COMPARATIVE FINE STRUCTURE AND IMMUNOCYTOCHEMICAL ANALYSIS OF THE CORPUS PINEALE RESPONSIBLE FOR THE REGULATION OF DAILY AND SEASONAL RHYTHM IN DIFFERENT VERTEBRATES

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INTRODUCTION

The corpus pineale is part of the diencephalon's topmost, epithalamical part, to which it joins with the habenules. In birds and mammals, the organ develops from two embryonic fields that are analogous to the area before and behind the recessus intrapinealis in mammals and humans. From Cyclostomes to reptiles the two fields remain separate and form two separate pineal organs. In principle, the organs' build-up is similar, beyond glial cells, they consist of cone-like retinal photoreceptor cells and neurons analogous to the retina's secondary neurons.

Contrary to the pinealocytes of Cyclostomes, fish and amphibians, the pinealocytes of birds and mammals are considered underdeveloped. As the human and mammalian pinealocytes - especially in adult age – do not have differentiated photoreceptor outer segment, most authors declare that they have lost their ability to sense light. Although photoreceptor molecules are present in mammalian pinealocytes, it is considered that light information for the regulation of daily and seasonal biorhythm is received from the retina, namely by means of sympathetic nerves, as sympathectomy inhibits pineal synthesis of melatonin.

From Cyclostomes to birds pineal organs are *non-visual photoreceptors,* they don't serve the vision of images, but the detection of circadian and circannual changes in the light intensity of the environment. The corpus pineale's synthesis of melatonin plays an important role in the regulation of circadian and circannual rhythm; light inhibits melatonin secretion, so most of melatonin is produced during the night. Melatonin has - among others - antigonadotropic effect, which influences the hormonal system directly, and regulates the body's daily and seasonal rhythm.

Basically, the normal daily rhythm of different organs is controlled by self-regulated genetic 'slave clocks', that dictate 24 hour, circadian rhythm. The daily rhythm of certain organs are coordinated by the hypothalamic nucleus suprachiasmaticus ('master clock'). Coordinated operation is set to the different biotopes' circumstances by the pineal organs, respectively the non-visual photoreceptors.

Besides visual light-sensitivity, retina is capable of non-visual photoreception. Cells containing cryptochromes, pinopsin and light-sensitive molecules detectable with OS-2 antibody are involved primarily in this function.

Deep encephalic photoreceptors, as the liquorcontact neurons of the recessus preopticus, or the septal photoreceptor cells have similar non-visual light-sensitivity in submammalian species. In these neurons pinopsin, other opsins, and other molecules of the photoreceptor cascade were detected. They play a role in the adjustment of reproductive periods and in the photoperiodic reactions of gonades. It is assumed that such deep encephalic photoreceptors are also present in mammals, primarily perinatally (for example, in mice there is encephalopsin in the area preoptica and in the nucleus paraventricularis).

Practical medical importance of daily rhythm is that night illumination inhibits pineal melatonin production, hence it disrupts the body's rhythm. Among other disorders, night illumination may cause breast cancer in women and colorectal and prostate carcinoma in men, as recent studies have shown. As short-wave light is the main inhibitor of melatonin production, it is justified to eliminate it from night-lighting and to use colour filtered glasses for night work.

Corpus pineale, which is responsible for the adjustment of the daily and seasonal rhythm, is examined most frequently in experimental animals, but has also significant pathological implications in humans. This justifies detailed comparison of

fine structure and immunocytochemistry under the different conditions in mammals and submammals as the topic of the present work.

OBJECTIVES

My main objective was the ***comparative developmental and fine-structure examination of pineal organs***, particularly in diversely differentiated species', including birds' and special mammals' specific structural conditions and the comparison of the organ's and the retina's histological structure .

As another objective, I chose ***examination of corpus pineale's neural, hormonal and non-synaptical connections.*** Autonomic fibres in the pineal organs' meningeal spaces, their relationship to the pineal neural tissue and the pineal blood vessels constitute a significant part of this section of the dissertation too.

Definition of the pineal organs' molecular components seems to be the most important objective that contributes to the better understanding of function, beyond the more accurate knowledge of fine structure, afferentation and efferentation. Our ***immunocytochemical investigations*** comparing different species address this problem.

Acervuluses can be observed in bird species aside from human and general mammalian appearances. In this work I compared corpus pineale, surrounding brain areas and retina from the aspect of calcification, and the relationship of calcification and photoreception.

METHODS AND MATERIALS

EXPERIMENTAL ANIMALS

For the fine-structure examination of the pineal organ of **birds** we used the following species:

Galliformes: Domestic Chicken, (white leghorn, Gallus domesticus), Helmeted Guineafowl (Numida meleagris), Ring-

necked Pheasant (Phasianus cochicus), Japanese Quail (Coturnix coturnix japonica), Wild Turkey (Meleagris gallopavo); *Columbiformes:* Rock Pigeon (Columba livia), *Anseriformes:* Greylag Goose (Anser anser), Mallard (Anas platyrhynchos); *Passeriformes:* House Sparrow (Passer domesticus), Zebra Finch (Taeniopygia guttata), Blackbird (Turdus merula) and Mistle Thrush (Turdus viscivorus), Canary (Serinus canaria), Great Tit (Parus major), *Psittaciformes:* Budgerigar (Melopsittacus undulatus); *Falconiformes:* Common Buzzard (Buteo buteo); *Struthioniformes:* Ostrich (Struthio camelus).

For corpus pineale and retina experiments performed on **bats** we used the following species: Lesser Mouse-Eared Bat (Myotis blythi oxygnathus); Greater Horseshoe Bat (Rhynolophus ferrumequinum), Long-winged Tomb Bat (Taphozous longimanus); Asian Yellow Bat (Scotophylus hethai), Temminck's Flying Fox (Pteropus temmincki); Greater Short-nosed Fruit Bat (Cynopterus sphinx); Egyptian Fruit Bat (Rousettus aegyptiacus).

Autonomic nerve fibers were examined on the following species: **mammals:** Brown rat (Rattus norvegicus), Syrian Hamster (Mesocricetus auratus), Least Weasel (Mustela nivalis); **birds:** Domestic Chicken (Gallus domesticus), Japanese Quail (Coturnix coturnix), Common Buzzard (Buteo buteo); **reptiles :** Common Wall Lizard (Lacerta muralis), Grass Snake (Natrix natrix), **amphibians:** Edible Frog (Rana esculenta), Great Crested Newt (Triturus cristatus), Smooth Newt (Triturus vulgaris), **fish:** Thornback Ray (Raja clavata), Prussian carp (Carassius auratus); **Cyclostomes:** European River Lamprey (Lampetra fluvatilis).

We received bats (except the Myotis species) from the Banaras Institute of Zoology. We collected the Myotis species in Hungarian caves. We bought the Rousettus species in the Budapest Zoo's animal breeding farm. We kept the animals

under normal laboratory light conditions. We own the following licences for the experimental use of the above animals: 22.1/3700/003/2008 (Budapest and Pest County Agricultural Office); 43327-2/2008 (Middle Danube Valley Environment, Nature Protection and Water Management Inspectorate), and 778 / 000/2005 (Veterinary and Food Control Station of Budapest).

ELECTRON MICROSCOPY STUDIES

Before fixation we used phenobarbital or ether anesthesia on the experimental animals. We carried out perfusion fixation through the aorta with Millonig buffer containing 0.1-2% glutaraldehyde and 2% paraformaldehyde (0.24 M phosphate buffer). After removing, we washed the pineal organs and retina in buffer, post-fixed for one hour with glutaraldehyde, dehydrated in ethanol, then embedded them in PolyBed 812 (Polysciences, St. Goar, Germany). We made sections with Reichert Ultracut S ultramicrotome. We contrasted the ultra-thin sections with uranyl acetate and lead citrate. We stained semi-thin sections with toluidine blue azure II. We carried out electron microscopic immune reactions on gold-plated ultra-thin sections placed to a nickel grid (postembedding staining).

TYROSINE HYDROXYLASE REACTION

To promote the differentiation of vegetative fibres we performed tyrosine hydroxylase immunological staining with polyclonal and monoclonal antibodies (Sigma, St. Louis, MO).

IMMUNOCYTOCHEMICAL DETECTION OF PINOPSIN

The chicken-pinopsin specific rabbit polyclonal antibody was made in our institute, using a C-terminal originated, 14 amino acid long peptide.

DETECTION OF RETINAL OPSINS

We used the following mouse monoclonal antibodies produced by means of chicken cones: OS-2, which is specific for blue and UV-sensitive photopigments, and COS-1, which is specific for green and red-sensitive pigments. We visualised the postembedding reaction with DAB.

DETECTION OF CALCIUM

In our own studies we stained light-microscopic corpus pineale sections by Kossa's staining protocol for calcium. For electron microscopic use we used pyroantimonate detection for calcium.

RESULTS

COMPARATIVE FINE-STRUCTURE EXAMINATION OF PINEAL ORGANS

In the examined Cyclostomes, bony and cartilaginous fish corpus pineale is only a simple, elongated, bladder-like extrusion of the top lamella of the epithalamus with a shaft. Its outer surface is covered by the epithalamical meninges, that are separated from the organ's tissue by pineal ependymal cells and membrana limitans gliae superficialis formed by the glial feet at the end of the glial cells' projections.

Inside the corpus pineale there is a cavity, which is analogous to the third brain ventricle's recessus pinealis and it is connected to the ventricle's cavity with the pineal shaft. Inside the organ, the cavity is wider, and it is closed by ependymal cells.

The pinealocytes' dendritic projection passes through the ependyma and enters the pineal cavity. Passing through, the ependyma is bound to the epithelial cells with cell-adhesion structures. In the pineal lumen, the dendrites of pinealocytes

expand and constitute an inner segment, from which an outer segment with a cilia-like basic structure derives.
In more differentiated species, the organ becomes gradually larger. At first, its wall forms several domes, that are, gradually growing, form secondary sinuses. Between pineal recessuses, pineal meninx penetrates deeply into the organ, carrying blood vessels and nerve fibres around the vessels.
Comparing the organ's enlargement and the outer segments' photoreceptor membranes, the number of the photoreceptor structures does not decrease, but increases.

Fine structure of the pineal body in birds

In birds, the pineal organ is located directly under the skull, and connected to the epithalamus with a longer pineal shaft. In the examined species the organ's wall forms sac-like extrusions. It's outer surface is covered by the diencephalical pia mater and arachnoidea. The meginges spread into the organ along the recessuses, but are separated from the pineal nerve tissue by the basal laminae.

The perikaryon of avian ***pinealocytes'*** is located near the lumenic surface of the pineal follicles'. Beyond the general cell organelles, granular vesicules in different sizes and numbers can be found in the cytoplasm. Pinealocytes , like neurons, are bipolar: they have receptor and effector projections cytologically differentiated adequately to their function.

The ***receptor projection*** of the pinealocytes is dendrit-like, contains cytoplasmic organelles, its end spreads into the pineal lumen between the ependymal cells. It is bound to the ependymal cells with junction structures. Similarly to the retinal cones and rods, the terminal thickening of the dendrit forms an inner segment. On the **inner segment** many microvilli and a sensory cilium can be found. Inside the inner segment many mitochondria, basal bodies and accessory basal

bodies are located. The sensory cilium (similarly to the retinal connector outer segment) - has 9 x 2 + 0 microtubule arrangement. The pineal "connecting part" in the examined birds is long, its distal end thickens and forms an outer segment similar to the one in the retina. **Pineal outer segments** are pear-shaped thickenings of the cilia, that consist of photoreceptor lamellae in various number, with granular cytoplasm between them.

The ***effector projection*** of the pinealocytes is axon-like, it contains parallel microtubules and granular vesicles beyond mitochondria. The axon of the pinealocytes can diverge, and it either ends on secondary pineal neurons or it forms a neurohormonal terminal on the surface of the pineal nervous tissue.

In the corpus pineale, similarly to the retina, ***secondary neurons*** can be found, that are either bipolar or multipolar. On the neurons not only pinealocytic axon terminals can be found, but also axon terminals that are not ribbon-like, and represent interneuronal connections or pinealopetal afferentation.

The ***presynaptic terminal*** of the pinealocytes ends on secondary pineal neurons. Pinealocytic axons form both axodendritic and axosomatic synapses that contain many synaptic vesicles and synaptic ribbons. Ribbons are associated to the thickened presynaptic membrane.

The cylindrical ***ependyma*** that covers the pineal lumen is the continuation of the third brain ventricle's ependyma. One part of the cells have moved away from the recessus pinealis that forms the diverticle of the third brain ventricle and is located in the pineal nervous tissue as an ***astrocyte*** or an ***oligodendrocyte.***

Examination of the corpus pineale in bats

The bats' corpus pineale and retina has a special structure because of the nocturnal nature of the animal.

The examined species' ***pinealocytes*** are polarised, the "receptor pole" is characterized by a 9 x 2 + 0 cilium structure. Similarly to the developing retinal outer segments, one part of the cilia can thicken and inside them vesicles can be found. Two basal bodies and some cilium roots belong to the cilium.

The ‘effector pole’ of the pinealocytes forms a branching, axon-like projection, and in the axon terminal there are synaptic and granular vesicles. The terminals end on secondary pineal neurons, some of them form neurohormonal terminals, and a substantial part of them end at the vascular surface of the corpus pineale on the lamina basalis. The extensions of the pinealocytes can contact the ventricular lumen between the recessus pinealis's ependymal cells. In the lumen thin axons are located that contain granular vesicles.

Pineal neurons - as opposed to pinealocytes - have clear cytoplasm, contain well-developed endoplasmic reticulum and granular vesicules. On the perikaryons similarly granulated somato-dendritic synapses can be found. In the intercellular pineal nervous fibre zones myelinated and unmyelinated axons and axodendritic synapses can be found.

COMPARATIVE FINE-STRUCTURE OF THE CORPUS PINEALE AND THE RETINA

The eyes of the examined ***microchiropteran*** bat species are small and their retina is underdeveloped. The outer segments are rod-type, and in the inner segments there are many mitochondria. The outer granular layer is relatively developed, while in the ganglionic layer there are only a few cells. Some of the large ganglion cells are found in the bipolar layer.

Unlike microchiropterans, fruit-eating ***megachiropterans*** have a developed photoreceptor layer with cells with long outer segments and spheruluses containing

synaptic ribbons. In these species the retina has characteristic morphology: the photoreceptor layer (outer and inner segments) forms folds and crypts. The internal synaptic zone, the ganglion cell layer and the optic nerve fibre layer remain unfolded.

NEURAL, HORMONAL AND NON-SYNAPTIC RELATIONSHIPS OF THE CORPUS PINEALE

Perivasal autonomic nerve fibres

In all species examined vegetative fibres come to the corpus pineale along blood vessels. They get inside the organ from the vessels meningeal sheath and in the organ's interfollicular-meningeal enclosures. Most fibres are unmyelinated but myelinated fibres can also be observed. Bundles are covered with Schwann-cells, and thin endo- and perineural connective tissue.

There are more nerve fibres in mammals, birds and reptiles than in amphibians, fishes and Cyclostomes. Nerve fibres arrive not only along the arteries supplying the corpus pineale, but along veins flowing into the vena cerebri magna.

Tracking the nerves on the serial sections, it can be observed that they do not enter the pineal nervous tissue itself, but stay in the meningeal enclosures and form nerve endings on the pineal arterioles' smooth muscle cells.

Both periarteriolar and perivenal fibres show tyrosine hydroxylase reaction. In addition, there are immunoreactive nerve fibres in the pineal nervous tissue that are (studied on serial sections) not connected to meningeal fibres, but come from intrapineal neurons. Intrapineal TH+ nerve cells' axons run to the pineal shaft.

Neurohormonal efferentation of the corpus pineale

On the one hand, the axonal projection that flows from the pinealocytes' effector pole forms axodendritic and axosomatic syapses on the pineal secondary neurons, but the

other part of it runs to the organ's surface. There the endings connect to the lamina basalis of pineal nervous tissue between the glial feet of the lamina limitans gliae perivascularis.

In the examined ***birds*** numerous pineal axons run to the surface, where they form a circumscribed neurohormonal area. The neurohormonal endings of pinealocytes are linked to the adjacent glial feet with cell-adhesion structures and they form a semidesmosome on the surface of the lamina basalis. Accumulation of synaptic vesicles can be detected around the semidesmosomes in some species (pigeon, hawk, ostrich).

There are neurohormonal terminals in ***mammals'*** corpus pineale too. In bats, the majority of pinealocytes' axonal endings form neurohormonal terminals. In some species, like in Myotis blythi oxignatus, the **venae cerebri internae** contact the lateral part of corpus pineale directly. Many axonal terminals - which contain granular and synaptic vesicles 60-120 nm in diameter and synaptic ribbons - are located in front of the veins.

NON-SYNAPTIC AFFERENTATION OF THE CORPUS PINEALE

Pineal liquor contact neuronal terminals

In inferior animals' pineal organs neurons similar to the retinal Landolt bipolar neurons can be found. Subependymal neurons of cartilaginous fish send ciliate dendrites to the pineal lumen. In the examined amphibians a group of pinealocytes stretches to the third brain ventricle's top part.

In some mammals the recessus suprapinealis and the recessus intrapinealis also contains pinealocytic dendrit endings that have a direct contact to the third brain ventricle's liquor. The axons of the cells can be followed to the habenular nuclei. In cats, small GABA-immunoreactive pineal neurons send dendrites to the recessus intrapinealis and to the recessus suprapinealis.

Ventricular connections of the pinealocytic dendrites

'Liquor contact' projections of the pinealocytes can come into contact with the ventricular lumen between the ependymal cells of the recessus pinealis. Based on structure some of these can be considered dendrites. Thin axons containing granular vesicles can also be found in the lumen of the recessus pinealis.

Pineal lumens derived from the third brain ventricle's recessus pinealis, are similar to retina's interphotoreceptor space, which develops from the third brain vertricle's recessus opticus. Contrary to the retinal interphotoreceptor space, pineal lumen keeps its connection with the third brain ventricle. Where the connection of recessus pinealis and the pineal lumen is embryonically terminated, it can be observed that the pinealocytic dendrites elongate to both the pineal lumen and the recessus pinealis.

Connections of the pineal interstitial space

Ependyma, lining pineal recessuses, forms cylindrical or cubic epithelium. Similarly to the third brain ventricle's ependyma - of which it is a direct continuation - it does not form tight cell-adhesion structures. The liquor space of the third brain ventricle is not isolated from the pineal neural tissue's intercellular fluid, which is relevant from the aspect of nonsynaptic signal transduction.

CYTOCHEMICAL EXAMINATION OF THE CORPUS PINEALE

In my studies I examined the expression of molecules included in the photoreception of the pineal organs, pinopsin and retinal photopigments.

Pinopsin is an opsin specific for corpus pineale with an absorption maximum of 470 nm. We examined the pinopsin's

fine structure localisation with self-made antiserum in diversely differentiated vertebrates. Immune reaction was localized to the pineal outer segment's photoreceptor membrane. Among the studied species, we found the strongest immune reaction in birds and reptiles, while in the more differentiated or less differentiated species (amphibians, fishes, Cyclostomes and mammals) the reaction was less intensive. We could not observe pinopsin immunoreaction in the pineal and parapineal organs of Lampetra (Cyclostoma). We got medium-weak reactions in the outer segment of fishes' pinealocytes. The parapineal organ of fishes and frontal eye of frogs gave a negative result.

We observed a strong reaction in some pinealocytes of all reptiles but the grass snake, but the parietal eye has proved to be negative. In birds, the majority of pinealocytes was positive, in contrast with the examined mammals, whose pinealocytes did not reacted with the antibody.

Comparing corpus pineale and the *retina,* reptiles' retinal cone-type photoreceptor outer segments and birds' retinal rod-type outer segments showed pinopsin immune reaction.

The presence of ***retinal photopigments*** in Submammalia was detectable in the **Cyclostome** Lampetra's pineal organ, in the pineal retina and antrum, and in the parapineal organ's ventromedial region. In **cartilaginous and bony fishes,** the majority of pinealocytic outer segments could be designated with *rodopsin* antibody. In the studied **frog species,** rod-type photoreceptor cells of the corpus pineale and the frontal body were both designated with rodopsin.

In birds, rodopsin immune reaction was observable in the photoreceptor membranes of all pinealocytes, but in the retina only rods were positive. The electron microscopic immune reaction showed specific designation of the photoreceptor membranes, and their similarity in the pineal

and retinal outer segments. In **mammals** some pinealocytes' perikaryon showed rodopsin immune reaction, some other perikaryon were negative.

In addition to rodopsin in the studied fishes and frogs corpus pineale's and frontal body's rod-type photoreceptor cells can be designated with **OS-2** monoclonal antibody and with **RET-2**. In Lacertalia the small pineal photoreceptor reacted with **COS-1** antibody, similarly to the outer segments of retinal rods.

PINEAL CALCIFICATION AND PHOTORECEPTIVE FUNCTION

We observed acervuluses in birds only among **Submammalia.** In **mammals** , we rarely found acervuluses in young (100 - 200 g) rats. In older, 400-500 g animals we could observe concrements in every case. Primarily meningeal-type acervuluses occured. Similar lime granules can be found in the meninx covering the corpus pineale of bats and minks.

The concentric structure of the corpora arenacea can be easily observed with an electron microscope. In the cells around the acervuluses Ca-pyroantimonate granules can be found in small vesicles. Decalcinating the segments with EDTA, granules lose their electron dense material, so their calcium content can be shown. In the studied species small microacervuluses can be found in the pineal tissue itself.

With the pyroantimonate method Ca-accumulation can be detected along the pinealocytes membrane. Similar localisation has also been detected in human corpus pineale. In some areas in the intercellular space a larger amount of Ca-pyroantimonate granules accumulate and a microacervulus-like condensation can be seen. From the particles on the cell membrane to the small and large concrements a continuous transition of all forms can be found, so we assume that the

primary source of calcium-accumulation is a kind of pineal cell-membrane activity.

CONCLUSIONS

Based on the comparative fine-structure studies, pinealocytes are not considered glial or endocrine cells, but neuronal elements with a structure and development similar to the retinal cones and rods that have a sensory cilium with a 9 x 2 + 0 structure and differentiate to photoreceptor outer segments in some mammals (for example in weasels) and submammals. Thickened dendritic projections of the pinealocytes' form inner segments similar to retinal photoreceptors, and spread to the pineal lumens derived from the third cerebral ventricle's recessus pinealis.

In the most Submammalia, pineal complex consists of two organs formed from the diencephalon's top lamina: in reptiles the corpus pineale and the parietal eye, in frog species the pineal organ and the frontal organ (frontal eye), in Cyclostomes and bony fishes the pineal and parapineal organ. The two organs can be originated in the even dorsal diencephalical eyes of the hypothetical vertebrate ancestor, which was similar to the European River Lamprey.

In addition to ependymal and glial elements, both pineal organs contain cone- and rod-like pineal photoreceptor cells. Pineal photoreceptors are polarized, bipolar cells. Their receptor pole is formed by a dendritic projection, on which there is an inner and an outer segment, both of which reach into the pineal lumen. The photoreceptor that forms the outer segment has a tubule-structure (9x2+0) that is typical to sensory cilia. In most examined species, the cilium of the outer segment contained various number of membrane multiplications.

During phylogenesis, the dorsal, parietal eyes of the vertebrate ancestor's four eyes differentiated to serve a photodosimeter function, in contrast with the lateral eyes' retina's 'locator' function. Retina remained unfolded, to receive the outer environments' two-dimensional images, projected on it by the lens and the cornea. Images turn into three-dimensional information due to the difference of the two eyes.

The parietal eye of reptiles provides directional light-sensing, because it has unfolded retinal part and lens attached. This allows of the visiting of sunny places, which is important for the reptiles' body temperature regulation. Frogs' front eyes have a similar function.

Contrary to the front and parietal eyes, the corpus pineale turned into a 'folded retina', which resulted in an increase of the number of photoreceptors and in the organ's light sensitivity. The follicles forming the folds are often thought to be endocrinal structures, but in fact they are spaces analogous to the retina's photoreceptor part, because of the photoreceptor inner and outer segments that reach into them.

Different pineal organs contain different photoreceptors, for example in frogs the extracranial frontal eye contains primarily cones. This can be related to the organ's termoregulational function. In turn, the intracranial pineal organ contains rod-type photoreceptors and its function is to detect the circadian and circannual changes of environmental lighting.

Visiting places with direct sunlight can be useful to ectotherm animals, but in endotherms and birds that have a high temperature it have lost its importance. Birds' odd pineal body is analogous to the ectotherms intracranial pineal body. Even corpus pineale that can be seen in quail embryos refers to the original, duplicated field of the organ.

In previous and recent literature it is widely believed that during evolution, the pinealocytes' photoreceptor outer

segments devoluted to endocrine cells, and in mammals they receive light information only from the retina, through sympathetic autonomic nerves. Contrary to this belief, immunocytochemical studies detected molecules known from retinal photo-transduction cascade not only in Cyclostomes, but in reptiles, birds and mammals. The detected molecules are pineal opsins, pinopsin, criptochrom, and OS - 2 shortwave-sensitive photoreceptor molecules. Usage of shortwave light at night disturbs the pineal melatonin production and biorhythm of the body less.

Another argument against the more differentiated species' corpus pineale's gradual transition into endocrine glands is that the neurohormonal axon terminals indicating neurohormonal activity can be found in one of the simplest vertebrates: in Cyclostomes. These neurohormonal nerve endings are formed by pinealocytes with well-developed photoreceptor outer segment, that argues against the antagonism of photoreception and neurohormonal activity.

Birds have a special status, because they evolved in parallel with mammals, so they can not be considered as a transition between reptiles and mammals. Electron microscopic studies showed that in birds there were more neurohormonal terminals in the corpus pineale than in mammals, thus avian corpus pineale had more significant secretory activity. It is more likely that the corpus pineale's fast neural or slower hormonal display in the given vertebrate class or species developed individually as a response to the need for adaptation in a special biotope.

Studies of Foster et al. (1993b) showed that in mutant mice with degenerative symptoms, circadian photoreception of cones and rods remained even in the absence of outer segments, based on the plasma membrane's opsin content. Regression of outer segment membranes is compensated by the increase in the number of pinealocytes in more

differentiated species' large and folded pineal organ ('folded retina'). Similarly, the longer activation time of the photoreceptor pinopsin molecule, the more sensitive to the light it is.

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