International Journal of Engineering, Science and Mathematics
Vol. 7, Special Issue 4(1), April 2018,
ISSN: 2320-0294 Impact Factor: 6.765
Journal Homepage: http://www.ijmra.us, Email: editorijmie@gmail.com
Double-Blind Peer Reviewed Refereed Open Access International Journal - Included in the International Serial Directories Indexed & Listed at: Ulrich's Periodicals Directory ©, U.S.A., Open J-Gage as well as in Cabell's Directories of Publishing Opportunities, U.S.A

Pineal Photoreceptor Molecules in Indian Major Carp *Catla catla*

Mohua Seth*

Abstract

In vertebrates, light is a major component of environment that regulates a large number of physiological and behavioural functions in rhythmic fashion. The present study shows the localization, dynamics and regulation of photoreceptor proteins namely pineal rod-like opsin and α-transducin in the pineal organ of Indian major carp *Catla catla*. The study showing diurnal rhythmic pattern in their expression with a peak at midday and fall at midnight reveals that the pineal organ may act as a transducer of the photic signal in carp. Notably, this rhythmicity has been dampened out following the exposure of fish to continuous illumination (LL) or continuous darkness (DD) as the expression of these photoreceptor proteins irrespective of the clock hours is higher in LL and lower in DD fish. In addition *in vitro* demonstration of the possible roles of different neuronal signals in the photic regulation of the expression of photoreceptor molecules in the carp pineal organ shows that cholinergic signals play a stimulatory role in the expression of both the studied photoreceptor proteins whereas the dopaminergic signals have an inhibitory influence.

Keywords:

Carp;
Pineal rod-like opsin;
α-Transducin;
Cholinergic signals;
Dopaminergic signals;

Author correspondence:

Dr. Mohua Seth
Department of Physiology, HMM College for Women,
Dakhineswar, Kolkata-700035, West Bengal, India
Email: mohuaseth@gmail.com

1. Introduction

The pineal organ or the "epiphysis cerebri" has been described as the greatest mystery in the physiology of animals. The structural and functional diversities of pineal in vertebrates have been provided high degree of evolutionary and adaptive plasticity (1). It serves sensory and/or neuroendocrine function depending on the vertebrate class. In reptiles and birds, it has mixed photoreceptive and secretory functions, but in fish it is an organ of direct photoreception and is regarded as a rudimentary third eye (2).

The environmental light has a major influence on the physiology of animals. Over the decades, attempts are made to understand the physiological mechanism in processing environmental light information in different groups of animals. In fish, the pineal organ is considered as the most influential extra-retinal light receptor. The pineal organ in teleosts is directly photosensitive in nature. Earlier immunocytochemical studies indicated that pineal photoreceptors share many molecular features with retinal photoreceptors and photosensitivity is provided by the photoreceptor molecules (like opsin, α subunit of transducin, recoverin and arrestin) which are present in the photoreceptor cells of the pineal organ in various teleosts (3). Despite this apparent homogeneity, the mechanisms by which the photic information is processed have been the matter of greatest interest. The importance of such information is very much appreciated and well proclaimed in the field of aquaculture.

An analysis of the existing literature clearly reflects an inadequacy of data on the influences of environmental light-dark conditions on the synthesis of photoreceptor molecules in the teleosts in general, and in any carp species in particular. Likewise, the neuronal mechanisms of photic signal transduction in the pineal organ in any low-latitude fish species remains unknown. The situation is poignant with regard to the

* Faculty, Department of Physiology, HMM College For Women, Dakhineswar, Kolkata-35, West Bengal, India.

Indian major carp *Catla catla*, which because of its natural surface dwelling habit maintains close contact with the environmental light, and has tremendous economic importance as the most preferred table fish in India. Therefore, an attempt has been made in the present treatise to gather basic information on the molecular mechanisms of photoreception in the pineal organ of a hitherto unstudied Indian major carp *Catla catla* that inhabits the sub-tropical zone (Lat. 23°39′ N., Long. 87°42′ E) in India. In *Catla catla*, the pineal complex consists of an antero-laterally elongated, dorso-ventrally flattened vesicular part, the end-vesicle and a very long posteriorly-directed pineal stalk , which is encircled inferiorly at the deeper level by highly folded plexiform saccular dorsal sac (4). Precisely, this investigative treatise embodies original findings on the localization and dynamics of photoreceptor molecules in the pineal organ of this free-living carp. An extensive *in vitro* study has been carried out to demonstrate the regulatory mechanisms of the expression of these proteins emphasizing the role of light-dark conditions and the neuronal signals in photo-signal transduction in the carp pineal.

2. Research Method

Adult *Catla catla* weighing in between 800 gm to 1000gm were collected from commercial sources and brought to the laboratory where they were maintained in large cement tanks with food and water *ad libitum* for acclimatization to laboratory conditions for a week. The pineal end vesicle from different freshly decapitated fish was collected at four different time points (corresponding to 06.00, 12.00, 18.00, and 24.00 clock hours) in a 24h cycle. The immunocytochemical localization of rod like opsin and α- transducin in the pineal end vesicle and the WB analysis of rod like opsin and α -transducin in pineal homogenates were done (5,6). The mouse monoclonal Anti-Opsin IgG (1:1000 dilution) ; and the rabbit Anti-Gt alpha (1:1000 dilution) (both purchased from Sigma Chemical Co. St. Louis, MO, USA) were used as primary antibodies for the immunocytochemical and WB analysis of rod like opsin and G protein transducin respectively. In addition in Western blot the membranes were separately incubated with the respective secondary antibody (Goat anti-mouse IgG-alkaline phosphatase conjugated for rod like opsin and Goat anti-rabbit IgG- alkaline phosphatase conjugated for G protein transducin purchased from Bangalore Genei Pvt. Ltd., Bangalore, India) at a dilution of 1:500 and the bands were visualized by treating the membrane with BCIP/NBT.

To resolve the question whether rhythmic functions of the photoreceptor molecules are driven by the endogenous circadian clock or solely by the environmental light signals independent of the clock, the adult carps weighing in between 800 gm to 1000gm were divided into three groups (each group containing 8 to 10 fishes) and exposed to either: (a) natural photoperiods (NP; varied in between 10-13h of light and 11-14h of darkness), or (b) continuous illumination (LL; 24L:0D), or (c) continuous darkness (DD; 0L:24D) for 10 days. Subsequently, the pineal tissues from each fish group were collected separately at each of the four different time points (corresponding to 06.00, 12.00, 18.00, and 24.00 clock hours) in a 24h cycle. The expression of the pineal rod-like opsin and α-transducin was studied following Western blot analysis of the collected pineal homogenates.

In each part of the study, the collection of sample during the dark phase was made under dim red light which does not influence the activity of the pineal gland (7).

The next part of investigation was aimed at *in vitro* demonstration of the possible roles of different neuronal signals in the photic regulation of the expression of photoreceptor molecules in the carp pineal organ. For this purpose, the adult *Catla catla* weighing in between 800gm to 1000gm were kept under continuous illumination for 10 days. After that the pineal organs were collected from these fish for organ culture study. The pineal organs were incubated in 1ml of RPMI 1640 culture medium in each well of the 24 well culture plates at 20±1°C in the presence of 95%O_2 and 5% CO_2 for 24h under the light (24L) either in the incubation medium alone (control), or separately in media containing any of the pharmacological agents which are known as specific agonists or antagonists of cholinergic and dopaminergic receptors. The illumination was provided with 20W white fluorescent lamp located within the incubator (8).

The following pharmacological agents were used separately at a specific dose in each of the 24 well culture plates. The drugs were dissolved in either sterile distilled water or ethanol according to their solubility.

a) Cholinergic Drugs: Each of the following cholinergic receptor agonists and antagonists were separately used at a dose of 100µM/ml (9):

i) Cholinergic Receptor Agonists
   1. Acetylcholine chloride (Ach)
   2. (±) Nicotine (Nicotinic Receptor Agonist)
   3. Oxotremorine M (Muscarinic Receptor Agonist)

ii) Cholinergic Receptor Antagonists:
   1. (+) Tubocurarine chloride hydrate (Nicotinic Receptor Antagonist)
   2. Atropine (Muscarinic Receptor Antagonist)

b) Dopaminergic Drugs: Each of the following dopaminergic receptor agonists and antagonists were separately used at a dose of 100µM/ml (10):

i) Dopaminergic Receptor Agonists
   1. Dopamine hydrochloride (DA)
   2. (±) SKF 38393 hydrochloride (D1 Receptor Agonist)

ii) Dopaminergic Receptor Antagonist
   R(+) SCH23390 hydrochloride (D1 Receptor Antagonist)

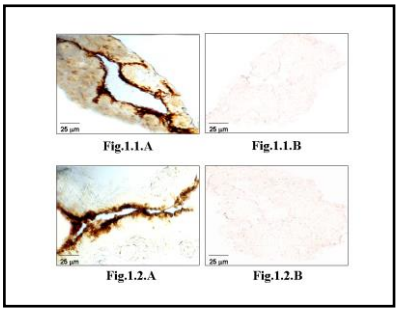
All the chemicals were purchased from Sigma Chemical Co. St. Louis, MO, USA.

At the end of the 24h light phase (24L), the cultured pineal organs were collected from each of the "control" and "treated" wells for further analysis. About 8-10 pineal EV collected from each well were homogenized together in 100mM Tris-Cl buffer (pH 7.2-7.4) containing 250mM sucrose, 0.1mM PMSF and 1% leupeptin hemisulphate and 20% homogenate was prepared. The pineal homogenate was then centrifuged at a low speed (1500 x g) for 10 min at 4°C. The initial pellets, which contained mostly unbroken cells and tissue debris, were discarded. The supernatant was collected and stored at -40^0C until used further for Western-blot analysis of photoreceptor proteins (5).

Statistical analysis of experimental data: The band intensities of pineal rod-like opsin and α-transducin were normalized by the intensity of β-actin (internal standard) in each sample and expressed as relative densitometric units (a ratio of the band intensities of rod-like opsin or α-transducin to β-actin for each sample) (11) by Image J software and expressed in line- diagram or bar diagram as Arbitrary Densitometric Units. Mean ± SE values of the relative densitometric data of each of the studied variables i.e., rod-like opsin and α-transducin immunoblot were also calculated and analyzed by one way analysis of variance (ANOVA), where F values indicated significance, and means were compared by a post-hoc multiple range test with $p<0.05$ taken as the statistically significant threshold.

3. Results

The immunocytochemical study clearly demonstrated the localization of both rod-like opsin and α subunit of the G protein transducin in the peripheral part of the end vesicle of the carp pineal organ (Figures 1.1-1.2).



Figures 1.1 – 1.2 : Photomicrographs of cryo-sections of the end vesicle of the pineal complex in *Catla catla* showing immuno-cytochemical localization of rod-like opsin (1.1A), and α-Transducin or α-TD (1.2A), while no positive reactions are detected in the corresponding control sections which were prepared in an identical manner but incubated in a medium without primary antibody of rod-like opsin (1.1B) or α-transducin (1.2B) respectively.

The Western-blot analysis of the rod-like opsin at each sampling point revealed four distinct bands, a closely spaced doublet of 39kDa and bands of 78 and 115kDa, and the immunoblot data of the α-transducin revealed two bands, one at 43kDa and another in the region of about 65kDa (Figures 2-3).

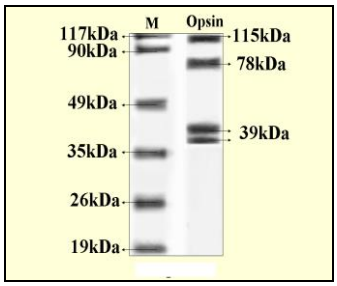


Figure 2: Western blot of rod-like opsin protein in the pineal organ of *Catla catla.* M' denotes the lane of pre-stained molecular weight marker.

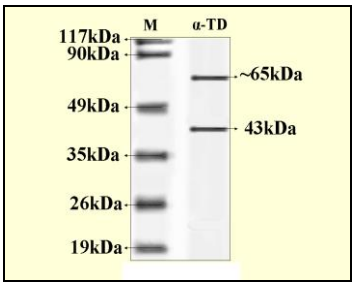


Figure 3: Western blot of α-transducin protein in the pineal organ of *Catla catla*. 'M' denotes the lane of pre-stained molecular weight marker.

Both the pineal photoreceptor molecules underwent diurnal variations with a peak at midday (12.00h) and fall at midnight (24.00h) (Figure 4).

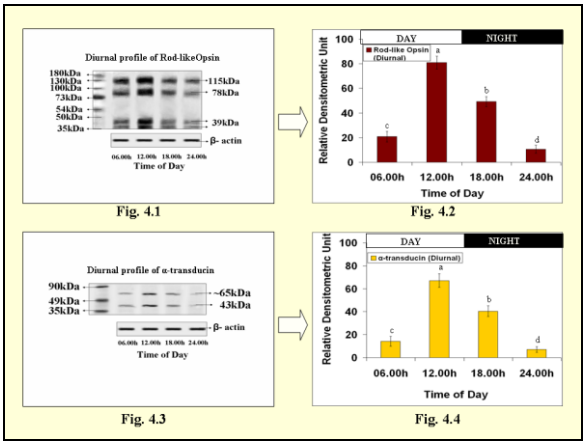


Figure 4: The representative immunoblot of rod-like opsin (Fig.4.1) and the mean values (± SE in vertical bars) of its relative densitometric data (Fig.4.2) showing the diurnal profiles (corresponding to 06.00h, 12.00h, 18.00h, and 24.00h) with maximum expression, compared to the expression of β-actin (in the lower panel), in midday (12.00h). Different alphabets in small scripts on the vertical bars indicate significant ($F=264.99$; $p<0.001$) difference between the sampling points following one way ANOVA (n=6).

The representative immunoblot of α-transducin (Fig.4.3) and the mean values (± SE in line diagram) of its relative densitometric data (Fig.4.4) showing the diurnal profiles (corresponding to 06.00h, 12.00h, 18.00h, and 24.00h) with maximum expression, compared to the expression of β-actin (in the lower panel), in midday (12.00h). Different alphabets in small scripts on the vertical bars indicate significant ($F=206.56$; $p<0.001$) difference between the sampling points following one way ANOVA (n=6).

Under altered photoperiodic condition it has been found that, irrespective of the clock hours in a 24h cycle, the expression of both pineal rod-like opsin (Figure 5) and α-transducin (Figure 6) remained high under LL and low under DD rather than showing a diurnal rhythmicity in their expression as noted in the pineal of NP fish.

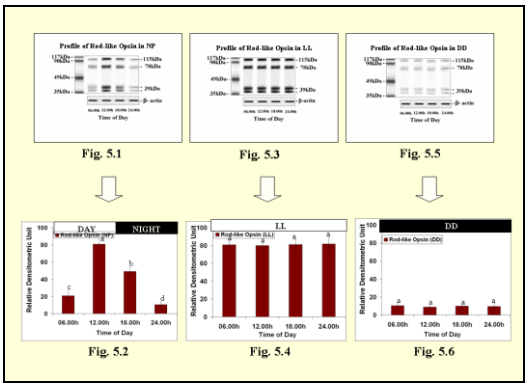


Figure 5: Western blot analysis of rod-like opsin in the pineal organs collected at four different time points (06.00h, 12.00h, 18.00h, and 24.00h) in a 24h cycle from the carp held under different light-dark conditions.

5.1and 5.2: The representative immunoblot of rod-like opsin in the pineal (Fig..5.1) and the mean values (± SE in vertical bars) of its relative densitometric data (Fig..5.2) showing the diurnal profile in natural photoperiodic (NP) fish with maximum expression, compared to the expression of β-actin (in the lower

panel), in midday (12.00h). Different superscripts on the bars indicate significant (F=264.99; p<0.001) difference between the sampling points following one way ANOVA (n=6).

5.3 and 5.4: The immunoblot of rod-like opsin (Fig. 5.3) and the mean values (± SE in vertical bars) of its relative densitometric data (Fig.5.4) in the pineal organ of carp held under constant illumination (LL) showing high expression compared to the expression of β-actin (in the lower panel), at each studied time points in a diurnal cycle. Identical superscript on the bars indicates that the variations between the sampling points are not statistically significant (F=0.178; p=0.908) following one way ANOVA (n=6).

5.5 and 5.6: The immunoblot of rod-like opsin (Fig. 5.5) and the mean values (± SE in vertical bars) of its relative densitometric data (Fig.5.6) in the pineal organ of carp held under constant darkness (DD) showing low expression compared to the expression of β-actin (in the lower panel), at each studied time points in a diurnal cycle. Identical superscript on the bars indicates that the variations between the sampling points are not statistically significant (F=0.195; p=0.897) following one way ANOVA (n=6).

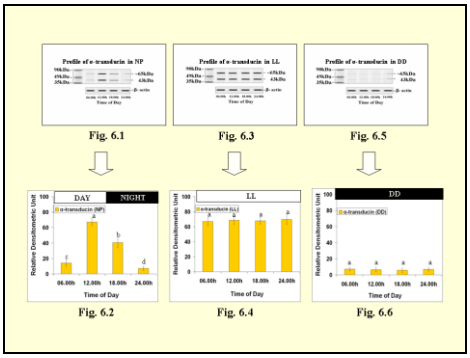


Figure 6: Western blot analysis of α subunit of the G protein transducin (α-TD) in the pineal organs collected at four different time points (06.00h, 12.00h, 18.00h, and 24.00h) in a 24h cycle from the carp held under different light-dark conditions.

6.1 and 6.2: The representative immunoblot of α-TD (Fig.6.1) and the mean values (± SE in vertical bars) of its relative densitometric data (Fig.6.2) showing the diurnal profile in natural photoperiodic (NP) fish with maximum expression, compared to the expression of β-actin (in the lower panel), in midday (12.00h). Different superscripts on the bars indicate significant (F=206.56; p<0.001) difference between the sampling points following one way ANOVA (n=6).

6.3 and 6.4: The immunoblot of α-TD (Fig.6.3) and the mean values (± SE in vertical bars) of its relative densitometric data (Fig.6.4) in the pineal organ of carp held under constant illumination (LL) showing high expression compared to the expression of β-actin (in the lower panel), at each studied time points in a diurnal cycle. Identical superscript on the bars indicates that the variations between the sampling points are not statistically significant (F=0.385; p=0.767) following one way ANOVA (n=6).

6.5 and 6.6: The immunoblot of α-TD (Fig.6.4) and the mean values (± SE in vertical bars) of its relative densitometric data (Fig.6.6) in the pineal organ of carp held under constant darkness (DD) showing low expression compared to the expression of β-actin (in the lower panel), at each studied time points in a diurnal cycle. Identical superscript on the bars indicates that the variations between the sampling points are not statistically significant (F=0.127; p=0.942) following one way ANOVA (n=6).

In *in vitro* study the analysis of the relative densitometric data of pineal rod-like opsin and α-transducin (α-TD) showed that incubation with acetylcholine (Ach;100µM /ml) or agonists of its nicotinic (NicAg;100µM/ml) or muscarinic (Mus Ag;100µM/ml) receptors, or a combination of NicAg (100µM/ml) and MusAg (100µM/ml) resulted in significant increase in the expression of opsin (F=1320.63; p<0.001) and α-TD (F=1775.66; p<0.001) relative to respective values in the samples incubated with nicotinic (NicAnt;100µM/ml) or muscarinic (MusAnt;100µM/ml) receptors antagonist. No expression of rod-like opsin or α-TD was found in presence of both NicAnt (100µM/ml) and MusAnt (100µM/ml) (Figure 7).

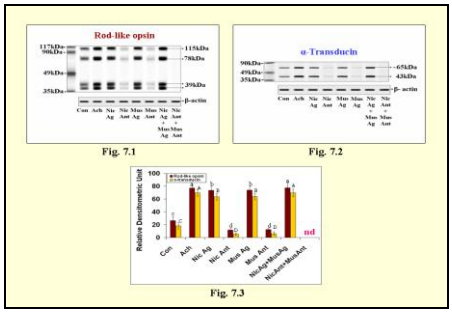


Figure 7: Cholinergic (Ach) signal mediated expression of photoreceptor proteins following incubation of the carp pineal organs with (i) Ach (ii) NicAg (iii) NicAnt (iv) MusAg (v) MusAnt (vi) NicAg and MusAg (vii) NicAnt and MusAnt.

7.1 , 7.2 and 7.3: The immunoblots of rod-like opsin (Fig. 7.1) and α-transducin (Fig. 7.2) and the mean (± SE in vertical bars) of their relative densitometric values (Fig. 7.3) in control and treated samples. In each sample, the expression of the proteins is compared separately to the expression of β-actin (in the lower panel). Different alphabets in small scripts and capital scripts on the bars indicate that the levels of rod-like opsin ($F=1320.63$; $p<0.001$) and α-transducin ($F=1775.66$; $p<0.001$) in different samples are statistically significant respectively following one-way ANOVA (n=6). Expressions of both of the photoreceptor proteins were not detectable (nd) in presence of NicAnt and MusAnt.

In contrast, incubation of pineal organs with dopamine (DA;100µM/ml) or its D1 receptor agonist (D1Ag;100µM/ml) significantly decreased the band intensities of both rod-like opsin ($F=638.24$; $p<0.001$) and α-TD ($F=645.56$; $p<0.001$) compared to those incubated with D1 receptor antagonist (D1Ant; 100µM/ml) (Figure 8).

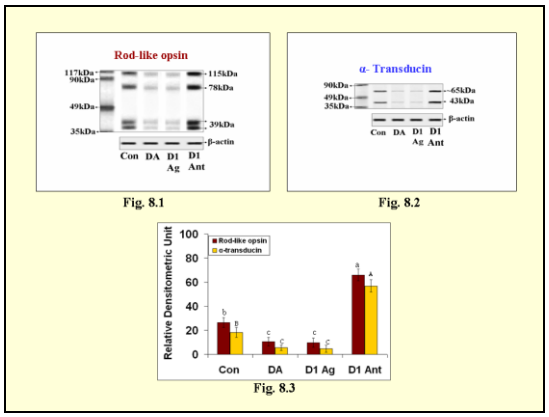


Figure 8: Dopaminergic (DA) signal mediated expression of photoreceptor proteins following incubation of the carp pineal organs with (i) DA (ii) D1Ag (iii) D1Ant.

8.1, 8.2 and 8.3: The immunoblots of rod-like opsin (Fig.8.1) and α-transducin (Fig. 8.2) and the mean (± SE in vertical bars) of their relative densitometric values (Fig. 8.3) in control and treated samples. In each case, the expression of the proteins is compared separately to the expression of β-actin (in the lower panel). Different alphabets in small scripts and capital scripts on the bars indicate that the levels of rod-like opsin ($F=638.24$; $p<0.001$) and α-transducin ($F=645.56$; $p<0.001$) in different samples are statistically significant respectively following one-way ANOVA (n=6).

4. Discussion

The present study demonstrated for the first time that the pineal organ in the Indian major carp *Catla catla* plays an important role in photoreception. Rod like opsin and α-transducin are the major photoreceptor molecules which are present in the peripheral part of the pineal end vesicle (Figures 1-3). These photoreceptor proteins exhibited a diurnal rhythm with a peak at midday and fall at midnight (Figure 4). The study showing dampened diurnal rhythms in the expression of pineal rod-like opsin and α-transducin in both LL and DD carps (Figure 5 and 6) provided the basis of a belief that the time bound changes in the profiles of the studied variables may be under the control of light-dark conditions, rather than the functions of endogenous circadian oscillator in the concerned pineal organ. In respect to the responsiveness mechanism to light-dark conditions, the studied features of pineal rod-like opsin and α-transducin in the carp share the characters of retinal photoreceptor proteins already reported in other fish species like zebrafish (*Danio rerio*) and other two teleosts (*Haplochromis burtoni*; *Astatotilapia burtoni*) (12-14). Accordingly, the study lends support to the suggestion that the studied molecules are important component of photoreception in the pineal organ in this species of teleost.

The pineal organ of teleost contains an impressive neuronal population. Neurones are postsynaptic to photoreceptors and their axons form the pineal tract that primarily innervates forebrain cell groups in the pre-tectum and thalamus, while smaller numbers project to the habenula, hypothalamus and pre-optic region. In fish, a large number of pinealocytes related to one large nerve cell shown in the pineal end vesicle may be a sign of a higher photosensitivity in this part of the pineal organ (15). A large population of AChE positive neurons have been identified in the pineal organ of the goldfish, *Carassius auratus* (16) . In teleosts like *Fugu pardale*, *Scomber japonica*, *Helicolenus hilgendorfi* and the shark *Triakis scyllia*, large multipolar cells are scattered throughout the pineal end-vesicle (17). In the pineal organ of pike, *Esox lucius*, AChE-positive nerve cells are distributed in the rostral and proximal part of the end-vesicle , whereas nerve cells are absent and the photosensitive structures of the pinealocytes are reduced in the intermediate and caudal parts of the end-vesicle (3). In the rainbow trout GABA ergic-large interneurons are present in the rostral end-vesicle, and small, centrally projecting neurons are present in the pineal stalk. The intermediate and caudal parts of

the end vesicle contain fairly evenly distributed AChE-positive pseudounipolar neurons, while the pineal stalk contains small, bipolar CSF-contacting neurons (3). Very small numbers of substance P-like immunoreactive unipolar neurons are present in the rostral end-vesicle (18). A population of small neurons immunoreactive for choline acetyltransferase (ChAT; the enzyme catalyzing synthesis of acetylcholine) is located in the dorsal wall of the rostral end-vesicle of the pineal in the minnow *Phoxinus phoxinus*, (3).CSF-contacting neurons have been identified in the pineal organ of different fish, such as, *Fugu niphobles*, *Scomber japonica* , *Fugu pardale*, *Chimaera monstrosa* (19).

Existing information on the neuronal and intracellular regulation of the synthesis of photoreceptor molecules is limited only to the retina of the higher vertebrates. No such studies were done in the pineal organ of any fish.The results of the present study depicted for the first time that both cholinergic and dopaminergic pathways are involved in the light-dependent expression of both rod-like opsin and α-transducin in the pineal organ in any fish. While cholinergic signals appeared to play a stimulatory role in the expression of both the studied photoreceptor proteins (Figure 7), the dopaminergic signals were found to have an inhibitory influence (Figure 8). The stimulatory effect of acetylcholine is mediated by both the nicotinic and muscarinic receptors. Whereas the inhibitory effect of dopamine is mediated by the D1 receptor. It is possible that both the stimulatory cholinergic effect and the inhibitory dopaminergic effect resulted respectively stimulated or inhibited photoinduced gene expression as well as the synthesis of the studied pineal photoreceptor proteins.

5. Conclusion

The study presented in the current treatise has provided basic but important information on the hitherto unknown aspects of the photoreceptive components of the pineal organ in a sub-tropical carp. This is the first report on localization, dynamics and regulation of photoreceptor proteins like pineal rod-like opsin and α-transducin in the pineal organ in any freshwater teleost. From the current study it is evident that photic condition of the environment is the most important element associated with the day-night rhythmicity in the synthesis of the photoreceptor molecules in the carp pineal rather than driven by the endogenous circadian clock. It is also important that role of the studied neuronal signals appeared diverse. The present study reports for the first time on role of cholinergic and dopaminergic signals in the regulation of the light mediated synthesis of photoreceptor molecules in the pineal organ in any fish in general, and in carp in particular. Nevertheless, there are certain gaps in the existing knowledge that need to be covered in future research. Some of the intriguing areas of further study may include: (a)search for a circadian clock, if at all present, in the carp pineal. (b) identification of specific genes associated with the expression of pineal photoreceptor molecules. (c) demonstration of the influences of different schedules of photoperiods on the expression of such genes in the pineal, and thereto the intracellular cascade mechanisms of photoreceptor molecule synthesis. (d) elaboration of the neuronal mechanisms involved in the photic signal transduction in the synthesis of photoreceptor proteins in the pineal organ in relation to the photoperiodic history and/or reproductive periodicity of the carp. (e) addressing the question whether the molecular mechanisms of photic signal transduction in the carp pineal organ and the retina are identical, if not, how do they differ from each other. In conclusion, the study included in the treatise led to the generation of several interesting and exciting data which provide a possible role of pineal photoreceptor molecules in the photic signal transduction mechanisms for the first time in any carp.

References

[1] Vigh, B., Manzano E Silva, M.J., Zadori, A., Frank, C.L., Lukats, A., Rohlich, P., Szel, A. and David C., "Nonvisual photoreceptors of the deep brain, pineal organs and retina,". *Histol Histopathol*, vol. 17, pp. 555-590, 2002.

[2] Vollrath, L. "The Pineal Organ", in *Handbuch der Mikroskopischen Anatomic des Menschen, Vol IV, part 10*,pp. 1-65, 1981.

[3] Ekstrom, P. and Meissl H., "The pineal organ of teleost fishes". *Rev Fish Biol Fisheries*, vol.7, pp.199-284, 1997.

[4] Bhattacharya, S., Dey, R., Basu, A., Maitra, S.K. and Banerji T.K., "The structure of the pineal complex in a common Indian teleost, *Catla catla*: Evidence for pineal-induced inhibition of testicular function within an annual reproductive cycle", *Endocrine Research*, vol. 29, pp.141-156, 2003.

[5] Sambrook, J., Fritsch, E.F. and Manniatis T., (1989) in *Molecular Cloning-A Laboratory Manual, Vol 1*, p 2.110, 1989.

[6] Tomas-Zapico, C., Boga, J.A., Caballero, B., Vega-Naredo, I., Sierra, V., Alvarez- Garcia, O., Tolivia, D., Rodriguez-Colunga, M.J. and Coto-Montes A., "Coexpression of MT_1 and $ROR\alpha_1$ melatonin receptors in the Syrian hamster Harderian gland", *J Pineal Res*, vol. 39, pp. 21-26, 2005.

[7] Maitra, S.K., Chattoraj, A. and Bhattacharyya S., "Implication of melatonin in oocyte maturation in Indian major carp *Catla catla*", *Fish Physiol Biochem*, vol. 31, pp. 201-207, 2005.

[8] Falcon, J., Galarneau, K.M., Weller, J.L., Ron, B., Chen, G., Coon, S.L. and Klein D.C., "Regulation of arylalkylamine *N*-acetyltransferase 2 (AANAT2, EC 2.3.1.87) in the fish pineal organ: evidence for a role of proteasomal proteolysis", *Endocrinology*, vol. 142, pp.1804–1813, 2001.

[9] Schomerus, C., Laedtke, E., Olcese, J., Weller, J.L., Klein, D.C. and Korf H.W., "Signal transduction and regulation of melatonin synthesis in bovine pinealocytes: impact of adrenergic, peptidergic and cholinergic stimuli", *Cell Tissue Res* , vol. 309, pp. 417–428, 2002.

[10] Santanavanich, C., Ebadi, M. and Govitrapong P., "Dopamine receptor activation in bovine pinealocyte via a cAMP-dependent transcription pathway", *J Pineal Res*, vol. 38, pp.170-175, 2005.

[11] Shao, R., Weijdegard, B., Fernandez-Rodriguez, J., Egecioglu, E., Zhu, C., Andersson, N., Thurin-Kjellberg, A., Bergh, C. and Billig H., "Ciliated epithelial-specific and regional-specific expression and regulation of estrogen receptor β2 in the fallopian tubes of immature rats: A possible mechanism of estrogen–mediated transport processs *in vivo*", *Am J Physiol Endocrinol Metab*, vol. 293, pp. E147-E158, 2007.

[12] Halstenberg, S., Lindgren, K.M., Samagh, S.P., Nadal-Vicens, M., Balt, S. and Fernald R.D., "Diurnal rhythm of cone opsin expression in the teleost fish *Haplochromis burtoni*", *Vis Neurosci*, vol. 22, pp.135–141, 2005.

[13] Li, P., Temple, S., Gao, Y., Haimberger, T.J., Hawryshyn, C.W. and Li L., "Circadian rhythms of behavioral cone sensitivity and long wavelength opsin mRNA expression: a correlation study in zebrafish", *J Exp Biol,* vol. 208, pp.497-504, 2005.

[14] Grone, B.P., Zhao, S., Chen, C. and Fernald R.D., "Localization and diurnal expression of melanopsin, vertebrate ancient opsin, and pituitary adenylate cyclase-activating peptide mRNA in a teleost retina", *J Biol Rhy* vol. 22, pp. 558-561, 2007.

[15] Ekstrom, P., Ostholm, T. and Holmqvist B., " Primary visual projections and pineal neural connections in fishes, amphibians and reptiles", in *Advances in Pineal Research, Vol 8*, pp 1–18, 1994.

[16] Wake, K., "Acetylcholinesterase-containing nerve cells and their distribution in the pineal organ of the goldfish, *Carassius auratus*", *Z Zellforsch*, vol. 145, pp. 287–298, 1973.

[17] Korf, H.W., "Acetylcholinesterase-positive neurons in the pineal and parapineal organs of the rainbow trout, *Salmo gairdneri* (with special reference to the pineal tract)", *Cell Tissue Res,* vol. 155, pp. 475-489, 1974.

[18] Ekstrom, P. and Korf, H.W., "Putative cholinergic elements in the photosensory pineal organ and retina of a teleost, *Phoxinus phoxinus* L. (Cyprinidae). Distribution of choline acetyltransferase immunoreactivity, acetylcholinesterase-positive elements, and pinealofugally projecting neurons", *Cell Tissue Res,* vol. 246, pp. 321–329, 1986.

[19] Ueck, M., Wake, K. and Kobayashi, H., "Nervous organization of the pineal complex in lower vertebrates", *Zool Sci,* vol. 6, pp. 817–831, 1989.