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ULTRASTRUCTURAL AND MORPHOMETRIC STUDY OF THE PINEAL COMPLEX IN INDIAN MAJOR CARP *CATLA CATLA* IN RESPONSE TO CONTINUOUS LIGHT AND DARKNESS

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SUMMARY

The present communication deals with the ultrastructural and morphometric study of the pineal complex in a hitherto unstudied Indian major carp *Catla catla*. In this fresh water teleost, the pineal complex is a discrete structure composed of a distal 'end vesicle' (EV), a long connecting 'pineal stalk' (PS), and a convoluted 'dorsal sac' (DS). Light microscopic study demonstrated that the parenchyma of the EV contains two types of cells, *viz.*, 'light cells', and 'dark cells'; of which only 'light cells' exhibit significant annual cyclic variations in the nuclear diameter. Consequently, attempts have been made to employ transmission electron microscopy for identification and characterization of the photosensory cellular components of the pineal complex in the carps following exposure to continuous illumination (LL; 24L) or continuous darkness (DD : 24D) for 30 days during the different phases of an annual reproductive cycle. The study also indicated the presence of two types of cells which shared the features of photoreceptor- and supporting cells in the EV of the pineal complex respectively. Significant changes were noted in the size and shape of various sub-cellular organelles in the photoreceptor-, but not in the supporting cells of the pineal EV following exposure to LL or DD schedules. While an increase in the nuclear diameter was noted in the photoreceptor cells of EV in the carp held under DD in each part of the annual gonadal cycle, significant regressive changes occurred in the photoreceptor cells of the EV in LL group of fish but only during the preparatory and the pre-spawning phases of theannual cycle. Commonly, it was found that an increase in the number of secretory granules, synaptic vesicles and synaptic ribbons, and hypertrophy of mitochondria occurred in the pinealocytes of carps held under DD, while regressive changes in the parenchymal cells of EV resulted in the pineal complex following exposure to LL. It was interesting to note that morphometric and ultrastructural changes in response to altered lighting conditions were demonstrable only in the EV, but not in the PS and DS of the pineal complex. Thus, it appeared conclusive that the pineal complex in *Catla catla* is directly associated with photoreception, and the EV is the only part of pineal complex that performs the alleged function. However, the etiology of differential responsiveness of the EV photoreceptor cells to LL in different parts of annual reproductive functions remains unknown.

Key words : Carp; *Catla catla;* Light; Darkness; Pineal complex; Ultrastructure.

INTRODUCTION

The pineal organ in lower vertebrates, in general, and in teleosts in particular, is a complex structure showing wide range of variations in structure and functions in

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relation to habit, habitat, and often phylogenetic status of the fish (1-3). India is quite rich in faunal diversity of fresh-water fishes. But available information on the structure and functions of pineal complex in different fishes in Indian sub-continent is frustratingly poor. Moreover, the studies so far undertaken on the pineal organ in Indian fish are not only very few, but also restricted mostly to their anatomy. It was quite surprising to note that none of the Indian major carps, despite tremendous economic importance, received any attention for the study of its pineal complex. A major breakthrough was the recent study on morpho-anatomy and structural organization of pineal complex in a surface dwelling Indian major carp *Catla catla* (4-6). The present communication is an attempt to employ transmission electron microscopy for identification and characterization of the photosensory cellular components of the pineal complex in the same species of carp following exposure to continuous illumination or to continuous darkness.

Morpho-anatomy of the pineal complex in *catla catla.*

Our recent studies (4-6) have shown that the pineal complex in *Catla catla* comprises of a distal End Vesicle (EV) and a long Pineal Stalk (PS) connecting the EV to the brain at the diencephalic region. The EV adhered to the ventral part of the parietal bone in a shallow depression (Fig. 1) behind the nasal orifice in straight line with the lateral eyes, perpendicular to the antero-posterior axis of the

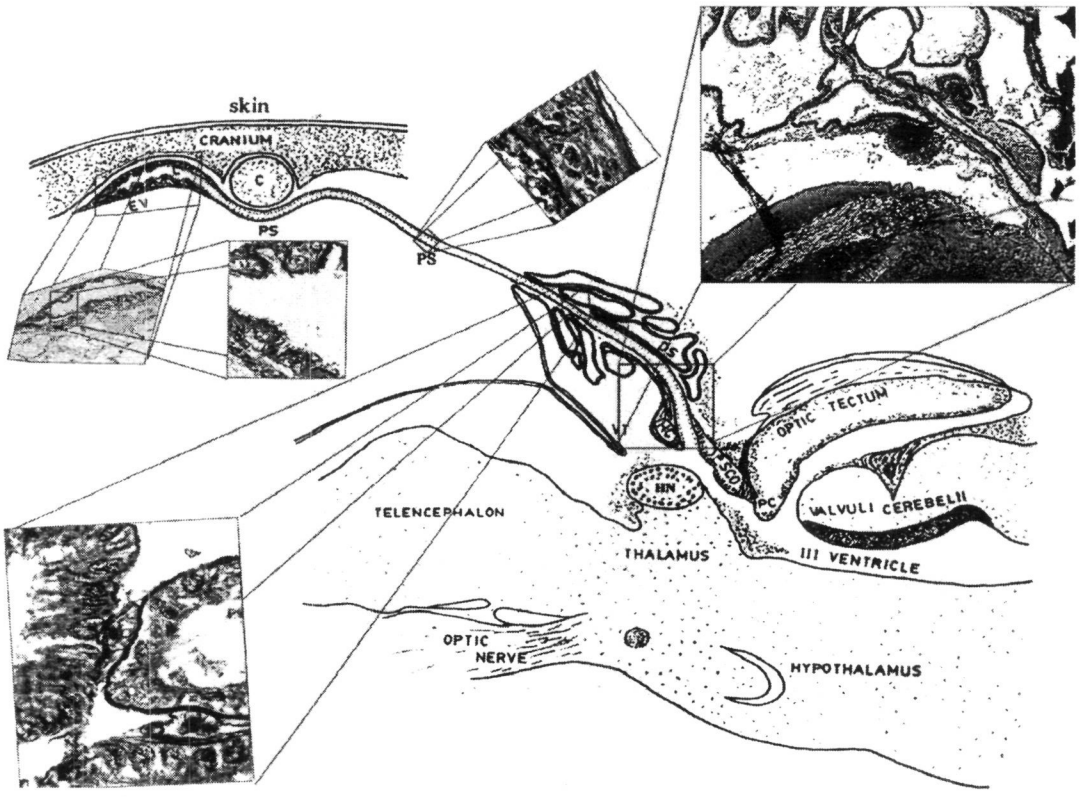


Fig.1. Schematic diagram representing the morpho-anatomy of the pineal complex and histology of their components (shown in insets) in *Catla catla.* A. Section of the End Vesicle; B. Section of the pineal stalk; C. Section of the Dorsal Sac; D. Section of the part of brain showing the opening of the pineal stalk into the Ilird ventricle. Legends: C - Cartilage, DS - Dorsal sac, EV - End vesicle, HC - Habenular commisure, HN - Habernular nucleus, PS - Pineal stalk, SCO-Sub-commisural organ, VT - Velum transversum.

skull. The thin cranial roof of the skull just above the pineal complex remained covered by thin dermis and epidermis. In an adult healthy fish, the PS (measuring about 1-1.2 cm in length) was shown to pass through the dorsal sac before entering into the brain. The parapineal organ, as described in most fish (1) was found to be absent in *Catla catla,* but a prominent Dorsal Sac (DS) was observed at the diencephalic roof of the brain.

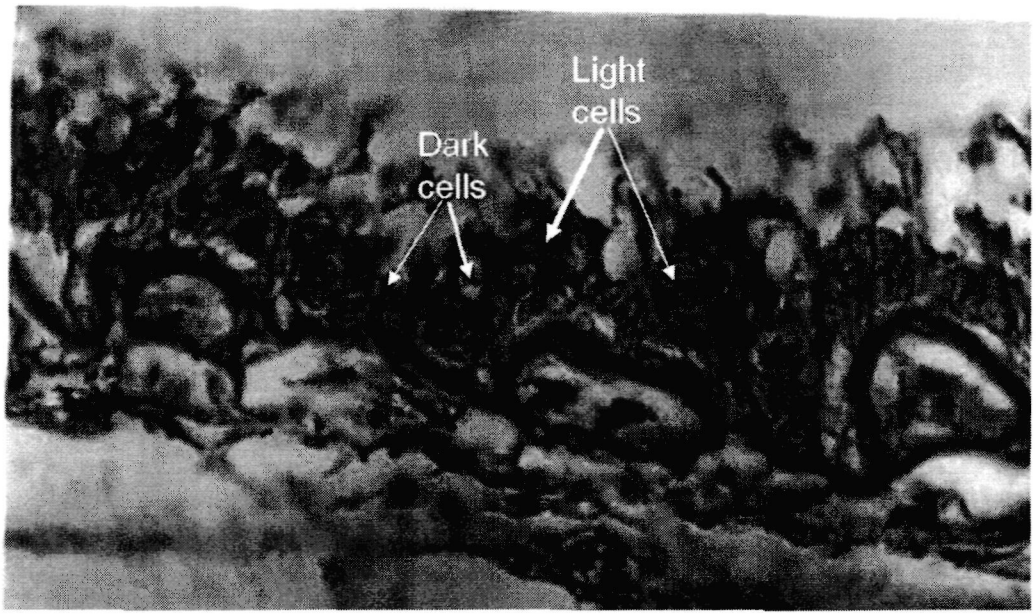


Fig. 2. Photomicrograph of the Masson's trichrome-stained section of ventral part of end vesicle of the pineal complex *of Catla catla* showing the 'light cells' at the luminal side and the 'dark cells' aggregated towards the basal lamina propria. X 400

Cellular organization of the pineal complex

At light microscopic level, the EV parenchyma presented a highly folded epithelial structure showing extensive vascularization. Tinctorially two types of cells, designated as 'light cells' (LC) and 'dark cells' (DC), respectively, were identified in the EV epithelium following staining with Masson's trichrome. The LC were characterized by their large size, translucent cytoplasm, basally located round or oval vesicular nucleus, and an apical long club shaped cytoplasmic outgrowth extending into the pineal lumen (7) showing marked affinity to haematoxyliri (Fig. 2). These cells were aggregated at the luminal side of the EV epithelium. Structurally the LC resembled the photoreceptor cells, as described in the pineal complex of other teleosts of temperate zones (7, 8).

The dark cells, which were interspersed throughout the vesicular epithelium, were characterized by irregular shaped, small, strongly basophillic nucleus, and scanty indiscernible cytoplasm (Fig. 2). These cells, which were concentrated towards the lamina propria (Fig. 2), resembled the supporting or ependymal cells (7,8). In addition to the 'light cells' and 'dark cells', a few nerve cells were also found in the EV.

The pineal stalk or PS predominantly consisted of nerve cells and fibers. Transverse sections of the PS appeared circular with a central lumen surrounded by a more or less uniformly thick cellular layer measuring about 12-14 *yxn.* (Fig. IB). The pineal stalk could be traced to the habenular and posterior commissures at the left habenular nuclear and third ventricular area in the longitudinal sections of the brain. The lumen of the stalk was found to be in open communication with the IIIrd ventricle of the brain (Fig. ID).

Saccus Dorsalis or the Dorsal Sac (DS) appeared as an evagination from the outer region of the diencephalic part of brain. It was found to remain attached to the Velum Transversum in between cerebral hemisphere and the habenular commissure. At light microscopic level, the dorsal sac presented a convoluted structure made up of a single layer of columnar epithelial cells arranged in a single layer on the underlying stroma of lamina propria (Fig. 1C). The pineal stalk was found to pass through the dorsal sac before opening into the IIIrd ventricle, but a direct communication between the pineal stalk and the dorsal sac was lacking.

Ultrastructure of the cellular elements of the pineal complex

From morphological standpoint, the pineal complex of all the studied teleosts basically consists of nervous tissue with abundant photoreceptor cells which exhibit considerable resemblance with the retinal cells (9,10) and remain interspersed between nerve cells and interstitial or supporting cells. Schematically, three principal cells make up the pineal parenchyma: the photoreceptor cells which are synaptically linked to the second order neurons (which constitute bineuronal chains), glial or interstitial cells, and the supportive cells (11).

At ultrastructural level, the EV parenchyma of pineal complex in *Catla catla* also found to be consisted of two different types of cells. One type of cells resembled the typical retinal photoreceptor cells. These cells comprised of an outer segment, an inner segment, a cell body with prominent nuclei and a synaptic

Fig. 3. TEM of the part of a photoreceptor cell (x 15K, reduced to 80%) showing the outer segment (OS), inner segment (IS) and the ellipsoid (E) in the end vesicle of the pineal complex of *Catla catla.* The outer segment comprises of lamellae or discs arranged perpendicular to the photoreceptor axis (inset).

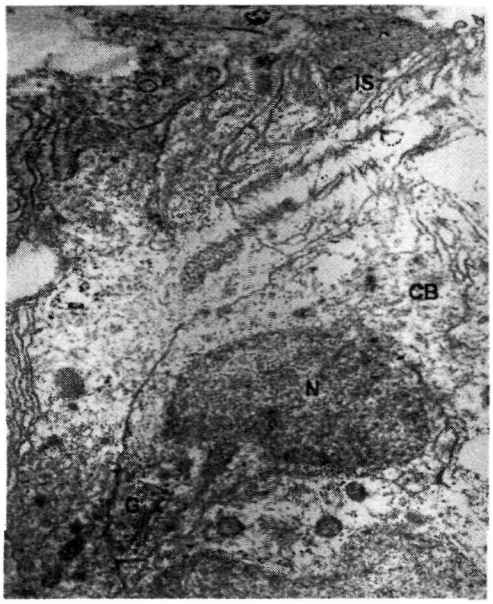


Fig. 4. TEM of a section of a photoreceptor cell (x 15K, reduced to 80%) in the end vesicle of the pineal complex of *Catla catla* showing part of the inner segment (IS), the cell body (CB) with the prominent nucleus (N) and the Golgi complex (G).

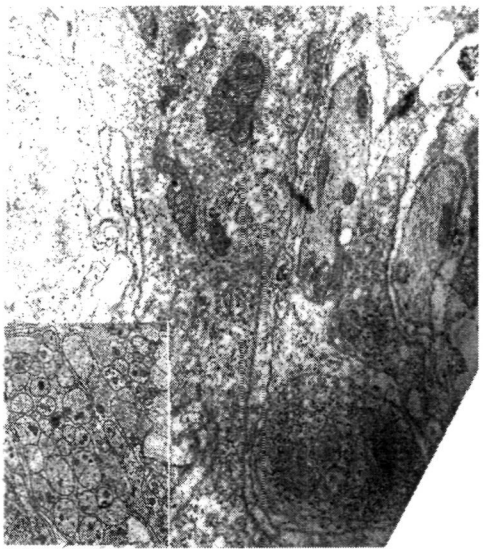


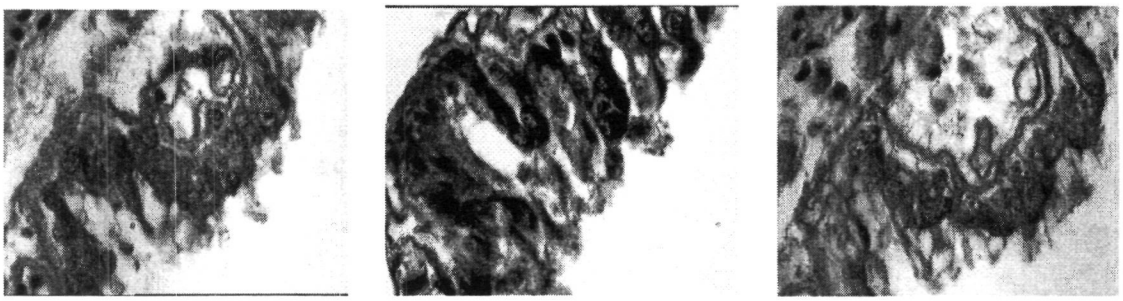
Fig. 5 TEM section of a photoreceptor cell (x 15 K, reduced to 80%) showing part of the synaptic pedicle containing the synaptic vesicle (inset) in the end vesicle of the pineal complex of *Catla catla.*

pedicle. The outer segment comprised of lamellae or discs arranged perpendicular to the photoreceptor axis at the luminal aspect of the EV (Fig. 3) and remained attached to the inner segment by a narrow cytoplasmic stalk, the centre of which is occupied by a cilium. The inner segment was characterized by the presence of an ellipsoid, consisting of accumulation of mitochondria intermingled with endoplasmic reticulum (Fig. 3). The cell body of these cells typically comprised of a weakly stained nucleus and a prominent Golgi apparatus (Fig. 4). It gave rise to a basal process called the synaptic pedicle characterized by the presence of synaptic vesicles (Fig. 5) and synaptic ribbons. Interspersed between the photoreceptor cells were the 'supporting cells'. These cells were found to be devoid of characteristic features of photoreceptor cells, and were concentrated at the basal aspect of the EV. The cell body of these cells consisted of a small irregularly shaped nucleus and dark cytoplasm.

The pineal stalk was found to be composed typically of nerve cells, glial cells and sympathetic nerve fibers. The dorsal sac comprised only of single layer of ciliated columnar epithelial cells on the lamina propria, as already noted under light microscope.

Pinealocyte response to continuous illumination and to continuous darkness

The pineal organ is generally considered as an important component of the photo-neuroendocrine system (2, 12) as it acts as a transducer of photic signals, responding to changing circumambient light by changing its rate of melatonin output, a conservative neuro-hormone in all the vertebrates. In teleosts, all the key functions of the photo-neuroendocrine system *i.e.* photoreception,



Control Continuous darkness (DD) Continuous illumination (LL)

Fig.6. Photomicrographs of the sections of end vesicle of the pineal complex of *Catla catla* showing the changes in the cellular morphology and in the shape and size of nucleus following exposure to continuous illuminations, or to continuous darkness compared to the features shown by the pineal complex in carp held under natural photoperiodic conditions (X 100).

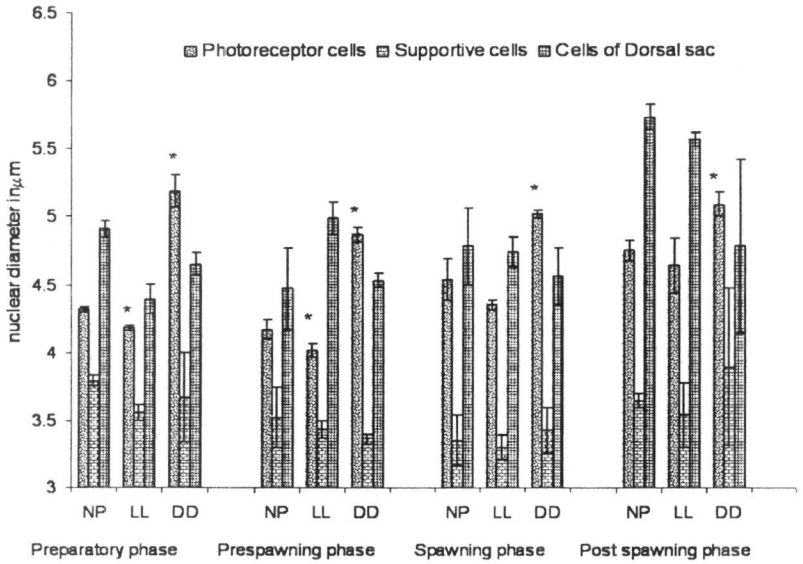


Fig. 7. Histogram representing mean values (± SE's in vertical bars) of nuclear diameter in photoreceptor and supportive cells of the end vesicle, and in the cells of dorsal sac in the pineal complex of *Catla catla* following exposure to NP, LL, or DD during the preparatory, pre-spawning, spawning and post-spawning phases in an annual cycle.

endogenous rhythm generation, and production of neuro-hormone are accomplished by a single type of 'photo-neuroendocrine cell' (2).

The present study was undertaken to characterize the photoreceptor cells in the pineal complex of hitherto unstudied Indian carp *Catla catla*. For this purpose, the carps of either sex were separately subjected to continuous

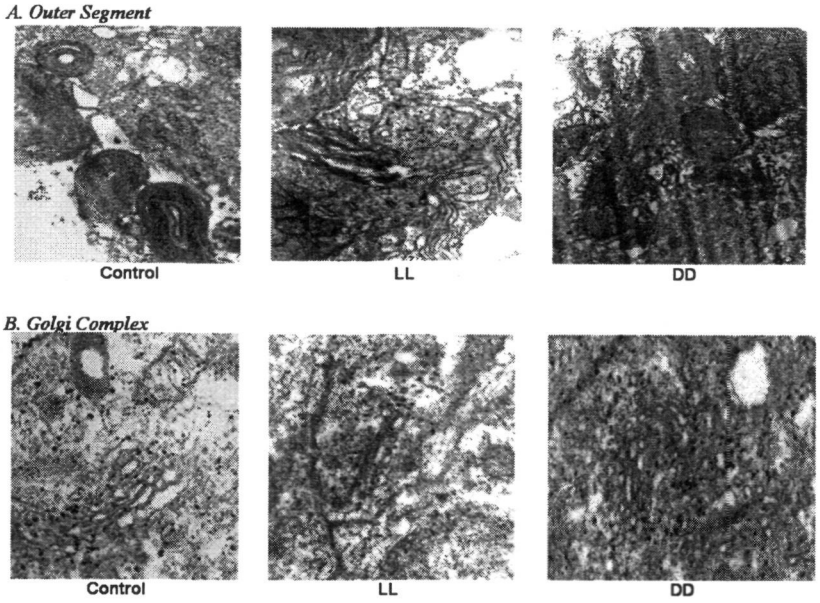


Fig. 8. TEM of the sections of end vesicle in the pineal complex of *Catla catla* held under NP, or LL, or DD showing responsiveness at the levels of outer segment (A) and Golgi complex (B) of the pinealocytes (X 15K, reduced to 80 %).

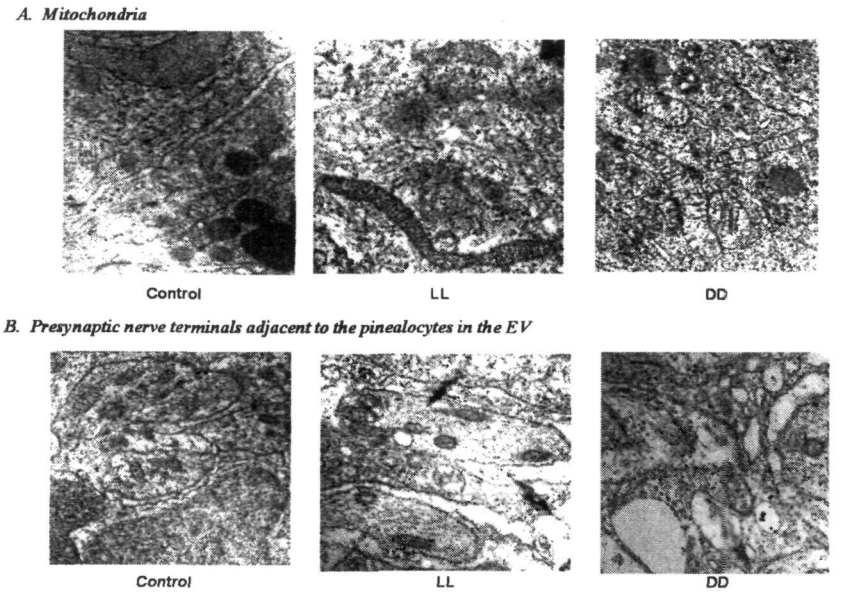


Fig. 9. TEM of the sections of end vesicle in the pineal complex of *Catla catla* held under NP, or LL, or DD showing responsiveness at the level of mitochondria (A) and pre-synaptic nerve terminals (B) (X 15K, reduced to 80 %).

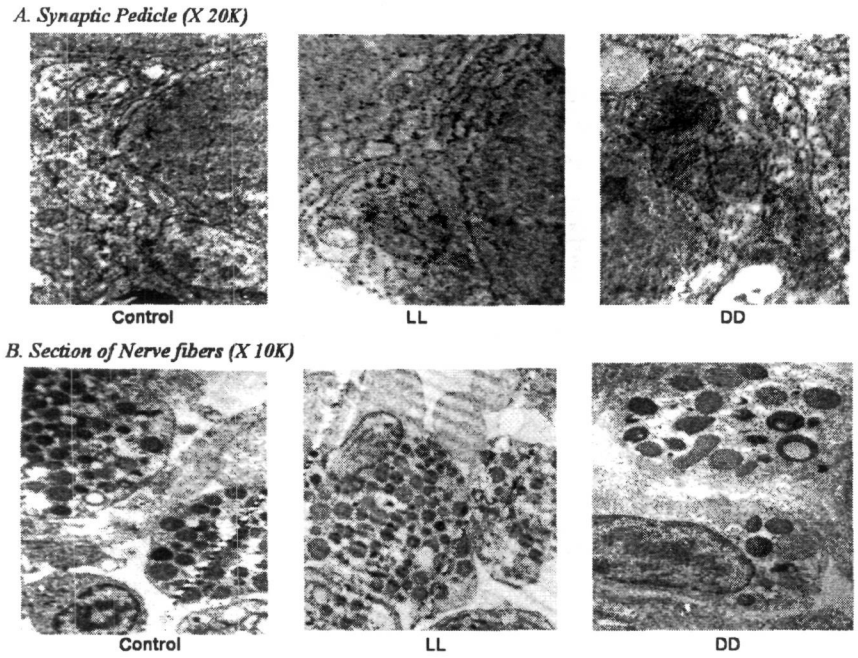


Fig. 10. TEM of the sections of end vesicle in the pineal complex of *Catla catla* held under NP, or LL, or DD showing responsiveness at the level of synaptic pedicle (A) and nerve fibres (B) (reduced to 80 %).

illuminations (LL) or to continuous darkness (DD) for 30 days, and the pinealocyte-response to such extreme light-dark regimens was evaluated comparing the ultrastructure of pinealocytes in carps maintained under natural photoperiods (NP; about 12L : 12D). An identical experimental regimen was followed during the pre-spawning, spawning, post-spawning and preparatory phases of the annual gonadal cycle (4).

The photoreceptor cells exhibited significant changes in the diameter of the nuclei and in the morphology of subcellular organelle following exposure to continuous LL or DD (Fig. 6). While a significant increase in the values of nuclear diameter of the photoreceptor cells was noted in the pineal complex of fish held under DD schedule in each reproductive phase, the diameter of nucleus in the photoreceptor cells became significantly decreased following exposure of fish to LL during the preparatory and pre-spawning phases, but not other phases, of the annual gonadal cycle (Fig. 7).

The morphological changes, which were noted in the pinealocytes of LL fish, at sub-cellular level, include decrease in the size of the outer segments of the photoreceptor cells, an increase in the number of vacuoles, decrease in the size of Golgi body and mitochondria (Fig. 8, 9A), and development of wide perinuclear space. On the contrary, an increase in the size of outer segments, Golgi body and mitochondria, and an increase in the number of secretory vesicles in synaptic pedicles (Fig. 8, 9A, 10A) were observed in the photoreceptors cells of the EV in the DD fish.

Earlier studies on other teleosts of high latitudes have indicated that the pineal complex in fish, in general, is directly photosensitive (13) and it elaborates the photoperiodic information via melatoninergic signals. The present study, thus showing notable changes in the size of the outer segment and the cellular organelles following exposure to diverse light-dark schedules provide persuasive support to the contention that the pinealocytes in the EV of pineal complex in *Catla*

catla share the features of typical photoreceptor cells reported with other teleosts from temperate zones.

The enlarged mitochondria with distended cristae and wide lumen, and the increased volume of Golgi complex in the pinealocytes of DD fish may be considered as the indices of an increased metabolic activity of the concerned cells. While the increase in the volume of the Golgi complex has been found to be related to active synthesis (14), the enlargement of the mitochondria is suggested as an indication of hydroxylation of tryptophan to 5-hydroxytryptophan leading to the formation of serotonin which occurs freely in the cytosol (15). Serotonin is known to be the precursor of the major pineal hormone melatonin. Studies have shown that the production and secretion of melatonin by the pineal organ is highest during the daily dark period and lowest during the daytime (16). Moreover, the secretory activity of the pineal organ in majority of fishes is known to be completely suppressed under the influence of constant illuminations (17). Thus, present study showing decreases in the size of mitochondria, in the volume of Golgi complex, and in the diameter of the nuclei in the pinealocytes of LL held *Catla catla* suggests inhibition of the cellular activities of pinealocytes under given conditions.

The features showing increases in the number of secretory vesicles in the synaptic pedicle of the photoreceptor cells of DD fish are generally considered as the indices of an increase in the neural activity under the influence of continuous darkness. Moreover, pre-synaptic nerve terminals associated with the pinealocytes exhibited signs of stimulation in the fish held under DD schedules, and signs of regression in LL fish (Fig.9B). Similar changes were also noted in the ultrastructure of nerve fibers in the EV of pineal complex in *Catla catla* following exposure to LL or DD (Fig. 10B). This study thus supports the conjecture that continuous darkness is stimulatory and continuous light is inhibitory to the functions of photoreceptor cells in the pineal complex of *Catla catla* (5).

CONCLUSION

Collectively, our study indicates that the photoreceptor cells in the EV of the pineal complex of *Catla catla* are associated with the transduction of light signals. These cells may also be the chief secretory cells of the pineal complex, secreting pineal hormones. It was also noted that the influences of DD on the pineal complex do not vary in relation to the reproductive status of the fish in an annual cycle. However, changes in photoreceptor cells of the pineal complex in response to continuous illuminations were noted to be restricted only to the preparatory and pre-spawning phases, with no changes during the remaining phases of the annual reproductive cycle. The etiology of such phenomena remains unknown. It deserves special mention that the morphology of the supporting cells of the EV in the pineal complex of various experimental groups of fish remained almost unaltered irrespective of the photo-schedules to which they were held, signifying the fact that the concerned cells may not have any function whatsoever in the transduction of light signals. Nonetheless, further studies are required to justify their physiological importance.

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