Revealing Melatonin's Mysteries: Receptors, signaling pathways, and therapeutics applications

Kulsoom Kulsoom[1], Wajahat Ali[2], Zainab Saba[3], Shabab Hussain[4], Muhammad Yasin[5], Samra Zahra[6], Maria Irshad[7], and Muhammad Ramzan[8]

[1]Bahauddin Zakariya University
[2]Xi'an Jiaotong University School of Basic Medical Sciences
[3]Khwaja Fareed University of Engineering & Information Technology
[4]University of Messina
[5]University of Baltistan
[6]COMSATS University Islamabad - Islamabad Campus
[7]University of Trieste
[8]The Islamia University of Bahawalpur Pakistan

June 27, 2023

Abstract

Melatonin (5-methoxy-acetyl tryptamine) sleep-inducing hormone, and the pineal gland produces it in response to the circadian clock of darkness. In the body, MT1 and MT2 receptors are mostly found, having an orthosteric pocket and ligand binding determinants. Melatonin acts by binding on melatonin receptors, intracellular proteins, and orphan nuclear receptors. It inhibits adenyl cyclase and activates phospholipase C, resulting in gene expression and an intracellular alteration environment. Melatonin signaling pathways are also associated with other intracellular signaling pathways, i.e., cAMP/PKA and MAPK/ERK pathways. Relative expression of different proteins depends on the coupling profile of G protein, accounting pharmacology of the melatonin receptor bias system, and mediates action in a Gi-dependent manner. It shows antioxidant, antitumor, antiproliferative and neuroprotective activity. Different types of melatonin agonists have been synthesized for the treatment of sleeping disorders. Researchers have developed therapeutics that target melatonin signaling, which could benefit a wide range of medical conditions. This review focuses on melatonin receptors, pharmacology, and signaling cascades; it aims to provide basic mechanical aspects of the receptor's pharmacology, melatonin functions in cancer and neurodegenerative diseases, and any treatments and drugs designed for these diseases. This will allow a basic comparison between the receptors in question, highlighting any parallels and differences that may exist and providing fundamental knowledge about these receptors to future researchers.

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[1]Department of Biochemistry, Bahauddin Zakariya University Multan, Pakistan https://orcid.org/0009-0003-9146-3069, kalsoomsana45@gmail.com

[2]School of Basic Medical Sciences, Xian Jiaotong University, China, https://orcid.org/0009-0008-5716-0597, aliwajahat@stu.xjtu.edu.cn

[3]Department of Optometry, Khawaja Fareed University of Engineering & Information Technology, Rahim Yar Khan, Pakistan https://orcid.org/0009-0004-3345-1569, zainabsaba34@gmail.com

[4]University of Messina Italy, Shabab.hussain@studenti.unime.it, https://orcid.org/0000-0002-2537-4792

[5] Department of Zoology, University of Baltistan Skardu, Pakistan, yasin@aup.edu.pk

[6]Department of Biosciences, COMSATS University Islamabad, Pakistan, samrazahra6@gmail.com

[7]University of Trieste, Italy, Department of Medical, Surgical and Health Sciences MARIA.IRSHAD@phd.units.it

[8]Department of Pharmacology, Islamia University Bahawalpur, Pakistan,https://orcid.org/0009-0005-9243-0028, dr_msramzan@yahoo.com

Abstract: Melatonin (5-methoxy-acetyl tryptamine) sleep-inducing hormone, and the pineal gland produces it in response to the circadian clock of darkness. In the body, MT1 and MT2 receptors are mostly found, having an orthosteric pocket and ligand binding determinants. Melatonin acts by binding on melatonin receptors, intracellular proteins, and orphan nuclear receptors. It inhibits adenyl cyclase and activates phospholipase C, resulting in gene expression and an intracellular alteration environment. Melatonin signaling pathways are also associated with other intracellular signaling pathways, i.e., cAMP/PKA and MAPK/ERK pathways. Relative expression of different proteins depends on the coupling profile of G protein, accounting pharmacology of the melatonin receptor bias system, and mediates action in a Gi-dependent manner. It shows antioxidant, antitumor, antiproliferative and neuroprotective activity. Different types of melatonin agonists have been synthesized for the treatment of sleeping disorders. Researchers have developed therapeutics that target melatonin signaling, which could benefit a wide range of medical conditions. This review focuses on melatonin receptors, pharmacology, and signaling cascades; it aims to provide basic mechanical aspects of the receptor's pharmacology, melatonin functions in cancer and neurodegenerative diseases, and any treatments and drugs designed for these diseases. This will allow a basic comparison between the receptors in question, highlighting any parallels and differences that may exist and providing fundamental knowledge about these receptors to future researchers.

Graphical Abstract:

Keywords: Melatonin Hormone, Synthesis of Melatonin, Mechanism of Melatonin, Melatonin Receptors, Receptor Structure, Receptor Signalling cascades/pathways, Therapeutic Application

Word count: 4843

Abbreviations

MT1 MT2 GPCRs. IP3 PLCB PI3K Bcl2 B-ARR Bax cGMP sGC PKG CREB cAMP SIRT ROS PDK Akt

1. Introduction:

Melatonin, chemically 5-methoxy-acetyl tryptamine, is a sleep-inducing hormone, and in 1958, it was extracted from a pineal gland (Lerner et al., 1958). Its concentration is high at night time in all species. Animals' endogenous circadian clock, which secretes the hormone melatonin at night, is synchronized by light and dark cycles in the suprachiasmatic nucleus of the hypothalamus. In a 24-hour cycle, the pineal gland releases the hormone melatonin. When the retina detects stimuli of darkness, it is perceived by the suprachiasmatic nucleus, which generates a signal in the form of a nerve impulse and sends it to the upper thoracic cord of the intermediolateral column, then perceived by the superior cervical ganglion. After adrenergic stimuli, pinocytes of the pineal gland synthesize the melatonin hormone intracellular. Neurological and physiological processes are regulated by melatonin. Photoperiodic species show seasonal changes regulated by the melatonin hormone in the hypothalamus, the pituitary pars tuberalis. It exerts direct action on the suprachiasmatic nucleus and entrains the circadian clock.

Circadian rhythm disorders, i.e., jet lag, shift work, blindness, and delayed or advanced sleep phase syndromes, are treated by the response exerted by the melatonin hormone (Hagan & Oakley, 1995). Dopamine

synthesis is repressed from the retinal amacrine cells by melatonin (A. S. L. Chan et al., 2002) and can promote vasoconstriction in the rat tail artery (Brydon et al., 1999). Melatonin has also played a well-established hypnotic action (Kräuchi et al., 2000). It triggers the opening of the sleep gate, which is circadian-dependent and initiates sleeping (Kräuchi et al., 2000) . Melatonin meditates action as an antioxidant and influences immune function. Sleep and wake rhythm are induced by melatonin (Reppert et al., 1994). The physiological processes, such as regulating the cardiovascular system, are controlled by melatonin (Reppert et al., 1995) and buffering of the immune system and neurodegenerative disorders (Dubocovich et al., 2010). In drug designing, the therapeutic agents are designed that target melatonin targets. Melatonin plays a role in cancer protection, confirmed by research, bone formation glucose maintenance (Sewerynek, 2002).

2. Synthesis of Melatonin hormone:

The synthesis of melatonin involves a two-step process from the pineal gland;

Arylalkylamine is used for serotonin acetylation by using acetyltransferase enzyme to produce acetylserotonin,

Melatonin is synthesized by the enzyme hydroxy indole-O-methyltransferase by methylation of the 5-hydroxy group.

3. Excretion of Melatonin:

Mainly in the liver, melatonin is metabolized most efficiently but to some extent in the kidney. Melatonin is oxidized into 6-hydroxy melatonin in the liver by the cytochrome P 450 enzyme, and 6-hydroxy melatonin is then conjugated with sulphuric acid to 6-sulfatoxymelatonin (Tan et al., 2015). In the form of 6-sulfatoxymelatonin, it is eliminated from the body through urination, which is a major melatonin metabolite used to access melatonin concentration in the plasma (Lynch et al., 1975).

Figure 1:

4. Mechanism of Melatonin effects:

In mammals, the effects of melatonin are revealed by four different mechanisms;

1. Melatonin acts as an antioxidant

2. Melatonin binds to plasma membrane receptors

3. Interaction of melatonin to intracellular proteins, i.e., calmodulin

4. Orphan nuclear receptors are targeting (Ekmekcioglu, 2006)

Intracellular proteins, i.e., calmodulin, calreticulin, and tubulin, interact with melatonin (Pandiperumal et al., 2008). The binding of calcium to calmodulin, an intracellular second messenger, is antagonized by melatonin (Ekmekcioglu, 2006; Pandiperumal et al., 2008). Showed regulation of antiproliferative effects in cancer. Melatonin shows immunomodulatory effects, which is mediated by retinoid-related Orphan nuclear receptor. Mononuclear cells secrete interleukins IL-2 and IL-6 owing to this modulation (Ekmekcioglu, 2006).

Figure 2:

5. Melatonin receptors:

Melatonin receptors present on the plasma membrane of different cells, i.e., cells of the immune system, cells of the coronary artery, cells of the cardiac ventricular wall, cells of the cardiovascular system, appendix vermiform, hepatocytes, gallbladder, duodenal enterocytes, aorta cells of the large intestinal cecum, colon, skin cells, fetal kidney, kidney, platelets, brain, retina, parotid gland, cells of cerebral arteries, exocrine pancreas, breast and prostate epithelial cells, placenta, epithelial cells of breast, cells of the ovary, myometrium, brown adipocytes, white adipocytes are morphology different from each other's (Tan et al., 2016; Uz et al., 2005). Jejunal and colonic mucosal cells possess melatonin receptors (Pandiperumal et al., 2008). Melatonin

Receptors are of four different types in living organisms in different cells. Three receptors are on the plasma membrane, while one is the nuclear receptor.

5.1. Type 1a receptor: These are primarily present in human skin cells, consisting of 351 amino acids and encoded by a gene on chromosome#4. It has five different receptor subtypes, i.e., MT1, MTNR1A, Mel1a, ML1a, and ML1 (Li et al., 2013). The binding of type 1 melatonin receptors to different types of GPCRs inactivates Adenyl cyclase (López-Canul et al., 2015). These receptors' expression is decreased in the cortex and suprachiasmatic nucleus during Alzheimer's and aging (Pandiperumal et al., 2008), suppressing protein secretion and neuronal discharge in the suprachiasmatic nucleus (Dubocovich, 2003).

5.2. Type 1b receptor: The gene for this receptor is present on chromosome#11, encoding a polypeptide of 363 amino acids. It has three different receptor subtypes, i.e., MTNR1B, ML1b, and MT2 (Li et al., 2013). The binding of this receptor to different GPCRs inactivates Adenyl cyclase and guanylyl cyclase (López-Canul et al., 2015). cAMP synthesis is decreased by inactivating adenyl cyclase (Chaste et al., 2011). These receptors are located in sweat glands and malign melanocytes (Pandiperumal et al., 2008). These receptors inhibit gamma amino butyric acid A receptors in rat hippocampus (Dubocovich, 2003). Showing antidepressant properties reveals that their expression decreased in Alzheimer's disease (Tan et al., 2016), depression & sleep diseases are associated with abnormal melatonin receptors, and pharmacology and Pathophysiology of Alzheimer's & anxiety diseases are associated with abnormality in these receptors. These receptors are the new target for hypotonic agents. Anxiety and sleep cycles are regulated by these receptors. MT1/MT2 doesn't possess hypotonic effects as compared to these receptors (López-Canul et al., 2015).

5.3. MTNR1C & Mel1c: These receptors are present in fish, birds, and amphibians but not in humans. The chicken MT1 and MT2 receptors are antagonistic to this receptor's circadian rhythm. In the daytime, it is present in high and low concentrations at night (Li et al., 2013).**5.4. MT3:** This receptor shows antioxidant properties due to the Quinone reductase-2 enzyme and inhibits the electrons transfer reaction of Quinone (Pandiperumal et al., 2008). Melatonin type 3 receptors and detoxification Quinone reductase 2-enzyme are present on the plasma membrane of muscle, brown fat tissue, liver, kidney, heart, lung, and intestinal cells. Intraocular pressure is regulated by it (Ekmekcioglu, 2006).**5.5. PZP/POPα:** These nuclear receptors help bind melatonin to transcription factors in the nucleus and belong to the retinoic acid receptor super T family (Hazlerigg & Loudon, 2008). This receptor consists of 618 amino acids encoded by chromosome#28 (Li et al., 2013). This receptor doesn't bind to melatonin and is present in all mammals (Ekmekcioglu, 2006); it helps bind melatonin to MT (Hirsch-Rodriguez et al., 2007).

5.6. Table:1

6.1 Melatonin type 1 and type 2 structure signaling complex:

2-iodomelatonin, a nonselective agonist (Dubocovich et al., 2010) & ramelteon (Kato et al., 2005) are used to obtain stable MT1 Gi-Protein complexes. Both of these compounds show high potency and affinity toward these receptors. Co-expression of G protein and receptor was studied in the insect cells. The resolution of 2-iodomelatonin and ramelteon was determined, showing global resolutions 3.1 and 3.3 Å, respectively. The assembled complex was purified for homogeneity, and cryo-EM studied their complexes for single particles. An atomic model consisting of ligands MT1, Gi, and scFv1627 was built, and relatively high-quality density maps were used. The side chain of melatonin receptor type 1 and G-inhibitory protein was explained in the structure. TM1 and TM7 possess extra density between their N-terminal portions, and as a cholesterol molecule, it was changed. The agonist-bound MT2-Gi complex was studied in the same manner. The reconstituted ramelteon-bound MT2-Gi-scFv16 complex was acquainted by cryo-EM.

Figure 3:

For high-resolution maps, the receptor stability was improved. According to previous findings, three thermostable mutations, F1293.41W, C1403.52L, and L108ECL1F , were introduced to MT2, which are not contagious to the coupling interface of G-protein and ligand binding pocket. The ligand interaction with receptor and G-proteins coupling interferes minimally with mutations (Johansson et al., 2019). An EM density

map was obtained at 3.5 nominal resolution by using the triple mutant complex of ramelteouun-MT2-Gi-scFv16, enabling to model ramelteon, scFv16, significant portions of the receptor, MT1 and MT2 Gi protein receptors are assembled similarly to Gi protein, GPCRS, and G-protein complexes. The ramelteon and 2-iodomelatonin ligands bound to orthostatic pockets of MT1 and MT2 receptors. MT1-Gi bound to ramelteon bound and 2-iodomelatonin are structurally identical, showing 1 A° root mean square deviation values indicating complexes of Ca atoms and 0.8 Å values indicating the Ca atoms of MT1. Ramelteon-bound MT2-Gi and MT1-Gi are structurally identical showing 1.4 roots mean square deviation of receptors Ca. The regions involved in the engagement of G-protein and the extracellular side are structurally different.

6.2 Ligand Selectivity Determinants and Orthosteric:

The orthosteric pocket created by TM3, TM5, TM6, TM7, and ECL2 in the structures of both MT1 and MT2 binds ramelteon and 2-iodomelatonin. (Fig. 4a, c). It is possible to superimpose the ramelteon's binding pose with that of inactive structures in active MT1 or MT2. But the active form of 2-iodomelatonin changes slightly from the inactive form, especially where the alkyl amide tail is concerned, where it approaches the W6.48 residue in functioning MT1, which acts as a "toggle switch." ECL2 consistently occupies the pocket's top position in both conformations, blocking ligand accessibility through the extracellular side (Fig. 4b, d). The only access point to the orthosteric-binding site in the active conformation has been discovered to be the lateral channel between TM4 and TM5. It was discovered that the only access point to the orthosteric-binding site in the active conformation is the lateral channel between TM4 and TM5. (Fig. 4b, d) The orthosteric pocket is more constrained in the center of active structures, but TM3, TM4, and TM5 in dormant structures make a large "longitudinal channel" that this fiber bundle grows to join. While the residues around the iodine group and alkyl amide tail (referred to as the R3 position in melatonin, Fig.5) match up well, the active pocket's structure might vary depending on the conformations of the residues flanking the solvent channel (Fig. 4e, f). A hydrogen bond is formed between the aromatic residue Y1875.38 in MT1 and N1624.60 in the active structure by rotating from the inactive structure's solvent-facing conformation toward TM4.

Furthermore, the hydrogen bond's diameter of the ligand entry is decreased, which may inhibit the unbinding of the bound agonist because the Y1875.38A mutation caused a high ligand dissociation rate. The functional relevance of this proton pair in MT1 activation was further demonstrated by the fact that the N1624.60A mutation rendered MT1 inactive. Homologous pair N1754.60 and Y2005.38 also underwent structural changes in MT2 during the transition. This hydrogen bond's absence demonstrates that MT2 does not require an entrance-restricting hydrogen bond similar to the one found in MT1 for activation, which is consistent with the earlier finding that the N1754.60 protein does not need such a hydrogen bond (Stauch et al., 2019). No functional consequences resulted from a mutation (Johansson et al., 2019). Because N4.60-Y5.38 can be altered in conformation thanks to a conserved proline (P4.59) located close by in MT1 and MT2, changing P4.59 impairs MT2's ability to bind ligands (Mazna et al., 2008).

Residue H5.46 (H1955.46 in MT1 and H2085.46 in MT2), two helical turns beneath Y5.38, distinguishing the pockets most clearly from the active and dormant structures (Fig. 4e, f). The pocket size of the residue H5.46 (H1955.46 in MT1 and H2085.46 in MT2) differs the most between the inactive and active forms (Fig. 4e, f). H5.46 avoiding bound ligand forms inactive complexes with TM4 (Fig. 4e, f). The toggle switch residue W6.48 (W2516.48 in MT1 and W2646.48 in MT2) and van der Waals contacts with the connected ligand's alkyl amide tail are formed when the ligand moves inside by 2.4 angstroms and flips its side chain in the active structures (Fig. 4e, f). H5.46's new conformation clarifies why the H2085.46A mutation dampened MT2 activity (Hagan & Oakley, 1995; Johansson et al., 2019).

Despite the fact that H5.46 experiences similar conformational changes in MT1 and MT2, its functional significance seems to vary between the two receptors, as the H2085.46 (MT2) mutation only slightly decreased MT2 function whereas the H1955.46A (MT1) mutation drastically impaired MT1 activity. Then, we docked to both receptors using the common ligands CTL 01-05-B-A0527 and 5-hydroxyethoxy-N-acetyltryptamine (5-HEAT) (Nonno et al., 2000). In contrast to melatonin, 5-HEAT and CTL 01-05-B-A05 have substitutions in the R1 position. 5-HEAT was able to keep a position superimposable to that of bound 2-iodomelatonin thanks to hydrogen bonds to MT1 residues N1624.60 and Y1875.38. (Fig. 4g). To conclude that 5-HEAT

is an MT1 agonist, we must first determine whether or not its molecular structure is consistent with the expected position of the active pocket necessary to activate MT1.

On the other hand, due to the dissimilar shapes of N1754.60 and Y2005.38, the MT2 antagonist 5-HEAT was not a good fit for docking in the active pocket of MT2. Induced-fit binding is probably used by 5-HEAT. Weak binding of CTL 01-05-B-A05 to MT2 was observed because the side chain of I2075.45 disrupted the stacking contact between these two molecules. (Fig. 4i). Notably, in light of our findings, further biopic ligand development is required to produce more focused MT1 agonists. In light of our findings, further development of the biopic ligand is required to produce more focused MT1 agonists. A feasible technique for optimizing the fit with the "longitudinal channel" would include specific substituents in the second unit.

Figure 4:

The region known as the sub-pocket, which is located around the R3 group of the ligand and was barely distinguishable in the inactive MT1 and MT2 pockets, became more distinct in the active structures. At position 7.40 in MT1, there is a tyrosine (Y2827.40). Lucien (L2957.40) is the equivalent residue in MT2 (Fig. 5j). When Y2827.40 is packed against TM1, the two adjacent residues Y2817.39 and Y2857.43 are pushed closer to the pocket's center than the corresponding residues Y2947.39 and Y2987.43 in MT2 (Fig. 4j). Since MT2 has a larger sub pocket; as a result, it can accommodate ligands with bulky R3 substituents, which is in line with the chemical architectures of the majority of MT2 selective agonists. (K. H. Chan et al., 2020; Zlotos et al., 2014). MT1 and MT2 receptors are structurally and functionally similar and also have unique features in their ligand binding pockets. The N4.60-Y5.38-H5.46 motif, the longitudinal channel, and the larger subpocket in melatonin receptor type 2 could all be used as targets for the designing of melatonin subtype-selective drugs.

Figure 5:

7.1. Receptor signaling:

The intrinsic melatonin receptor affinity for different types of G proteins is not yet known. The relative expression of different proteins is dependent on the coupling profile of the G protein, accounting pharmacology of the melatonin receptor bias system. MT1 and MT2 receptors inhibit Adenyl cyclase after coupling to G inhibitory proteins. Melatonin receptor type 1 co-immunoprecipitated with Gαi3 and Gαi2 inhibitory proteins, has the least affinity for Gq/11 proteins and doesn't couple to Gαi1, Gαz, Gαo, Gα12, or Gαs proteins in HEK293 cells (Brydon et al., 1999). Gα16 protein is expressed in hematopoietic cells, which illustrates the bias system (Amatruda et al., 1991). Melatonin receptors type 1 and type 2 couple to Gα16 protein through Jun N-terminal kinase. In COS-7 cells, the melatonin signaling pathway is initiated (A. S. L. Chan et al., 2002). The concentrations of inositol triphosphate, diacylglycerol, Ca2+, and cAMP are regulated by melatonin receptors in the cells (Brydon et al., 1999). In tissues and cells, Gq/11 couples to melatonin receptors endogenously in the myometrium, prostate (Steffens et al., 2003), pancreatic cells and epithelial cells (Bähr et al., 2012) and mesenchymal stem cells of humans (Lee et al., 2014), cells from non-mammalian organisms (Hotta et al., 2000) and cells which express recombinants (MacKenzie et al., 2002). Ion channels and multiple pathways are regulated by melatonin. Muscle contraction is modulated by melatonin in arteries (Masana et al., 2002). Melatonin controls the myometrium's conductance of K+ channels that Ca2+ activates, and the activation of the Gi/cAMP/PKA & Gq/PLC/Ca2+ signaling pathways modulates the function of these channels. Activation of gene transcription & inhibition of transcriptional factor cAMP responsive element binding protein takes place through extracellular-signal-regulated kinase pathway at the transcriptional level. Melatonin receptors type 1 and type 2 are different only in the inhibition of cGMP synthesis during signaling. Melatonin receptor type 2 synthesizes cGMP, which is studied in human non-pigmented ciliary epithelial cells (Petit et al., 1999).

7.2. Signalling cascades and Effects:

Regulation of circadian rhythm has been extensively studied and based on system bias (Dubocovich et al., 2010). Melatonin affects the master clock and hypothalamic suprachiasmatic nucleus neurons and mediates

in a cAMP-independent manner but a Gi-dependent manner. G protein-coupled receptors are activated, rectifying K-channels, i.e., Kir3 in melatonin receptors type 1 (Nelson et al., 1996), and melatonin receptor type-2 mediates action through the PKC signaling pathway (McArthur et al., 1997). Both receptors modulate neuronal actions through induced cAMP synthesis by pituitary Adenyl cyclase activating peptides (PACAP) in the suprachiasmatic nucleus (Pfeffer et al., 2012). Melatonin mediates action in a Gi-dependent manner and modulates gene expression in the striatum (Imbesi et al., 2009). Melatonin type 1 receptor can affect the rate of activation of cerebellar Purkinje cells by inhibiting P-type Ca2+ channels via Gi/G/PI3K/PKC signaling (Zhang et al., 2015). Synchronizing effects in the hypophyseal pars tuberalis with SCN are mediated by melatonin. In order to control the production of mPer1, mCry1, clock, and Bmal1 genes, melatonin activates a heterologous repressive mechanism via MT1 and adenosine A2B receptors and sensitizes the cAMP pathway (Von Gall et al., 2002). This signaling cascade is mediated by NPAS4, a transcription factor with a Per-Arnt Sim domain, and G protein regulators (West et al., 2013). It is not fully understood how melatonin regulates circadian rhythm, but it appears to vary on cell type (Mühlbauer et al., 2009), including Clock Gene Transcription and Post-Translational Regulation (Vriend & Reiter, 2015). Melatonin regulates the clock machinery of the retina. The melatonin signaling mechanism in retinal physiology is still unknown (Dinet et al., 2007).

Figure 6 :

Melatonin receptors of knockout mice showed variations in the expression of genes that control clock rhythm and other genes' expression (Hiragaki et al., 2014). Melatonin mediates action dependent on MT1/MT2 heteromers, activating Gq/PLC/Ca2+ pathway and controlling light sensitivity in the retina at night (Hiragaki et al., 2014); regulation of photoreceptor is dependent on the Akt/FOXO1 signaling pathway (Gianesini et al., 2016). During pathological and physiological conditions, the viability of neurons is regulated by melatonin. Melatonin shows neuroprotective and antiapoptotic and different signaling pathways. Melatonin helps in cell survival, maturation, and differentiation in the stem cells and is prevented by luz indole, a competitive receptor antagonist (Ramírez-Rodríguez et al., 2009). Melatonin stimulates neural development in pluripotent stem cells by activating the PI3K/Akt pathway, while luz indole inhibits this process (Shu et al., 2016). Melatonin increases the glucose transporter GLUT1's activity, activating the PI3K/Akt and ERK pathways in ES cells to promote pluripotency (X. Wang et al., 2011; H. Wu et al., 2017) Luz indole is vulnerable to neurons and is the main cause of neuron cell death in MT1-silenced cells. Melatonin upregulates different antioxidant enzymes, i.e., SOD1 and glutathione peroxidase, and plays antiapoptotic and antioxidant roles in ischemia or reperfusion (Parada et al., 2014). Neuroprotective effects of melatonin and ago melatonin in cerebral ischemia via up-regulation of nuclear factor erythroid related factor 2 and down-regulation of reactive oxygen species (Ding et al., 2014). Neu-P11 ligand, which acts on both the 5-HT and melatonin receptors, activates multiple pathways critical to neuronal survival. These include the PI3K/Akt, ERK, and JAK2 pathways (Buendia et al., 2015) (Figure#7). Mitochondrial function and dynamics are responsible for the antioxidant and antiapoptotic effects of melatonin (Tan et al., 2016). It activates caspase-3 and, prevents cytochrome c from being released & regulates Bcl-2 and Back expression (Radogna et al., 2008). Bax/Bcl-2 translocation is induced by the JAK2/STAT3 pathway in cardiomyocytes (Duan et al., 2013). By inducing ERK activation and blocking p38 MAPK in monocytes, an antiapoptotic effect is produced (Hardeland, 2012). Sirtuin histone deacetylase (SIRTs) is activated by melatonin through mitochondrial signaling pathways (Mayo et al., 2017), i.e., In hepatocytes, AMP-activated protein kinase (AMPK), sirtuin 3 (SIRT3), superoxide dismutase (SOD2), and sirtuin (Guo et al., 2014). The transcription factor PGC-1α is controlled by the MT1 receptor in retinal cells (Kunst et al., 2015). The nuclear factor kappa B (NF-kB) pathway is inhibited by sirtuin 1 (SIRT1), which in turn causes the anti-inflammatory effects of melatonin (Tajes et al., 2009). Melatonin-induced MT1-dependent regulation of mitochondrial function in mice models is used to treat the neurodegenerative diseases of Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis (Li et al., 2013). Cytochrome c is inhibited by melatonin in brain mitochondria through mitochondrial MT1 receptors (X. Wang et al., 2011). A cell-permeable melatonin receptor agonist was employed to distinguish between the mitochondrial Gi/cAMP cascade generated by MT1 and the rest of the cell (Gbahou et al., 2017). Melatonin's neuroprotective effects are under-studied and is associated with mitochondrial

MT1 signaling pathways. In the cancer field, melatonin impacts system bias on melatonin receptor cascades and shows antitumor properties by inducing apoptosis and inhibiting proliferation. MT1 receptors inhibit the phosphorylation of AKT, ERK, and PKC molecules in breast cancer models and show antitumor activity (Hill et al., 2015). In these cells, melatonin activates the p53 DNA pathway dependent on the receptor**,** Akt, p38 MAPK and mTOR pathways are inhibited by melatonin in ovarian cancer.

Figure 7 : .

8. Therapeutic application:

The regulatory effects of melatonin on the sleep-wake cycle and circadian rhythm are crucial for a wide range of melatonin therapeutic applications. Due to its potential therapeutic effects, melatonin hormone is used to treat a variety of disorders, including jet lag, insomnia, circadian rhythm disorders, mood disorders, cancer, cardiovascular diseases, and neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, as well as other seasonal affective disorders.

Figure 8:

8.1. Cardiovascular system:

8.1.1. Lipids metabolism: Melatonin reduces high serum total cholesterol and triglyceride levels in the blood. It improves lipid metabolism by decreasing low-density lipoprotein and hence maintains lipid profile (Koziróg et al., 2011).

8.1.2. Blood Pressure: Melatonin intake decreases systolic and diastolic blood pressure at night. It also decreases nocturnal systolic blood pressure (Borghi & Cicero, 2017).

8.1.3. Ischemia/reperfusion injury: Consuming melatonin reduces the severity of cardiac marker injury and myocardial infarction size. It lowers heart rate by increasing ejection fraction. Melatonin intake improves blood pressure, glycaemic, and lipid in patients suffering from chronic heart diseases (Baker & Kimpinski, 2018).

8.1.4. Heart Failure: Owing to antioxidant and antiapoptotic properties, melatonin intake decreases cardiac fibrosis in nonischemic heart failure. Its intake prevents myocardial infarction (Nduhirabandi & Maarman, 2018).

8.2. Nervous System

8.2.1. Ischemic stroke: Melatonin intake is helpful in decreasing infarct volume and brain edema and improving neurologic score (Liu et al., 2019).

8.2.2. Alzheimer's disease: Melatonin intake is helpful in improving sleep quality and cognitive function in the brain. Its intake treats sleep disorders associated with amnesia, sundowning, and Alzheimer's diseases (Lin et al., 2013).

8.2.3. Parkinson's disease: As it delays the degeneration of dopaminergic neurons in the substantia nigra, melatonin intake is beneficial in the treatment of Parkinson's disease. Cognitive dysfunction, anxiety, depression, and sleep quality all improve as a result (Medeiros et al., 2007).

8.2.4. Epilepsy: Melatonin improves sleep behavior in epilepsy patients (Gupta et al., 2004).

8.2.5. Migraine: Melatonin is used to treat migraine and prophylaxis in both adults and children (Fallah et al., 2015).

8.2.6. Chronic tension-type headache: Melatonin intake improves sleep quality and prevents headache and tension (A. B. Danilov et al., 2020).

8.2.7. Traumatic brain injury and spinal cord injury: Melatonin is used to treat sleep disturbances in patients with traumatic brain injury who report feeling anxious (Grima et al., 2018).

8.3. Reproductive system

8.3.1. Erectile dysfunction: Consuming melatonin enhances the corpus cavernosum's ability to contract and relax. It improves endothelial density and erectile function (Tavukçu et al., 2014).

8.3.2. Female reproductive system: Melatonin intake improves fertilization rate. It increases progesterone production in the corpus luteum (Hu et al., 2020).

8.4. Gastrointestinal system

8.4.1. Gastroesophageal reflux disease and Gastrointestinal ulcer: Melatonin provides protection against mucosa oxidative damage in different types of gastrointestinal tract ulcers, reduces relaxation duration, and increases gastrin (Kandil et al., 2010).

8.4.2. Irritable bowel syndrome: Melatonin decreases abdominal pain, bloating, and constipation and increases rectal pressure (Chojnacki et al., 2013).

8.4.3. Hepatic steatosis: Melatonin lowers liver cholesterol, triglycerides, serum AST and ALT levels in hepatic steatosis patients (Bahrami et al., 2020).

8.4.4. Hepatoprotective effects: Haemorrhagic shock, ischemia-reperfusion injury, liver damage, ionizing radiation, and Schistosoma mansoni infection are all diminished by melatonin (Mathes, 2010).

8.5. Renal system

8.5.1. Reno protective effects: Melatonin protects against radiation-, folic acid-, aminoglycoside-, contrast-mediated-, and nephrotoxicity-induced by these agents. It reduces creatinine and blood urea nitrogen levels (Kim et al., 2019).

8.5.2 Sepsis-induced renal injury: Melatonin intake decreases inflammasome activation (Dai et al., 2019).

8.6. Dermatology: Melatonin protects against UV-light-induced damage by preventing the production of free radicals, erythema caused by natural sunlight, and radioprotective effects. It exhibits antiaging properties, enhances hydration, and lessens the roughness of the skin (Rusanova et al., 2019).

8.7.1. Fibromyalgia: Melatonin intake improves pain level and fibromyalgia (A. Danilov & Kurganova, 2016).

8.8.1. Autism spectrum disorder: Consuming melatonin lowers mid-sleep awakenings, improves sleep quality, and lengthens total sleep time (Maras et al., 2018).

8.8.2. Mood disorder: Melatonin diminishes sleep disorders linked to a depressed mood (Serfaty et al., 2010).

8.9. Oncology

8.9.1. Breast cancer: Due to its antiestrogenic effects, which lessen unwanted side effects, melatonin adjuvant therapy is used for patients who are at risk of developing ER-positive breast cancer (González-González et al., 2018).

8.9.2. Ovarian cancer: During chemotherapy, melatonin provides protection to ovaries and fertility preservation (N. Wang et al., 2020).

8.9.3. Lung cancer: Melatonin lessens harm to the ileum, colon, liver, and lungs. It lessens radiation-induced lung injury and modulates the effectiveness of DNA repair in humans as well as the genotoxic activity of irinotecan (Tahamtan et al., 2015).

8.9.4. Colorectal cancer: Because ursolic acid has antiproliferative and pro-apoptotic effects on colon cancer cells, melatonin is used to treat this disease (J. Wang et al., 2013).

8.10. Viral syndromes

8.10.1. Respiratory Syncytial Virus: The acute lung oxidative injury brought on by respiratory syncytial virus is lessened by melatonin (Huang et al., 2010).

8.10.2. Viral myocarditis: Melatonin is used for the preservation of cardiac functions and for repression of virus-induced cardiomyocyte apoptosis. It inhibits apoptosis, regulates the rate of autophagy, and maintains mitochondrial dysfunction (Ouyang et al., 2019).

8.10.3. Venezuelan equine encephalitis: Melatonin intake increases survival rate by reducing virus load in the brain and serum (Montiel et al., 2015).

8.10.4. Encephalomyelitis virus: Melatonin is used to treat encephalomyelitis by preventing death and paralysis (Boga et al., 2012).

8.10.5. Semliki forest virus: Melatonin lowers viremia and postpones disease and death (Ben-Nathan et al., 1995).

8.10.6 COVID-19: Consuming melatonin prolongs survival time by reducing oxidative damage and slowing down the release of cytokines (Cross et al., 2021).

Future Prospects:

Future directions in studying the signaling pathways and receptors for the melatonin hormone appear promising. To better comprehend the signaling pathways underlying melatonin action, the structure of the MT1 and MT2 receptors in association with various ligands and signaling molecules must be determined. Recent research has focused on the implicit role of the MT1 and MT2 receptors in a variety of sleep, cancer, and metabolic disorders. Creating new medications and adjusting melatonin's signaling pathways to treat complaints. Just two examples of the signaling pathways that interact with melatonin signaling are the cAMP and mitogen-activated protein kinase (MAPK) pathways. To find new signaling pathways, researchers are examining how the melatonin hormone binds to receptors that are similar to orphan GPCRs. More study is required to determine new therapeutic targets for melatonin and its analogs. More research is required to fully comprehend its mechanisms of action and to maximize its therapeutic application. Down streaming signaling mechanism is still unknown. All studies about melatonin effects were assessed in in-vivo and in-vitro testing and preclinical trial but lacked significant clinical trials. So there's a huge need to conduct clinical trials to fully understand long-term melatonin's physiological effects. The signaling mechanism of melatonin receptors, their structures and their interaction with the melatonin hormone are still unknown, so there are a lot of unexplored future directions for researchers. Researchers may be able to develop potent new treatments for a variety of diseases and disorders if they learn more about the receptors involved and the signals they send. Melatonin's regulatory effects on several physiological systems make it promising for a variety of therapeutic purposes.

Conclusion:

Melatonin, a hormone produced by the pineal gland, controls circadian rhythms, sleep-wake cycles, and other physiological processes. G-protein-coupled receptors (GPCRs), of which two are known as MT1 and MT2, mediate melatonin's effects on the body. Molecular modeling and X-ray crystallography are two methods that have been used to determine the structures of the melatonin type 1 and type 2 receptors. Each of these receptors has seven transmembrane domains that connect the intracellular and extracellular domains together. A conformational change that occurs when melatonin binds to its receptors causes downstream signaling pathways to become active. Recent research continues to focus on the receptors and signaling pathways that melatonin mediates. Due to the multifactorial pathophysiology of melatonin, the majority of the studies were preclinical, in vitro, with small sample sizes, and concentrated on short-term results. Additionally, more research is required because inadequate study methods constrain decision-making regarding this new use for this medication. Before applying this knowledge to clinical practice, more clinical trials with larger sample sizes, precise dosages, and longer durations are required to confirm the long-term side effects of melatonin. The investigation of the receptors and signaling mechanisms that melatonin uses to exert its beneficial therapeutic effects.

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Graphical Abstract:

Figure 1: Synthetic and Metabolic Pathway of Melatonin

Figure 2: Synthesis of Melatonin through the neurologic pathway from the pineal gland and its effects.

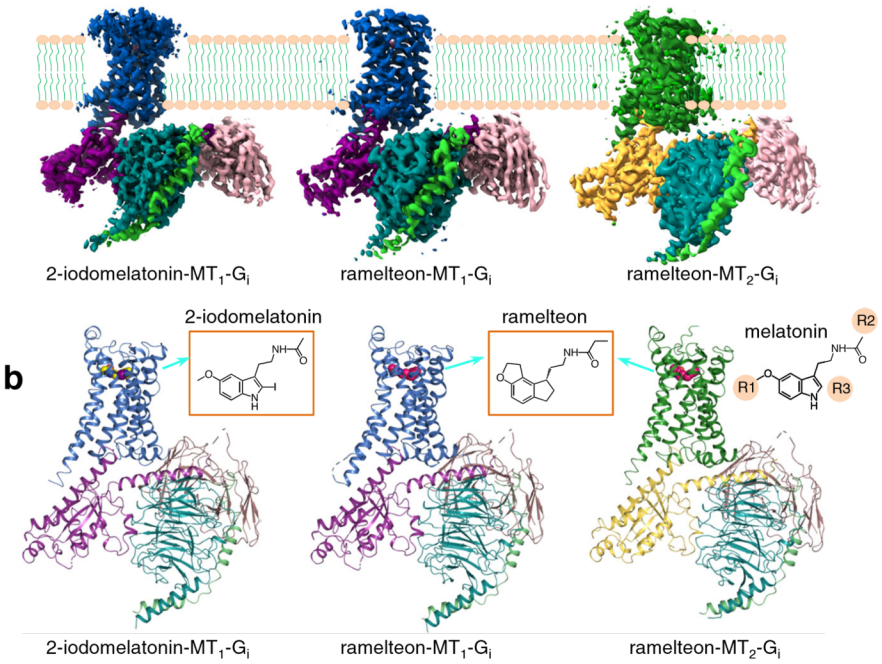

Figure 3: MT1-Gi and MT2-Gi complex structures: The complexes are shown by Cryo-EM density maps. blue colour represents melatonin type 1 receptors; green colour represents melatonin type 2, green; purple represent Gαi in MT1; yellow represent Gαi in melatonin type 2; scFV16, violet is colour code. Gβ, teal; Gγ, light green (b) showed the cryo-EM structure of MT1-Gi and MT2-Gi. The left side showed 2-iodomelatonin-bound MT1-Gi; the middle side showed ramelteon-bound MT1-Gi; the right side showed ramelteon-bound MT2-Gi. The right side shows the structure of ligands and melatonin molecules

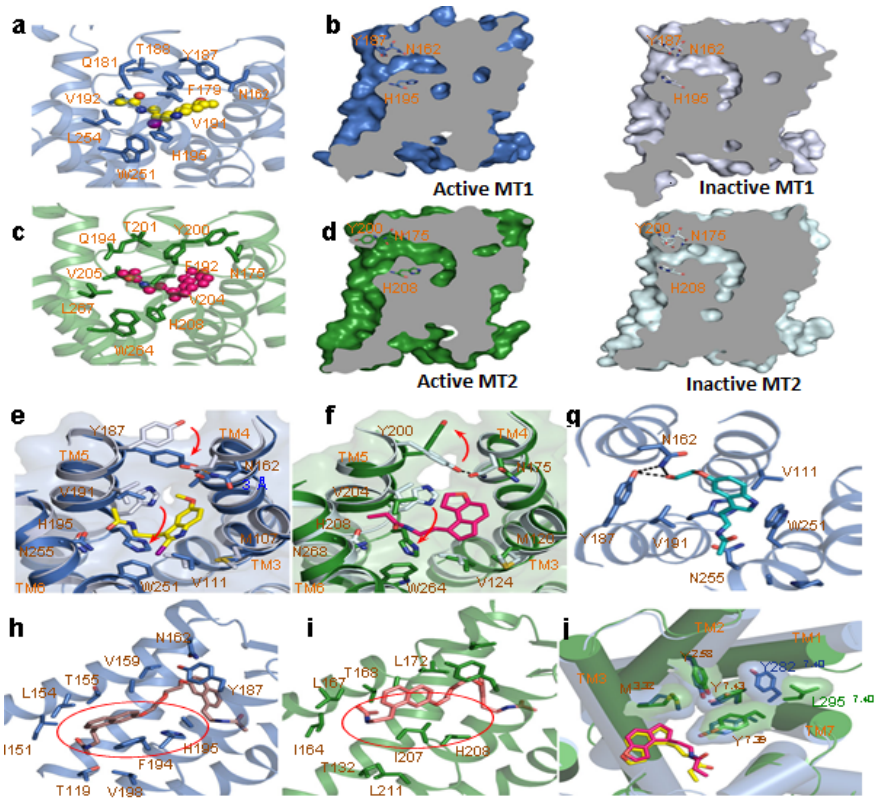

Figure 4: Ligand-binding pocket and selectivity of determinants. MT1 has a ligand-binding pocket (yellow) where 2-iodomelatonin (yellow) is bound (blue). B Active (left) and inactive (right) forms of the ligand access channel MT1 are shown in the slab view (right, light blue, PDB ID: 6ME4). Ramelteon (pink) is attached to the ligand-binding pocket in MT2 (green). Schematic slab views of the active (left) and inactive (right) forms of the MT2 ligand access channel (right, light cyan, PDB ID: 6ME9). Ligand-binding residues of MT1 are shown in blue, while those of inactive ligand-binding pockets are shown in a lighter shade of blue. Red arrows indicate alterations of note. N162 and Y187 form a 3-f orbital distance hydrogen bond. The ligand-binding pockets of active (green) and inactive (light cyan) MT2 are contrasted. Red arrows indicate changes of significance. Active MT1 has g 5-HEAT docked in it (cyan). Key residues that are causing problems in this pocket are shown as sticks. In the open MT1, salmon CTL 01-05-B-A05 has successfully spawned. The red circle denotes the hydrophobic packing of the naphthalene group and F1945.45. MT2 was open when salmon CTL 01-05-B-A05 swam in. The Naphthalene group and I2075.45 are packed incompatible, as indicated by the red circle. j Comparison of the sub pockets from MT1 and MT2 that are active and bound to ramelteon (red in MT1, yellow in MT2). Sticks represent important distinct residues.

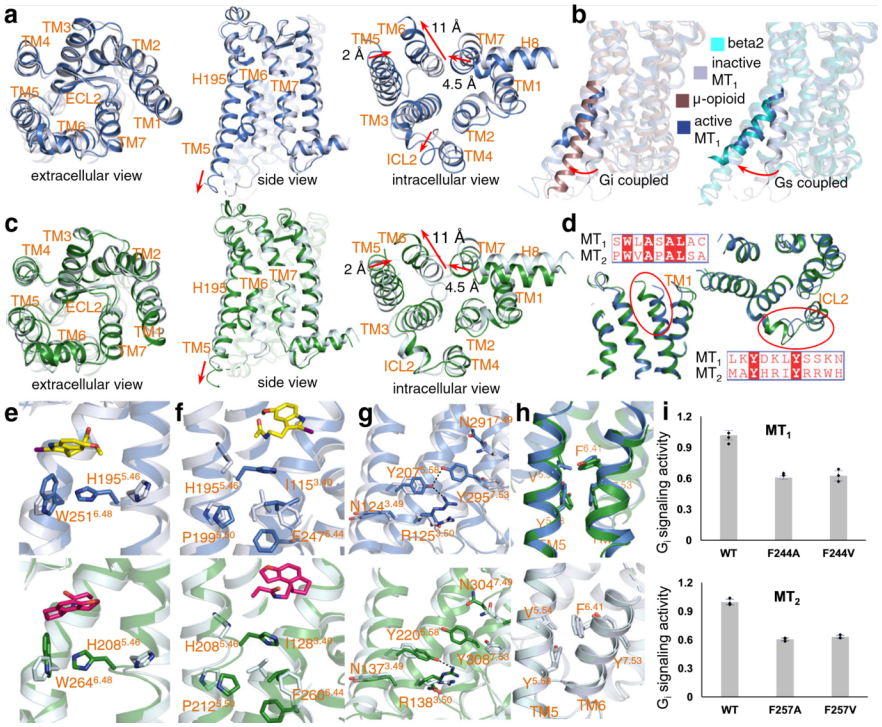

Figure 5: Activation of MT1 and MT2 Receptors; a. The active structure of the MT1 receptor (blue) and an inactive type 1 receptor (light blue) are shown and contrasted. There are three perspectives available. In this image, the TM6 conformation of the Gs-coupled beta2 receptor (right) and the Gi-coupled -opioid receptor (left) is compared to that of the MT1 molecule. Structures of active (green) and inactive (light green) MT2 are shown side by side. We see three different perspectives here. These images show the differences between MT1's TM1 (left) and MT2's ICL2 (right). E–g There are conformational changes in MT1 motifs and other critical residues during receptor activation. Structures of active and inactive melatonin receptors 1 and 2 revealed F6.41 conformations. Gi signaling pathways were visible in I F6.41 from both MT1 and MT2 mutants. The findings are presented as the means standard deviations of three separate experiments in which wild-type receptors were used as a reference.

Figure 6 Signalling Pathway: Melatonin activates MT1 receptors and activates Gαi decreasing cAMP second messenger and activates PI3K/Akt, PKC, and ERK pathways dependent on Gβγ. Intracellular Ca2+ concentration is increased. PLC is activated by Gq coupling to melatonin. Potassium and calcium on channels are activated by melatonin and modulate neuronal action mediated and inhibit Ca2+ entry through Gβγ subunits. MT2 receptors are activated by the ERK signaling pathway & Gαi-dependent cAMP & inhibited cyclic guanosine monophosphate synthesis. Recruitment of β-arrestin is induced by melatonin, down streaming signaling mechanism is still unknown.

Figure 7 Signalling pathways: Different signaling pathways are activated by melatonin depending on the presence of cell stressors or cell types. MT1 receptors are mainly involved in these signaling pathways, and MT2 receptors also participated in these pathways and were studied in neurodegenerative disorders and under oxidative stress conditions, involving melatonin modulation of mitochondrial signaling mechanisms, i.e., translocation of SIRT proteins and Bcl2/Bax is regulated. The Akt/FOXO1, ERK, and JAK2 complexes activated by melatonin induce the survival of cells and regulate stem cell differentiation. These signaling pathways are inhibited by melatonin in cancer cells. Anti-inflammatory and anti-oxidative effects are regulated by the transcription factor, i.e., Nrf2, PGC1α, and NF-ϰB, which depends on the activation of SIRT1. In hematopoietic cells, the JNK pathway is triggered by the coupling of MT1 to G16 protein.

Expression of different miRNAs is regulated by melatonin in different types of cells, i.e., cancer cells.

Figure 8: Physiological action of Melatonin hormone

Table#1: MT1 and MT2 Receptor's distribution in Human

Receptor Type		**Tissues**	**Tissues**	**Reference**
hMT1	Brain	Cerebellum Occipital cortex Parietal cortex Temporal cortex Thalamus Frontal cortex Hippocampus	(Mazzucchelli et al., 1996) (Al-Ghoul et al., 1998) (Mazzucchelli et al., 1996) (Mazzucchelli et al., 1996) (Mazzucchelli et al., 1996) (Mazzucchelli et al., 1996)	(Mazzucchelli et al., 1996) (Al-Ghoul et al., 1998) (Mazzucchelli et al., 1996) (Mazzucchelli et al., 1996) (Mazzucchelli et al., 1996) (Mazzucchelli et al., 1996)
	Peripheral tissues	SCN Retina Brown and white adipose tissue Fetal kidney Coronary artery Granuloma cells Myometrium Pancreatic alpha and beta cells Testis	(Savaskan et al., 2007) (Reppert et al., 1995) (Brydon et al., 2001) (Drew et al., 1998) (Soares et al., 2003) (Lanoix et al., 2006) (Sharkey et al., 2009) (Blodgett et al., 2015) (Rossi et al., 2014)	(Savaskan et al., 2007) (Reppert et al., 1995) (Brydon et al., 2001) (Drew et al., 1998) (Soares et al., 2003) (Lanoix et al., 2006) (Sharkey et al., 2009) (Blodgett et al., 2015) (Rossi et al., 2014)
hMT2	Brain	Cerebellum Hippocampus SCN	(Al-Ghoul et al., 1998) (Savaskan et al., 2007) (Y.-H. Wu et al., 2013)	(Al-Ghoul et al., 1998) (Savaskan et al., 2007) (Y.-H. Wu et al., 2013)
	Peripheral tissues	Retina Brown and white adipose tissue Fetal kidney Granulosa cells Placental tissues Myometrium Pancreatic alpha and beta cells Testis	(Reppert et al., 1995) (Brydon et al., 2001) (Drew et al., 1998) (Soares et al., 2003) (Lanoix et al., 2006) (Sharkey et al., 2009) (Kato et al., 2005) (Rossi et al., 2014)	(Reppert et al., 1995) (Brydon et al., 2001) (Drew et al., 1998) (Soares et al., 2003) (Lanoix et al., 2006) (Sharkey et al., 2009) (Kato et al., 2005) (Rossi et al., 2014)