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Spectral Characteristics of Visible Radiation Penetrating into the Brain and Stimulating Extraretinal Photoreceptors

Transmission Recordings in Vertebrates

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Summary. Supravital recordings of spectral transmission in the brains of two species of teleosts (*Anguilla anguilla*, *Ictalurus nebulosus*), an amphibian (*Rana temporaria*), a reptile (*Lacerta muralis*), two species of birds (*Passer domesticus*, *Columba livia*), and a mammal (*Phodopus sungorus*) indicate that photons of longer wavelengths (700–750 nm) penetrate approximately 1,000 times more effectively into the hypothalamus than photons of shorter wavelengths (400–450 nm). The decrease in transmission from 750 to 400 nm is slightly interrupted by a plateau around 500 to 540 nm because of the transmission characteristics of hemoglobin. There is a small, ill-defined transmission minimum around 430 nm corresponding to the transmission minimum of melanin and hemoglobin (soret band). The high light sensitivity of deep diencephalic photoreceptors involved in the control of photoneuroendocrine events characteristic of some non-mammalian vertebrates suggests the occurrence of photopigment-containing receptors and nerve cells summating the input of several photoreceptors. However, in addition to photopigments, there may also exist other photosensitive compounds that mediate non-visual photoneuroendocrine responses.

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Introduction

Vertebrates, with the apparent exception of mammals, have photosensitive areas in the diencephalon that are most probably located in the vicinity of the third ventricle. Stimulation of the "deep diencephalic photoreceptor" in blinded and pinealectomized European minnows, *Phoxinus phoxinus* (L.), results in a color change (von Frisch, 1911) or in light dependent motor reactions (light-dependent conditioned reflexes, Scharrer, 1928; for the role of the pineal organ in color change mechanisms of *Phoxinus phoxinus,* see also Schäfer, 1964). Van Veen et al. (1976) showed that in blinded and pinealectomized European eels, *Anguilla anguilla* (L.), deep diencephalic photoreceptors are responsible for (1) the synchronization of circadian motor activity with an external photoperiod and (2) for the control of photonegative behavior. In birds, the photoperiodic control of gonadal growth is apparently mediated by the deep diencephalic photoreceptor and not by the rods and cones of the lateral eyes (for review, see Benoit, 1970; Menaker, 1971a, b; Farner, 1973; Yokoyama et al., 1978). There is very little information on the nature and location of the deep diencephalic photosensitive elements and their connections with neuroendocrine and motor effectors. Hartwig (1975), using microspectrophotometric techniques, detected in *Phoxinus phoxinus* a circumscribed ependymal area covering the antero-dorsal hypothalamus that contained a photosensitive compound with an ill-defined absorption maximum between 550 and 580 nm. This region is rich in nerve cells contacting the cerebrospinal fluid with bulbous cilia of the 9+0 type, thus resembling early developmental stages of retinal and pineal photoreceptor cells

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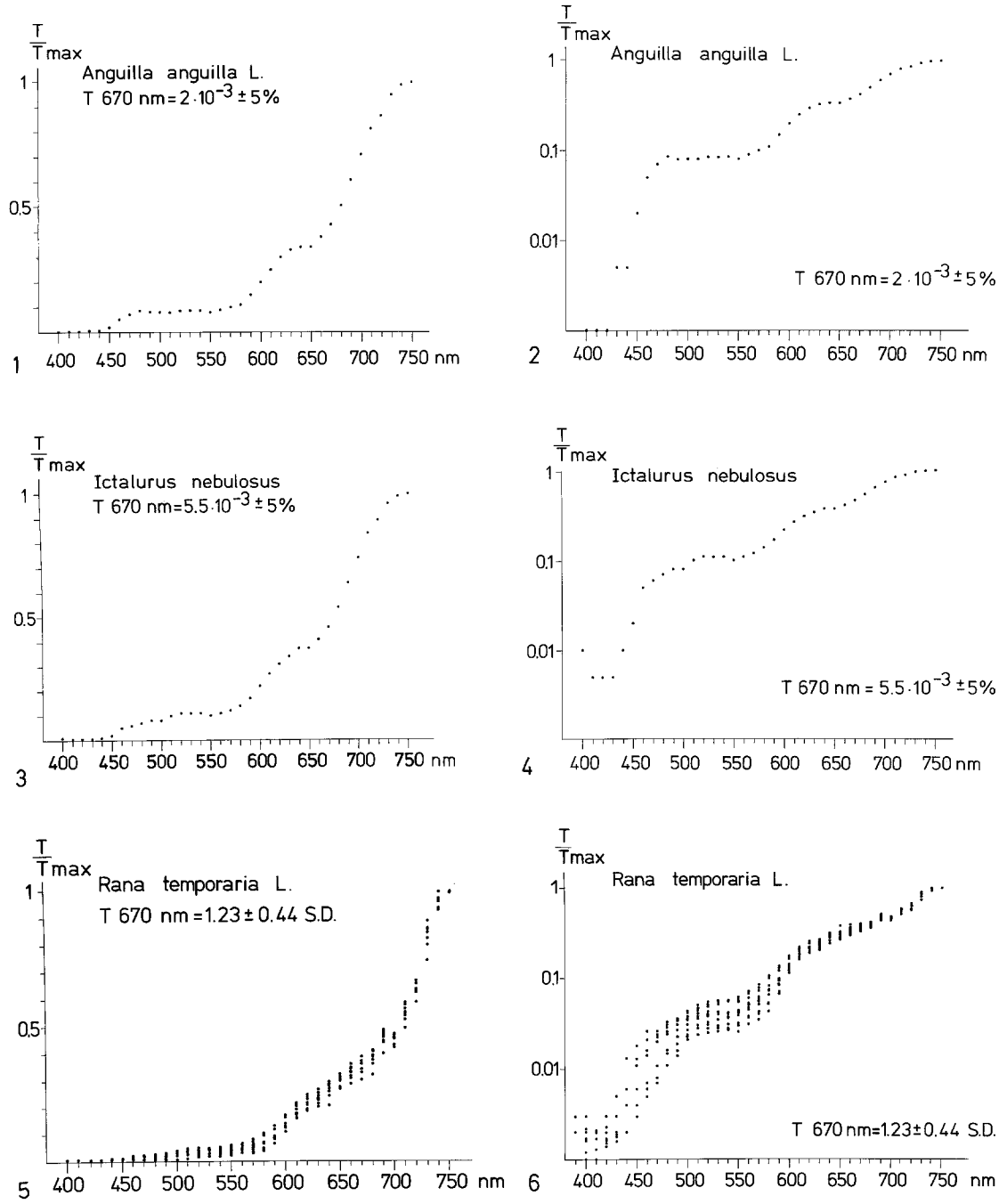
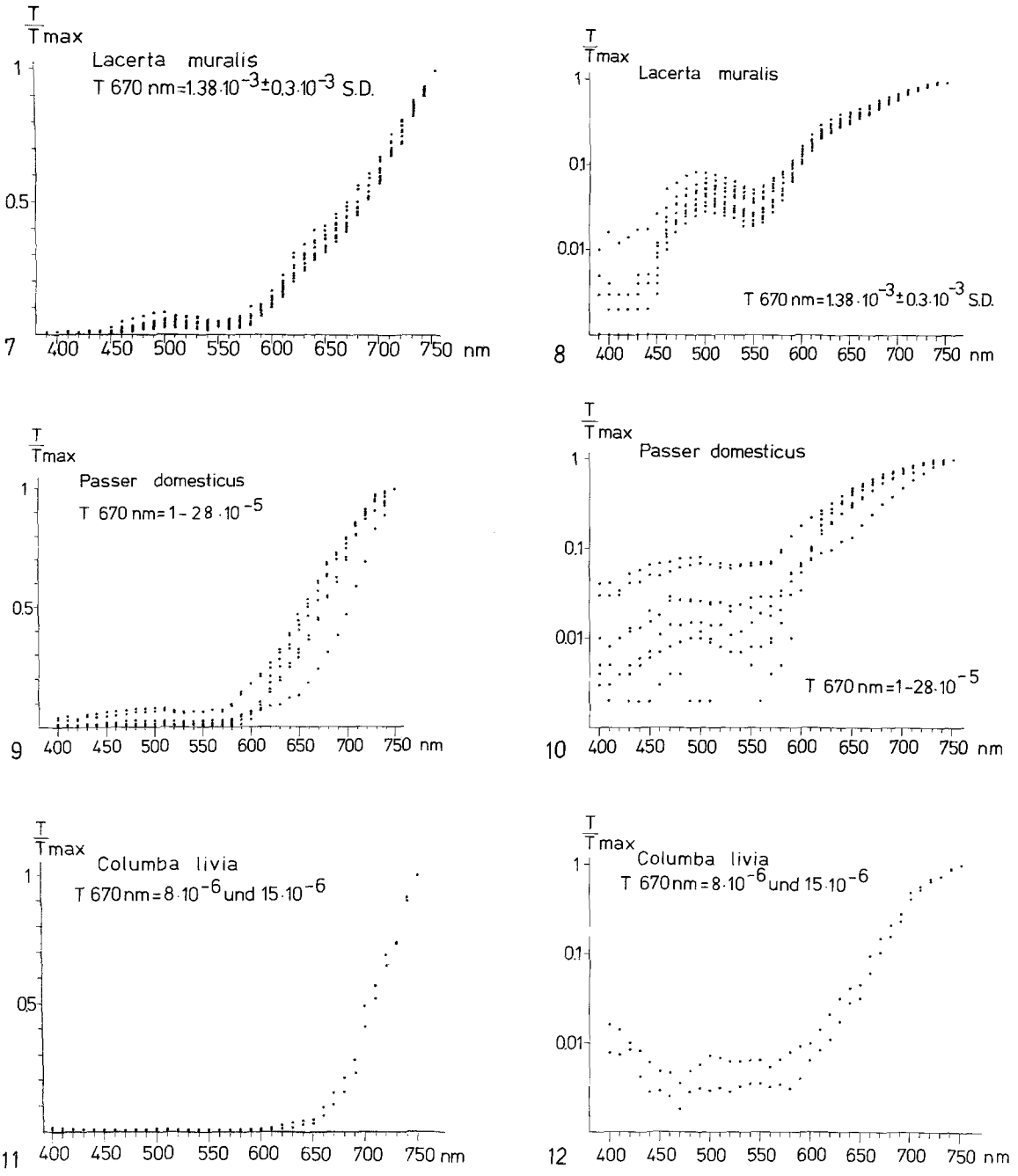


Figs. 1–6. Spectral transmission recordings of light penetrating into the hypothalamus in teleosts (*Anguilla anguilla, Ictalurus nebulosus,* $n=10$) and amphibians (*Rana temporaria,* $n=10$) exhibiting a melanophore index of 3–4. In Figs. 1–4 each dot represents the mean of 10 individual measurements. In Figs. 5 and 6 each dot represents a single measurement. Figures show corrected instrument readings expressed as T (transmission) divided by T_{max} (maximal transmission value). Absolute values of transmission are given at 670 nm. Transmission values are plotted in a linear (Figs. 1, 3, 5) or in a log (Figs. 2, 4, 6) scale

(Oksche and Hartwig, 1975). Light reaching the diencephalon must penetrate skin, bony skull, meningeal connective tissue, blood vessels, and brain substance. On its way the light is (1) scattered and (2) the spectral composition is modified by a color-filter effect (e.g. hemoglobin, melanin) of the penetrated tissue. Since deep diencephalic photoreceptors that mediate photoperiodic responses analyze the amount of light reaching the brain at different phases of the internal clock (for references, see Yokoyama et al., 1978), it is worthwhile to measure (1) the amount of light that reaches the diencephalon and (2) the



Figs. 7–12. Spectral transmission recordings of light penetrating into the hypothalamus in lizards (*Lacerta muralis*, $n=12$) and in birds (*Passer domesticus*, $n=8$; *Columba livia*, $n=2$). Each dot represents one single measurement. The figures show corrected instrument readings expressed as T (transmission) divided by T_{max} (maximal transmission value). Absolute values of transmission are given at 670 nm. Transmission values are plotted in a linear (Figs. 7, 9, 11) or in a log (Figs. 8, 10, 12) scale

spectral absorption characteristics of the tissues that cover the diencephalon.

Materials and Methods

Spectral transmission was recorded supravitally in the brains of male and female species of 1) teleosts (10 European eels, *Anguilla anguilla*, of about 60 g body weight and 10 bullheads, *Ictalurus nebulosus*, 20 g); 2) amphibians (10 frogs, *Rana temporaria*, 23–40 g); 3) reptiles (12 lizards, *Lacerta muralis*, 3–6 g); 4) birds (8 House sparrows, *Passer domesticus*, 19–28 g and 2 white feathered pigeons, *Columba livia*, 267 and 340 g); and 5) one mammal (a male dsungarian dwarf hamster, *Phodopus sungorus*, 25 g). Animals were killed by decapitation. The dorsal aspect of the skull (skin, bones and tissues) was left intact. The ventral surface of

the brain was carefully removed of the covering tissues and subsequently the hypophysis and the caudal-most part of the infundibulum were exposed. Spectral transmission was recorded with a modified microspectrophotometer (Beckman Instruments MS 1206) equipped with a Xenon high-pressure lamp (Osram XBO 450) and interchangeable photomultiplier tubes (spectral response type S-20 and spectral response type S-13). The power supply of the Xenon arc and of the photomultiplier tube exhibited a high stability (uncontrollable changes less than 0.6%). The illumination microscope of the microspectrophotometer was modified to produce a homogeneously illuminated area of 3×3 cm at the level of the object stage. The specimens were placed upside down on a glass slip on the homogeneously illuminated stage and the previously exposed ventral brain surfaces inspected using the observation microscope of the instrument by means of epiillumination. In a field covering an area from the caudal border of the transversal commissure (teleosts) or the optic chiasma (amphibians, reptiles, birds, mammals) to the dissected infundibulum, the penetrating light was measured in 10 nm intervals from 400 to 750 nm. The spectral band width was 10 nm. In measurements at wavelengths longer than 640 nm in addition to the manufacturer's filter for suppression of undesired harmonics of the grating monochromator, a blocking filter (Schott OG 630) was used to eliminate false light output. After an adaptation of the high tension supplying the photomultiplier tube, the measurements were performed with a stabilized high tension (500–800 V in small sized specimens and 1,200–1,400 V in large sized specimens). Uncorrected instrument readings showed values between 0.5–155 units (total scale range: 0.1–164 units). Correcting factors were obtained in the following way: by means of a variable diaphragm inserted into the illuminating system at a point of ray parallelism the intensity of the illuminated area was reduced to values recordable with the high tension selected for the recording of the specimen. Reduction of illumination intensity did not interfere with the spectral composition or with the aperture of the illuminating system. Corrected instrument readings were expressed as T (transmission) divided by T_{max} (maximal transmission value). Finally, absolute values of transmission were obtained at 670 nm using a calibrated series of neutral density filters (Schott).

Results

General Aspects

In all species investigated visible radiation of longer wavelengths penetrates into the diencephalon approximately 1,000 times more effectively than photons of shorter wavelengths. The longest wavelength investigated (750 nm) shows the best penetration to the hypothalamus. The shape of the transmission curve (plotted in a linear scale of T values) in general depends on the absolute size of the skull and brain. In large specimens (mammal, bird) the decrease of transmission values is more rapid in comparison to small specimens (teleost, amphibian). The decrease of transmission values is slightly interrupted at wavelengths between 500–540 nm. Between 420 and 440 nm there exists a small, ill-defined transmission minimum. In large specimens the transmission curve plotted in a linear scale of T values decreases rapidly from maximal values at 750 nm to values near zero around 600 to 650 nm. In transmission curves plotted in a logarithmic scale of T values the further decrease of transmission values is clearly visible (c.f. Figs. 1–12).

Special Aspects

Anguilla anguilla, Ictalurus nebulosus, Rana temporaria. In the two species of teleosts and in the anuran the absolute number of photons that penetrate into the hypothalamus depends strongly on the melanophore index (MI) of the specimen investigated. In a pilot study some animals were placed for several days prior to investigation on a white or a black continuously illuminated background. The melanophore index (MI) was recorded according to Hogben and Slome (1931) on the pectoral fins (teleosts) or the interdigitatal web (*Rana temporaria*) by means of a stereo microscope. In animals adapted to a white background (MI = 1–2) the amount of light reaching the hypothalamus is about 100 times higher than in animals adapted to a black background (MI = 4–5). However, the adaptation to a white or to a black background does not interfere with the general pattern of the transmission curves. The results shown in Figs. 1–6 are obtained in animals exhibiting a MI of about 3–4 (for transmission data of tissues covering the pineal organ in *Salmo irideus, Rana temporaria* and *Rana esculenta*, see Morita, 1966).

Passer domesticus, Columba livia, Phodopus sungorus. In these larger animals it was necessary to record longer wavelengths with a photomultiplier of the spectral response type S-20 and shorter wavelengths with a photomultiplier tube of the spectral response type S-13. Moreover, in the pigeon and in the dsungarian dwarf hamster only animals bearing either white feathers or a white winter fur could be measured with the required precision. In large specimens bearing dark feathers or a dark fur only photons of wavelengths longer than 600 nm could be detected due to technical limitations of the instrument used in this investigation. However, in these specimens (recordings in three dark feathered pigeons, in two dark feathered chickens and in a dsungarian dwarf hamster bearing a dark summer fur) the decrease of transmission values followed the same principles as in small specimens.

House sparrows were trapped and investigated in January. Thus, the reported values deal with animals that are known to be maximally photosensitive. Recordings in the *Phodopus sungorus*, bearing a white

winter fur exhibit transmission values in the range of 10^{-7}–10^{-8} for wavelengths between 650–750 nm. Values recorded below 550 nm are characterized by a poor signal to noise ratio. Therefore, the data are not shown in a diagram.

Discussion

It is well known that measurable quantities of visible radiant energy penetrate the skull into the hypothalamus, even in mammals (Ganong et al., 1963). In the House sparrow, *Passer domesticus*, the light intensity of a full moon night (moon not covered by clouds) is sufficient to stimulate the photoreceptor located inside the brain (for review, see Menaker, 1971a, b; for threshold data of light sensitive pineal organs, see Dodt, 1973). Menaker and coworkers (McMillan et al., 1975) demonstrated that electromagnetic radiation between 600 and 700 nm characterized by an energy of only 0.15 erg/cm^2/s measured at the head surface of *Passer domesticus* is capable of penetrating feathers, skin, bony skull and brain tissue and inducing gonadal growth. In blinded ducks Benoit (for review, see Benoit, 1970) found that longer wavelengths stimulated gonadal growth more effectively than shorter wavelengths when the radiation was applied externally.

The light sensitivity of the deep diencephalic photoreceptors demonstrated in *Passer domesticus* by McMillan et al. (1975) is comparable to that of retinal and pineal photoreceptors which contain photopigment molecules. In experiments dealing with the spectral sensitivity of deep diencephalic photoreceptors, it is necessary to know the spectral transmission values of the tissues covering the diencephalon, because the number of photons reaching the diencephalon per unit time differs with the wavelengths applied. The presented transmission recordings show that in all species investigated longer wavelengths from 700 to 750 nm penetrate 100–1,000 times more effectively into the diencephalon than shorter wavelengths from 400 to 450 nm. Double-beam transmission recordings performed in isolated skin pieces of *Anguilla anguilla* and in protein microdroplets containing synthetic melanin, revealed that the black pigment melanin has a characteristic absorption spectrum with a high transmission of long wavelengths and a low transmission of short wavelengths. Moreover, a less distinct transmission minimum was observed between 420 to 440 nm (Hartwig and van Veen, unpublished). In general, the transmission spectrum of melanin resembles the spectra of this study. The small plateau between 500–540 nm found in the present recordings most probably is due to hemoglobin transmission maxima, characteristic for this range (for general considerations concerning the influence of blood transmission characteristics on the spectral sensitivity of retinal photoreceptors see Dodt, 1958; Peregrin, 1974). Three factors seem to be responsible for the slope of the transmission spectra observed: (1) photons of long wavelengths are known to be scattered to a lesser extent by biological tissues and thus to penetrate deeper than photons of shorter wavelengths (c.f. Seliger and McElroy, 1965), (2) melanin and (3) hemoglobin (erythrocytes of the blood vessels) show a strong absorption in the visible range of the spectrum. Hemoglobin and melanin both absorb short wavelengths to a greater degree than long wavelengths. The absolute number of photons penetrating into the diencephalon per unit time is a function of the size of the animal and of the density of melanin in the tissues covering the brain.

The absolute amount of environmental light reaching deep diencephalic photoreceptors must be higher in living animals than the values reported in this investigation for the following reasons: 1) Measurements were performed at the ventral surface of the hypothalamus and not at probable sites of the diencephalic photoreceptors. 2) Deep diencephalic photoreceptors are most probably located in the vicinity of the third ventricle; thus from a dorsal direction environmental light must penetrate only the relatively thin layers covering the dorsal diencephalic roof; the cerebrospinal fluid absorbs only negligible amounts of photons in the visible range of the spectrum. 3) Environmental light penetrates the skull not only from dorsal but also from lateral, anterior and posterior directions whereas in this investigation, due to technical reasons, only the dorsal skull surface could be illuminated. However, for experiments dealing with the spectral sensitivity of diencephalic photoreceptors in intact animals the knowledge of the spectral transmission characteristics of the tissues covering the photoreceptor is more important than the knowledge of the absolute number of photons penetrating into the brain. Finally it should be noted that in aquatic animals daylight is modified by the transmission and reflectance characteristics of water.

In vertebrates lamellated photoreceptor cells have been found only in the retina and in the pineal organ (Teleostei, Amphibia, Lacertilia). Retina and epiphysis cerebri have developed phylogenetically as diencephalic evaginations. Most probably a circumscribed region of the diencephalic primordium is the only area in vertebrates capable of forming photoreceptor cells. This assumption is strongly supported by a report of Sacerdote (1971) who showed differentiation of ectopic retinal structures in the hypothalamo-hypophyseal area of *Triturus cris-*

tatus induced by a methylene blue-soaked barrier at the level of the median eminence. Photoreceptor cells containing regularly lamellated outer segments only exist in connection with specialized supporting cellular elements (e.g. pigment epithelium in the retina; c.f. Hollyfield and Witkovsky, 1974). Regularly lamellated outer segments are responsible for a high optical density of photopigments in a circumscribed structural area.

This condition is mandatory in an image-analyzing photoreceptor system. The deep diencephalic photoreceptor does not serve to analyze spatial intensity differences. It records the number of photons reaching the diencephalon. This task can be fulfilled by unlamellated outer segments distributed over a large space, whereas in the retina one photon reaching one single outer segment must be detected in order to attain optimal spatial analysis at low intensities.

In considering the exact structure, function and location of deep diencephalic photoreceptors, one cannot preclude the possibility that additional mechanisms of photosensitivity may be of importance. Such possibilities include (1) photosensitive enzymes (Hug et al., 1971), (2) oscillatory behavior of enzymic activity induced by radiant energy (Comorosan et al., 1969), and (3) enzyme activation or inhibition by photolabile chromophores (e.g. carotenoids, hemoproteins; Deal et al., 1969). However, as far as is known the latter mechanisms would have a decided disadvantage in that their photosensitivity is much lower than that of photopigments.

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