Journal of the Anatomical Society of India 66 (2017) 58–66

Contents lists available at ScienceDirect

Journal of the Anatomical Society of India

journal homepage: www.elsevier.com/locate/jasi

Original Article

Expanding the horizons of melatonin use: An immunohistochemical neuroanatomic distribution of MT1 and MT2 receptors in human brain and retina

Tulika Gupta[a,*], Daisy Sahni[a], Richa Gupta[a], S.K. Gupta[b]

[a] Department of Anatomy, Postgraduate Institute of Medical Education & Research, Sector-12, Chandigarh 160012, India
[b] Department of Neurosurgery, Postgraduate Institute of Medical Education & Research, Sector-12, Chandigarh 160012, India

ARTICLE INFO

Article history:
Received 21 April 2017
Accepted 13 May 2017
Available online 19 May 2017

Keywords:
Brain stem
Cerebral cortex
Hypothalamus
Melatonin
Retina
Thalamus

ABSTRACT

Introduction: There is increasing evidence implicating the active role of melatonin beyond regulation of the human biological clock and reproduction. Its therapeutic use has been extended to neurodegenerative disorders, psychiatric disturbances, memory as well as a host of other neurological manifestations. This study was thus designed to identify regions of brain and retina for the expression of different types of melatonin receptors.

Method: Six unfixed brains and 10 retinae were studied. Tissue samples were taken from 14 sites in the brain. Immuno-histochemical staining was done using antibody against Melatonin 1and Melatonin 2 receptors.

Result: In the cerebral cortex, MT 1 receptor presence was mostly detected in layers 4 and 5 while MT2 receptors were mapped in all the layers. The frontal and occipital poles were devoid of both the receptors. The suprachiasmatic nuclei of the hypothalamus had immunoreactivity for both MT1 and MT2 while the larger cells of the supraoptic nuclei showed positivity for MT1 receptors. The pyramidal and granule cells of the cerebellar cortex showed the presence of MT2 receptors while the pons and the medullary reticular formation stained positive for MT1 and MT2.

Discussion: Differential and comparative characterization of MT1 and MT 2 receptors in different regions of brain and retina has led to virtual creation of a neuroanatomical map localizing potential areas susceptible to interventions specifically targeting melatonergic pathways.

1. Introduction

Melatonin hormone is synthesized and produced by the pineal gland and affects the circadian rhythms, day/night cycles, and reproduction.[1] Melatonin has a direct action on hypothalamic suprachaismatic nucleus, the central biological clock of the brain.[2–4] A strong expression of melatonin has been observed in the human hypothalamic-pituitary axis, especially the pars tuberalis, providing a neurobiological basis of its role in the regulation of various hypothalamic and pituitary functions.[5,6]

In clinical practice melatonin has been effectively used for long to adjust the body's internal clock. It is commonly prescribed for jet lag, for adjusting sleep-wake cycles in people who work in changing shifts, in helping blind people establish a day night cycle and in insomnia associated with various disorders.[6–9]

Apart from these established roles, in recent years, its use has been recommended in patients with a plethora of neurological disorders such as Alzheimer's disease, schizophrenia, migraine, tinnitus, epilepsy, involuntary movement disorders, delirium etc. There is thus a growing body of evidence implicating melatonin in various physiologic processes and disorders beyond its traditional chronobiotic role.[7,10–12] It is possible that there may be others locations in the human nervous system secreting melatonin, or there may be widespread distribution of melatonin binding sites in the brain through which melatonin may exert its influence.

Exogenous melatonin modulates processes and physiological responses via activation of the melatonin 1 (MT1) and melatonin 2 (MT2) receptors.[13] There have been many animal studies,[1–4,13–15] and few human studies,[5,16,17] limited to selected areas, for locating the melatonin receptors in the brain, but a specific detailed study

* Corresponding author.
E-mail address: tulikag11@yahoo.com (T. Gupta).

http://dx.doi.org/10.1016/j.jasi.2017.05.007
0003-2778/

mapping the neuroanatomical location of different kinds of melatonin receptors in the human brain is not available.

The present study was thus designed to identify melatonin 1 (MT 1) and melatonin 2 (MT2) receptors in different regions of the brain by using specific antibodies. This may be of use in future pharmacological targeting of disease specific neural areas.

2. Materials and methods

This is a cross-sectional immune-histochemical study on human brain and retina.

2.1. Material

Six adult unfixed brains and 10 retinal tissues were sampled within 3–4 hours of death. In each brain the tissue was taken from the following 14 areas:

- Orbito frontal cortex
- Medial frontal cortex
- Pre- frontal cortex
- Frontal cortex
- Parietal cortex
- Occipital cortex
- Temporal cortex
- Hippocampus
- Thalamus
- Hypothalamus
- Mid brain
- Pons
- Medulla
- Cerebellum

Specimens with history of neurological disease, head injury, ocular disease or ocular trauma, were excluded from the study. Ethical clearance was taken from the institutional ethical committee.

2.2. Methods

The tissue samples were fixed in 10% buffered formalin followed by paraffin embedding, block making and sectioning. 4 μm thick sections were taken on the Poly L-lysine coated slides for the immuno-histochemistry (IHC). For the IHC Avidine Biotine peroxidise complex (ABC) technique was followed: The paraffin sections were deparaffinised, then hydrated gradually and washed in de-ionised water. Endogenous blocking, to quench the activity of endogenous peroxidise was done. It was followed by antigen retrieval. The slides were then incubated with primary antibodies followed by secondary antibody. The sections were treated with DAB (3,3′-diaminobenzidine) and counter stained with haematoxylin. Following primary antibodies (Abcam) were used:

Anti Melatonin Receptor 1 (MT1) antibody (Polyclonal) Ratio 1:100

Anti Melatonin Receptor 2 (MT2) antibody (Polyclonal) 1:200

For positive control retinal and pituitary tissue was used while negative control was obtained by omitting the primary antibody. Micro photograph was taken with the help of Ci-L Pentahead Nikon microscope (700857) with camera (MC 30). The MT1 and MT2 receptor immune stain was evaluated semi quantitatively as staining intensity 1,2, 3 indicating mild, moderate and severe staining respectively.

3. Results (Table 1)

In the present study the term white matter refers to neurites of the cells as well as fiber tracts passing through.

3.1. Cerebral Cortex (Figs. 1 and 2)

Separate samples were taken from 6 areas; Prefrontal, Medial frontal, Frontal, Parietal, Temporal, and Occipital areas of the cerebral cortex. Frontal and occipital pole did not show any immuno reactivity (IR).

Table 1
Distribution of MT1 & MT2 receptors in different areas of brain.

Tissue Area	Reaction to MT1 antibody				Reaction to MT2 antibody			
	Neurons	WM	Vessels	Glial cells	Neurons	WM	Vessels	Glial cells
Hippocampus	Pyramidal cells +ve Granule cells −ve SI:1-2	+ve SI: 2	+ve Few & patchy	−ve	Pyramidal cells +ve Granule cells +ve in deep layer only SI: 2	+ve SI: 1	+ve Few & patchy	−ve
Hypothalamus	Large neurons +ve SI: 3	+ve SI: 1	+ve Few & patchy	−ve	Both large neurons and 60% of small neurons are +ve SI: 3	+ve SI: 2	+ve Few & patchy	−ve
Thalamus	−ve excpt for occasional neuron	+ve SI: 3	+ve Few & patchy	−ve	40–50% neurons +ve SI: 3	+ve SI: 3	+ve Few & patchy	−ve
Cerebral Cortex	10%-60% (more in deeper layers) pyramidal cells +ve SI: 1-3	+ve SI: 1-3	+ve Few & patchy	−ve	100% cells +ve SI: 3	+ve SI: 3	+ve Few & patchy	+ve in Temporal & Parietal areas
Cerebellum	−ve	–	+ve Few & patchy	−ve	Pyramidal cells all +ve Molecul L −ve Granule L 40% cells +ve SI: 1-2	+ve SI: 1	−ve All	–
Brain stem	Pontine neurons +ve SI: 1-2 Medullary RF neurons +ve SI: 2	+ve SI: 1-2	+ve Few & patchy	+ve	Similar to MT1 but SI: 3			

L: layer, RF: reticular formation, SI: staining intensity, 1: mild, 2: moderate, 3: severe, +ve: positive, −ve: negative, WM: white matter.

3.1.1. MT1

The white matter (WM) was showing moderate staining (+2) with very small dot like areas (size of tip of pin at 40×), of intense staining (+3) dispersed in all the cerebral layers in the frontal, parietal and temporal areas. In the occipital area staining was mild with interspersed streaks of severe intensity. In frontal and parietal tissue both the pyramidal and granule cells were immune negative, except for 2–3 small areas, present in cortical layer 4 and 5, in which pyramidal as well as granule cells were stained. In the cells the staining was regular cytosolic type and predominantly moderate in intensity. In the temporal cortex similar regular type staining of moderate intensity was observed in 50–60% of cells present in all the six layers. In occipital cortex IR was shown only by few cells, dispersed across all the six cortical layers. The reaction was mild and patchy. In the all the locations the vessels were immune positive for both the antibodies while glial cells did not take the stains.

3.1.2. MT2

Tissue samples from all locations showed moderate neuronal (both pyramidal & granule cells) staining in 80% of neurons. It is seen in all the cortical layers. WM staining of moderate intensity was seen with pattern similar to MT1. Percentage of cells demonstrating IR increased in layer 4 and 5 of cerebral cortex in most tissue type. IR for both WM and neurons was more, deeper to the calcarine sulcus. MT2 IR in glial cells was observed in temporal and occipital cortex. The vessels showed staining.

3.2. Hippocampus (Fig. 2c, d)

3.2.1. MT1

WM staining varies from mild (+1) in some areas to moderate (+2) in other, but in each area staining intensity is consistent across different cerebral layers. In areas with moderate WM staining, the large pyramidal cells present from layer I to layer VI. In light staining areas about 10% of pyramidal cells demonstrated staining of mild intensity in the layers II, III and IV; number of stained cells marginally increased in the deeper layers. The cytosolic staining observed in the pyramidal cells was stippled, covering from 1/3 to total cell area; for further description in this paper we will refer to this type of cytosolic IR, as regular type. The granule cells and glial cells were immune negative. Vessels were immunopositive.

3.2.2. MT2

Moderate staining of WM seen in whole tissue. Pyramidal cells present in all the layers showed regular cytosolic stain of moderate intensity. In the deeper layers cytosolic area stained in each cell was much more as compared to superficially placed cells. Few granule cells were stained in the deeper cortical layers while in rest of the layers they were unstained. Glial cells were unstained while vessels took the stain.

3.3. Diencephalon (Fig. 3)

3.3.1. MT1

In thalamus the white matter bundles showed intense immune reactivity with no IR in the neurons and glial cells except for few

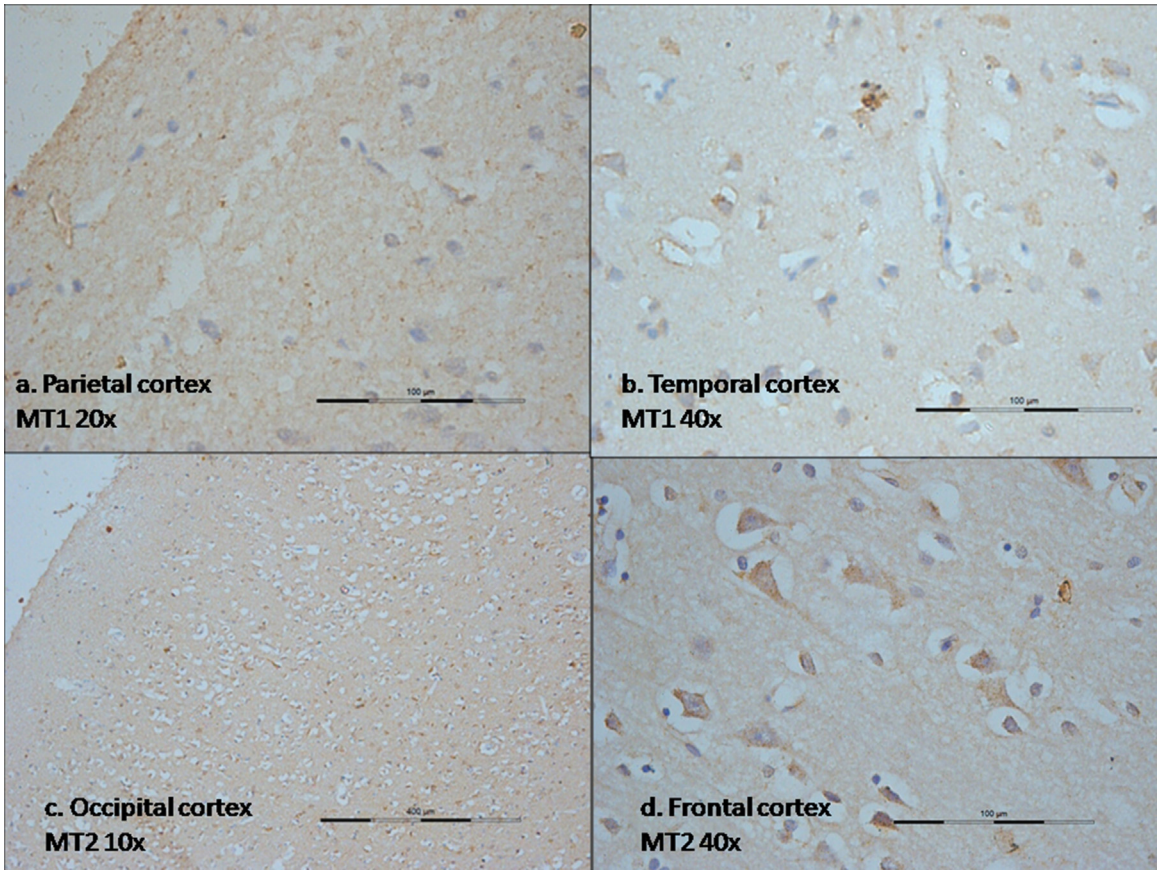

Fig. 1. Cerebral cortex **a.** Parietal cortex showing mid staining of white matter wi8th MT1 antibody. The neurons are immune negative. MT1 40× **b.** The pyramidal cells present in the layer V demonstrate immunopositivity. MT1 40× **c.** Moderate intensity staining with MT2 antibody evident in all the layers of occipital cortex. MT2 10× **d.** MT2 antibody positivity present in both pyramidal and granule cells of frontal cortex. MT2 40×.

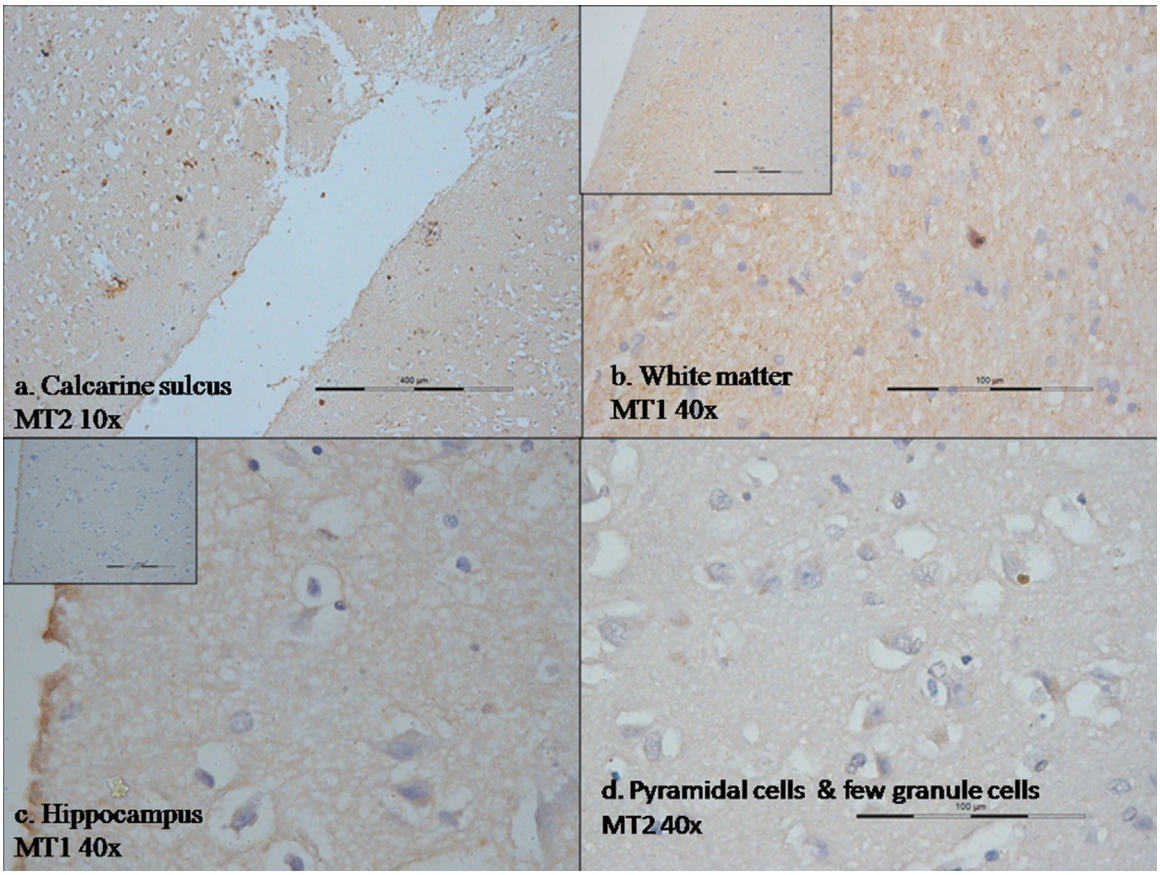

Fig. 2. **a.** Immunopositivity for MT2 antibody seen in all the cortical layers deep to the calcarine sulcus. MT2 10× **b.**The pattern of white matter staining with MT1 antibody. Small dots of severe intensity are interspersed among the fibers. MT1 40× The inset shows that this staining was seen across the whole cortical thickness. 20× **c.** MT1 immunopositive field in the hippocampus with immunoreactivity seen in large pyramidal cells. MT1 40×. The inset shows this staining in the layers I, III and IV. 20× **d.** Few granule cells are also stained in the deeper cortical layers along with the pyramidal cells MT2 40×.

isolated neurons with mild staining. In hypothalamus large neurons had polar stippled staining of severe intensity. Fibres demonstrate very mild staining.

3.3.2. MT2

In thalamus intense immunoreactivity is seen in whole tissue specimen in both white matter as well as neurons. About 40–50% of the neurons show stippling at one pole of the cell. In hypothalmus about 40% of small sized neurons were also stained along with the large neurons and intensity of staining was more.

Glial cells and vessels also exhibit immunopositivity for both antibodies.

3.4. Brain stem (Fig. 4)

Tissue samples from medulla, pons and midbrain were taken separately from the ventral aspect. First two were taken near the ponto-medullary junction.

3.4.1. MT1

The white matter showed mild & moderate staining but the glial cells did not show any immunoreactivity (IR). In the medulla area of reticular formation was identified with magnocellular cells showing IR of moderate intensity, stippled in characteristic and spanning 1/2 to 2/3 of the total area. The most of the pontine cells were large pyramidal shaped multipolar cells with large vesicular nucleus and prominent nucleoli and most of them were IR positive with variable staining intensity. The neurites of the cells also showed IR. Glial cells and vessels also show immunoreactivity.

3.4.2. MT2

Staining pattern was similar to that seen in MT1. However the staining intensity was more intense (3) both in the white matter as well as in the neurons. Moreover IR was seen in the cytoplasm of many glial cells. In the pons there were many areas which were IR negative; these areas were devoid of neurons.

3.5. Cerebellum (Fig. 5)

3.5.1. MT1

Immunoreactivity for MT1 is almost absent except for very faint and patchy staining in the white matter in the molecular and granule cell layers. None of the neurons show IR. Vessels do not show staining.

3.5.2. MT2

Occasional cell demonstrate mild intensity staining in the molecular layer. Very patchy and mild staining was seen on the pyramidal cell surface. 30–40% cells in the granule cell layer show mild to moderate staining of the cell membrane. Most of the white matter show mild staining. Cerebellar vessels show moderate intensity staining in all the layers.

3.6. Retina and choroid (Fig. 6)

3.6.1. MT1

Moderate to severe (2 – +3) intensity staining was seen in the inner limiting membrane (ILM), ganglion cell layer (GCL), inner plexiform layer (IPL), outer plexiform layer (OPL), layers of rods and

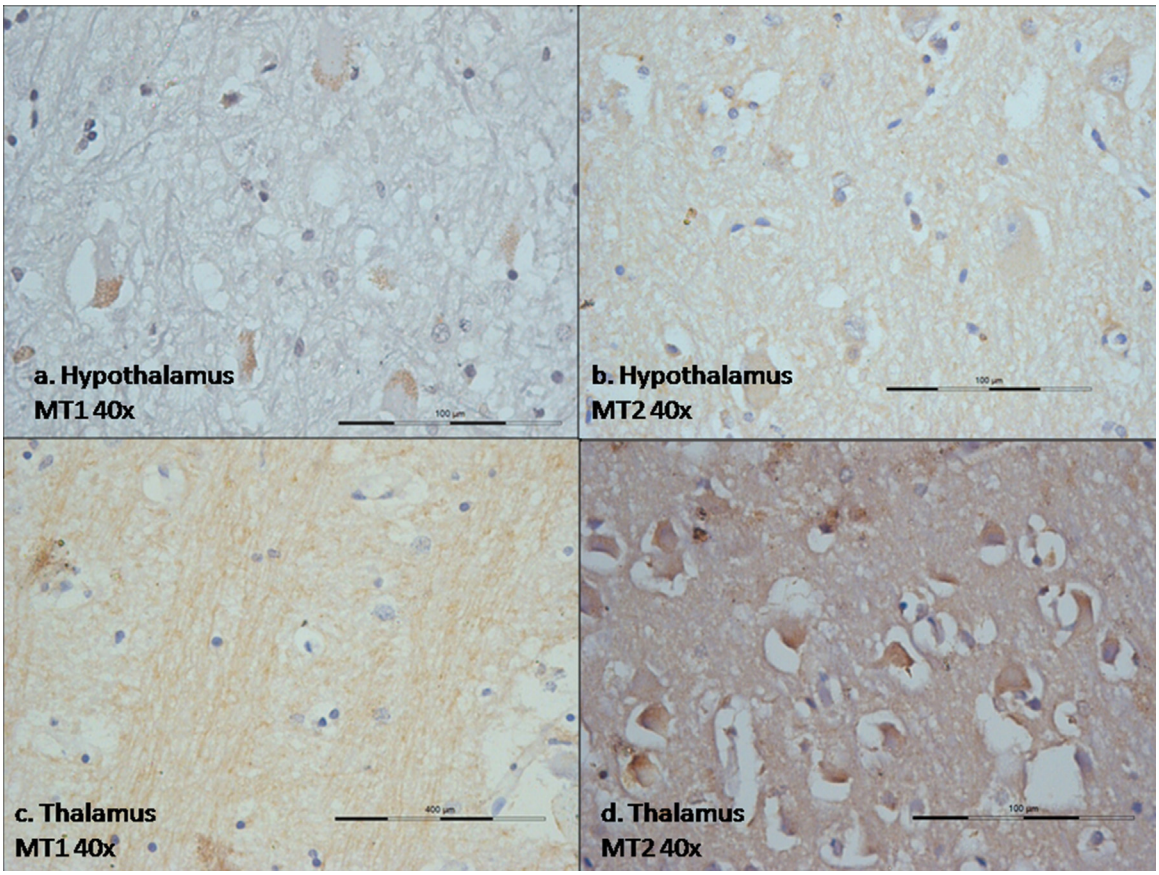

Fig. 3. Diencephalon **a.** Hypothalamus with large neurons having polar stippled staining of severe intensity. Fibers demonstrate very mild staining. MT1 40×. **b.** Area of neuron stained by MT2 antibodies and intensity of white matter staining is more as compared to that seen in MT1. Some of the small sized neurons also display immunoreactivity. MT2 40×. **c.** MT1 immunoreactivity is positive for fiber bundles only. MT1 40× **d.** Thalamus display MT2 immunopositivity in neurons and white matter. MT2 40×.

cones and retinal pigment epithelium. Cell nuclei present in the outer nuclear layer (ONL) and inner nuclear layer (INL) and the inner limiting membrane (ILM) did not show any IR though the fibers of the OPL and IPL show positive staining. Nuclei in the GCL showed patchy staining, which was most likely present on the nuclear membrane, though haematoxylin staining of the nuclei was visible in almost all the cells except few in which stain completely covered the nucleus. The IPL and OPL were uniformly stained. Layer of rods and cones demonstrated maximum staining on the outer segment. The intensity of staining and area covered was more in the rods as compared to the cones. The cell membrane of the RPE did not take any stain but the granules present in the RPE cytoplasm were darkly stained. The Bruch's membrane was unstained. The choroidal vessels and choroidal connective tissue were moderately stained (2) while the choroidal pigment showed severe intensity staining (+3).

3.6.2. MT2

Staining characteristics were similar to those observed for the MT1 antibodies.

4. Discussion

This data provides first detailed neuro-anatomical map of both MT1 and MT2 receptors across all the regions of the brain including retina. In addition to localization of the receptor positive regions on the brain and retina, a comparative analysis of immunoreactivity with MT1 and MT2 receptor antibody is described. In all the regions studied, histological characterization of the receptor sites has also been done.

4.1. Role of melatonin in health and disease

There is an ongoing effort to establish the physiological role of melatonin and determining its mechanism and site of action. In recent years there have been many studies substantiating the role of melatonin beyond its traditional chronobiotic and hypnotic actions. The activation of the melatonin receptors have been found to reduce the symptoms and neurochemical changes observed in the depressive disorders. Melatonin antagonists improve the memory by long term potentiation. Melatonin has also been found to modulate responses to drugs of abuse. Melatonin has potential utility both in slowing normal brain aging and in treatment of neurodegenerative conditions. These properties are made more attractive by the low cost of melatonin and its very low toxic hazard.[10,18,19] All these facts prove the presence of melatonin beyond the pineal gland, throughout the whole neural axis and underline the importance of a detailed knowledge of melatonin receptors.

The melatonin modulates processes and responses in the central nervous system via activation of the MT1 and/or MT2 melatonin receptors. Hence detailed description of the melatonin receptor sites, in different parts of the cerebral cortex, thalamus, hypothalamus, pituitary, pineal gland, brain stem and cerebellum; in the present study is an essential step in increasing the knowledge of melatonin behavior in the human neural tissue.

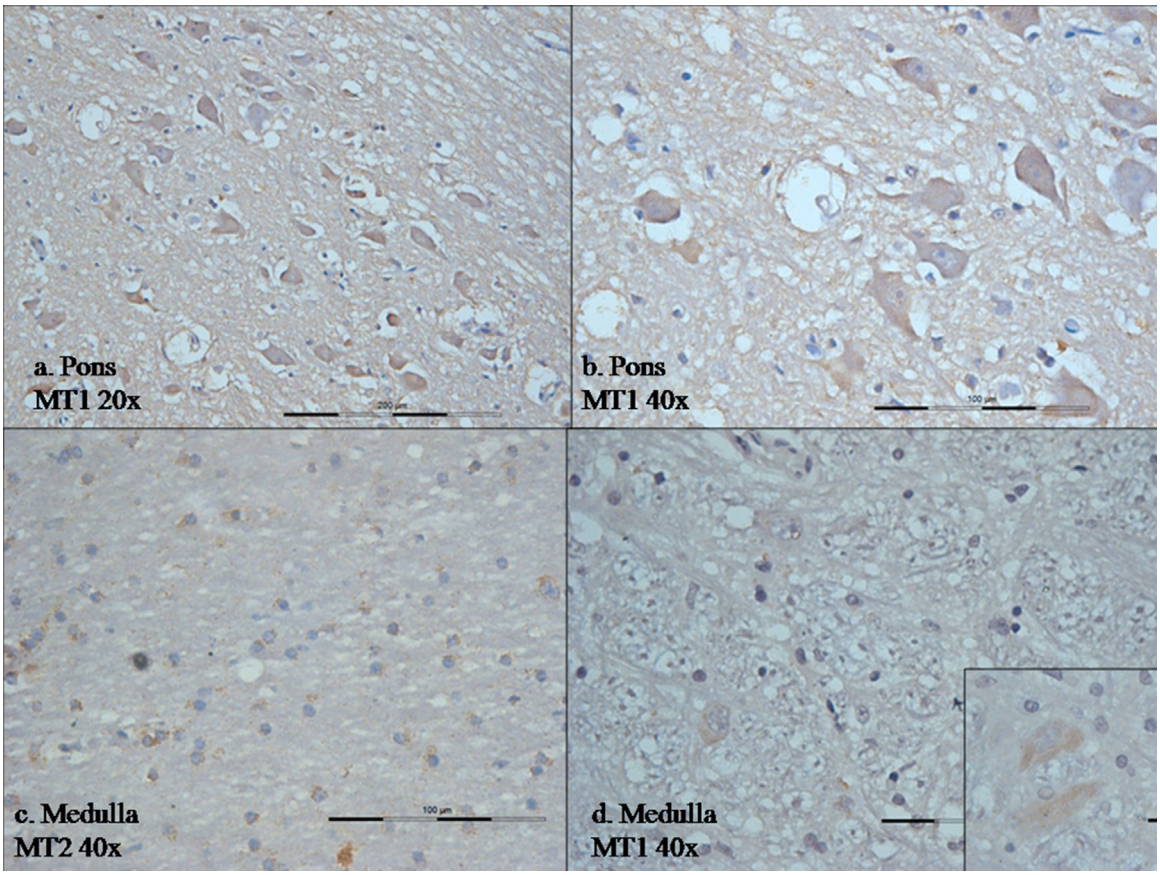

Fig. 4. Brain stem **a.** Immunopositive pontine neurons lying deep to pontocerebellar fibers seen on the right superior corner. MT1 20× **b.** Group of pontine neurons is seen at 40× MT1 **c.** The medullary white matter. MT2 40× **d.** Magnocellular cells of the medullary reticular formation showing immunoreactivity of moderate intensity. MT1 40×.

Application of this knowledge may lead to pharmacological approaches mediated via the two receptor subtypes.

A): Cerebral cortex: Both MT 1 and MT 2 receptors were identified in all lobes of the brain, with the exception of the frontal polar and occipital polar regions. The intensity of staining was more for the MT 2 receptor antibodies. A significant observation was the consistent finding of selective staining of layers 4 and 5 for MT1 in most of the cortical regions. These layers have predominant pyramidal and granule cells. On the other hand, MT2 receptors were localized in all the cortical layers. Axons of pyramidal cells situated in layer 5 form the corticospinal tract, the main pathway for voluntary motor control, while the granular cells in layer 4 are the main target of thalamocortical connections as well as intra hemispheric cortico-cortical connections. These findings further give weight to the hypothesis that melatonin has a significant role in modulating motor activity as well as transfer and modulation of sensory input.

Age related decline in melatonin may increase oxidative stress and mitochondrial dysfunction, which may be the common pathophysiological phenomena associated with neurodegenerative disorders such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD). Attempts to compensate for age and disease-dependent melatonin deficiency have shown that administration of this compound can improve sleep efficiency in AD and PD and, to some extent, cognitive function in AD patients.[10] Many effects of melatonin, are mediated by membrane receptors MT1 and MT2. Hirsch-Rodriguez et al.[20] believe that the pattern of melatonin receptor expression in the brain may influence antidepressant treatment. Demonstration of melatonin receptor sites across various cortical sites strengthens this hypothesis. Attempts to selectively target the MT positive sites through vector mediated targeted drug delivery may provide a therapeutic breakthrough in the management of cognitive decline.

4.2. Hypothalamus

Two different staining patterns were observed in the hypothalamic region. Large neurons, specific to the supraoptic region, stained only for MT1 receptors while in the suprachiasmatic region having small size neurons, both MT1 and MT 2 positivity was demonstrated. Presence of both MT 1 and MT2 receptors in the suprachiasmatic nuclei (SCN) of mice has been reported previously.[1,21] Contrary to these studies, Lacoste et al.[15] while confirming the presence of MT1 receptors in the SCN, could not detect significant amounts of the MT2 protein in this region of rat brain. In the present study the hypothalamic tissue from the suprachiasmatic region showed positivity for both MT1 and MT2 receptors on the WM as well as on neurons. Curious localisation of signal to one pole of the neuron was noticed. This staining pattern was different from cerebral neurons which showed IR all over the cell.

Melatonin affects the phase of circadian rhythms by a direct action on the biological clock present within the hypothalamic suprachiasmatic nucleus. Activation of MT2 melatonin receptors in rat SCN phase has been shown to advance the circadian clock. Melatonin receptor activation decreases neuronal firing through activation of the MT1 receptor in the SCN and areas of the limbic system, which may mediate the sleep-promoting properties of melatonin.[3] The latter showed polar stippled staining of severe intensity in the cytoplasm which is similar to non nuclear granular staining localised to the cytoplasm only in the part of the neuron as observed by Wu et al.[5] in human hypothalamus.

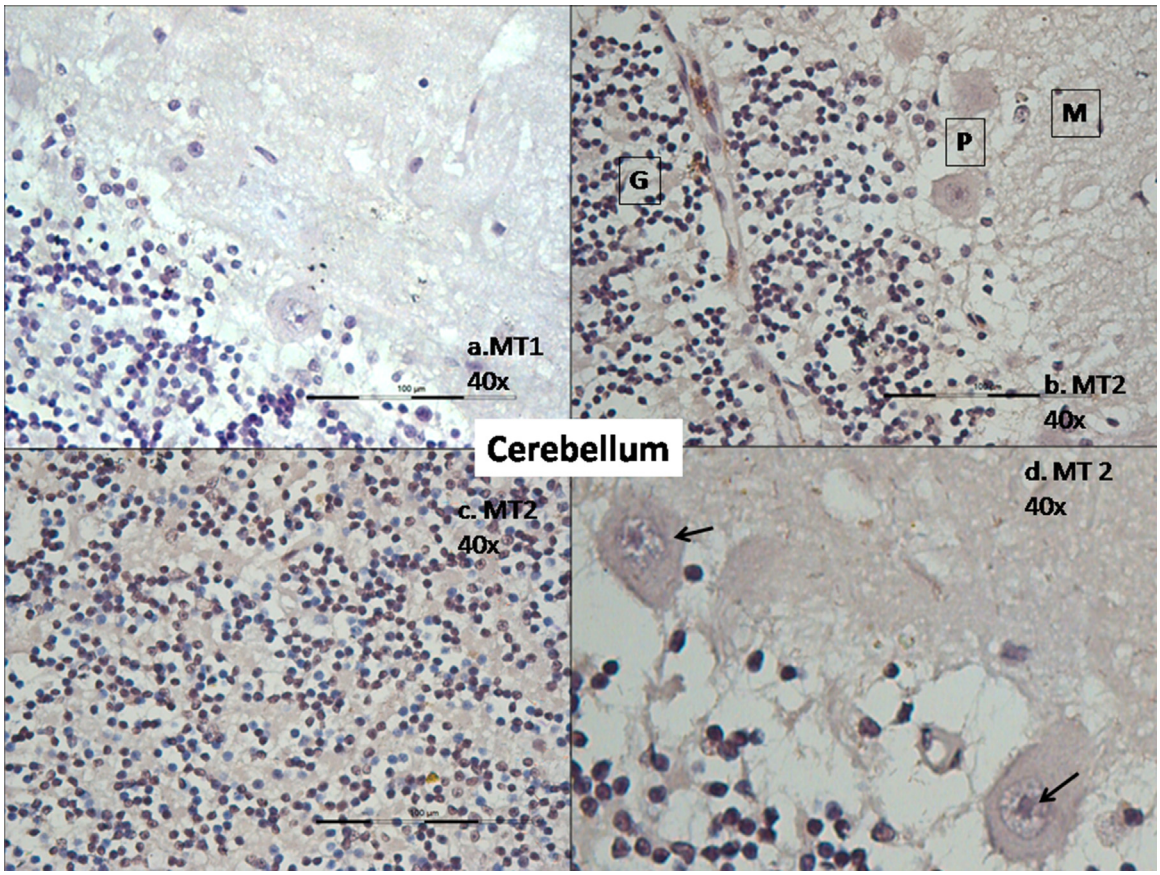

Fig. 5. Cerebellum **a.** Immunonegative for MT1 except for mild staining of white matter. MT1 40× **b.** Immunoreactivity for MT2 antibody seen in about 40% of neurons in the granule cell layer. MT2 40× **c.** All three layers of cerebellar cortex; molecular (M), purkinje (P) & granule cell layer (G), exhibit staining of mild intensity with MT2 antibody. 40× **d.** Purkinje cells MT2 40×.

Selective presence of MT1 receptors in the supraoptic region provides evidence for its possible role in production of vasopressin.

4.3. Hippocampus

Memory formation is driven by long-term potentiation (LTP) in the hippocampus, a process by which associations between neurons are cooperatively and selectively strengthened by increased synaptic activity. Melatonin appears to inhibit the LTP in the dendritic layer of mouse hippocampal brain slices through MT2 receptors.[22,23] The clinical significance of the melatonin receptors in dementia is strongly supported by postmortem histology studies in hippocampus from Alzheimer's disease patients showing increased MT1 and decreased MT2 receptor immunoreactivity. Authors reported MT2 IR in pyramidal and granular neurons in hippocampus of normal elderly controls. The overall intensity of the MT2 staining was distinctly decreased in AD cases.[16,17] In the present study pyramidal cells show mild to moderate intensity staining for MT1 as well as MT2 receptors in all the cerebral layers while most of the granule cells were immune negative for both MT1 and MT2, with the exception of a few granule cells in deep layers which were immunopositive for MT2 only.

4.4. Cerebellum and pons

The demonstration of MT2 receptors in the pyramidal cells of the cerebellum and IR for both MT1 and MT2 in the pontine region gives rise to the possibility of the existence of a melatonergic pathway parallel to the corticospinal tracts from the pyramidal cell layer of the frontal lobe.

4.5. Retina

In the present study we found positive immunoreactivity to both MT1 and MT2, proving the role of both the receptors in retinal circuitry. The outer segments of the rods were better stained then those of cones. This might be related to the scotopic vision provided by the rods. Some authors believe that melatonin protects the photoreceptor outer segment from photo-oxidative stress.[24,25] Previous studies have reported the presence of MT1 receptors in rods photoreceptor cells in human retina. In the present study we found that segments of both the photoreceptors present in the layer of rods and cones demonstrate immunopositivity for MT1 as well as MT2 receptors, though the reaction was more extensive in rods. It has been suggested that the activation of melatonin receptors present on the ganglion cells contributes to the modulation of retino-hypothamic transmission of the light–dark signal.[6] We have found positive IR for both MT1 and MT2 on the ganglion cells. In the ONL MT1 and MT2 mRNAs were detected in almost all the nuclei of the photoreceptors in mouse retina by authors.[26] We did not find IR for either of the receptor on the cell membranes in the ONL, though the surrounding white matter stained positive. They detected MT1 and MT2 mRNA both in the ONL and INL and only MT2 mRNA in the GCL of mouse retina. Our findings of positive IR for both the melatonin receptors in GCL might be due to species specific variations; which further points to

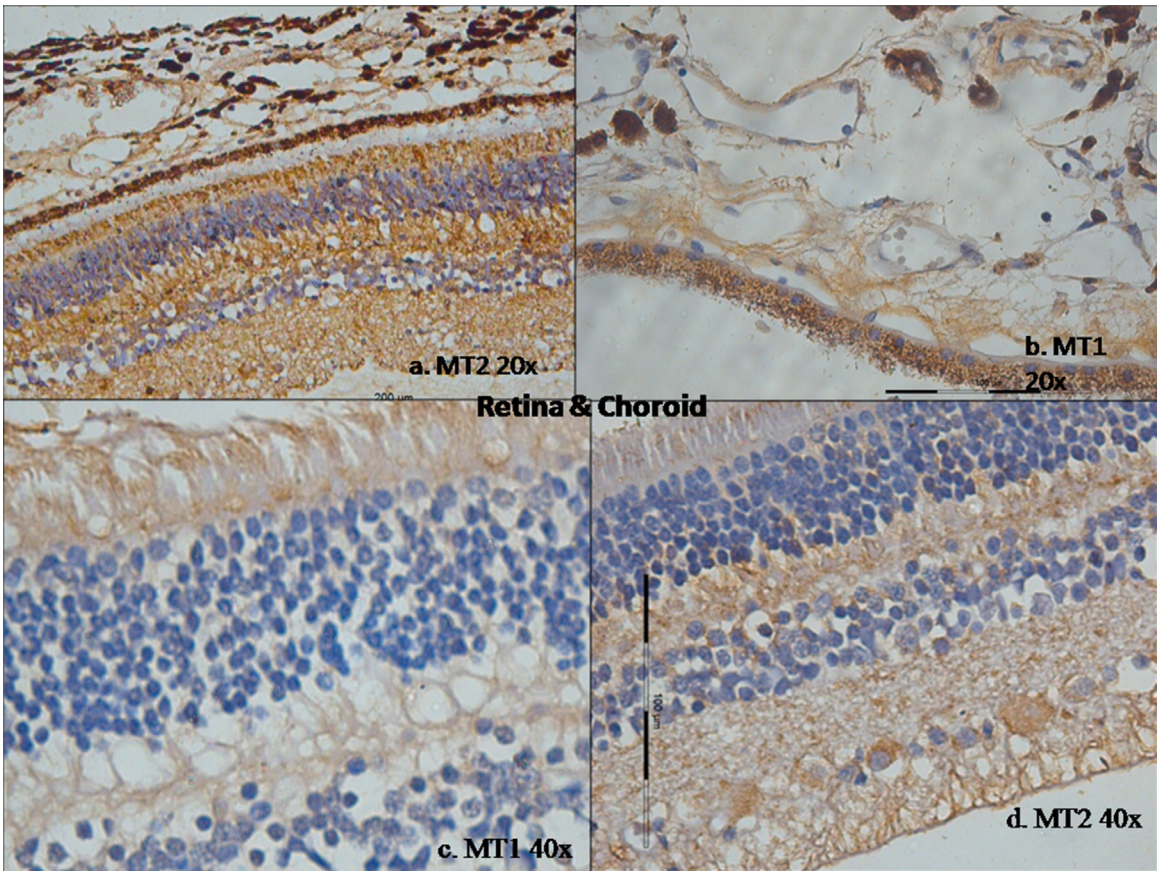

Fig. 6. Retina & Choroid **a.** Immunopositivity to MT2 antibody can be seen in retina and choroid 20× MT2 **b.** Reinal pigment epithelium, choroidal vessels and pigment all stain positive, while bruch's membrane (BM) is unstained. MT1 20× **c.** Layer of rods and cones demonstrated maximum staining on the outer segment. The intensity of staining and area covered is more in the rods as compared to the cones. MT1 40× **d.** Immunopositivity of ganglion cells, inner plexiform and outer plexiform layer is evident. MT2 40×.

the urgent need for human tissue based studies, so that potential advantages of this molecule can be clinically utilized.

It is interesting to note positive immunoreactivity to both the melatonin receptors in pigment present in the cytoplasm of the retinal pigment epithelium and in the choroid. RPE has been identified as one of the retinal sites for melatonin production. This melatonin is believed to affect the ocular physiology[27] and counteract ischemic injury to chick embryo RPE cells.[28] The IR for both MT1 and MT2 have been found to be complementary to each other in most of the tissues studied. This is in concordance to the findings by Gall et al.[1] who reported that the binding characteristics are generally similar between the two receptors in human.

5. Conclusion

This study has documented the presence of melatonin receptors in extensive areas of brain and retina. It is highly conceivable that there exists a melatonergic pathway connecting the cortical regions, diencephalon, hippocampus, brainstem and cerebellum modulating not only the human circadian rhythm and reproductive physiology but also behavior patterns, cognition and locomotion. Topographic localization of melatonin receptors as well as their differential expression may be helpful in pharmacological manipulation of these anatomic sites specific to the disease processes. This opens an exciting door for optimism for many hitherto untreatable conditions.

References

1. von Gall C, Stehle JH, Weaver DR. Mammalian melatonin receptors: molecular biology and signal transduction. *Cell Tissue Res*. 2002;309:151–162.
2. Hunt AE, Al-Ghoul WM, Gillette MU, Dubocovich ML. Activation of MT2 melatonin receptors in rat suprachiasmatic nucleus phase advances the circadian clock. *Am J Physiol Cell Physiol*. 2001;280:C110–C118.
3. Liu C, Weaver DR, Jin X, Shearman LP, Pieschl RL, Gribkoff VK, Reppert SM. Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. *Neuron*. 1997;19:91–102.
4. Poirel VJ, Masson-pevet M, Pevet P, et al. MT1 melatonin receptor mRNA expression exhibits a circadian variation in the rat suprachiasmatic nuclei. *Brain Res*. 2002;946:64–71.
5. Wu YH, Zhou JN, Balesar R, et al. Distribution of MT1 melatonin receptor immunoreactivity in the human hypothalamus and pituitary gland: colocalization of MT1 with vasopressin, oxytocin, and corticotropin-releasing hormone. *J Comp Neurol*. 2006;499:897–910.
6. Dubocovich ML, Markowska M. Functional MT1 and MT2 melatonin receptors in mammals. *Endocrine*. 2005;27(2):101–110.
7. Pala D, Lodola A, Bedini A, Spadoni G, Rivara S. Homology models of melatonin receptors: challenges and recent advances. *Int J Mol Sci*. 2013;14:8093–8121.
8. Sánchez-Barceló EJ, Mediavilla MD, Tan DX, Reiter RJ. Clinical uses of melatonin: evaluation of human trials. *Curr Med Chem*. 2010;17:2070–2095.
9. Spadoni G, Bedini A, Rivara S, Mor M. Melatonin receptor agonists: new options for insomnia and depression treatment. *CNS Neurosci Ther*. 2011;17:733–741.
10. Srinivasan V, Pandi-Perumal SR, Cardinali DP, Poeggeler B, Hardeland R. Melatonin in Alzheimer's disease and other neurodegenerative disorders. *Behav Brain Funct*. 2006;2:15.
11. Liu RY, Zhou JN, van Heerikhuize J, Hofman MA, Swaab D. Decreased melatonin levels in postmortem cerebrospinal fluid in relation to aging, Alzheimer's disease, and apolipoprotein E-ε4/4 genotype. *J Clin Endocrinol Metab*. 1999;84:323–327.
12. Mishima K, Tozawa T, Satoh K, Matsumoto Y, Hishikawa Y, Okawa M. Melatonin secretion rhythm disorders in patients with senile dementia of Alzheimer's type with disturbed sleepwaking. *Biol Psychiatry*. 1999;45:417–421.

13. Ishii H, Tanaka N, Kobayashi M, Kato M, Sakuma Y. Gene structures, biochemical characterization and distribution of rat melatonin receptors. *J Physiol Sci*. 2009;59:37–47.
14. Dubocovich ML, Rivera-Bermudez MA, Gerdin MJ, Masana MI. Molecular pharmacology, regulation and function of mammalian melatonin receptors. *Front Biosci*. 2003;8:1093–1108.
15. Lacoste B, Angeloni D, Dominguez-Lopez S, et al. Anatomical and cellular localization of melatonin MT1 and MT2 receptors in the adult rat brain. *J Pineal Res*. 2015;58(4):397–417.
16. Savaskan E, Wirz-justice A, Olivieri G, et al. Distribution of melatonin MT1 receptor immunoreactivity in human retina. *J Histochem Cytochem*. 2002;50:519–526.
17. Savaskan E, Ayoub MA, Ravid R, et al. Reduced hippocampal MT2 melatonin receptor expression in Alzheimer's disease. *J Pineal Res*. 2005;38:10–16.
18. Emet M, Ozcan H, Ozel L, Yayla M, Halici Z, Hacimuftuoglu A. A review of melatonin, its receptors and drugs. *Eurasian J Med*. 2016;48:135–141.
19. de Bodinat C, Guardiola-Lemaitre B, Mocaer E, Renard P, Mu noz C, Millan MJ. Agomelatine, the first melatonergic antidepressant: discovery, characterization and development. *Nat Rev Drug Discov*. 2010;9:628–642.
20. Hirsch-Rodriguez E, Imbesi M, Manev R, Uz T, Manev H. The pattern of melatonin receptor expression in the brain may influence antidepressant treatment. *Med Hypotheses*. 2007;69(1):120–124.
21. Dubocovich ML, Delagrange P, Krause DN, Sugden D, Cardinali DP, Olcese J. International Union of Basic and Clinical Pharmacology. LXXV. Nomenclature, classification, and pharmacology of G protein-coupled melatonin receptors. *Pharmacol Rev*. 2010;62:343–380.
22. Wang LM, Suthana NA, Chaudhury D, Weaver DR, Colwell CS. Melatonin inhibits hippocampal long-term potentiation. *Eur J Neurosci*. 2005;22:2231–2237.
23. Larson J, Jessen RE, Uz T, Arslan AD, Kurtuncu M, Imbesi M, Manev H. Impaired hippocampal long-term potentiation in melatonin MT2 receptor-deficient mice. *Neurosci Lett*. 2006;2006(393):23–26.
24. Marchiafava PL, Longoni B. Melatonin as an antioxidant in retinal photoreceptors. *J Pineal Res*. 1999;26:184–189.
25. Siu AW, Reiter RJ, To CH. Pineal indoleamines and vitamin E reduce nitric oxide-induced lipid peroxidation in rat retinal homogenates. *J Pineal Res*. 1999;27:122–128.
26. Baba K, Benleulmi-Chaachoua A, Journé AS, et al. Heteromeric MT1/MT2 melatonin receptors modulate photoreceptor function. *Sci Signal*. 2013;6 (296):10.
27. Acuña Castroviejo D, López LC, Escames G, López A, García JA, Reiter RJ. Melatonin-mitochondria interplay in health and disease. *Curr Top Med Chem*. 2011;11(2):221–240.
28. Ogino N, Matsumura M, Shirakawa H, Tsukahara I. Phagocytic activity of cultured retinal pigment epithelial cellsfrom chick embryo: inhibition by melatonin and cyclic AMP, and its reversal by taurine and cyclic GMP. *Ophthalmic Res*. 1983;15:72–89.