. 6A–D. Achromatic responses of the frontal organ of *Xenopus laevis*. **A** Action potentials recorded from the frontal-organ nerve. Light stimulation as indicated by upward deflection of the lower beam. **B** Integrated spike frequency of mass spike potentials recorded from the frontal-organ nerve. **C** Relative spectral sensitivity of the achromatic response determined by measurements of the relative quanta of light flashes of 1 sec duration necessary for the just-perceptible inhibition of the impulse activity in the frontal-organ nerve. Continuous line indicating Dartnall's nomogram absorption curve of visual pigment (v.p.) 520 nm. Different animals represented by different symbols. **D** Dark-adaptation curves obtained by a threshold criterion response. First measurement of each curve obtained a few sec after cessation of a 20 min light exposure to white light (▲ ■, 4.9 mW/cm^2), to 516 nm (◆, 57 µW/cm^2) and to 587 nm (●, 86 µW/cm^2)

organ. Sixteen out of 36 specimens of stage 65/66 and 15 out of 45 juvenile specimens showed direct light sensitivity of the frontal organ. No recordings were obtained from the frontal-organ nerve of adult animals. The prominent physiological feature of the frontal organ of younger animals (stage 65/66 and juveniles) is a maintained spike activity in the absence of light. Stimulation by light caused two types of responses: *achromatic* and *chromatic*.

The *achromatic units* respond to light stimuli of white light (Fig. 6A, B) and light of all wavelengths between 400 and 750 nm by a decrease or inhibition of the firing rate. The inhibition by light is occasionally followed by off-discharges (Fig. 6B). The spectral sensitivity of the achromatic response revealed one maximum only located at about 520 nm (Fig. 6C) similar to the visual pigment 523 (porphyropsin) extracted from retinal photoreceptors of *X. laevis* (Dartnall 1956). During dark adaptation up to 2 h, there was no indication of a Purkinje shift (cf. Dodt and Heerd 1962).

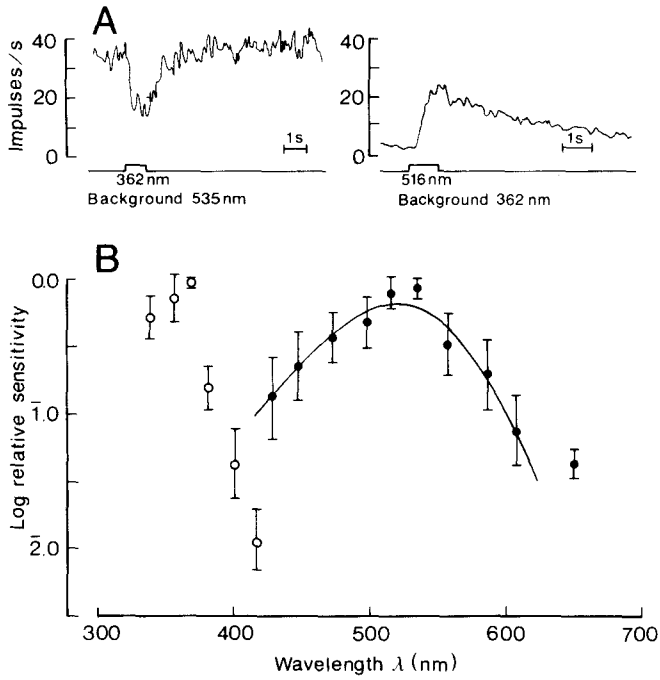


Fig. 7A, B. Chromatic response of the frontal organ of *Xenopus laevis*. **A** Impulse rate-time histogram for the inhibitory and excitatory component of the chromatic response. Both components were measured against an antagonistic background illumination. The radiation energy of background illumination was 35 μW/cm² (535 nm) and 8.3 μW/cm² (362 nm) and the energy of the test stimuli was 9.4 μW/cm² (362 nm) and 57 μW/cm² (516 nm). **B** Relative spectral sensitivity of the chromatic response: inhibitory action spectrum (*open circles*); excitatory spectrum (*dots*). The light threshold of the excitatory component is 2.2 log units lower than the inhibitory threshold. The curve drawn in full represents Dartnall's nomogram v.p. 520 nm (Dartnall 1953)

After exposure to strong white or green light, the dark-adaptation curve of the achromatic response consisted of one branch only (Fig. 6D). The total sensitivity change during dark adaptation amounts to approximately 5.0 log units. The absolute threshold of the photic response of the frontal organ, as defined by the minimum strength of stimulus intensity required to produce an even perceptible decrease of impulse frequency in the dark-adapted state, was 12.3×10^{-3} μW/cm². This is more than 10 times the absolute threshold of the frontal organ of *R. temporaria* (Dodt and Morita 1964).

The *chromatic units* revealed the presence of an opponent color mechanism showing inhibition for short wavelengths and excitation for medium and longer wavelengths. Inhibition and excitation are of longer duration than the stimulus up to several minutes. Therefore, chromatic responses were studied during conditioning with either inhibitory (ultraviolet) or excitatory (green) background illumination of small radiation intensity, following the procedure of Meissl and Donley (1980). The test stimulus was superimposed on this steady background (Fig. 7A). Maximum sensitivity of the inhibitory event is obtained at about 360 nm (Fig. 7B, open circles). The action spectrum of the excitatory component peaks at about

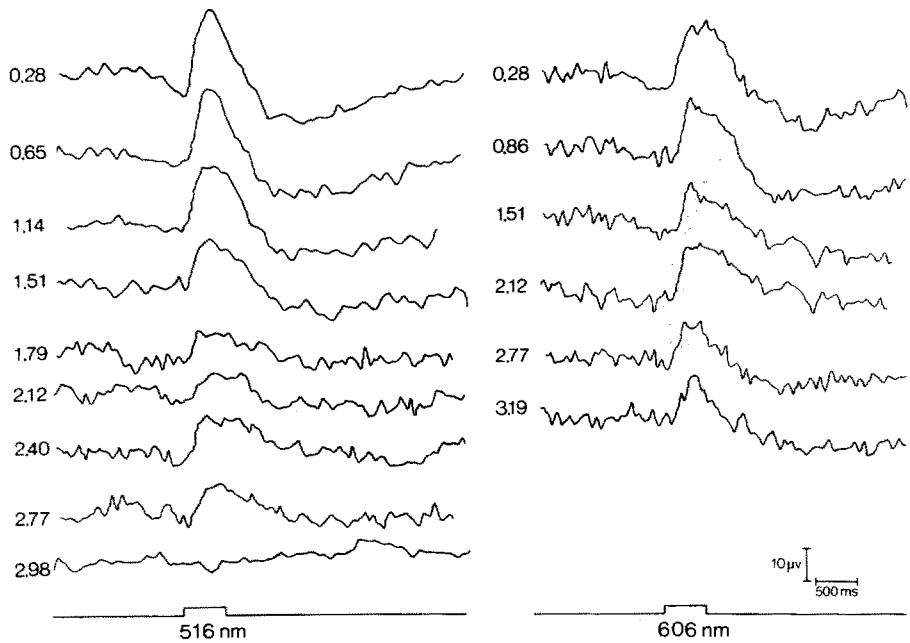


Fig. 8. Slow potential changes to flashes (516 nm and 606 nm, 0.5 sec). Log radiation intensity of stimulus is indicated to the left of each curve. 0.0 is equal to 57 $\mu W/cm^2$ for the 516 nm stimulus and to 155 $\mu W/cm^2$ for the 606 nm stimulus

520 nm (Fig. 7B, dots); its sensitivity spectrum closely following the absorption spectrum of Darnall's nomogram v. p. (visual pigment) 520 nm. The light threshold of the excitatory component is 2.2 log units lower than the inhibitory threshold. Therefore, using white light of the Xenon arc for stimulation, the inhibitory component dominates the responses.

The majority of the frontal organs *X. laevis* of stage 65/66 and of juvenile animals shows in contrast to Ranidae (cf. Dodt and Heerd 1962) only one type of response: chromatic *or* achromatic.

In addition to the impulse activity, the frontal organ of *X. laevis* generates a mass slow potential passively conducted over the frontal-organ nerve and originating most probably from the photoreceptor elements (cf. Donley and Meissl 1979) (Fig. 8).

In a few cases, recordings from single nerve fibers or nerve cells of the intracranial *epiphysis cerebri* of adult *X. laevis* were successful. In 2 out of 22 animals, the spike activity was found to be strong enough to measure the type of response and action spectra. In six further preparations, a direct photosensitivity was conspicuous, however, with a signal-to-noise ratio of the impulse discharges not sufficient for measurement. All these units were achromatic; no chromatic responses were observed. The epiphysis is active in darkness and responds to direct illumination by a decrease or inhibition of the firing rate. The action spectrum peaks at about 500 nm (Fig. 9) and closely resembles the sensitivity spectrum of the epiphysis of *R. esculenta.*

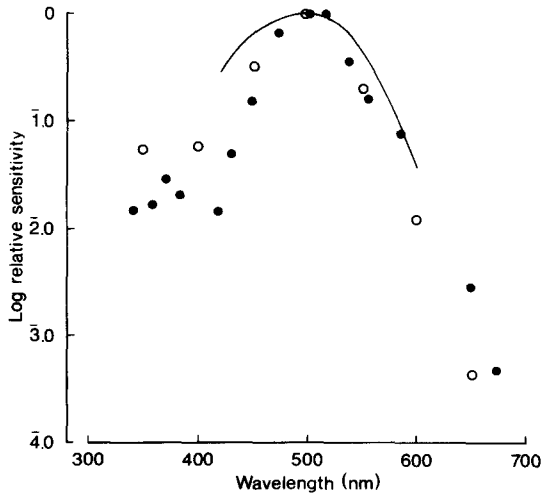


Fig. 9. Relative spectral sensitivity of a single ganglion cell in the epiphysis of an adult specimen of *Xenopus laevis* (*dots*). For comparison, the action spectrum of the epiphysis of *Rana esculenta* (Dodt and Morita 1964) is indicated (*open circles*). The curve drawn in full represents Dartnall's nomogram v.p. 500 nm

Discussion

An essential morphological finding of the present study is the demonstration of an intact frontal organ in six out of nine adult *X. laevis* investigated. This result is in contrast to former studies on the pineal complex of *X. laevis* which appeared to indicate that in this species the frontal organ undergoes complete degeneration during the course of the postlarval period (von Haffner 1950). Using light microscopic methods, von Haffner observed an accumulation of lipid droplets in the frontal organ of postmetamorphic animals and interpreted these inclusions as signs of degeneration. However, the staining method used by von Haffner may have stained the lipid component of membrane structures in sections of outer segments displaying flattened or whorl-like lamellae or in aggregations of mitochondria located in the so-called ellipsoid of the inner segment. On the other hand, different strains of *X. laevis* may have been used in the two studies. Using the histochemical reaction for acetylcholinesterase and transmission electron microscopy, the authors of the present study could clearly show that in a considerable number of adult animals the frontal organ of *X. laevis* exhibits regularly shaped outer segments, AChE-positive neurons and a nervous pathway. On the other hand, the frontal organ was degenerated in three specimens, indicated by a loss of AChE-positive nerve cells and the frontal-organ nerve. Thus, no fundamental differences exist between *X. laevis* and the Ranidae with respect to the degeneration of the frontal organ during the course of the postmetamorphic development.

Moreover, the morphologic pattern of the pineal complex of *X. laevis* resembles that of Ranidae except for the finer topography of the pineal tract and the number of nervous elements. In the frontal organ of *R. esculenta* ~ 60 neurons display a positive AChE-reaction (Wake et al. 1974), and ~ 160 nerve fibers were counted in the frontal-organ nerve (Oksche and von Harnack 1963), whereas in *X. laevis* ~ 30 neurons could be demonstrated by means of the AChE-reaction and ~ 50 fiber elements were counted in the frontal-organ nerve. Similar quantitative differences were found with respect to the neuronal apparatus of the epiphysis of *X. laevis* and *R. esculenta* (cf. Wake et al. 1974).

It is somewhat surprising that no impulse activity could be recorded from the frontal-organ nerve of adult *X. laevis* and some juvenile animals. For this the following interpretations may be offered: 1) All these animals belonged to a group displaying a final stage of degeneration of the frontal-organ nerve; 2) in comparison to Ranidae the number of nerve fibers in the frontal-organ nerve of *X. laevis* is very small; and 3) the connective tissue layer intimately encompassing the nerve fibers may establish unfavorable conditions for electric recordings. Thus, negative physiological evidence must not *per se* speak in favor of a degeneration of the frontal organ.

The results obtained in *X. laevis* further support previous findings in amphibian species indicating that the neuronal organization of the pineal system is less complex than that of the retina. The neuropil areas of the epiphysis of *X. laevis* display simple, dyad, triad and tangential types of synaptic contacts. Occasionally, conventional synapses were found. These findings correspond to results obtained from the epiphysis of *Bombina variegata* (Bayrhuber 1972), *Diemictylus viridescens viridescens* (Flight 1973), and *Ambystoma tigrinum* (Korf 1976). The presynaptic elements of the conventional synapses probably represent processes of local interneurons; on the other hand, some of these elements may belong to pinealopetal fiber systems (cf. Ueck 1979; Omura and Ali 1980).

The neuropil areas of the frontal organ first described by Oksche and von Harnack (1963) have not been investigated in detail until the present time. The frontal organ of anurans is known to generate chromatic and achromatic responses (Dodt and Heerd 1962), whereas the epiphysis predominantly generates achromatic responses (Dodt and Jacobson 1963). To date, there is only a limited structural basis for the understanding of the origin of chromatic and achromatic responses. Thus, it seemed promising to compare the neuropil areas of the frontal organ with those of the intracranial epiphysis in an attempt to discover differences that might account for the differing types (i.e., chromatic and achromatic) of responses. However, the arrangement and the pattern of the neuropil areas in the frontal organ strongly resemble those in the pineal organ proper. This finding may speak against the assumption that different types of neuronal connections are an essential prerequisite for generation of chromatic or achromatic responses in pineal sense organs.

The achromatic response of the pineal complex is probably due to the inhibitory action of sensory cells containing one or two photopigments onto the second order neurons (Dodt et al. 1971). The photopigments responsible for the achromatic response exhibit sensitivity maxima at 520 nm (frontal organ) and at 500 nm (epiphysis) and thus, closely resemble the photosensitive material extracted from retinal photoreceptor cells of *X. laevis* containing a mixture of approximately 8 % of visual pigment 502 and 92 % of a photopigment similar to visual pigment 523 (cf. Dartnall 1956). The chromatic response requires a different mechanism. From single fiber recordings in the frontal-organ nerve of the frog, evidence was gained that the opposing effects of short and long wavelengths interact at the same ganglion cell (Dodt and Heerd 1962). Such interactions may indicate that two different pineal receptor populations synapse on a common ganglion cell using different (inhibitory or excitatory) types of transmitters (cf. Hamasaki 1970). On the other hand, this interaction may be based on the photointerconversion of two

states of a single visual pigment (Dodt 1963; Eldred and Nolte 1978) without involvement of multiple receptor types.

The achromatic response probably acts as a dosimeter for solar radiation, since constant illumination of the epiphysis of the frog reveals a linear relation between spike rate and the logarithm of luminance (Morita and Dodt 1965). The properties of the frontal organ of the frog also satisfy the requirements of a dosimeter function (Hamasaki and Eserman 1976). Thus, the frequency of nerve impulses originating both from the frontal organ and the epiphysis, and conducted to the brain, is related to the ambient light level (Hamasaki and Eder 1977) and presumably represents an important chronobiological mechanism.

Generally, the manifestation of a chromatic response in the pineal complex of lower vertebrates is paralleled by a separation of the primordium of the parietal organs into an extracranial (i.e., frontal organ, parietal eye) and an intracranial component (pineal organ proper or epiphysis). Favored by its superficial location, the frontal organ is capable of the perception of light belonging to the ultraviolet range of the visible spectrum.

The chromatic response permits a color discrimination between long and short wavelengths of the visible spectrum. Meissl and Donley (1980) showed that the output of the frontal organ depends on the balance between opposing inhibitory and excitatory processes. This balance provides a very sensitive mechanism for the measurement of variations in the spectral distribution of the visible light. Daylight (natural photoperiod) shows cyclic variations both in light intensity and spectral composition. The change in the spectral distribution is most prominent during twilight (Munz and McFarland 1977). A decrease in a certain part of the visible spectrum is capable of shifting the chromatic response to another state of activation. Thus, it is reasonable to assume that the chromatic system of the frontal organ provides an important switching mechanism during twilight. In addition to the achromatic system present in the frontal organ and in the epiphysis, it may act as a refined mechanism synchronizing physiological and behavioral activities.

The hypothesis presented implicates that, in anurans, the frontal organ and the epiphysis act as a functional unit. The finding that nerve projections of the frontal organ to the brain resemble those of the epiphysis (Eldred et al. 1980; cf. Paul et al. 1971) may speak in favor of this interpretation. Furthermore, investigations on the complex functional relationship between the pineal complex and the subcommissural organ of *R. esculenta* and *R. temporaria* (Diederen 1975) indicate that the frontal organ and the epiphysis may produce similar effects with respect to the secretory activity of the subcommissural organ.

The assumption that the frontal organ is the more subtle sensor of this functional unit might explain why the degeneration of the frontal organ occurring in adult specimens of some anuran species is not followed by drastic changes in the behavior of these animals, since they still possess the other component of this photoneuroendocrine unit, the pineal organ proper (= epiphysis cerebri).

References

Bagnara JT (1964) Independent actions of pineal and hypophysis in the regulation of chromatophores of anuran larvae. Gen Comp Endocrinol 4:299–303

Bagnara JT (1965) Pineal regulation of body blanching in amphibian larvae. Progr Brain Res 10:489–506
Bayrhuber H (1972) Über die Synapsenformen und das Vorkommen von Acetylcholinesterase in der Epiphyse von *Bombina variegata* L (Anura). Z Zellforsch 126:278–296
Dartnall HJA (1953) The interpretation of spectral sensitivity curves. Br med Bull 9:24–30
Dartnall HJA (1956) Further observations on the visual pigments of the clawed toad, *Xenopus laevis*. J Physiol 134:327–338
Denton EJ, Pirenne MH (1954) The visual sensitivity of the toad *Xenopus laevis*. J Physiol 125:181–207
Diederen JHB (1975) A possible functional relationship between the subcommissural organ and the pineal complex and the lateral eyes in *Rana esculenta* and *Rana temporaria*. Cell Tissue Res 158:37–60
Dodt E (1963) Reversible Umsteuerung lichtempfindlicher Systeme bei Pflanzen und Tieren. Experientia 19:53–56
Dodt E, Heerd E (1962) Mode of action of pineal nerve fibers in frogs. J Neurophysiol 25:405–429
Dodt E, Jacobson M (1963) Photosensitivity of a localized region of the frog's diencephalon. J Neurophysiol 26:752–758
Dodt E, Morita Y (1964) Purkinje-Verschiebung, absolute Schwelle und adaptives Verhalten einzelner Elemente der intracraniellen Anurenepiphyse. Vision Res 4:413–421
Dodt E, Ueck M, Oksche A (1971) Relation of structure and function: The pineal organ of lower vertebrates. In: Kruta V (ed) J Purkinjě Centenary Symposium Prag 1969. Brno University JE Purkinjě
Donley CS, Meissl H (1979) Characteristics of slow potentials from the frog epiphysis (*Rana esculenta*); possible mass photoreceptor potentials. Vision Res 19:1343–1349
Eakin RM (1961) Photoreceptors in the amphibian frontal organ. Proc Natl Acad Sci (Wash) 47:1084–1088
Eldred WD, Nolte J (1979) Pineal photoreceptors: evidence for a vertebrate visual pigment with two physiological active states. Vision Res 18:29–32
Eldred WD, Finger TE, Nolte J (1980) Central projections from the frontal organ of *Rana pipiens*, as demonstrated by the anterograde transport of horseradish peroxidase. Cell Tissue Res 211:215–222
Flight WFG (1973) Observations on the pineal ultrastructure of the urodele, *Diemictylus viridescens viridescens*. Proc Kon Ned Akad Wet Ser C 76
Haffner K von (1950) Über die progressive und regressive Entwicklung der Pinealblase (Parietalorgan) des Krallenfrosches (*Xenopus laevis* Daud). Verh Zool Gesell, pp 93–100
Hamasaki DI (1970) Interaction of excitation and inhibition in the stirnorgan of the frog. Vision Res 10:307–316
Hamasaki DI, Esserman L (1976) Neural activity of the frog's frontal organ during steady illumination. J comp Physiol 109:279–285
Hamasaki DI, Eder DJ (1977) Adaptive radiation of the pineal system. In: Crescitelli F (ed) Handbook of Sensory Physiology. Vol VII/5 The visual system in vertebrates. Springer, Berlin Heidelberg New York, pp 497–548
Hartwig HG, Baumann Ch (1974) Evidence for photosensitive pigments in the pineal complex of the frog. Vision Res 14:597–598
Hogben L, Slome D (1931) The pigmentary effector system. VI. The dual character of endocrine coordination in amphibian color change. Proc Roy Soc B 108:10–53
Karnovsky MJ, Roots L (1964) A "direct coloring" thiocholine method for cholinesterase. J Histochem Cytochem 12:219–221
Korf HW (1976) Histological, histochemical and electron microscopical studies on the nervous apparatus of the pineal organ in the tiger salamander, *Ambystoma tigrinum*. Cell Tissue Res 174:475–497
Kreht H (1940) Die markhaltigen Fasersysteme im Gehirn der Anuren und Urodelen und ihre Myelogenie; zugleich ein kritischer Beitrag zu den Flechsigschen myelogenetischen Grundgesetzen. II. Kleinhirn, Mittelhirn, Zwischenhirn und Endhirn. Z mikr anat Forsch 48:191–286
Meissl H, Donley CS (1980) Change of threshold after light-adaptation of the chromatic response of the frog's pineal organ (stirnorgan). Vision Res 20:379–383
Morita Y, Dodt E (1965) Nervous activity of the frog's epiphysis cerebri in relation to illumination. Experientia 21:221–222
Munz FW, McFarland WN (1977) Evolutionary adaptations of fishes to the photic environment. In: Crescitelli F (ed) Handbook of Sensory Physiology. Vol VII/5 The visual system in vertebrates. Spinger, Berlin Heidelberg New York, pp 193–274

Nieuwkoop PD, Faber J (1956) Normal table of *Xenopus laevis* Daudin. North Holland Publ Comp, Amsterdam

Oksche A (1955) Untersuchungen über die Nervenzellen und Nervenverbindungen des Stirnorgans, der Epiphyse und des Subkommissuralorgans bei anuren Amphibien. Morph Jb 95:393–425

Oksche A (1971) Sensory and glandular elements of the pineal organ. In: Wolstenholme GEW, Knight J (eds) The pineal gland. A Ciba Foundation Symposium. Churchill-Livingstone, Edinburgh London, pp 127–146

Oksche A, von Harnack M (1963) Elektronenmikroskopische Untersuchungen am Stirnorgan von Anuren (Zur Frage der Lichtrezeptoren). Z Zellforsch 59:239–288

Oksche A, Vaupel-von Harnack M (1965) Elektronenmikroskopische Untersuchungen an den Nervenbahnen des Pinealkomplexes von *Rana esculenta* L. Z Zellforsch 68:389–426

Omura Y, Ali MA (1980) Responses of pineal photoreceptors in the brook and rainbow trout. Cell Tissue Res 208:111–122

Parker GH (1948) Animal colour changes and their neurohumors. Univ Press Cambridge

Paul E, Hartwig HG, Oksche A (1971) Neurone und zentralnervöse Verbindungen des Pinealorgans der Anuren. Z Zellforsch 112:466–493

Ueck M (1968) Ultrastruktur des pinealen Sinnesapparates bei einigen Pipidae und Discoglossiden. Z Zellforsch 92:452–476

Ueck M (1979) Innervation of the vertebrate pineal. Progr Brain Res 52:45–88

Wake K, Ueck M, Oksche A (1974) Acetylcholinesterase containing nerve cells in the pineal complex and subcommissural area of the frogs, *Rana ridibunda* and *Rana esculenta*. Cell Tissue Res 154:423–442

Accepted December 12, 1980