International Journal of Genetics and Genomics
2022; 10(1): 21-31
http://www.sciencepublishinggroup.com/j/ijgg
doi: 10.11648/j.ijgg.20221001.14
ISSN: 2376-7340 (Print); ISSN: 2376-7359 (Online)

Neuropsin (OPN5): A Non-visual UV Sensitive Photoreceptor Gene Expression Pattern Among Mega and Micro Bats

Steffi Christiane Ramesh[1], Subbian Baskaran[1], Uthandakalaipandian Ramesh[2], Thangavel Karuppudurai[1, *]

[1]Department of Animal Behaviour & Physiology, School of Biological Sciences, Madurai Kamaraj University, Madurai, India

[2]Department of Molecular Biology, School of Biological Sciences, Madurai Kamaraj University, Madurai, India

Email address:

tkdurai@gmail.com (T. Karuppudurai)

[*]Corresponding author

To cite this article:

Steffi Christiane Ramesh, Subbian Baskaran, Uthandakalaipandian Ramesh, Thangavel Karuppudurai. Neuropsin (OPN5): A Non-visual UV Sensitive Photoreceptor Gene Expression Pattern Among Mega and Micro Bats. *International Journal of Genetics and Genomics*. Vol. 10, No. 1, 2022, pp. 21-31. doi: 10.11648/j.ijgg.20221001.14

Received: January 20, 2022; **Accepted**: February 11, 2022; **Published**: February 19, 2022

Abstract: Bats are nocturnal animals with functional eyes and M/L and S opsin genes in the majority of the species. These genes are prerequisite for daylight, UV and dichromatic colour vision. Several studies suggest that other non-visual light-sensitive pigments are also involved in the UV light perception in animals. Recent behavioural, molecular and immunohistochemical evidence supports that the opsin-like gene, neuropsin (OPN5), is identified in humans, mice and birds, where it serves as a G protein-coupled UV-sensitive photoreceptor. Based on its low sequence homology with other opsin groups, OPN5 is classified as an independent group. While the roles of non-visual light-sensitive pigment OPN5 in bats remain an open question, here we report that bat's neuropsin (OPN5) encoded by OPN5 gene shares 89-96% amino acid identity and similar domain organization with human and mouse OPN5. By PCR amplification, we confirm that all the mega and micro bats express the OPN5 gene in their genome. The expression of OPN5 is detectable only in the brain, eye and retina and not in the heart, kidney, liver, lungs and testis. This result suggests that OPN5 gene expression is neural specific in bats. OPN5 gene expression level is significantly higher in tree-roosting bats compared to cave-roosting bats. Since, the tree-roosting bats received slightly more sunlight every day when compared to cave-roosting bats. In captive conditions, the expression levels of OPN5 in the neural tissues are significantly lower than those of wild bats. Our preliminary results suggest that the opsin-like gene, neuropsin (OPN5) is involved in UV light perception in bats.

Keywords: Bats, UV-vision, Opsin Genes, Non-visual Pigments, Neuropsin (OPN5)

1. Introduction

Most animals experience visual sensitivity due to the presence of photoreceptors in the eye's retina. These photoreceptors act as initiators of the vision process when a molecule of visual pigment absorbs a photon [1]. Vision plays a vital role in the animal kingdom, for example in mate selection, communication, foraging, predator avoidance and navigation [2]. Many animals employ light cues to achieve many physiological processes, including vision and circardian clock regulation, while light is likely the most crucial signal for acquiring the process of vision [3]. This innate response to light is due to the presence of rods and cones in the eye's retina. Rod and cone photoreceptors have light sensitive pigments called rhodopsin and iodopsin respectively, through which light is absorbed and converted into neural signals [4]. Color vision in terrestrial mammals is based on two spectrally different visual pigments located in two types of retinal cone photoreceptors: long/middle wavelength (L/M or red/green) opsin and a short wavelength (S or blue) opsin [5]. Besides color vision, UV vision plays major roles in guiding navigation and orientation, detecting food and potential predators, supporting high-level tasks such as mate assessment and intra-specific communication [6].

Most flying insects, birds and some mammals utilize sunlight's ultraviolet (UV) for environmental cues. UV visual pigments are made up of opsin proteins that detect light in conventional photoreceptors [6] and UV vision is mediated by rhodopsin, the visual pigment of both invertebrates and vertebrates. The existence of UV sensitive genes may be beneficial for flower discrimination and orientation/navigation in insects, as well as for mate selection and parent-offspring communication in birds [7-9]. UV-sensitive photoreceptors are present in the eyes of these animals consistently, and among the classical visual opsin genes, the short wavelength sensitive 1 (SWS1) is known to be responsible for UV vision in animals [10]. Among mammals, UV vision was initially thought to be rare in only a few species, such as bats, marsupials, eulipotyphlans and rodents. According to genomic sequencing, UV-sensitive SWS1 pigment, which is responsible for UV perception, is more broadly spread in mammals, with violet sensitive pigments being derived several times [11].

Opsins, the typical light sensing membrane proteins, weigh around 30-50 kDa and are responsible for visual and non-visual photoreception in animals [12]. Opsin based photosensitive pigments (opsin pigments) help many animals capture light information. The opsin pigment consists of a protein moiety, opsin, and a retinal chromophore. It evolved from a non-photosensitive G protein coupled receptor (GPCR) that binds to and activates a range of G proteins (guanine nucleotide-binding proteins) [3]. Opsins are heptahelical proteins with seven transmembrane helices that are connected by intra-cellular and extracellular loops. The chromophore binding site is found in the seventh helix of both cone and rod opsins, a region that has remained highly constant during divergent evolutionary history [13]. Opsins are not photosensitive by themselves, and it is only when they are combined with 11-*cis* retinal that they absorb visible light. Photoisomerization of 11 cis retinal to all trans-retinal occurs as a result of light absorption by retinal. This results in a conformational change in the protein moiety, which activates the G protein [12].

Bats are the most abundant and diverse order of mammals, with approximately 1,331 species divided into two suborders the Megachiroptera and the Microchiroptera [14]. They are found all over the world except the Artic and Antarctic regions, and they have a great diversity of habitat utilisation, behaviour, morphology, and nutrition [15]. Though bats are popular for their special sensory adaptations, the importance of vision has been overlooked due to the fact that most of them have small eyes [2, 16, 17]. Most bat species have functional eyes and functional M/L and S opsin genes, making them the only mammals that have evolved active flight and can navigate through complete darkness [18]. These genes are required for daylight, UV, and dichromatic colour vision. Various studies suggest that other non-visual light-sensitive pigments may potentially have a role in UV light perception in animals. Megachiropteran bats have fairly large eyes, while microchiropteran bats have small eyes with retinae that are dominated by rods [19]. Microbats, on the other hand, orient themselves in complete darkness using echolocation, and vision is essential for their flights between daytime roosts, feeding areas, and migration across greater distances [20-22]. Many bat species employ visual cues for long-range orientation and navigation at twilight and dawn, as well as on brightly moonlight nights. Predator avoidance and prey detection are also assisted by vision [15, 23].

Recent behavioural, molecular and immunohistochemical evidence supports that the opsin-like gene, neuropsin (OPN5), is identified in humans, mice and birds, where it acts as a G protein-coupled UV-sensitive photoreceptor. OPN5, a Gi- coupled UV sensor having similar properties to a bistable pigment, is one of the most recently identified opsin groups that is responsible for non visual photoreception in animals [24]. It has been reported that the OPN5 showed 25-30% amino acid identity with the other opsin families [25] and hence OPN5 is classified into a separate group based on its low sequence homology [24, 26, 27]. Mice and chicken OPN5 exhibit an absorption maximum of 380 and 360 nm, respectively, when reconstituted with 11 *cis* retinal. Most bat species use classical visual opsins for color vision [28]. In addition to visual opsins, they also utilize a non visual opsin that aids in UV vision. Recent research findings support that bats perceive UV and exhibit an absorption spectrum at 380 nm. It was reported that a non visual opsin like UV sensitive photoreceptor gene plays a role in the UV vision of mouse and human [25]. To our knowledge, no previous systematic study has examined the expression of non-classical opsin OPN5 in bats and also whether this gene plays a role in UV perception in bats. Therefore, the main objective of the study was to determine the non-visual UV sensitive photoreceptor gene (OPN5) expression pattern among mega and micro bats.

2. Materials and Methods

2.1. Bats Capture

The study was carried out between December 2016 and April 2017. The fruit eating bats, *Cynopterus sphinx* and *Pteropus giganteus* (Megachiropterans), were captured in and around Madurai, Tamil Nadu, South India (lat: 9° 58’ N; long: 78° 10’ E). Bats were captured using nylon mist nets of 9 m x 2.6 m with a mesh size of 38 mm (Avinet-Dryden, New York, USA). The mist nets were placed away from illuminated areas to avoid the bats' visual detection. Mist nets covered a height of up to 4 m from the ground level. The bats, which were trapped in the mist nets were removed immediately with gloved hands and placed in cloth bags [29].

In Madurai, the insectivorous bats (Microchiropterans) were mainly present in three different hill regions: the Samanar Hills in Keela kuyil kudi village, Thidiyan Hills, and the Panniyan Hills. Four species (*Megaderma lyra, Hipposideros speoris*, *Hipposideros bicolor*, and *Rhinopoma hardwickei)* of microchiropteran bats were captured from Panniyan Cave. The cave is situated in the Panniyan hill complex (09°58’N, 78°10’E), ca. 10 km NW of the MKU campus. It occupies the

southern slope of the hill, about 200 m above the adjacent plain. The cave mouth has a diameter of 2.4 m and faces the zenith. The internal passage goes on both west (up to 69 m) and east (ca. 62 m) from the entrance of the cave. Daylight penetrates to a distance of about 10 m on either side of the cave entrance. *R. hardwickii* roosts at the cave entrance, *H. speoris* occupies the middle part, *M. lyra* roosts deep inside, and *H. bicolor* exists in all parts of the cave. In addition, the cave has numerous labyrinthine ramifications, and the bats use several of them as their roosting sites. A stable temperature of 27 ± 0.5°C and a relative humidity of 85 ± 5% prevails inside the cave throughout the year.

2.2. Collection of Tissue and Blood Samples from Wild Bats

For the tissue excision (4 mm^2), a medical punch was used and, to avoid injury, care was taken to place the punch in an area between the blood vessels (wing membranes healed within 3-4 weeks) [30]. The punched hole and the punch was disinfected with 70 % ethanol after each sampling. There were no detrimental consequences on the bats' health as a result of this treatment. The tissue samples were collected and stored on ice, transported to the lab, and kept at -20 °C until DNA/RNA extraction [30-32].

2.3. Collection of Tissue Samples from Experimental Bats (Blocking the Sunlight)

To analyse the OPN5 gene expression pattern between wild and experimental conditions, the *C. sphinx* bats were captured using nylon mist nets in and around MKU Botanical Garden. The captured bats were released into a free flight room (3×5 m long × 2×4 m wide × 3×5 m height). Sunlight was blocked as it serves as the main source of UV rays. During the study period, the bats were provided with fruits (banana and guava) every night. The left over fruits and faeces were removed the following day at 7 AM. After two months, the bats were sacrificed using chloroform and their organs like lungs, liver, heart, kidney and testis, and neural tissues including brain, eye, and retina were harvested and stored at -80°C until RNA extraction. During the dissection of bats, we followed the Institutional Ethical and Bio-safety Committee Guidelines of Madurai Kamaraj University.

2.4. RNA Isolation and Tissue Expression of OPN5

Total RNA was isolated from ten different bat species using TriRNA reagent according to the manufacturer's instructions. Single stranded cDNA was synthesised using Thermo Scientific's Revert Aid First Strand cDNA Synthesis Kit. Primers were designed to amplify a 229 bp fragment (OPN5F: 5' TCATCTGGGCCTATGCTTCCT 3'; OPN5R: 5' TGGAGGAGGACTTAACCTTGG 3') in order to analyze the tissue expression pattern of OPN5 gene among different bat species. PCR was carried out in a 25 µl reaction containing 100 ng of template cDNA, 20 µl of 2X PCR buffer, 2 µl of 2 µM primers and 2 µl of H_2O. All DNA amplifications were performed using an Eppendorf PCR machine, with following cycling conditions: initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 58°C for 45 sec, extension at 72°C for 1 min, and final extension at 72°C for 10 min. The amplified products were eluted using a QIAquick gel extraction kit, and the eluted fragment was then sequenced (SciGenome, Kerala, India) using OPN5 primers. The sequences were analysed using the BioEdit Sequence Alignment Editor and the A Plasmid Editor (ApE) version 2.0.49.10.

2.5. Expression of OPN5 Gene Using Semi Quantitative RT-PCR

After reverse transcription, all the samples were diluted and used for polymerase chain reaction (PCR) with primer pair specific for *OPN5* and housekeeping gene *β*-actin to accurately determine the level of expression in mega and microbats. Gene specific primers corresponding to PCR targets were designed using the required specifications in ApE software. The primers are (OPN5F: 5' TCATCTGGGCCTATGCTTCCT3'; OPN5R: 5' TGGAGGAGGACTTAACCTTGG3' and *β*-actinF: 5'CATCCAGGCTGTGCTGTGCTGTCCCT3'; *β*-actinR: 5'TGCCAATAGTGATGACCTGGC 3). The conditions for semi quantitative RT-PCR reactions were, initial denaturation at 94°C for 5 min, followed by 94°C for 30 s, annealing for 45 s at 58°C and extension at 72°C for 1 min (35 cycles). The products were analyzed on 1.2% agarose gels, the images were captured with Gel-Doc XR system (Bio-rad, CA, USA), and the amplified RT-PCR products were normalized to amplified *β*-actin gene as a control.

2.6. Domain Organization and Multiple Sequence Alignment of OPN5 Gene

The sequences that are required for comparison were retrieved from the NCBI database. The OPN5 protein sequences of various organisms were downloaded. The organism's selection rationale was based on the widely used model organisms for bats like mice, humans and chicken. Using the data that comes along with the sequences, the domains were organized, keeping the mouse OPN5 protein sequence as a reference and were represented pictorially. Multiple sequence alignment was done using Clustal Omega software with the Needleman-Wunsch algorithm. The sequence similarity and percentage identity across the species and organisms were also identified.

2.7. Multiple Sequence Alignment and Phylogeny Tree Construction

The sequences that are required for comparison were retrieved from the NCBI database. The OPN5 gene sequences for various organisms were downloaded. The organism's selection rationale was based on its close evolutionary lineage to bats like the mouse, human, and chicken. The downloaded nucleotide sequences were selected and a multiple sequence alignment was performed using Clustal Omega software. The sequence similarity and

percentage identity were analysed across the species and organisms. The phylogenetic tree construction was done for the OPN5 gene using the Clustal Omega alignment software.

3. Results

3.1. Bats OPN5 Share Similar Sequence Homology and Domain Organization with Human, Mouse and Chicken OPN5

A bioinformatics approach was used to identify the domain organisation and amino acid identity of bats OPN5 gene. Alignment of the bats' OPN5 protein sequence with other model organisms such as mouse, human and chicken OPN5 protein sequences reveals that several key features are conserved (Figure 1). Seven transmembrane domains and a lysine residue at position 296 are required to form a Schiff base with the chromophore, which is considered diagnostic for the opsin family. A counterion in the third transmembrane domain, a tyrosine residue at position 109 in mice and humans, balances the positively charged Schiff base. The OPN5 amino acid sequences of bats and mice share a significant degree of similarity. The predicted bat OPN5 amino acid sequence is 89-96% similar to mouse and human OPN5 protein sequences, and 4-5% similar to chicken OPN5 protein sequences. (Figure 1). These results suggest that the bats' OPN5 shares similar sequence homology and domain structures with mouse and human OPN5.

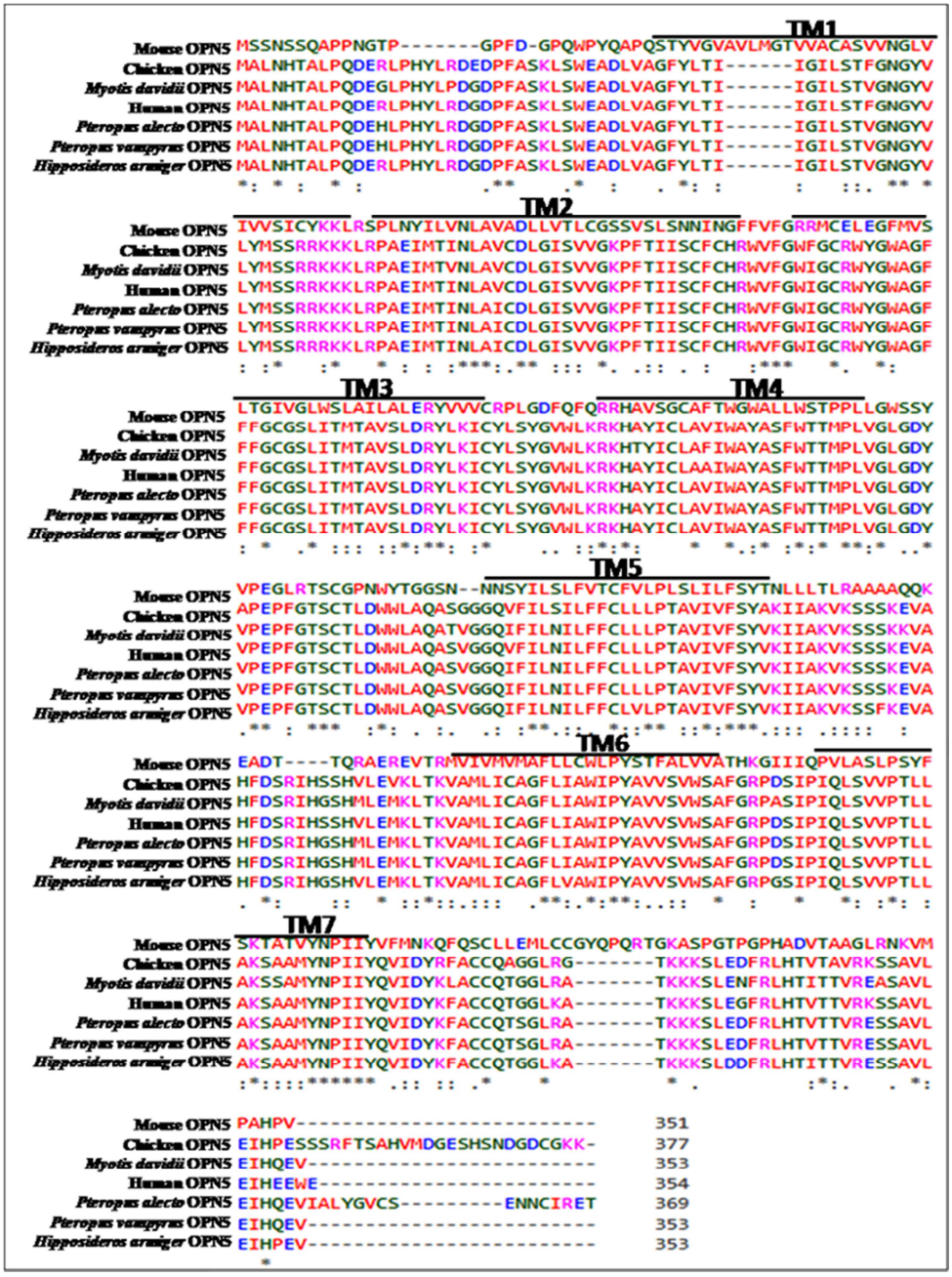

Figure 1. *Multiple sequence alignment of bats, human, mouse and chicken OPN5 protein sequences indicating seven transmembrane domains.*

3.2. Sequence Analysis and Phylogenetic Position of Bats OPN5

BLAST was used to align the OPN5 gene sequence with corresponding OPN5 gene sequences from other taxa in the database. A general global alignment technique that follows the Needleman-Wunsch algorithm was adapted to perform multiple sequence alignment. After truncating the sequences to the length of the shortest sequence in the particular alignment data using the neighbor-joining method, phylogenetic trees were generated with the concept that bats' OPN5 gene is related to that of other creatures' OPN5 gene. Bats' OPN5 genes appeared to be most closely related to other bat species and the human OPN5 clade (Figures 2).

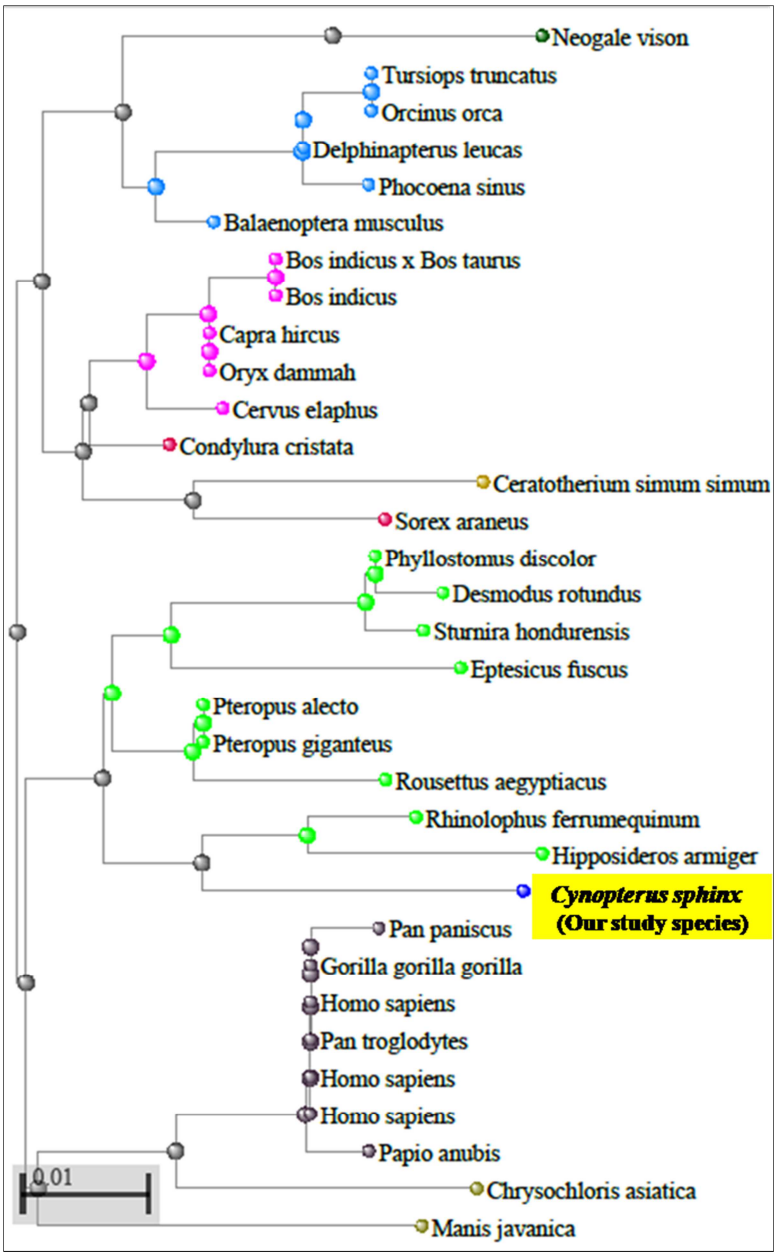

Figure 2. *Phylogenetic tree construction using partial nucleotide sequences of OPN5 gene. Dendrogram was constructed by the neighbour-joining method using bats, mouse and human OPN5 nucleotide sequences. Yellow coloured is the query sequence which is a sequenced our bat species Cynopterus sphinx. It is closely related with the other bats species and also with human OPN5 and other species.*

3.3. OPN5 Tissue Expression Pattern

The OPN5 gene expression pattern was analysed in a panel of eight tissues (brain, eye, heart, kidney, liver, lungs, retina and testis). RT-PCR results showed that the expression is detectable only in the brain, eye and retina. But, no expression was observed in the heart, kidney, liver, lungs and testis. (Figure 3). This result suggests that OPN5 gene expression is neural specific in bats.

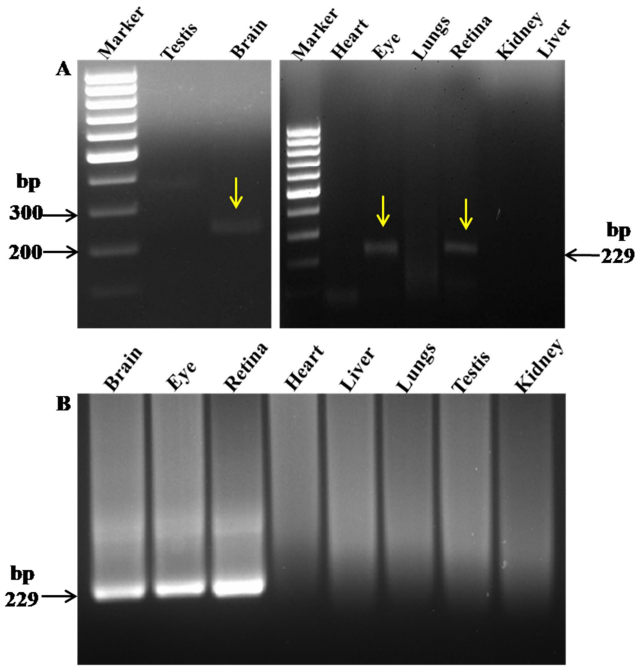

Figure 3. *Expression of OPN5 gene in a panel of eight (brain, eye, heart, kidney, liver, lungs, retina and testis) bat tissues using RT-PCR. The OPN5 gene expression is observed only in the brain, eye and retina and the expression is not detectable in other tissues. Representative gel photos are shown.*

3.4. Expression Pattern of OPN5 Gene Between Tree Roosting and Cave Roosting Bats

We determined the OPN5 expression level between tree-roosting and cave-roosting bats. We therefore collected tissue samples from tree-roosting and cave roosting bats and performed semi-quantitative RT-PCR analysis on two tree roosting and four cave roosting bats and then measured the amount of OPN5 transcripts in comparison to GAPDH, which served as an internal control. The expression level of the OPN5 gene of tree roosting bats was significantly higher compared to cave roosting bats (Figure 4). Since, the tree-roosting bats received slightly more sunlight every day when compared to cave-roosting bats. These results suggest that the bats' OPN5 may contribute to the UV light detection.

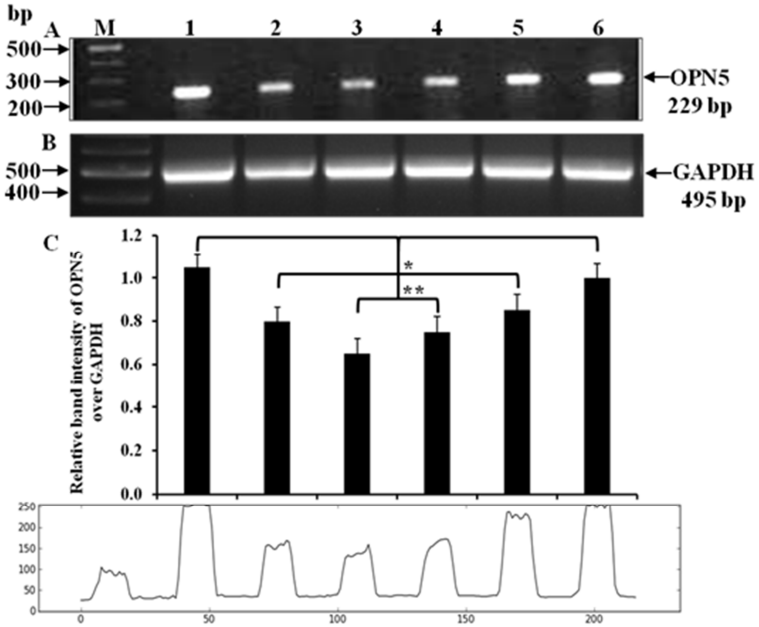

Figure 4. *OPN5 gene expression using semiquantitative RT-PCR between tree and cave-roosting bats. (A) Expression of GAPDH mRNA served as internal control. (B) Normalized OPN5 gene with GAPDH shown in arbitrary values. (C) Lanes M) 100 bp ladder, 1) C. sphnix (Fruit bat), 2) M. lyra (Insect bat), 3) H. fulvus (Insect bat), 4) H. speoris (Insect bat), 5) R. hardwickii (Insect bat), and 6) P. giganteus (Fruit bat). Values are mean ± SD, P<0.05). Representative gel photos are shown.*

3.5. Expression Pattern of OPN5 Gene Between Wild and Captive Condition Bats

Next, we determined whether captive conditions influenced the OPN5 gene expression in bats, and to examine the relative abundance of OPN5 genes between wild and captive bats, we captured the bats from the wild and held them in captivity for two months. After two months, the tissue samples were collected and semi-quantitative RT-PCR analysis was performed in a panel of eight tissues (brain, eye, heart, kidney, liver, lungs, retina and testis) and quantified the amount of OPN5 transcripts in comparison to *β-actin,* which serves as an internal control. In captive bats, the neural tissues expressed lower levels of OPN5 than those of wild bats. Statistically, there were significant differences between wild and captive condition bats (Figure 5; *P>0.05*). These results suggest that the opsin-like gene, neuropsin (OPN5) is also involved in UV light perception in bats.

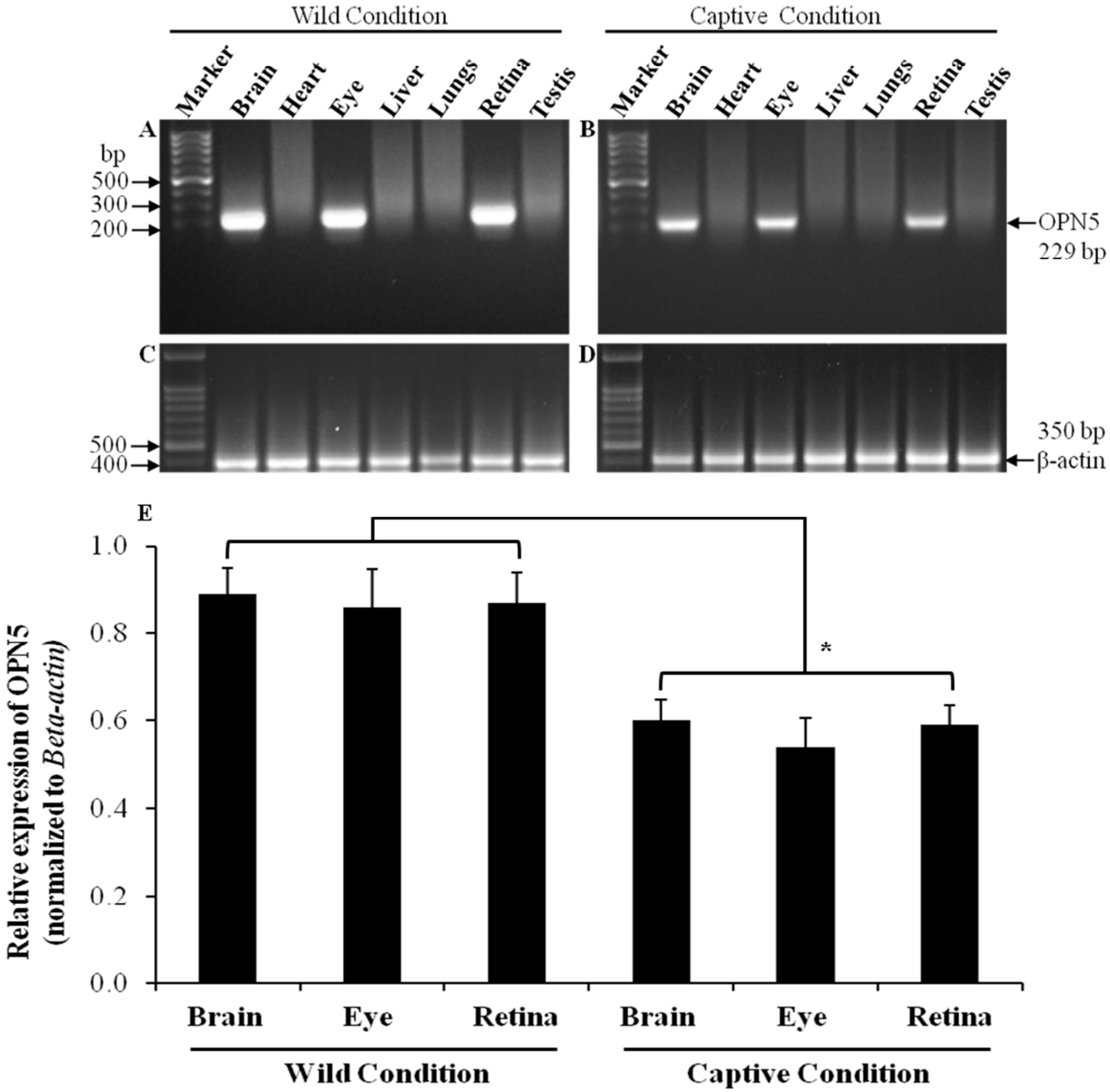

Figure 5. *Relative expression of OPN5 gene using semiquantitative RT-PCR between wild and captive condition bats. Seven tissue samples from C. sphinx wild (A) and captive (B) condition bats were analyzed for OPN5 gene expression by RT-PCR analysis using specific primer pairs, where the expression of β-actin mRNA served as internal reference for wild (C) and captive (D) condition bats. Normalized OPN5 gene with β-actin shown in arbitrary values (E). Values are mean ± SD, P<0.05). Representative gel photos are shown.*

	S.No.	Scientific Name	Common Name	Roost Type
Fruit-eating bats	1.	*Cynopterus sphinx*	Indian short-nosed fruit bat	Tree
	2.	*Pteropus giganteus*	Indian flying fox	Tree
Insect-eating bats	3.	*Hipposideros fulvus*	Fulvus round leaf bat	Cave
	4.	*Hipposideros speoris*	Schneider's leaf-nosed bat	Cave
	5.	*Megaderma lyra*	Indian false vampire bat	Cave
	6.	*Pipistrellus mimus*	Indian pygmy bat	Cave

Figure 6. *Selected bat species for study the OPN5 gene expression pattern representing both fruit (S.No: 1 and 2) and insectivorous (S.No: 3-6) bats.*

4. Discussion

All organisms use light as an adaptive advantage to interact with the external world. Due to the reliable rhythms of light and dark, life has evolved [33]. Light has a wide-ranging influence on human physiology and behaviour, from synchronising sleep–wake cycles to generating daily changes in body temperature and energy metabolism [34]. The visual system acts as a decisive interface between many organisms and their environment [35]. Most mammals possess two cone types with spectrally different visual pigments (opsins) that have absorption maxima at short wavelengths (Ss, blue or ultraviolet light) and middle/long wavelengths (M/Ls, green or red light). Most species of bats have functional eyes with M/L and S opsin genes. These genes are required for daylight, UV, and dichromatic colour vision [15]. UV visual sensitivity has a wide range of ecological and adaptive significance across the animal kingdom and is used in a variety of ecological processes in bats, including foraging, partner selection, navigation, and social communication. [15]. OPN5 is one of the most recently discovered opsin groups in animals and is responsible for non-visual photoreception [24]. Recent research in bats has mainly focused on the non-visual UV sensitive pigments involved in UV perception in bats.

A number of behavioural, molecular and immunohistochemical evidences support that the opsin-like gene, neuropsin (OPN5) identified in human, mouse and birds serves as a G protein-coupled UV-sensitive photoreceptor. Short-wavelength light photoentrains circadian rhythms in the skin of mice and can also induce clock gene expression in vitro and in vivo via an Opn5-dependent mechanism. These findings show that mammals may use local photic cues in peripheral tissue to synchronise their circadian rhythms. [36]. More recent bioinformatics studies have overlooked the types of opsins expressed in human skin types and their roles in skin physiology. These findings suggest that opsins produced by numerous human cell types play a variety of roles, including mediating wound healing, skin photoaging, and hair development. [37]. Spectrophotometric analysis and radionucleotide-binding assay revealed that OPN5, a UV-sensitive bistable pigment found in birds, directly binds to all-trans-retinal to activate G protein. OPN5 expression was found to be higher in the non photoreceptive adrenal gland, while the brain and retina showed lower expression. Thus, the non-mammalian type opsin 5 found in birds serves as a second UV sensor in the photoreceptive organs, but it might also serve as a chemosensor in non-photoreceptive organs [26]. In quail, OPN5 is reported as a deep brain photoreceptive molecule. Immunohistochemistry identified that OPN5 in neurons translates photoperiodic information into neuroendocrine responses. Also, short wavelength light (between UV-B and blue light) induced photoperiodic responses in eye patched

pinealectomized quail, suggesting that OPN5, which is a deep brain photoreceptive molecule, controls seasonal reproduction in birds. This suggests that OPN5 regulates seasonal reproduction in birds [38]. In our study, we confirmed that both fruit and insect-eating bats provide evidence to suggest the OPN5 gene is expressed and involved in UV perception and non-image forming responses to light.

RT-PCR studies using cDNA isolated from chicken embryonic retina revealed three different OPN5 related genes in the chicken genome: *cOPN5m* (*chicken opsin 5, mammalian type*), *cOPN5L1* (*chicken opsin 5-like 1*), and *cOPN5L2* (*chicken opsin 5-like 2*). According to In situ hybridization analysis, *cOpn5m* is specifically expressed in differentiating ganglion cells and amacrine cells, suggesting that the mammalian type OPN5 may contribute to retinal cell development in chicken. [27]. Recent research has discovered that the OPN5 dopamine circuit controls optic axis clearance in preparation for visual function, with violet light serving as a developmental time cue [39]. Our RT-PCR analysis showed that OPN5 in bats is observed only in the brain, eye and retina. These findings suggest that OPN5 expression in bats is neural specific.

OPN5 expressed in the hypothalamic preoptic area (POA) regulates thermogenesis in brown adipose tissue (BAT) in mice through a light sensing pathway. OPN5 POA neurons project to bats and decreases their activity, in response to chemogenetic stimulation. When cold challenged, the mice that lack OPN5 show overactive BAT, increased body temperature, and exaggerated thermogenesis. Moreover, violet photostimulation decreases BAT temperature in wild-type mice but not in Opn5-null mice after cold exposure. Thus, a violet light-sensitive deep brain photoreceptor that generally reduces BAT thermogenesis has been identified [40]. Recent studies suggest that OPN5 inhibits Brown Adipose Tissue (BAT) activity, which results in increased core temperature in mice. It was identified that OPN5 responds to violet light while the mutant mice are insensitive. In control animals, violet light decreased BAT activity and core temperature [34]. There has been a little evidence to demonstrate OPN5 is involved in UV vision in the order Chiroptera. Therefore, in this study, we have utilised both bioinformatics and molecular approaches to study the role and expression pattern of a photoreceptor protein gene *neuropsin* (OPN5) among mega and micro bats. Our preliminary, bioinformatics analysis suggests that the bats' OPN5 shares a similar sequence homology and domain organisation with human and mouse OPN5. The predicted bats OPN5 amino acid sequence shows around 89-96% identity to mouse and human OPN5 and 4-5% identity to chicken OPN5 protein sequences.

Mammalian neuropsin (OPN5), encoded by the OPN5 gene is a UV sensitive photoreceptor. OPN5 expression in mouse was found to be constrained to the eye, brain, spinal cord and testes. In humans, OPN5 expression has been detected in the brain and testis and has been sub-localised within the eye, retina and RPE. Further, OPN5 expression was observed within neural tissues [25]. Our findings also agree with previous reports. In this study, we observed both fruit and insect-eating bats expressed the OPN5 gene in their genome and predominantly expressed in neural tissues such as the brain, eye and retina. OPN5 expression in neural tissues suggests that they may serve as neural photoreceptors and prompt further studies to uncover their physiological roles in bats. OPN5 gene in bats may contribute to UV light detection, and we could not exclude the possibility that OPN5 might be mainly expressed in the testis or other tissues. Mouse testis also reported the expression of OPN5 mRNA [25]. Further characterization of the sites of expression of OPN5 within the brain, eye and retina will provide insight into the potential role of this OPN5 gene.

Recently, the spectral properties of OPN5 have been reported in three independent papers: mouse and human OPN5s have absorption maxima in the UV-A region (380 nm) [9], quail OPN5 is violet-sensitive with maximal sensitivity at around 420 nm [38], and chicken OPN5 is UV-sensitive with an absorption maximum at around 360 nm [41]. It is unclear whether bats' OPN5 is violet- or UV-sensitive since the absorption spectra of OPN5 differ to a large extent between avian and mammalian species. According to our captive study, OPN5 in bats may contribute to UV light detection in bats. The physiological role of mammals, birds, and bats may be related to changes in OPN5 molecular properties and expression patterns. From these results, we conclude that bats require OPN5 for daylight, UV, and dichromatic colour vision.

UV-sensitive pigments may be beneficial for bats in visual orientation during dusk, predator avoidance, and detection of UV-reflecting flowers for those that feed on nectar. According to a previous study, several bat-pollinated neotropical plant species have violet blooms that reflect UV light to a significant degree [42]. Fascinatingly, ambient light at dawn and dusk contains a mostly high proportion of short wavelengths, notably UV light [43], though it needs further extensive behavioural and electrophysiological studies. Despite their crucial role in vision, opsins also play various physiological roles in animals. Bats are the ideal model organisms to study the various roles of OPN5. To maximize the benefits of OPN5 in bats, further exploration into the mechanisms is necessary.

5. Conclusion

Vision plays a vital role in the animal kingdom, and this innate response to light is due to the presence of rods and cones in the eye's retina. In addition to color vision, UV vision plays a major role in guiding navigation and orientation, detecting food, potential predator avoidance and intra-specific communication. The present study deals with determining the non-visual UV sensitive photoreceptor gene (OPN5) expression pattern among mega and micro bats. In this study, we have utilised both bioinformatics and molecular approaches to show that bats' neuropsin (OPN5) encoded by the Opn5 gene shares 89-96% amino acid

identity and similar domain organization with human and mouse OPN5. The expression of OPN5 is detectable only in the brain, eye and retina. In addition, we identified that the expression levels of OPN5 in the neural tissues were significantly lower than those of wild bats in captive conditions. This result suggests that OPN5 gene expression is neural specific in bats and the opsin-like gene, neuropsin (OPN5) is involved in UV light perception in bats. The OPN5 gene may be beneficial for bats in visual orientation during dusk, predator avoidance, and detection of UV-reflecting flowers for those that feed on nectar. Fascinatingly, ambient light at dawn and dusk contains a high proportion of short wavelengths, notably UV light, though it needs further extensive behavioural and electrophysiological studies. Despite their crucial role in vision, opsins also play various physiological roles in animals. Further studies on the mechanisms will be crucial to expand the benefits of OPN5 in bats. Moreover, our results suggest that OPN5 gene expression in neural tissues may serve as neural photoreceptors and prompt further studies to uncover their physiological roles in bats.

References

[1] Kefalov, V. J. (2012). Rod and cone visual pigments and phototransduction through pharmacological, genetic, and physiological approaches. Journal of Biological Chemistry, 287: 1635-1641.

[2] Fujun, X., Kailiang, H., Tengteng, Z., Paul, R., Xuzhong, W. and Yi, S. (2012). Behavioral evidence for cone-based ultraviolet vision in divergent bat species and implications for its evolution. Zoologia (Curitiba), 292: 109-114.

[3] Shichida, Y. and Matsuyama, T. (2009). Evolution of opsins and phototransduction. Philosophical Transactions of the Royal Society B: Biological Sciences, 364: 2881–2895.

[4] Yau, K. W. (1994). Phototransduction mechanism in retinal rods and cones. Investigative Ophthalmology and Visual Science, 35: 9-32.

[5] Ahnelt, P. K. and Kolb, H. (2000). The mammalian photoreceptor mosaic-adaptive design. Progress in Retinal and Eye Research, 19: 711-777.

[6] Cronin, T. W. and Bok, M. J. (2016). Photoreception and vision in the ultraviolet. Journal of Experimental Biology, 219: 2790-2801.

[7] Eisner, T., Silberglied, R. E., Aneshansley, D., Carrel, J. E. and Howland, H. C. (1969). Ultraviolet video viewing: the television camera as an insect eye. Science, 166: 1172–1174.

[8] Froy, O., Gotter, A. L., Casselman, A. L. and Reppert, S. M. (2003). Illuminating the circadian clock in monarch butterfly migration. Science, 300: 1303–1305.

[9] Kojima, D., Mori, S., Torii, M., Wada, A., Morishita, R. and Fukada, Y. (2011). UV-Sensitive Photoreceptor Protein OPN5 in Humans and Mice. PLoS One, 6: e26388.

[10] Yokoyama, S. (1997). Molecular genetic basis of adaptive selection: examples from color vision in vertebrates. Annual Review of Genetics, 31: 315-336.

[11] Li, L., Chi, H., Liu, H., Xia, Y., Irwin, D. M., Zhang, S. and Liu, Y. (2018). Retention and losses of ultraviolet-sensitive visual pigments in bats. Scientific Reports, 8 (1): 11933. doi: 10.1038/s41598-018-29646-6.

[12] Terakita, A. (2005). The opsins. Genome Biology, 6: 213.1-213.9.

[13] Unger, V. M. and Schertler, G. F. (1995). Low resolution structure of bovine rhodopsin determined by electron cryo-microscopy. Biophysical Journal, 68: 1776-1786.

[14] Muller, B., Butz, E., Peichl, L. and Haverkamp, S. (2013). The rod pathway of the microbat retina has bistratified rod bipolar cells and tristratified all amacrine cells. Journal of Neuroscience, 33: 1014-1023.

[15] Eklof, J. and Jones, G. (2003). Use of vision in prey detection by brown long-eared bats, *Plecotus auritus*. Animal Behaviour, 66: 949-953.

[16] Speakman, J. (2001). The evolution of flight and echolocation in bats: another leap in the dark. Mammal Review, 31: 111-130.

[17] Zhao, H. B., Rossiter, S. J., Teeling, E. C., Li, C. J., Cotton, J. A. and Zhang, S. Y. (2009). The evolution of color vision in nocturnal mammals. Proceedings of the National Academy of Sciences, 106: 8980-8985.

[18] Kim, T. J., Jeon, Y. K., Lee, J. Y., Lee, E. S. and Jeon, C. J. (2008). The photoreceptor populations in the retina of the greater horseshoe bat *Rhinolophus ferrumequinum*. Molecules and Cells, 26: 373-379.

[19] Muller, B., Glosmann, M., Peichl, L., Knop, G. C., Hagemann, C. and Ammermuller, J. (2009). Bat eyes have ultraviolet-sensitive cone photoreceptors. PLoS One, 4: e6390 doi: 10.1371/journal.pone.0006390.

[20] Griffin, D. R. (1970). Migration and homing of bats. In: Biology of bats (Wimsatt WA, ed) New York: Academic, p. 233–264.

[21] Suthers, R. A. (1970). Vision, olfaction, taste. In: Biology of bats (Wimsatt WA, eds) New York: Academic, p. 265-304.

[22] Eklof, J. (2003). Vision in echolocating bats. Doctoral thesis. Department of Zoology, Göteborg University, Sweden.

[23] Altringham, J. D. and Fenton, M. B. (2003). Sensory ecology and communication in the chiroptera. In: Bat ecology (Kunz TH, Fenton MB, eds), Chicago: The University of Chicago Press, p. 90-127.

[24] Yamashita, T., Ono, K., Ohuchi, H., Yumoto, A., Gotoh, H. and Tomonari, S. et. al. (2014). Evolution of mammalian OPN5 as a specialized UV-absorbing pigment by a single amino acid mutation. Journal of Biological Chemistry, 289: 3991-4000.

[25] Tarttelin, E. E., Bellingham, J., Hankins, M. W., Foster, R. G. and Lucas, R. J. (2003). Neuropsin (OPN5): a novel opsin identified in mammalian neural tissue. Federation of European Biochemical Societies Letter, 554: 410-416.

[26] Ohuchi, H., Yamashita, T., Tomonari, S., Fujita-Yanagibayashi, S., Sakai, K., Noji, S. and Shichida, Y. (2012). A non-mammalian type opsin 5 functions dually in the photoreceptive and non-photoreceptive organs of birds. PLoS One, 7: e31534.

[27] Tomonari, S., Migita, K., Takagi, A., Noji, S. and Ohuchi, H. (2008). Expression patterns of the *opsin 5*–related genes in the developing chicken retina. Developmental Dynamics, 237: 1910–1922.

[28] Terakita, A., Koyanagi, M., Tsukamoto, H., Yamashita, T., Miyata, T. and Shichida, Y. (2004). Counterion displacement in the molecular evolution of the rhodopsin family. Nature Structural and Molecular Biology, 11: 284-289.

[29] Gaisler, J. (1973). Netting as possible approach to study bat activity. Periodicum Biologorum. 75: 129-134.

[30] Karuppudurai, T., Sripathi, K., Gopukumar, N., Elangovan, V., Marimuthu, G. (2007). Genetic diversity within and among populations of the Indian short-nosed fruit bat *Cynopterus sphinx* assessed through RAPD analysis. Current Science, 93: 942-950.

[31] Wilmer, J. W., Barratt, E. (1996). A non-lethal method of tissue sampling for genetic studies of chiropterans. Bat Research News, 37: 1-3.

[32] Karuppudurai, T. and Sripathi, K. (2010). Molecular genetic analysis of male alternative strategy and reproductive success in the polygynous mating bat *Cynopterus sphinx.* Journal of Cell and Animal Biology, 4: 042–050.

[33] Upton, B. A., Díaz, N. M., Gordon, S. A., Van Gelder, R. N., Buhr, E. D. and Lang, R. A (2021). Evolutionary constraint on visual and non-visual mammalian opsins. Journal of Biological Rhythms, 36: 109-126.

[34] Schwartz, G. J. (2020). Light-activated neurons can alter body heat. Nature, 585: 351-352.

[35] Hauser, F. E. and Chang, B. S. W. (2017). Insights into visual pigment adaptation and diversity from model ecological and evolutionary systems. Current Opinion in Genetics and Development, 47: 110–120.

[36] Buhr, E. D., Vemaraju, S., Diaz, N., Lang, R. A. and Van Gelder, R. N. (2019). Neuropsin (OPN5) mediates local light-dependent induction of circadian clock genes and circadian photoentrainment in exposed murine skin. Current Biology, 29: 3478–3487.

[37] Suh, S., Choi, E. H. and Mesinkovska, N. A. (2020). The expression of opsins in the human skin and its implications for photobiomodulation: A Systematic Review. Photodermatology Photoimmunology and Photomedicine, 36: 329-338.

[38] Nakane, Y., Ikegami, K., Ono, H., Yamamoto, N., Yoshida, S., Hirunagi, K. et. al. (2010). A mammalian neural tissue opsin (Opsin 5) is a deep brain photoreceptor in birds. Proceedings of the National Academy of Sciences, 107: 15264–15268.

[39] Nguyen, M. T., Vemaraju, S., Nayak, G., Odaka, Y., Buhr, E. D. et al. (2019). An Opsin 5-dopamine pathway mediates light-dependent vascular development in the eye (2019). Nature Cell Biology, 21: 420–429.

[40] Zhang, K. Z., D'Souza, S., Upton, B. A., Kernodle, S., Vemaraju, S., et. al. (2020). Violet-light suppression of thermogenesis by opsin 5 hypothalamic neurons. Nature, 585: 420-425.

[41] Yamashita, T., Ohuchi, H., Tomonari, S., Ikeda, K., Sakai, K. and Shichida, Y. (2010). OPN5 is a UV-sensitive bistable pigment that couples with Gi subtype of G protein. Proceedings of the National Academy of Sciences, 107: 22084-22089.

[42] Winter, Y., López, J. and von Helversen, O. (2003). Ultraviolet vision in a bat. Nature*,* 425, 612-614.

[43] Lythgoe, J. N. (1979). The ecology of vision. Oxford, Clarendon Press, New York, Oxford University Press, pp. 244.