

Update on Melatonin Receptors. IUPHAR Review.

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▶ **To cite this version:**

Ralf Jockers, Philippe Delagrange, Margarita L Dubocovich, Regina P Markus, Nicolas Renault, et al.. Update on Melatonin Receptors. IUPHAR Review.: Melatonin Receptors . British Journal of Pharmacology, 2016, 10.1002/BPH.13536 . inserm-01335656

HAL Id: inserm-01335656

https://inserm.hal.science/inserm-01335656

Submitted on 22 Jun 2016

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Title: Update on Melatonin Receptors. IUPHAR Review

Short title: Melatonin Receptors

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/BPH.13536

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Abstract

Melatonin receptors are seven transmembrane-spanning proteins belonging to the G protein-coupled receptor super-family. In mammals, two melatonin receptor subtypes exit MT_1 and MT_2 encoded by the *MTNR1A* and *MTNR1B* genes, respectively. The current review provides an update on melatonin receptors by the corresponding sub-committee of the International Union of Basic and Clinical Pharmacology. We will highlight recent developments of melatonin receptor ligands, including radioligands and give an update on the latest phenotyping results of melatonin receptor knockout mice. The current status and perspectives of the structure of melatonin receptor structures will be summarized. The physiological importance of melatonin receptor dimers and biologically important and type 2 diabetes-associated genetic variants of melatonin receptors will be discussed. The role of melatonin receptors in physiology and disease will be further exemplified by its functions in the immune system and the central nervous system. Finally, antioxidant and free radical scavenger properties of melatonin and its relation to melatonin receptors will be critically addressed.

Keywords (max. 10): GPCR oligomerization, sleep disorders, circadian rhythm disorders, depression, neuroprotection, genetic variants, type 2 diabetes, neurodegenerative diseases, free radical scavenger.

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Abbreviations:

ADHD, attention-deficit/ hyperactivity disorder;

ASD, autism spectrum disorders;

AD, Alzheimer's disease;

BRET, bioluminescence resonance energy transfer;

CaMKII, Ca2+/Calmodulin (CaM)-dependent kinase II;

cAMP, cyclic adenosine monophosphate;

CNS, central nervous system;

ERG, electroretinogram;

fMLP, N-formyl-l-methionyl-l-leucyl-l-phenylalanine;

FPG; fasting plasma glucose;

GPCRs, G protein-coupled receptors;

HD, Huntington disease;

ICR, Imprinting Control Region;

IL-2, interleukin 2;

IOP, intraocular pressure;

IUPHAR, international union of basic and clinical pharmacology;

KO, knockout;

LPS, lipopolysaccharide;

MLT, melatonin;

MTR, melatonin receptor

MS, multiple sclerosis;

NREM, non-rapid eye movement;

PD, Parkinson disease;

REM, rapid eye movement;

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SCN, suprachiasmatic nucleus;

QR2, quinone reductase 2;

T2D, type 2 diabetes.

Links

Targets

GPCRs[b]
5-HT2C
β2-adrenergic receptor
A2A adenosine receptor
GPR50
MT1 receptor
MT2 receptor
Channels
Cav2.2
Transporters[e]
GLUT1
Enzymes[d]
CaMKII
ERK1

Ligands
6-chloromelatonin
[^{125}I]-DIV880
5-HEAT
6-hydroxymelatonin
2-iodomelatonin
2-[125I]iodomelatonin
[3H]-melatonin
2-methoxy-α,β-didehydro-agomelatine
5-methoxyluzindole
4P-PDOT
[^{125}I]-SD6
[^{125}I]-S70254
agomelatine
BOMPPA
cAMP
CBOBNEA
CIFEA
Difluoroagomelatine

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dopamine
EFPPEA
fMLP
GR 196429
GR 128107
(hydroxymethyl)phenyl agomelatine
IIK7IL-2
K185
Luzindole
MCA-NAT
N-acetyl-serotoninLPS
Melatonin
ramelteon
S22153
S24014
S24773
S26131
S26284
tasimelteon
TNF
TIK 301
UCM 454
UCM 765
UCM 793

Introduction

The hormone melatonin (MLT) is mainly produced by the pineal gland following a circadian rhythm, with high levels during the subjective night. MLT can also be produced by extra-pineal sites like the retina, the gastrointestinal tract and the innate immune system. MLT regulates a variety of physiological and neuroendocrine functions through activation of two G protein-coupled melatonin receptors (MTR) called MT_1 and MT_2 in mammals. Both receptors are typically coupled to $G_{i/o}$-type proteins and MT_1 in addition to G_q-type proteins. In humans, the *MTNR1A* gene encoding MT_1 is located on chromosome 4q35.1 and the *MTNR1B* gene encoding MT_2 on chromosome 11q21-q22.

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This article will review and discuss recent updates by the MTR sub-committee of the International Union of Basic and Clinical Pharmacology (IUPHAR) database (http://www.guidetopharmacology.org/GRAC/FamilyDisplayForward?familyId=39), which include the development of new MTR ligands, radioligands and structural perspectives of MTRs. The discovery of MTR dimers with physiological function *in vivo* as well as genetic variants and mutants of MTRs will be discussed as they provide a new dimension to understand MTR pharmacology and function. An update on the latest results obtained with MTR knockout (KO) mouse models will be provided. Among the multiple physiological effects of MTRs, we chose to focus on those of the immune system and the central nervous system (CNS). At the end, MTR-independent effects, including antioxidant and free radical scavenger properties of MLT will be critically addressed.

For more complete or other specific aspects of MTR the reader is referred to other recent expert reviews (Dubocovich *et al.*, 2010; Jockers *et al.*, 2008; Liu *et al.*, 2016; Markus *et al.*, 2013; Tosini *et al.*, 2014; Zlotos *et al.*, 2014).

Melatonin receptor ligands

MT_1 and MT_2 share a high degree of sequence homology and bind both the natural ligand, MLT, with high affinity. Important progress has been made in the identification of synthetic MTR antagonists and agonists and subtype-selective ligands by diversifying the chemical scaffolds. Indeed, MTR ligands from different structural classes show distinct structure activity relationships on native and recombinant MTRs (Dubocovich *et al.*, 2010; Dubocovich *et al.*, 1997; Spadoni *et al.*, 2011; Zlotos, 2012; Zlotos *et al.*, 2014). The methoxy group and the acetamido side chain of MLT determine the intrinsic activity and the binding affinity, respectively, at both hMT_1 and hMT_2 (Audinot *et al.*, 2003; Browning *et al.*, 2000; Dubocovich *et al.*, 1997). Replacement of the amide methyl group by ethyl and propyl

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substituents enhances affinity (Sugden *et al.*, 1995). Exchange of the indole ring by various aromatic scaffolds maintains high binding and agonist potency.

Substitutions at the 2-position with a halogen or a phenyl group generate agonists with ~10-fold increased binding affinity. The majority of nonselective MT_1-MT_2 ligands, including drugs used in humans, i.e., **ramelteon** (Rozerem®, (Kato *et al.*, 2005; Mini *et al.*, 2007; Rawashdeh *et al.*, 2011)), **agomelatine** (Valdoxan®, (de Bodinat *et al.*, 2010)), and **tasimelteon** (Hetlioz®, (Lavedan *et al.*, 2015; Rajaratnam *et al.*, 2009)), are agonists (Figure 1). Ramelteon and tasimelteon are MTR selective, while agomelatine is also an antagonist at the 5-HT_{2c} receptors, a pharmacological property believed to contribute to its antidepressant action. The therapeutic effects of approved drugs acting on hMT_1 and/or hMT_2 as agonists was recently reviewed (Liu *et al.*, 2016). Other nonselective MTR agonists include **6-chloromelatonin, 6-hydroxymelatonin, 2-iodomelatonin, GR 196429** (Audinot *et al.*, 2003; Browning *et al.*, 2000; Dubocovich *et al.*, 1997), **UCM 793** (Rivara *et al.*, 2007) and **2-methoxy-α,β-didehydro-agomelatine** (Morellato *et al.*, 2013). This latter ligand shows the highest affinity for hMT_1 (K_i = 0.03 nM) and hMT_2 (K_i = 0.07 nM) receptors and ~3,500-fold greater potency than MLT in the melanophore aggregation assay. **TIK 301** (Mulchahey *et al.*, 2004) acts also as an antagonist at the serotonin receptor subtypes 5-HT_{2C} and 5-HT_{2B}, (Landolt *et al.*, 2009). **5-HEAT** has a unique pharmacological profile acting as a full agonist at the hMT_1 and antagonist at the hMT_2 (Nonno *et al.*, 2000). **EFPPEA**, a high-affinity hMT_1 (K_i = 0.062 nM) and hMT_2 (K_i = 0.420 nM) agonist, decreases the percentage of wakefulness and increases the percentage of slow wave sleep in cats (Koike *et al.*, 2011). The competitive MTR antagonist **luzindole** lacks the methoxy group, which led to the suggestion that this group is necessary for intrinsic activity (Dubocovich, 1988). Similarly, **S22153** acts as a partial agonist (Audinot *et al.*, 2003). Luzindole, with a 15- to 25-fold higher affinity for hMT_2 than for hMT_1, is widely used for pharmacological characterization of functional

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MTRs (Browning *et al.*, 2000; Dubocovich *et al.*, 2010; Dubocovich *et al.*, 1997; Dubocovich *et al.*, 1998).

A ligand is considered selective for a specific receptor type when its affinity or potency is at least 100-times higher than that for the other(s) receptor types in the family (Dubocovich *et al.*, 2010). This concept holds true for *in vitro* studies where ligand concentrations can be easily adjusted. However, ligand selectivity might be more difficult to reach *in vivo*, in the body fluids reaching the receptors. Depending on ligand dose and pharmacokinetics, concentrations could easily raise to levels activating on both receptors (e.g., MT_1 and MT_2). This is particularly of concern for MLT and synthetic MTR ligands activating receptors at picomolar concentrations (Audinot *et al.*, 2003; Browning *et al.*, 2000; Dubocovich *et al.*, 1997). Therefore caution should be taken when interpreting selective MTR activation *in vivo* using MTR-selective ligands, unless pharmacological selectivity or lack of is confirmed by KO models with deletion of each receptor type.

Numerous ligands with high selectivity for hMT_2 over hMT_1 have been identified (Zlotos *et al.*, 2014). MT_2 possess a lipophilic pocket close to the N1−C2 binding region of MLT, which is absent in MT_1 (Rivara *et al.*, 2005). Accordingly, most MT_2-selective ligands bear a flexible bulky hydrophobic substituent in a position equivalent to C2 or N1 of MLT (Figure 2). The tetrahydroquinoline analogue **UCM1014** is the most potent MT_2-selective ligand reported to date. It shows picomolar binding affinity (K_i = 0.001 nM) at hMT_2, > 10,000-fold selectivity over hMT_1, and full agonist profile in the GTPγS test (Spadoni *et al.*, 2015). Other agonists with approximately 800-fold hMT_2 selectivity are **BOMPPA** (Chan *et al.*, 2013; Heckman *et al.*, 2011; Hu *et al.*, 2013), and **CIFEA** (Koike *et al.*, 2011). In imprinting control region (ICR) mice, **CIFEA** reeintrainment effects to a new light/dark cycle indicate the involvement of MTRs in the regulation of chronobiotic activity (Koike *et al.*, 2011). The dose of CIFEAA used in this study most likely reached μM concentrations,

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which would activate both MT_1 and MT_2, precluding any conclusion about the specific receptor type involved in the regulation of chronobiotic processes. Similarly, doses of the MT_2-selective antagonist 4P-PDOT (90 µg/mouse s.c.) used to block the MLT-mediated phase advance of circadian activity rhythms in mice (Dubocovich *et al.*, 1998) may have resulted in micromolar circulating 4P-PDOT concentrations hence blocking both MT_1 and MT_2.

Two moderately selective MT_2 ligands, the agonist **IIK7** (Faust *et al.*, 2000) and the partial agonist **UCM 765** (Rivara *et al.*, 2007) have been used to examine the role of each MTR type in the modulation of sleep architecture. **UCM 765** promoted non-rapid eye (NREM) movement sleep in rodents and this effect was blocked by the MT_2 antagonist **4P-PDOT** (Ochoa-Sanchez *et al.*, 2011). In contrast, the non-selective MT_1-MT_2 agonist **UCM793** decreased sleep onset without having an effect on NREM sleep maintenance suggesting that dual MT_1 and MT_2 agonistic activity accounts for the effect on sleep onset, whereas selectivity for MT_2 has an additional effect on NREM sleep maintenance. **IIK7** was also reported to reduce NREM sleep onset latency and transiently increase the time spent in NREM sleep in rats without altering REM sleep latency or the amount of REM sleep (Fisher *et al.*, 2009).

Among the hMT_2-selective partial agonists **GR 128107**, **5-methoxyluzindole**, **S 24014**, **S 24773** (Audinot *et al.*, 2003; Dubocovich *et al.*, 1997), and **isoamyl agomelatine**, the latter shows the highest affinity (K_i = 0.01 nM) and selectivity (7,200-fold) (Ettaoussi *et al.*, 2012). **4P-PDOT,** an hMT_2-selective antagonist with 300- to 1,500-fold higher affinity for hMT_2, is still considered the gold standard for pharmacological characterization of MTRs (Dubocovich *et al.*, 1997). Other MT_2-selective antagonists, such as **K185** (Faust *et al.*, 2000; Sugden *et al.*, 1999), **UCM 454** (Rivara *et al.*, 2005), and **2-(indolin-1yl) melatonin** (Zlotos *et al.*, 2009) display ~100-fold higher affinity for hMT_2. For **(hydroxymethyl)phenyl**

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agomelatine, the affinity for hMT_2 is 750-times higher than for hMT_1 (Poissonnier-Durieux *et al.*, 2008).

Discovery of MT_1-selective ligands remains a challenge, and only few compounds with preference for hMT_1 have been reported (Zlotos *et al.*, 2014). Ligands preferentially binding to hMT_1 reach maximally 100-fold selectivity, and, when investigated, this selectivity is significantly reduced in functional *in vitro* studies (Figure 3). A common structural feature conferring MT_1 selectivity is a bulky, hydrophobic ether replacing the methoxy group. The first hMT_1-selective agents were obtained by connecting two agomelatine units via their ether oxygens by $(CH_2)_3$- and $(CH_2)_4$-linker to give **S 26131** (antagonist) and **S 26284** (partial agonist), both displaying ~100-fold selectivity (Audinot *et al.*, 2003; Descamps-Francois *et al.*, 2003). A similar approach led to the **UCM 793 dimer** with 100-fold hMT_1 selectivity and partial agonist activity (Spadoni *et al.*, 2011). Monomeric ligands, such as **CBOBNEA** (Mèsangeau et al., 2010), and **AAE M PBP amine** (Rivara *et al.*, 2012) are partial agonists showing similar, ~100-fold hMT_1 selectivity. ***N*-Acetyl-O-phenoxypropyl serotonin** is a full agonist obtained by exchange of the methoxy group of MLT with an $O(CH_2)_3OPh$ moiety. Although it shows only 10-fold binding preference toward hMT_1, its MT_1-MT_2 binding ratio and hMT_1 affinity were higher than that for the MT_1-selective reference **S 26131** that was retested under the same experimental conditions (Markl *et al.*, 2011). A 140-fold MT_1 selectivity could be accomplished by introduction of two fluorine atoms into the *N*-acetyl group of agomelatine. The resulting **difluoroagomelatine** shows high hMT_1 binding (K_i = 0.03 nM), and is a nonselective MT_1-MT_2 full agonist (Ettaoussi *et al.*, 2012). Very recently, **tetrafluoro S26131**, **the difluoroacetamide analogue of S 26131,** has been reported to show higher affinity and selectivity toward hMT_1 than the parent ligand (Zlotos *et al.*, 2015).

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In summary, while numerous MTR ligands selective for the MT_2 subtype are available, discovery of ligands with at least >100-fold selectivity for MT_1 remains a challenging task. None of the MT_1-selective ligands has been tested *in vivo*. Future progress on the elucidation of the structure of MTRs will hopefully foster the discovery of such ligands. The MT_1-MT_2 nonselective receptor antagonist **luzindole** and the MT_2-selective antagonists **4P-PDOT** are still considered the gold standards for pharmacological characterization of MTRs.

Radioligands - update

Radioactive- and fluorescent-labeled ligands are indispensable tools for the pharmacological characterization of G protein-coupled receptors (GPCRs). A major breakthrough in the field of MTR research was the labelling of 2-iodomelatonin with ^{125}I at carbon 2 resulting in a high affinity radioligand with high specific activity (Vakkuri *et al.*, 1984) for use in the localization (Vanecek, 1988) and pharmacological characterization of MTR in tissues (Dubocovich *et al.*, 1987). The radioligand, 2-[^{125}I]iodomelatonin (2-[^{125}I]-MLT), has been extensively used as a high-affinity radioliogand for both MT_1 and MT_2 , which was until now the only available radioligand for the characterization and localization of MLT binding sites in native tissues (Figure 4). Studies performed with [^{3}H]-melatonin ([^{3}H]-MLT) established the pharmacological profile of the human recombinant MT_1 and MT_2, as being identical to that established using 2-[^{125}I]-MLT as a radioligand. However due to the rather low specific activity of this ^{3}H-MLT its use to characterize and/or localize MLT sites in tissues with low MTR density is hampered (Browning *et al.*, 2000).

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Three new iodinated radioligands have been recently characterized for use in the pharmacological characterization and localization of MTRs (Figure 4). These radioligands are: SD6 (N-[2-(5-methoxy-1Hindol-3-yl)ethyl]iodoacetamide), S70254 (2-iodo-N-2-[5-methoxy-2-(naphthalen-1-yl)-1H-pyrrolo[3,2-b]pyridine-3-yl])acetamide and DIV880 2-(2-[(2-iodo-4,5-dimethoxyphenyl)methyl]-4,5-dimethoxy phenyl) (Legros *et al.*, 2016; Legros *et al.*, 2013). [^{125}I]-SD6 has a similar pharmacological profile than 2-[^{125}I]-MLT with the same affinity for MT_1 and MT_2. On the contrary, the two other radioligands [^{125}I]-S70254 and [^{125}I]-DIV880 show selectivity for MT_2 with pK_d values of 9.6 and 9.7, respectively, in the absence of any specific binding to MT_1. All radioligands are agonists, either partial agonists ([^{125}I]-S70254, [^{125}I]-DIV880) or full agonists (2-[^{125}I]-MLT, [^{125}I]-SD6, [^{3}H]-MLT) which means that their K_d values not only depend on the affinity of the ligand for the receptor but also on the activation of the G protein in the ternary Ligand-Receptor-G protein complex.

The extensive pharmacological characterization of these three new radioligands in comparison with 2-[^{125}I]-MLT and [^{3}H]-MLT on membrane preparations from CHO-K1 cell lines stably expressing hMT_1 or hMT_2 showed that [^{125}I]-S70254 and [^{125}I]-DIV880 mainly differ from 2-[^{125}I]-MLT in its dissociation kinetics, which are faster for [^{125}I]-S70254 and [^{125}I]-DIV880 than for 2-[^{125}I]-MLT (Legros *et al.*, 2016). Interestingly, [^{125}I]-SD6 labeled only approximately half of the binding sites detected with 2-[^{125}I]-MLT in cells expressing hMT_1 while comparable amounts were detected in cells expressing hMT_2 (Legros *et al.*, 2013). This suggests the existence of different receptor sub-populations for hMT_1 of which [^{125}I]-SD6 labels a more restricted number than 2-[^{125}I]-MLT. In contrast, for hMT_2 similar sub-populations would be detectable by both radioligands. The nature of these receptor sub-populations is currently unknown but could be related to the differential engagement of hMT_1 into complexes with different G proteins or ß-arrestins following the binding of these

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agonistic radioligands. [^{125}I]-SD6 detected as 2-[^{125}I]-MLT binding sites in sheep retinal membranes, while the MT_2-specific ligands [^{125}I]-S70254 and [^{125}I]-DIV880 failed to do so.

The MT_2-specific [^{125}I]-S70254 was successfully used for autoradiography studies in rat and sheep brain and retina slices (Legros *et al.*, 2016). A similar labeling pattern to 2-[^{125}I]-MLT (detecting MT_1 and MT_2) was observed in several areas but also distinct labeling in others. Absence of labeling by [^{125}I]-S70254 in regions that are labeled by 2-[^{125}I]-MLT can be explained by low(undetectable) MT_2 expression levels. Absence of 2-[^{125}I]-MLT labeling in regions labeled by [^{125}I]-S70254 could be due to the detection of different receptor complexes (see above).

Altogether, the new radioligands considerably expand the repertoire of pharmacological tools for MTRs with the development of MT_2-specific radioligands and radioligands detecting distinct receptor populations revealing a previously unrecognized diversity. The availability of a radiolabeled antagonist would largely contribute in a better characterization of these different populations. Further advances can be expected from the development of fluorescent-labeled ligands.

Structural perspectives for melatonin receptors

Currently, crystal structures of MT_1 and MT_2 are not available. Despite a sequence identity lower than 30% between MTRs and the closest crystallized GPCRs, several 3D models have produced some structural hypothesis for binding of (non)selective MT_1 or/and MT_2 agonists (Table I). According to site-directed mutagenesis data, most of these models corroborate the importance of both serine residues 3.35 and 3.39 in MT_1 as well as His5.46 in both MT_1 and MT_2. Although His5.46 seems to be an anchoring residue for polar interactions with the methoxy or amide group of MLT, only a few MT_1 models display a direct

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participation of serine residues in MLT binding (Chugunov *et al.*, 2006; Farce *et al.*, 2008), which could be otherwise involved in an essential bending of helix 3 for binding site plasticity. Models take also into account several receptor-ligand interactions with amino acids conserved within GPCRs and known to play a role in aromatic switch activation (F5.47, W6.48).

Such homology modelling methods make predictions of flexible receptor regions difficult. Although not directly proven for MTRs, E2 and I3 loops are known to be key features for ligand accessibility and G protein binding of GPCRs. Moreover amino acid sequences of MTRs show several singularities like the presence of a $^{3.49}$NRY$^{3.51}$ motif instead of the classical $^{3.49}$DRY$^{3.51}$ motif of other rhodopsin-like GPCRs. Another specificity is the replacement of the proline by an alanine residue in the conserved $^{7.49}$NPXXY$^{7.53}$ motif. Buried in the vicinity of the cytoplasmic surface, these marked differences are likely to impact on receptor activation and/or signalling specificity of MTRs rather than the ligand binding process.

Whereas 3D models of MTRs were up to now dedicated to the discovery and optimization of new efficient drugs, the next generation of 3D models should be expanded towards larger, multimeric systems and not be restricted to receptor monomers. Computation of the energy landscape of GPCRs by enhanced molecular dynamics simulations, together with NMR and X-ray studies, provided valuable molecular insights on the dynamics of ligand recognition, receptor activation and oligomerization (Johnston *et al.*, 2014). Depicting free energy landscapes of MTRs should address biasing molecular dynamics simulations from the inactive apoform transiting toward the active trimeric L-R-Gi or L-R-arrestin forms of receptors (Figure 5). As ligands modulate these free energy landscapes (Dror *et al.*, 2013; Provasi *et al.*, 2011), *in silico* optimization of new efficient ligand structures could be explored by predicting its functional selectivity through arrestin or G_i-mediated pathways.

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These approaches also open the way for the exploration of homo- and heterodimers, particularly MT_1/MT_2 and MT_1/GPR50 complexes, as discussed in the following section.

Melatonin receptor dimers

MTRs are part of dynamic signaling complexes that contain proteins involved in receptor biosynthesis, export, signaling, desensitization, internalization and cytoskeleton modulation (Daulat *et al.*, 2007; Maurice *et al.*, 2008) (IntAct database, http://www.ebi.ac.uk/intact/search/do/search?searchString=pubid:26514267). The core of these complexes is often composed of receptor dimers, either homodimers of the same receptor or heterodimers composed of two different receptors (see (Ferre *et al.*, 2014; Maurice *et al.*, 2011) for review). Initial observations have been made in 2002 in transfected HEK293 cells demonstrating the capacity of MT_1 and MT_2 to form homo- and heterodimers (Ayoub *et al.*, 2002) with MT_1/MT_2 heterodimers showing a pharmacological profile distinct from MT_2 homodimers (Ayoub *et al.*, 2004). Shortly after, MT_1 and MT_2 were reported to form heterodimers with the orphan GPR50, which completely abolished the function of MT_1 in MT_1/GPR50 heterodimers (Levoye *et al.*, 2006a). Sporadic reports on Western blots with endogenously expressed MTRs in chicken astrocyte cultures (Adachi *et al.*, 2002) and *Xenopus* tectal cells (Prada *et al.*, 2005) further indicated the possible existence of MTR homodimers. However, the physiological relevance of these dimers remained largely unclear (Levoye *et al.*, 2006b) until 2013 when compelling *in vivo* evidence for the functional significance of MT_1/MT_2 heterodimers was obtained. In retinal photoreceptor cells, MLT enhances the light sensitivity during the night. The phenotype of MT_1 KO ($MT_1^{-/-}$) and MT_2 KOs ($MT_2^{-/-}$) mice, the use of type-selective ligands and over-expression of a dominant negative form of MT_2 in photoreceptor cells of transgenic mice indicated the exclusive involvement of MT_1/MT_2 heterodimers in this physiological effect of MLT (Baba *et al.*,

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2013). Interestingly, this effect was dependent on the activation of the G_q/PLC pathway by MLT, an observation that could be confirmed *in vitro* in cells co-expressing MT_1 and MT_2.

Whether MTR heterodimers could become novel drug targets remains an open question. Recent evidence on the antidepressant agomelatine suggests this possibility (Kamal *et al.*, 2015). Previous studies showed that agomelatine is a high affinity agonist for MT_1 and MT_2 and an antagonist with moderate affinity for serotonin 5-HT_{2C} receptors (Audinot *et al.*, 2003; Millan *et al.*, 2003). Of note, the antidepressant effect of agomelatine involves both pathways in a synergistic manner (Racagni *et al.*, 2011). Formation of MT_2/5-HT_{2C} heterodimers was demonstrated in transfected HEK293 cells and these heterodimers are targeted by agomelatine (Kamal *et al.*, 2015). Agomelatine behaved as a biased ligand, activating the G_i/cAMP pathway and antagonizing the G_q/PLC pathway. Whether the MT_2/5-HT_{2C} heterodimer participates in the antidepressant effect of agomelatine remains to be shown.

Formation of receptor dimers offers the possibility to design dimeric ligands targeting receptor dimers. Several dimeric ligands with two identical pharmacophores have been synthesized for MTRs and their binding properties have been determined (Audinot *et al.*, 2003; Descamps-Francois *et al.*, 2003; Journe *et al.*, 2014; Mesangeau *et al.*, 2010; Spadoni *et al.*, 2011). Binding of the two pharmacophores of these dimeric ligands to the two protomers of the same receptor dimer has been only shown in one study using a bioluminescence resonance energy transfer (BRET) approach (Journe *et al.*, 2014). Compounds linked through 22–24 atom spacers were able to bind to MT_1 and MT_2 protomers in pre-existing homo- and heterodimers and to induce conformational changes detected by BRET. Induction of receptor dimerization was not observed. The functional properties of these compounds remain to be studied. Taken together, the existence and physiological relevance of MTR dimers is increasingly recognized but its functional role and

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pharmacological exploitation are still ongoing.

Genetic variants and mutants of melatonin receptors

The existence of multiple rare variants in the human population was discovered in recent genome sequencing programs. The 1000 human genome project detected 38 million variants (Abecasis *et al.*, 2012) and 172 variants, including 46 non-synonymous variants, have been identified on average per GPCR in a population of 14002 individuals (Karamitri *et al.*, 2014; Nelson *et al.*, 2012). Numerous variants have been identified in the *MTNR1A* and *MTNR1B* genes, encoding MT_1 and MT_2 receptors, respectively. Here, only non-synonymous variants, modifying the amino acid sequence of the receptors, will be considered (Figure 6). Variants with altered receptor function can potentially participate in disease development. Ebisawa *et al.* (1999) were searching for variants in *MTNR1A* and *MTNR1B* genes in patients with circadian disorders. Two non-synonymous variants were identified in the *MTNR1A* gene (R54W, A157V) which were 3-fold and 2-fold more frequent in people with non-24-hour sleep-wake syndrome (Table II) (Ebisawa *et al.*, 1999). Due to small sample size (N=22) statistical significance was not reached.

Alteration of MLT synthesis has been reported in autism spectrum disorders (ASD) triggering the search for variants in *MTNR1A* and *MTNR1B* genes in 295 patients with ASD, 362 controls and 284 individuals from the human genome diversity panel (Chaste *et al.*, 2010). Six non-synonymous mutations were identified for *MTNR1A* and ten for *MTNR1B* (Table II, III). The majority of these mutants showed altered receptor function. Particularly deleterious mutants were MT_1-I49N, which is devoid of any MLT binding and cell surface expression, and MT_1-G166E and MT_1-I212T, which showed severely impaired cell surface expression and biased behavior towards the ERK1/2 pathway. No significant difference in the

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prevalence of these mutations was found indicating that they do not represent major risk factor for ASD.

Four non-synonymous mutations were identified for *MTNR1A* and four for *MTNR1B* in a cohort of 101 individuals with attention-deficit/ hyperactivity disorder (ADHD) (Table II, III), however, none of them was enriched in ADHD individuals as compared to the general population (Chaste *et al.*, 2011). The MT_1-Y170X non-sense mutation was only detected in one ADHD patient and introduced a premature STOP codon resulting in complete loss of receptor function.

MTR variants have been most extensively searched in studies focused on type 2 diabetes (T2D) based on the discovery of several frequent polymorphisms associated with increased fasting plasma glucose (FPG) and T2D risk close to the *MTNR1B* gene in genome-wide association studies (Bouatia-Naji *et al.*, 2009; Prokopenko *et al.*, 2009). Sequencing of the coding region of the *MTNR1B* gene revealed six non-synonymous variants (G24E, L60R, V124I, R138C, R231H, K243R) of which none was associated with T2D risk. The common 24E variant was associated with increased body mass and decreased FPG (Andersson *et al.*, 2010), an observation that was not replicated in a later study (Bonnefond *et al.*, 2012). Whereas only subtle changes in the capacity of G24E and V124I to activate a GαΔ6qi4myr chimeric G protein, the L60R variant was completely inactive in transfected COS cells. A more extensive sequencing study discovered 40 non-synonymous variants in the coding region of *MTNR1B* (Table II, III, IV) (Bonnefond *et al.*, 2012) of which 36 very rare mutants associated with T2D risk. Functional analysis of the 40 variants revealed intact cell surface expression for all variants, complete loss of MLT binding in 4 very rare cases (A42P, L60R, P95L, Y308S) and partially and severely blunted signaling (Gαqi9 chimera and ERK1/2 activation) in 1 rare cases (R138C) and 9 very are cases (W22L, A52T, A74T, R138H,

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R138L, L166I, R222H, R330W, I353T). Carriers of the 13 very rare loss-of-function variants showed increased T2D risk establishing a functional link between *MTNR1B* and T2D (for review see (Karamitri *et al.*, 2013)).

In conclusion, the genetic variability of the *MTNR1B* gene in terms of non-synonymous variants has now been well defined and an association of very rare variants with T2D risk established. Less is known about the variability of the *MTNR1A* gene in terms of non-synonymous variants.

Melatonin receptor mouse models - update

$MT_1^{-/-}$ mice were created in the late 90s' followed by the generation of $MT_2^{-/-}$ mice in 2003 (Jin *et al.*, 2003; Liu *et al.*, 1997). Studies using these mice have provided important insights on the role that MTRs play in the modulation of many different biological functions. In $MT_1^{-/-}$ mice, but not in $MT_2^{-/-}$ mice, the inhibitory effect of MLT on neuronal activity in the suprachiasmatic nucleus (SCN) is impaired suggesting the involvement of MT_1. In contrast, in SCN slices from $MT_1^{-/-}$ mice MLT (1-10 pM) phase-shifts the peak of circadian rhythms of neuronal firing by approximately 3 h suggesting the involvement of MT_2 (Dubocovich *et al.*, 2005; Liu *et al.*, 1997). Blockade of this effect using the MT_2 selective 4P-PDOT antagonist confirmed the latter conclusion shaping a pathway where MT_2 phase-shifts the peak of neuronal firing through PLC-PKC signaling pathway (Dubocovich *et al.*, 2005; Hunt *et al.*, 2001) (Mc Arthur *et al.*, 1997). Liu *et al*. (1997) reported that the phase shift of neuronal firing rhythms induced by 2-iodomelatonin (10 pM) was of smaller magnitude in the SCN slice from $MT_1^{-/-}$ than in WT mice suggesting a role for the MT_1 in this response (see detailed discussion in (Dubocovich, 2007)). Together these findings suggest a potential role for both MT_1 and MT_2 in the phase shift of circadian rhythms of neuronal firing in the SCN slice *in vitro*. The use of $MT_1^{-/-}$ mice demonstrated that MT_1 is required for the MLT-meditated

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phase-shift of the onset of overt circadian rhythm of locomotor activity (Dubocovich *et al.*, 2005). An independent study demonstrated that C3H/HeN mice (MLT-proficient) entrained faster to a phase advance of dark onset than the C57BL/6J mice (MLT-deficient), suggesting a facilitating role of endogenous MLT on circadian reentrainment (Pfeffer *et al.*, 2012). However, we should note that faster entrainment could also result from genetic differences between the two mouse strains rather than different endogenous melatonin levels (Adamah-Biassi *et al.*, 2013). In a mice strain producing endogenous melatonin the faster entrainment to an abrupt advance of dark onset persisted in $MT_1^{-/-}$ C3H/HeN mice but was lost in $MT_2^{-/-}$ and double KOs ($MT_1^{-/-}/MT_2^{-/-}$) suggesting again the involvement of MT_2. This apparent contradiction could be explained by the activation of MT_2 and MT_1 by endogenous and exogenous MLT, respectively, at different periods of sensitivity (subjective night vs. subjective day, respectively). Changes in efficacy could also result from desensitization and/or internalization of MTRs in response to exposure to physiological and supraphysiological MLT concentrations as demonstrated by the phase shift of the peak of neuronal firing in the SCN by physiological levels of MLT, which involved the desensitization of MT_2 (Gerdin *et al.*, 2004).

MTR KO mice have been also used to elucidate the role played by these receptors in the regulation of the sleep/wake cycle. In $MT_2^{-/-}$ NREM sleep is decreased during the light phase (i.e., during the time that mice normally sleep), whereas $MT_1^{-/-}$ mice showed an increase in the amount of NREM sleep during the dark phase (i.e., during active phase) (Ochoa-Sanchez *et al.*, 2011). Further analysis of the data indicated that MT_1 signaling is implicated in the modulation of the daily rhythm of REM sleep (Ochoa-Sanchez *et al.*, 2011). An additional study in which double KOs ($MT_1^{-/-}/MT_2^{-/-}$) were used indicated that removal of both receptors induced an increase in wakefulness, and a reduction in REM sleep (Comai *et*

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al., 2013). Hence these data seem to indicate that removal of MTRs may affect wakefulness rather than sleep.

The effect of MTR removal has been also investigated in the mouse retina, where these receptors are widely distributed (Baba *et al.*, 2013; Baba *et al.*, 2009). Removal of either receptor has profound effects on photoreceptors function as it abolishes the daily rhythms in the scotopic and photopic electroretinogram (ERGs) (Alcantara-Contreras *et al.*, 2011; Baba *et al.*, 2009; Sengupta *et al.*, 2011). Such a result also indicates that MT_1 and MT_2 form heterodimers in mouse photoreceptors (Baba *et al.*, 2013). Further studies have also demonstrated that removal of MTRs in addition affects the viability of the photoreceptors and retinal ganglion cells during aging (Alcantara-Contreras *et al.*, 2011; Baba *et al.*, 2009; Gianesini *et al.*, 2016) as well as corneal biology (Baba *et al.*, 2015).

As mentioned before, recent studies have also implicated MTRs in the pathogenesis of T2D in humans (Bonnefond *et al.*, 2012; Bouatia-Naji *et al.*, 2009; Lyssenko *et al.*, 2009). Thus a few studies used MTR KO mice to determine the mechanisms by which these receptors contribute to regulation of glucose homeostasis and insulin sensitivity (Contreras-Alcantara *et al.*, 2010; Muhlbauer *et al.*, 2009; Stumpf *et al.*, 2008). Mice lacking MT_1 exhibit higher mean blood glucose levels than controls (Muhlbauer *et al.*, 2009), and tend to be more glucose intolerant and insulin resistant than WT and $MT_2^{-/-}$ mice (Contreras-Alcantara *et al.*, 2010). Furthermore, removal of MT_1 or MT_2 abolishes the daily rhythm in blood glucose levels (Owino *et al.*, 2016).

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Finally, it is important to mention that although the reproductive system of mice is not sensitive to photoperiod, the development of MTR KO mice provided an important tool for dissecting the mechanisms by which MLT regulates reproduction in photoperiodic species. For example, MT_1 signaling controls the rhythmic expression of the clock gene *Period 1* in the pituitary gland (von Gall *et al.*, 2002) and further studies have shown that the rhythmic expression of several other clock genes (*Per1, Per 2, Bmal1, and Cry 1*) in the mouse *Pars tuberalis* depends on MT_1 signaling as well (Jilg *et al.*, 2005). MT_1 signaling has been also reported to be crucial for the photoperiodic response of gene expression in the ependymal cell layer and thus for the photoperiodic regulation of gonadal activity (Sheynzon *et al.*, 2006; Yasuo *et al.*, 2009). Finally, we should mention that a recent study reported that MT_1 signaling plays a key role in photoperiodic programming of serotonergic neurons as well as depression- and anxiety-related behaviors in mice (Green *et al.*, 2015).

In conclusion studies in the last twenty years using MTR KO mice have greatly helped to understand the role(s) played by these receptors in the regulation of many physiological functions and they have provided important insights on the mechanisms by which MLT signaling affects these functions.

Functional role of melatonin receptors in physiology and pathophysiology

MTRs are involved in many physiological processes that will however not all be covered by this review but can be consulted in other reviews (Dubocovich *et al.*, 2010; Johnston *et al.*, 2015; Karamitri *et al.*, 2013; Tosini *et al.*, 2012; Tosini *et al.*, 2014). Here, we focused our attention on two major systems, the immune and the central nervous system. Important progress has been made recently in both fields and links to diseases have been established justifying a review of our current knowledge on these aspects. Finally, we will make a critical assessment of reports of receptor-independent effects of MLT, such as binding

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of MLT to additional binding site and intrinsic antioxidant and free radical scavenger properties of MLT.

- Melatonin receptors in the immune system

The role of MLT as a player in immunity, first proposed by Berman in 1926, is now well accepted (Carrillo-Vico *et al.*, 2013). Several reports demonstrated that MLT produced either by the pineal gland or immune cells can regulate the activation of an immune response. MLT derived from activated human lymphocytes induces the synthesis of interleukin 2 (IL-2) and IL-2 receptors ((Carrillo *et al.*, 2004; Carrillo-Vico *et al.*, 2013). Luzindole and targeted deletion of the *MNTR1A* gene (Lardone *et al.*, 2006; Lardone *et al.*, 2010) block the effect of lymphocyte-derived MLT. Interestingly, daily rhythms of plasma MLT and IL-2 are transiently lost in non-infectious human inflammatory conditions and the recovery of the IL-2 rhythm follows the restoration of the daily MLT rhythm (Pontes *et al.*, 2007). In addition, the daily and seasonal variation of MLT production contributes to the seasonality of some diseases. In multiple sclerosis (MS) MLT blocks the differentiation of Th17 cells and boosts the generation of protective Tr1 cells by a MT_1-dependent mechanism, resulting in the seasonal variation on MS symptoms (Farez *et al.*, 2015). Seasonality of regular immunity is also related to changes in the MLT system (Weil *et al.*, 2015). In the spleen of several species extended light exposure decreases MT_1 expression (Lahiri *et al.*, 2009; Maestroni, 1993; Yadav *et al.*, 2013). In healthy conditions rolling and adhesion of neutrophils to the endothelial cell layer is inhibited by activation of MT_2 and ligands binding to the putative *MT3* binding site, respectively (Lotufo *et al.*, 2001). In contrast, other effects of MLT such as the inhibition of transcription factors that mediate acute inflammation induced by lipopolysaccharides (LPS) (Tamura *et al.*, 2010) or N-formyl-l-methionyl-l-leucyl-l-

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phenylalanine (fMLP) (Cernysiov *et al.*, 2015) are not blocked by luzindole suggesting a MTR-independent action mode.

MTRs also play an important role in promoting engulfing of bacteria, fungi and parasites. MLT facilitates the invasion of erythrocytes by *Plasmodium falciparum* (Hotta *et al.*, 2000) the invasion of macrophages by *Leishmania amazonensis* (Laranjeira-Silva *et al.*, 2015) and the phagocytosis of zymosan by colostrum polymorphonuclear and mononuclear cells (Pires-Lapa *et al.*, 2013) and the RAW 264.7 macrophage cell line (Muxel *et al.*, 2012). The entrance of different microorganisms in polymorpho and monuclear cells, including colostral and lineage established cell lineages is blocked by luzindole. Indeed, parasites, bacteria and fungi activate the NF-κB pathway in these two cell models resulting in the expression of arylalkyl-N-acetyltransferase and the synthesis of MLT. Luzindole and 4P-PDOT blocked the expression of dectin-1, a protein that is important for phagocytosis, suggesting the participation of MT_2 in this effect (Muxel *et al.*, 2016; Muxel *et al.*, 2012; Pires-Lapa *et al.*, 2013). Thus, the evaluation of binding parameters and functional states of MTRs in immune competent cells need to consider the masking effect of on-demand synthesized MLT.

Although complex, the role of MLT on the immune system is beginning to be understood. MT_1 and MT_2 receptor types appear to play different roles, MT_1 is the main target in acquired immune response, and MT_2 the target for innate immune responses.

- Melatonin receptors in the CNS

MTRs are widely expressed throughout the CNS, and are particularly well characterized in the SCN of the hypothalamus, where they are known to inhibit neuronal firing and mediate the phase-shifting effect of MLT on circadian rhythms (see above). In addition to its chronobiotic effect, MLT participates in the modulation of neuronal functions,

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neurodevelopment at early and late stages (Kong et al., 2008; Chen et al., 2014) and affects brain structures underlying sleep regulation (Ochoa-Sanchez *et al.*, 2011), drug-related learning (Savaskan *et al.*, 2006; Wang *et al.*, 2005) and reward (Clough *et al.*, 2014; Hutchinson *et al.*, 2012). MTRs have been shown to mediated the MLT-induced increase in dendrite length, thickness and complexity of hippocampal neurons, as these effects were partially blocked by luzindole (Dominguez-Alonso *et al.*, 2015). Similarly, MLT-induced differentiation and maturation of adult neural stem cells was almost abrogated in the presence of luzindole (de la Fuente Revenga *et al.*, 2015). A recent study using $MT_2^{-/-}$ mice reveals that MT_2 is essential for axogenesis and for the formation of functional synapses (Liu *et al.*, 2015). MT_2 is also involved in MLT-induced protection against oxidative stress and memory impairment in a mice model of aging (Shin *et al.*, 2015). Recent advances in the understanding of presynaptic MTRs and their role in neurodegenerative diseases are discussed in the following chapters.

Presynaptic melatonin receptors. The role of MLT on the regulation of calcium-dependent dopamine release from axon terminals in brain and amacrine cells in the retina was shown in the early 1980's (Dubocovich, 1983; Zisapel *et al.*, 1982). However, more direct and global proof for the presence of presynaptic MLT heteroreceptors (i.e. receptor for a transmitter or hormone other than the neuron's own neurotransmitter) capable of regulating neurotransmitter release was still insufficient. A recent protein interaction network analysis established that MT_1, but not MT_2, is expressed on presynaptic axon terminal membranes in the hypothalamus, striatum, cortex, and hippocampus, where it is part of the presynaptic protein network (Benleulmi-Chaachoua *et al.*, 2016). Notably, this study shows a strong physical association between MT_1 and presynaptic proteins such as synapsin, SNAP25, Munc-18 and voltage-gated Cav2.2 channels. Interaction with the latter was responsible for constitutive inhibition of calcium entry by MT_1 in a Gβγ-dependent manner (Benleulmi-

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Chaachoua *et al.*, 2016).

These recent findings provide strong support for the involvement of MTRs in synaptic functions, particularly in neurotransmitter release as indicated by previous studies. Indeed, activation of MTRs has been implicated in the inhibition of ^{3}H-dopamine release from the ventral hippocampus, medulla pons, preoptic area and hypothalamus (median and posterior) (Dubocovich, 1983; Zisapel *et al.*, 1982). This effect followed a diurnal rhythm in the hypothalamus with a maximum and a minimum observed at ZT 5 and ZT 13-15, respectively (Zisapel *et al.*, 1985). 6-Chloromelatonin-mediated modulation of norepinephrine turnover via activation of presynaptic MLT heteroreceptors was demonstrated in hypothalamus (Fang *et al.*, 1990). In this model, luzindole, applied during the night when MLT levels are high, accelerated norepinephrine turnover suggesting the involvement of MTRs stimulated by endogenous MLT (Fang *et al.*, 1990). The presence of presynaptic MLT heteroreceptors on retino-hypothalamic fibers innervating superficial retinorecipient layers of the avian optic tectum has been inferred by the presence of 2-[^{125}I]-IMLT binding sites and its decrease following transsection of the retinotectal pathway (Krause *et al.*, 1992; Krause *et al.*, 1994). The function of these presynaptic MTR is currently unknown but a modulatory role of light input to visual and circadian target responses is likely. Recent electrophysiological evidence suggest that MLT acting through presynaptic MTRs increases glutamatergic neurotransmission in the habenula, an effect blocked by luzindole (Evely *et al.*, 2016). Finally, it is worth mentioning in this context that MTRs have been first shown to be involved in the inhibition of depolarization-evoked calcium-dependent neurotransmitter (dopamine) release from amacrine cells in the chick and rabbit retina (Dubocovich, 1985; Dubocovich, 1983). These mammalian functional presynaptic heteroreceptors were used to establish the first structure-activity-relationship for MTR ligands, which correlated with the pharmacological profile of MT_2 (Dubocovich *et al.*, 1997), and to identify and

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pharmacologically characterize the first competitive MTR ligands, luzindole and 4P-PDOT (Dubocovich, 1988).

In summary, proteomic studies of the MT_1 interactome revived interest in the function of presynaptic MTRs and reinforced previous functional studies indicating the role of presynaptic MTRs in neurotransmitter release. Use of MTR KO mouse models will be particularly instrumental in this context, as they will clarify the respective roles of MT_1 and MT_2. Based on current data, a predominant role of MT_1 in presynaptic functions, like neurotransmitter release, and a potential role of MT_2 in axogenesis and synapse formation can be postulated.

Melatonin receptors in neurodegenerative diseases. Altered expression of MTRs has been frequently reported in neurodegenerative diseases and psychiatric disorders, including Alzheimer's disease (AD), Parkinson disease (PD), Huntington disease (HD) and ASD. In AD patients, MT_1 expression in the SCN and MT_2 expression in the hippocampus are reduced compared to control subjects in *post-mortem* brains (Wu *et al.*, 2007). Intriguingly, higher expression of MT_1 was detected in hippocampal arteries of AD brains (Savaskan *et al.*, 2002), which might be due to a compensatory response to the low levels of circulating MLT in these patients (Zhou *et al.*, 2003). These observations suggest that the expression of MTRs under pathological conditions can be differentially regulated depending on the brain area. In PD patients, down-regulation of MT_1 and MT_2 expression was observed in the substantia nigra and amygdala, the two most relevant areas in PD pathogenesis (Adi *et al.*, 2010). Small case-control studies accessing MT_1 and MT_2 expressions in HD patients showed no changes in the SCN (van Wamelen *et al.*, 2013), while decreased expression of MT_1, but not of MT_2, was detected in the striatum (Wang *et al.*, 2011). Interestingly, the progressive loss of MT_1 correlates with HD severity, also confirmed in a mice model of HD (Wang *et al.*, 2011). In

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ASD patients no information on MT_1 and MT_2 expression is available but several MT_1 and MT_2 mutants with strongly reduced function have been identified (Chaste *et al.*, 2010).

Additional evidence supports the emerging concept of MTR dysfunction as a permissive condition favoring the development and/or progression of neurodegenerative diseases. The neuroprotective effect of endogenous and exogenous MLT has been demonstrated in different systems (reviewed by (Escribano *et al.*, 2014)). In a neuroinflammatory model induced by LPS administration, cerebellar neuronal death was observed only in animals pre-treated with luzindole (Pinato *et al.*, 2015). Similarly, depletion of endogenous MLT by pinealectomy caused spontaneous neuronal loss in the hippocampal CA1 area, which was prevented by treatment with agomelatine (Tchekalarova *et al.*, 2016). The requirement of MTRs for the neuroprotective action of MLT has also been elegantly demonstrated in a series of *in vitro* studies in which luzindole treatment or siRNA-mediated knockdown of MT_1 enhanced neuronal vulnerability to cell death (Wang *et al.*, 2011). Different cell stressor conditions such as temperature shift or treatments with hydrogen peroxide, TNF or with the HD-related protein huntingtin, resulted in reduced levels of MT_1. Accordingly, it has also been shown that the AD-related neurotoxic amyloid beta peptide (Aβ) impairs the function of MTRs (Cecon *et al.*, 2015), implying that MTRs and MLT signaling are among the primary molecular targets affected in the course of AD.

Insights in the impact of MTRs on cognitive functions are also obtained from MTR KO mice. $MT_2^{-/-}$ mice show impaired long-term potentiation and performance in memory tests (Larson *et al.*, 2006). However, the double KO $MT_1^{-/-}/MT_2^{-/-}$ mice show no clear differences to WT mice in memory test performances and show increased long-term potentiation responses, even though the deletion of MTRs negatively affected the expression of important proteins for synaptic activity, such as phospho-synapsin and spinophilin (O'Neal-Moffitt *et al.*, 2014). The relevance of MTRs for cognitive performance was

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undoubtedly evidenced using an AD mice model lacking MT_1 and MT_2, in which MLT treatment failed to improve mice performance on hippocampal-dependent spatial learning tasks, as observed in the AD mouse model in the presence of MT_1 and MT_2. Impressively, the lack of MTRs *per se* markedly increased the mortality in young AD mice (O'Neal-Moffitt *et al.*, 2015). Finally, the therapeutic use of MLT has been proposed and tested in a number of mice models and clinical trials in several neurodegenerative conditions, including AD (Cardinali *et al.*, 2010; Olcese *et al.*, 2009; Peng *et al.*, 2013; Wade *et al.*, 2014; Zhang *et al.*, 2016), amyotrophic lateral sclerosis (Weishaupt *et al.*, 2006; Zhang *et al.*, 2013), PD (Medeiros *et al.*, 2007; Naskar *et al.*, 2015; Zhang *et al.*, 2016) and HD (van Wamelen *et al.*, 2015). The therapeutic use of MLT is usually associated with sleep improvement and better alignment of circadian parameters, and its beneficial effect on neuroprotection and cognitive performance is starting to be recognized (Joshi *et al.*, 2015; Wade *et al.*, 2014). Dysfunction or down-regulation of MTRs is likely to be part of the primary pathophysiological mechanisms rather than a consequence of advanced neurodegeneration and, thus, prophylactic hormonal replacement and/or early-stage intervention strategies to restore MTR expression and function might provide the most efficient result.

Taken together, the subcellular localization and role of MTRs in neuron functions and their participation in neurodegererative diseases are now starting to be understood and suggest a broad modulatory role of MLT in neuronal function, development and plasticity.

- Melatonin as antioxidant and free radical scavenger

The IUPHAR classifies only clearly identified pharmacological targets in mammals. However, some effects of MLT persist even in the absence of MT_1 and MT_2 or upon complete pharmacological blockage of MTRs indicating the existence of MTR-independent

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mechanism, which are still not fully understood. In addition, MTR-dependent and -independent mechanisms can participate simultaneously, as demonstrated by O'Neal-Moffitt *et al.* (2015) regarding the antioxidant and pro-cognitive effects of MLT on AD mice models, for example. Two predominant mechanisms have been put forward to explain MLT's antioxidant and free radical properties: MLT binding to the *MT3* binding site (Dubocovich *et al.*, 2003; Nosjean *et al.*, 2000) and to the cytosolic enzyme quinone reductase 2 (QR2) (Dubocovich *et al.*, 2003; Nosjean *et al.*, 2000), and MLT scavenging of free radicals, which has been suggested to be an electron donor (for a review see (Tan *et al.*, 2015). Binding of MLT to intracellular targets is readily achieved due to the hydrophilic nature of this indolamine. MLT binds with nanomolar affinity to *MT3/QR2* binding sites but shows a pharmacological profile distinct from MT_1 and MT_2. The order of affinities for the *MT3* binding site is 2-iodomelatonin > N-acetyl-serotonin > MLT (Dubocovich, 1995; Nosjean *et al.*, 2000), the one for MT_1 and MT_2 is 2-iodomelatonin > MLT >>>> N-acetyl-serotonin. MCA-NAT (5-methoxycarbonylamino-N-acetyltryptamine), prazosin and N-acetyltryptamine are selective ligands for the membrane MT_3 binding site (Dubocovich, 1995; Molinari *et al.*, 1996; Nosjean *et al.*, 2000). Nosjean *et al.* (2000) showed that a cytosolic binding site identified as QR2 has the pharmacological characteristics of the membrane *MT3* binding site. QR2 is a cytosolic flavin adenine dinucleotide (FAD)-dependent flavoprotein that reduces menadione and other quinones by using *N*-ribosyl- and *N*-alkyldihydronicotinamides as the co-substrates (Liao *et al.*, 1961) thus acting as a detoxifying enzyme to increase the antioxidant defense (Jockers *et al.*, 2008). There are still open questions as to whether the MLT binding site on QR2 corresponds to the *MT3* binding site, in particular regarding those sites that are membrane associated.

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Several physiological effects of MLT such as inhibition of leukocytes adhesion to rat endothelial cell layer were reported to be mimic by *MT3* agonists (Lotufo *et al.*, 2001). Similar observations were made for the expression of adhesion molecules by granulocytes (Cernysiov *et al.*, 2015), the increase in dopamine levels in chick retina (Sampaio Lde *et al.*, 2014)

and the reduction of intraocular pressure (IOP) in rabbits (Alarma-Estrany *et al.*, 2009). However, it has been questioned whether the functional effects of MCA-NAT are indeed mediated by QR2, as the lack of QR2 did not prevent the MCA-NAT-induced reduction on IOP, and overexpression of QR2 did not promote receptor-like responses (Vincent *et al.*, 2010). In addition, MCA-NAT turned out to be a partial agonist for MT_1 and MT_2 at submicromolar concentrations suggesting the possibility that some of the MCA-NAT effects might be mediated by MT_1 and/or MT_2 (Vincent *et al.*, 2010).

MLT and metabolites with or without open ring structures have been described to be potent electron donors. Cyclic-3-hydroxymelatonin, N1-acetyl-5-methoxykynuramine (secondary metabolite) (AMK, tertiary metabolite), and N-acetyl-N-formyl-5-methoxykynuramine (AFMK, quaternary metabolite) scavenge free radicals neutralizing reactive oxygen and nitrogen species (Ressmeyer *et al.*, 2003; Tan *et al.*, 2007; Zavala-Oseguera *et al.*, 2014). Hence one MLT molecule and its associated metabolites are believed to scavenge a large number of reactive species and thus the overall antioxidant capacity of MLT is believed to be superior of other well known antioxidants (e.g. vitamin C, vitamin E, etc.,) under *in vitro* or *in vivo* conditions (Gitto *et al.*, 2001; Ortiz *et al.*, 2013; Sharma *et al.*, 2006). However, the ability of MLT in reducing oxidative stress does not only rely on donating electrons. Indeed, by acting on MT_1 and MT_2, low pM and low nM concentrations of MLT increase the expression or activity of enzymes such as superoxide dismutase, catalase and glutathione peroxidase, which are involved in oxygen detoxification (Rosen *et al.*, 2009).

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Thus, depending on the dose of exogenous or endogenous MLT, receptor-dependent or -independent mechanisms may be involved. A further twist in the interplay between receptor-dependent and -independent processes could arise from the fact that MLT by changing the redox state of the cell might influence receptor mediated functions. Indeed the function of several GPCRs has been shown to be sensitive to the cellular redox state. Whether this is also the case for MTRs, has to be addressed in future studies. Although endogenous MLT levels are typically considered to range from low pM to low nM concentrations much higher concentrations may be reached locally in the brain (Legros *et al.*, 2014) and in activated immune cells (Conti *et al.*, 2000). In addition, melatonin can be actively taken up through the GLUT1 glucose transporter (Hevia *et al.*, 2015). In conclusion, the role of the *MT3* binding site is still not fully understood and warrants further attention. Concerning the free radical scavenging properties of MLT, it is surprising that still opposing opinions are in the literature. Overall, the antioxidant effects of MLT appear to be complex, relying on a mixture of MTR-dependent and -independent processes.

Acknowledgements:

This work was supported by the Agence Nationale de la Recherche (ANR 2011 - BSV1-012-01 "MLT2D" and ANR-2011-META "MELA-BETES", ANR-12-RPIB-0016 "MED-HET-REC-2"), the Fondation de la Recherche Médicale (Equipe FRM DEQ20130326503), Institut National de la Santé et de la Recherche Médicale (INSERM), Centre National de la Recherche Scientifique (CNRS) and the "Who am I?" laboratory of excellence No.ANR-11-LABX-0071 funded by the French Government through its "Investments for the Future" program operated by The French National Research Agency (ANR) under grant No.ANR-11-IDEX-0005-01 (to R.J.); USPHS Grants MH-42922, MH 52685, DA021870, NS 061068 (to MLD); the National Institutes of Health Grants

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EY022216 and EY020821 and by 5U54NS083932, S21MD000101, G12-RR03034, U54RR026137 (to G.T.), the São Paulo Research Foundation (FAPESP – Thematic Project - 2013/13691-1), Brazilian National Council of Research and Innovation (CNPq – 480097) (to RPM). PD is an employee of the pharmaceutical company Institut de Recherches Servier.

Author contributions:

All authors contributed equally in the writing and proofreading of the review and RJ supervised and edited the different contributions.

Competing Interests' Statement: None

References

Abecasis, GR, Auton, A, Brooks, LD, DePristo, MA, Durbin, RM, Handsaker, RE, *et al.* (2012) An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**(7422)**:** 56-65.

Adachi, A, Natesan, AK, Whitfield-Rucker, MG, Weigum, SE, Cassone, VM (2002) Functional melatonin receptors and metabolic coupling in cultured chick astrocytes. *Glia* **39**(3)**:** 268-278.

Adamah-Biassi, EB, Stepien, I, Hudson, RL, Dubocovich, ML (2013) Automated video analysis system reveals distinct diurnal behaviors in C57BL/6 and C3H/HeN mice. *Behav Brain Res* **243:** 306-312.

Adi, N, Mash, DC, Ali, Y, Singer, C, Shehadeh, L, Papapetropoulos, S (2010) Melatonin MT1 and MT2 receptor expression in Parkinson's disease. *Med Sci Monit* **16**(2)**:** BR61-67.

Alarma-Estrany, P, Crooke, A, Pintor, J (2009) 5-MCA-NAT does not act through NQO2 to reduce intraocular pressure in New-Zealand white rabbit. *J Pineal Res* **47**(2)**:** 201-209.

Alcantara-Contreras, S, Baba, K, Tosini, G (2011) Removal of melatonin receptor type 1 increases intraocular pressure and retinal ganglion cells death in the mouse. *Neurosci Lett* **494**(1)**:** 61-64.

Andersson, EA, Holst, B, Sparso, T, Grarup, N, Banasik, K, Holmkvist, J, *et al.* (2010) MTNR1B G24E variant associates With BMI and fasting plasma glucose in the general population in studies of 22,142 Europeans. *Diabetes* **59**(6)**:** 1539-1548.

This article is protected by copyright. All rights reserved.

Audinot, V, Mailliet, F, Lahaye-Brasseur, C, Bonnaud, A, Le Gall, A, Amosse, C, *et al.* (2003) New selective ligands of human cloned melatonin MT1 and MT2 receptors. *Naunyn Schmiedebergs Arch Pharmacol* **367**(6)**:** 553-561.

Ayoub, MA, Couturier, C, Lucas-Meunier, E, Angers, S, Fossier, P, Bouvier, M, *et al.* (2002) Monitoring of ligand-independent dimerization and ligand-induced conformational changes of melatonin receptors in living cells by bioluminescence resonance energy transfer. *J Biol Chem* **277**(24)**:** 21522-21528.

Ayoub, MA, Levoye, A, Delagrange, P, Jockers, R (2004) Preferential formation of MT1/MT2 melatonin receptor heterodimers with distinct ligand interaction properties compared with MT2 homodimers. *Mol Pharmacol* **66**(2)**:** 312-321.

Baba, K, Benleulmi-Chaachoua, A, Journe, AS, Kamal, M, Guillaume, JL, Dussaud, S, *et al.* (2013) Heteromeric MT1/MT2 melatonin receptors modulate photoreceptor function. *Sci Signal* **6**(296)**:** ra89.

Baba, K, Davidson, AJ, Tosini, G (2015) Melatonin Entrains PER2::LUC Bioluminescence Circadian Rhythm in the Mouse Cornea. *Invest Ophthalmol Vis Sci* **56**(8)**:** 4753-4758.

Baba, K, Pozdeyev, N, Mazzoni, F, Contreras-Alcantara, S, Liu, C, Kasamatsu, M, *et al.* (2009) Melatonin modulates visual function and cell viability in the mouse retina via the MT1 melatonin receptor. *Proc Natl Acad Sci U S A* **106**(35)**:** 15043-15048.

Benleulmi-Chaachoua, A, Chen, L, Sokolina, K, Wong, V, Jurisica, I, Emerit, MB, *et al.* (2016) Protein interactome mining defines melatonin MT1 receptors as integral component of presynaptic protein complexes of neurons. *J Pineal Res* **60**(1)**:** 95-108.

Bonnefond, A, Clement, N, Fawcett, K, Yengo, L, Vaillant, E, Guillaume, JL, *et al.* (2012) Rare MTNR1B variants impairing melatonin receptor 1B function contribute to type 2 diabetes. *Nat Genet* **44**(3)**:** 297-301.

Bouatia-Naji, N, Bonnefond, A, Cavalcanti-Proenca, C, Sparso, T, Holmkvist, J, Marchand, M, *et al.* (2009) A variant near MTNR1B is associated with increased fasting plasma glucose levels and type 2 diabetes risk. *Nat Genet* **41**(1)**:** 89-94.

Browning, C, Beresford, I, Fraser, N, Giles, H (2000) Pharmacological characterization of human recombinant melatonin mt(1) and MT2 receptors. *Brit J Pharmacol* **129**(5)**:** 877-886.

Cardinali, DP, Furio, AM, Brusco, LI (2010) Clinical aspects of melatonin intervention in Alzheimer's disease progression. *Curr Neuropharmacol* **8**(3)**:** 218-227.

Carrillo, VA, Calvo, JR, Abreu, P, Lardone, PJ, Garcia, MS, Reiter, RJ, *et al.* (2004) Evidence of melatonin synthesis by human lymphocytes and its physiological significance: possible role as intracrine, autocrine, and/or paracrine substance. *Faseb Journal* **18**(1).

Carrillo-Vico, A, Lardone, PJ, Alvarez-Sanchez, N, Rodriguez-Rodriguez, A, Guerrero, JM (2013) Melatonin: buffering the immune system. *Int J Mol Sci* **14**(4)**:** 8638-8683.

This article is protected by copyright. All rights reserved.

Cecon, E, Chen, M, Marcola, M, Fernandes, PA, Jockers, R, Markus, RP (2015) Amyloid beta peptide directly impairs pineal gland melatonin synthesis and melatonin receptor signaling through the ERK pathway. *FASEB J* **29**(6)**:** 2566-2582.

Cernysiov, V, Mauricas, M, Girkontaite, I (2015) Melatonin inhibits granulocyte adhesion to ICAM via MT3/QR2 and MT2 receptors. *Int Immunol* **27**(12)**:** 599-608.

Chan, KH, Hu, Y, Ho, MK, Wong, YH (2013) Characterization of substituted phenylpropylamides as highly selective agonists at the melatonin MT2 receptor. *Curr Med Chem* **20**(2)**:** 289-300.

Chaste, P, Clement, N, Botros, HG, Guillaume, JL, Konyukh, M, Pagan, C, *et al.* (2011) Genetic variations of the melatonin pathway in patients with attention-deficit and hyperactivity disorders. *J Pineal Res* **51:** 394-399.

Chaste, P, Clement, N, Mercati, O, Guillaume, JL, Delorme, R, Botros, HG, *et al.* (2010) Identification of pathway-biased and deleterious melatonin receptor mutants in autism spectrum disorders and in the general population. *PLoS One* **5**(7)**:** e11495.

Chugunov, AO, Farce, A, Chavatte, P, Efremov, RG (2006) Differences in binding sites of two melatonin receptors help to explain their selectivity to some melatonin analogs: a molecular modeling study. *J Biomol Struct Dyn* **24**(2)**:** 91-107.

Clough, SJ, Hutchinson, AJ, Hudson, RL, Dubocovich, ML (2014) Genetic deletion of the MT1 or MT2 melatonin receptors abrogates methamphetamine-induced reward in C3H/HeN mice. *Physiol Behav* **132:** 79-86.

Comai, S, Ochoa-Sanchez, R, Gobbi, G (2013) Sleep-wake characterization of double MT(1)/MT(2) receptor knockout mice and comparison with MT(1) and MT(2) receptor knockout mice. *Behav Brain Res* **243:** 231-238.

Conti, A, Conconi, S, Hertens, E, Skwarlo-Sonta, K, Markowska, M, Maestroni, JM (2000) Evidence for melatonin synthesis in mouse and human bone marrow cells. *J Pineal Res* **28**(4)**:** 193-202.

Contreras-Alcantara, S, Baba, K, Tosini, G (2010) Removal of melatonin receptor type 1 induces insulin resistance in the mouse. *Obesity (Silver Spring)* **18**(9)**:** 1861-1863.

Conway, S, Mowat, ES, Drew, JE, Barrett, P, Delagrange, P, Morgan, PJ (2001) Serine residues 110 and 114 are required for agonist binding but not antagonist binding to the melatonin MT(1) receptor. *Biochem Biophys Res Commun* **282**(5)**:** 1229-1236.

Daulat, AM, Maurice, P, Froment, C, Guillaume, JL, Broussard, C, Monsarrat, B, *et al.* (2007) Purification and identification of G protein-coupled receptor protein complexes under native conditions. *Mol Cell Proteomics* **6**(5)**:** 835-844.

de Bodinat, C, Guardiola-Lemaitre, B, Mocaer, E, Renard, P, Munoz, C, Millan, MJ (2010) Agomelatine, the first melatonergic antidepressant: discovery, characterization and development. *Nat Rev Drug Discov* **9**(8)**:** 628-642.

This article is protected by copyright. All rights reserved.

de la Fuente Revenga, M, Fernandez-Saez, N, Herrera-Arozamena, C, Morales-Garcia, JA, Alonso-Gil, S, Perez-Castillo, A, *et al.* (2015) Novel N-Acetyl Bioisosteres of Melatonin: Melatonergic Receptor Pharmacology, Physicochemical Studies, and Phenotypic Assessment of Their Neurogenic Potential. *J Med Chem* **58**(12)**:** 4998-5014.

Descamps-Francois, C, Yous, S, Chavatte, P, Audinot, V, Bonnaud, A, Boutin, JA, *et al.* (2003) Design and Synthesis of Naphthalenic Dimers as Selective MT1 Melatoninergic Ligands *J Med Chem* **46**(7)**:** 1127-1129.

Dominguez-Alonso, A, Valdes-Tovar, M, Solis-Chagoyan, H, Benitez-King, G (2015) Melatonin stimulates dendrite formation and complexity in the hilar zone of the rat hippocampus: participation of the Ca++/Calmodulin complex. *Int J Mol Sci* **16**(1)**:** 1907-1927.

Dror, RO, Green, HF, Valant, C, Borhani, DW, Valcourt, JR, Pan, AC, *et al.* (2013) Structural basis for modulation of a G-protein-coupled receptor by allosteric drugs. *Nature* **503**(7475)**:** 295-299.

Dubocovich, ML (1985) Characterization of a retinal melatonin receptor. *J Pharmacol Exp Ther* **234**(2)**:** 395-401.

Dubocovich, ML (1988) Luzindole (N-0774): a novel melatonin receptor antagonist. *J Pharmacol Exp Ther* **246**(3)**:** 902-910.

Dubocovich, ML (1983) Melatonin is a potent modulator of dopamine release in the retina. *Nature* **306**(5945)**:** 782-784.

Dubocovich, ML (1995) Melatonin receptors: Are there multiple subtypes? *Trends in Pharmacological Sciences* **16**(2)**:** 50-56.

Dubocovich, ML (2007) Melatonin receptors: role on sleep and circadian rhythm regulation. *Sleep Med* **8 Suppl 3:** 34-42.

Dubocovich, ML, Delagrange, P, Krause, DN, Sugden, D, Cardinali, DP, Olcese, J (2010) International Union of Basic and Clinical Pharmacology. LXXV. Nomenclature, classification, and pharmacology of G protein-coupled melatonin receptors. *Pharmacol Rev* **62**(3)**:** 343-380.

Dubocovich, ML, Hudson, RL, Sumaya, IC, Masana, MI, Manna, E (2005) Effect of MT1 melatonin receptor deletion on melatonin-mediated phase shift of circadian rhythms in the C57BL/6 mouse. *J Pineal Res* **39**(2)**:** 113-120.

Dubocovich, ML, Masana, MI, Iacob, S, Sauri, DM (1997) Melatonin receptor antagonists that differentiate between the human Mel1a and Mel1b recombinant subtypes are used to assess the pharmacological profile of the rabbit retina ML1 presynaptic heteroreceptor. *Naunyn Schmiedebergs Arch Pharmacol* **355**(3)**:** 365-375.

Dubocovich, ML, Rivera-Bermudez, MA, Gerdin, MJ, Masana, MI (2003) Molecular pharmacology, regulation and function of mammalian melatonin receptors. *Front Biosci* **8:** d1093-1108.

This article is protected by copyright. All rights reserved.

Accepted Article

Dubocovich, ML, Takahashi, JS (1987) Use of 2-[125I]iodomelatonin to characterize melatonin binding sites in chicken retina. *Proc Natl Acad Sci U S A* **84**(11)**:** 3916-3920.

Dubocovich, ML, Yun, K, AlGhoul, WM, Benloucif, S, Masana, MI (1998) Selective MT2 melatonin receptor antagonists block melatonin-mediated phase advances of circadian rhythms. *FASEB J* **12**(12)**:** 1211-1220.

Ebisawa, T, Kajimura, N, Uchiyama, M, Katoh, M, Sekimoto, M, Watanabe, T, *et al.* (1999) Alleic variants of human melatonin 1a receptor: Function and prevalence in subjects with circadian rhythm sleep disorders. *Biochem Biophys Res Commun* **262**(3)**:** 832-837.

Escribano, BM, Colin-Gonzalez, AL, Santamaria, A, Tunez, I (2014) The role of melatonin in multiple sclerosis, Huntington's disease and cerebral ischemia. *CNS Neurol Disord Drug Targets* **13**(6)**:** 1096-1119.

Ettaoussi, M, Sabaouni, A, Rami, M, Boutin, JA, Delagrange, P, Renard, P, *et al.* (2012) Design, synthesis and pharmacological evaluation of new series of naphthalenic analogues as melatoninergic (MT1/MT2) and serotoninergic 5-HT2C dual ligands (I). *Eur J Med Chem* **49:** 310-323.

Evely, KM, Hudson, RL, Dubocovich, ML, Haj-Dahmane, S (2016) Melatonin receptor activation increases glutamatergic synaptic transmission in the rat medial lateral habenula. *Synapse* **70**(5)**:** 181-186.

Fang, JM, Dubocovich, ML (1990) Activation of melatonin receptor sites retarded the depletion of norepinephrine following inhibition of synthesis in the C3H/HeN mouse hypothalamus. *J Neurochem* **55**(1)**:** 76-82.

Farce, A, Chugunov, AO, Loge, C, Sabaouni, A, Yous, S, Dilly, S, *et al.* (2008) Homology modeling of MT1 and MT2 receptors. *Eur J Med Chem* **43**(9)**:** 1926-1944.

Farez, MF, Mascanfroni, ID, Mendez-Huergo, SP, Yeste, A, Murugaiyan, G, Garo, LP, *et al.* (2015) Melatonin Contributes to the Seasonality of Multiple Sclerosis Relapses. *Cell* **162**(6)**:** 1338-1352.

Faust, R, Garratt, PJ, Jones, R, Yeh, LK, Tsotinis, A, Panoussopoulou, M, *et al.* (2000) Mapping the melatonin receptor. 6. Melatonin agonists and antagonists derived from 6H-isoindolo[2,1-a]indoles, 5,6-dihydroindolo[2,1-a]isoquinolines, and 6,7-dihydro-5H-benzo[c]azepino[2,1-a]indoles. *J Med Chem* **43**(6)**:** 1050-1061.

Ferre, S, Casado, V, Devi, LA, Filizola, M, Jockers, R, Lohse, MJ, *et al.* (2014) G protein-coupled receptor oligomerization revisited: functional and pharmacological perspectives. *Pharmacol Rev* **66**(2)**:** 413-434.

Fisher, SP, Sugden, D (2009) Sleep-promoting action of IIK7, a selective MT2 melatonin receptor agonist in the rat. *Neurosci Lett* **457**(2)**:** 93-96.

Gerdin, MJ, Masana, MI, Rivera-Bermudez, MA, Hudson, RL, D.J., E, Gillette, MU, *et al.* (2004) Melatonin desensitizes endogenous MT2 melatonin receptors in the rat

This article is protected by copyright. All rights reserved.

suprachiasmatic nucleus: relevance for defining the periods of sensitivity of the mammalian circadian clock to melatonin. *Faseb J* **18**(14)**:** 1646-1656.

Gerdin, MJ, Mseeh, F, Dubocovich, ML (2003) Mutagenesis studies of the human MT2 melatonin receptor. *Biochem Pharmacol* **66**(2)**:** 315-320.

Gianesini, C, Hiragaki, S, Laurent, V, Hicks, D, Tosini, G (2016) Cone Viability Is Affected by Disruption of Melatonin Receptors Signaling. *Invest Ophthalmol Vis Sci* **57**(1)**:** 94-104.

Gitto, E, Karbownik, M, Reiter, RJ, Tan, DX, Cuzzocrea, S, Chiurazzi, P, *et al.* (2001) Effects of melatonin treatment in septic newborns. *Pediatric Research. Dec* **50**(6)**:** 756-760.

Green, NH, Jackson, CR, Iwamoto, H, Tackenberg, MC, McMahon, DG (2015) Photoperiod programs dorsal raphe serotonergic neurons and affective behaviors. *Curr Biol* **25**(10)**:** 1389-1394.

Heckman, D, Attia, MI, Behnam, MAM, Mohsen, AMY, Markl, C, Julius, J, *et al.* (2011) 2-[(1,3-Dihydro-2H-isoindol-2yl)methyl]-melatonin- a novel MT2-selective melatonin receptor antagonist. *Med Chem Commun* **2:** 991-994.

Hevia, D, Gonzalez-Menendez, P, Quiros-Gonzalez, I, Miar, A, Rodriguez-Garcia, A, Tan, DX, *et al.* (2015) Melatonin uptake through glucose transporters: a new target for melatonin inhibition of cancer. *J Pineal Res* **58**(2)**:** 234-250.

Hotta, CT, Gazarini, ML, Beraldo, FH, Varotti, FP, Lopes, C, Markus, RP, *et al.* (2000) Calcium-dependent modulation by melatonin of the circadian rhythm in malarial parasites. *Nat Cell Biol* **2**(7)**:** 466-468.

Hu, Y, Zhu, J, Chan, KH, Wong, YH (2013) Development of substituted N-[3-(3-methoxylphenyl)propyl] amides as MT(2)-selective melatonin agonists: improving metabolic stability. *Bioorg Med Chem* **21**(2)**:** 547-552.

Hunt, AE, AlGhoul, WM, Gillette, MU, Dubocovich, ML (2001) Activation of MT2 melatonin receptors in rat suprachiasmatic nucleus phase advances the circadian clock. *Amer J Physiol Cell Physiol* **280**(1)**:** C110-C118.

Hutchinson, AJ, Hudson, RL, Dubocovich, ML (2012) Genetic deletion of MT(1) and MT(2) melatonin receptors differentially abrogates the development and expression of methamphetamine-induced locomotor sensitization during the day and the night in C3H/HeN mice. *J Pineal Res* **53**(4)**:** 399-409.

Jilg, A, Moek, J, Weaver, DR, Korf, HW, Stehle, JH, von Gall, C (2005) Rhythms in clock proteins in the mouse pars tuberalis depend on MT1 melatonin receptor signalling. *Eur J Neurosci* **22**(11)**:** 2845-2854.

Jin, XW, von, GC, Pieschl, RL, Gribkoff, VK, Stehle, JH, Reppert, SM, *et al.* (2003) Targeted disruption of the mouse Mel(1b) melatonin receptor. *Mol Cell Biol* **23**(3)**:** 1054-1060.

Accepted Article

This article is protected by copyright. All rights reserved.

Jockers, R, Maurice, P, Boutin, JA, Delagrange, P (2008) Melatonin receptors, heterodimerization, signal transduction and binding sites: what's new? *Br J Pharmacol* **154**(6)**:** 1182-1195.

Johnston, JD, Skene, DJ (2015) 60 YEARS OF NEUROENDOCRINOLOGY: Regulation of mammalian neuroendocrine physiology and rhythms by melatonin. *J Endocrinol* **226**(2)**:** T187-198.

Johnston, JM, Filizola, M (2014) Beyond standard molecular dynamics: investigating the molecular mechanisms of G protein-coupled receptors with enhanced molecular dynamics methods. *Adv Exp Med Biol* **796:** 95-125.

Joshi, N, Biswas, J, Nath, C, Singh, S (2015) Promising Role of Melatonin as Neuroprotectant in Neurodegenerative Pathology. *Mol Neurobiol* **52**(1)**:** 330-340.

Journe, AS, Habib, SAM, Dodda, BR, Morcos, MNF, Sadek, MS, Tadros, SAA, *et al.* (2014) N1-linked melatonin dimers as bivalent ligands targeting dimeric melatonin receptors. *Med. Chem. Commun* **5:** 792-796.

Kamal, M, Gbahou, F, Guillaume, JL, Daulat, AM, Benleulmi-Chaachoua, A, Luka, M, *et al.* (2015) Convergence of melatonin and serotonin (5-HT) signaling at MT2/5-HT2C receptor heteromers. *J Biol Chem* **290**(18)**:** 11537-11546.

Kang, Y, Zhou, XE, Gao, X, He, Y, Liu, W, Ishchenko, A, *et al.* (2015) Crystal structure of rhodopsin bound to arrestin by femtosecond X-ray laser. *Nature* **523**(7562)**:** 561-567.

Karamitri, A, Jockers, R (2014) Exon Sequencing of G Protein-Coupled Receptor Genes and Perspectives for Disease Treatment. In: *Methods in Pharmacology and Toxicology series, G Protein-Coupled Receptor Genetics: Research and Methods in the Post-Genomic Era*, Stefens, CW (ed), pp 313-332. New York: Springer Science.

Karamitri, A, Renault, N, Clement, N, Guillaume, JL, Jockers, R (2013) Minireview: Toward the Establishment of a Link between Melatonin and Glucose Homeostasis: Association of Melatonin MT2 Receptor Variants with Type 2 Diabetes. *Mol Endocrinol* **27**(8)**:** 1217-1233.

Kato, K, Hirai, K, Nishiyama, K, Uchikawa, O, Fukatsu, K, Ohkawa, S, *et al.* (2005) Neurochemical properties of ramelteon (TAK-375), a selective MT1/MT2 receptor agonist. *Neuropharmacology* **48**(2)**:** 301-310.

Koike, T, Hoashi, Y, Takai, T, Nakayama, M, Yukuhiro, N, Ishikawa, T, *et al.* (2011) 1,6-Dihydro-2H-indeno[5,4-b]furan derivatives: design, synthesis, and pharmacological characterization of a novel class of highly potent MT(2)-selective agonists. *J Med Chem* **54**(9)**:** 3436-3444.

Kokkola, T, Foord, SM, Watson, MA, Vakkuri, O, Laitinen, JT (2003) Important amino acids for the function of the human MT1 melatonin receptor. *Biochem Pharmacol* **65**(9)**:** 1463-1471.

Krause, DN, Siuciak, JA, Dubocovich, ML (1992) Optic nerve transection decreases 2-[125I]iodomelatonin binding in the chick optic tectum. *Brain Res* **590**(1-2)**:** 325-328.

This article is protected by copyright. All rights reserved.

Krause, DN, Siuciak, JA, Dubocovich, ML (1994) Unilateral optic nerve transection decreases 2-[125I]-iodomelatonin binding in retinorecipient areas and visual pathways of chick brain. *Brain Res* **654**(1)**:** 63-74.

Lahiri, S, Haldar, C (2009) Response of melatonin receptor MT1 in spleen of a tropical Indian rodent, Funambulus pennanti, to natural solar insolation and different photoperiodic conditions. *Chronobiol Int* **26**(8)**:** 1559-1574.

Landolt, HP, Wehrle, R (2009) Antagonism of serotonergic 5-HT2A/2C receptors: mutual improvement of sleep, cognition and mood? *Eur J Neurosci* **29**(9)**:** 1795-1809.

Laranjeira-Silva, MF, Zampieri, RA, Muxel, SM, Floeter-Winter, LM, Markus, RP (2015) Melatonin attenuates Leishmania (L.) amazonensis infection by modulating arginine metabolism. *J Pineal Res* **59**(4)**:** 478-487.

Lardone, PJ, Carrillo-Vico, A, Naranjo, MC, De Felipe, B, Vallejo, A, Karasek, M, *et al.* (2006) Melatonin synthesized by Jurkat human leukemic T cell line is implicated in IL-2 production. *J Cell Physiol* **206**(1)**:** 273-279.

Lardone, PJ, Rubio, A, Cerrillo, I, Gomez-Corvera, A, Carrillo-Vico, A, Sanchez-Hidalgo, M, *et al.* (2010) Blocking of melatonin synthesis and MT(1) receptor impairs the activation of Jurkat T cells. *Cell Mol Life Sci* **67**(18)**:** 3163-3172.

Larson, J, Jessen, RE, Uz, T, Arslan, AD, Kurtuncu, M, Imbesi, M, *et al.* (2006) Impaired hippocampal long-term potentiation in melatonin MT2 receptor-deficient mice. *Neurosci Lett* **393**(1)**:** 23-26.

Lavedan, C, Forsberg, M, Gentile, AJ (2015) Tasimelteon: a selective and unique receptor binding profile. *Neuropharmacology* **91:** 142-147.

Legros, C, Brasseur, C, Delagrange, P, Ducrot, P, Nosjean, O, Boutin, JA (2016) Alternative Radioligands for Investigating the Molecular Pharmacology of Melatonin Receptors. *J Pharmacol Exp Ther* **356**(3)**:** 681-692.

Legros, C, Chesneau, D, Boutin, JA, Barc, C, Malpaux, B (2014) Melatonin from cerebrospinal fluid but not from blood reaches sheep cerebral tissues under physiological conditions. *J Neuroendocrinol* **26**(3)**:** 151-163.

Legros, C, Matthey, U, Grelak, T, Pedragona-Moreau, S, Hassler, W, Yous, S, *et al.* (2013) New Radioligands for Describing the Molecular Pharmacology of MT1 and MT2 Melatonin Receptors. *Int J Mol Sci* **14**(5)**:** 8948-8962.

Levoye, A, Dam, J, Ayoub, MA, Guillaume, JL, Couturier, C, Delagrange, P, *et al.* (2006a) The orphan GPR50 receptor specifically inhibits MT(1) melatonin receptor function through heterodimerization. *EMBO J* **25**(13)**:** 3012-3023.

Levoye, A, Dam, J, Ayoub, MA, Guillaume, JL, Jockers, R (2006b) Do orphan G-protein-coupled receptors have ligand-independent functions? New insights from receptor heterodimers. *EMBO Rep* **7**(11)**:** 1094-1098.

This article is protected by copyright. All rights reserved.

Liao, S, Williams-Ashman, HG (1961) Enzymatic oxidation of some non-phosphorylated derivatives of dihydronicotinamide. *Biochem Biophys Res Commun* **4:** 208-213.

Liu, C, Weaver, DR, Jin, X, Shearman, LP, Pieschl, RL, Gribkoff, VK, *et al.* (1997) Molecular dissection of two distinct actions of melatonin on the suprachiasmatic circadian clock. *Neuron* **19**(1)**:** 91-102.

Liu, D, Wei, N, Man, HY, Lu, Y, Zhu, LQ, Wang, JZ (2015) The MT2 receptor stimulates axonogenesis and enhances synaptic transmission by activating Akt signaling. *Cell Death Differ* **22**(4)**:** 583-596.

Liu, J, Clough, SJ, Hutchinson, AJ, Adamah-Biassi, EB, Popovska-Gorevski, M, Dubocovich, ML (2016) MT1 and MT2 Melatonin Receptors: A Therapeutic Perspective. *Annu Rev Pharmacol Toxicol* **56:** 361-383.

Lotufo, CM, Lopes, C, Dubocovich, ML, Farsky, SH, Markus, RP (2001) Melatonin and N-acetylserotonin inhibit leukocyte rolling and adhesion to rat microcirculation. *Eur J Pharmacol* **430**(2-3)**:** 351-357.

Lyssenko, V, Nagorny, CL, Erdos, MR, Wierup, N, Jonsson, A, Spegel, P, *et al.* (2009) Common variant in MTNR1B associated with increased risk of type 2 diabetes and impaired early insulin secretion. *Nat Genet* **41**(1)**:** 82-88.

Maestroni, G (1993) Th immunoneuroendocrine role of melatonin. *Journal of Pineal Research* **14:** 1-10.

Markl, C, Clafshenkel, WP, Attia, MI, Sethi, S, Witt-Enderby, PA, Zlotos, DP (2011) N-Acetyl-5-arylalkoxytryptamine analogs: probing the melatonin receptors for MT(1) - selectivity. *Arch Pharm (Weinheim)* **344**(10)**:** 666-674.

Markus, RP, Cecon, E, Pires-Lapa, MA (2013) Immune-Pineal Axis: Nuclear Factor ŒʃB (NF-kB) Mediates the Shift in the Melatonin Source from Pinealocytes to Immune Competent Cells. *Int J Mol Sci* **14**(6)**:** 10979-10997 %! Immune-Pineal Axis: Nuclear Factor ŒʃB (NF-kB) Mediates the Shift in the Melatonin Source from Pinealocytes to Immune Competent Cells %@ 11422-10067.

Maurice, P, Daulat, AM, Broussard, C, Mozo, J, Clary, G, Hotellier, F, *et al.* (2008) A generic approach for the purification of signaling complexes that specifically interact with the carboxy-terminal domain of G protein-coupled receptors. *Mol Cell Proteomics* **7**(8)**:** 1556-1569.

Maurice, P, Kamal, M, Jockers, R (2011) Asymmetry of GPCR oligomers supports their functional relevance. *Trends Pharmacol Sci* **32**(9)**:** 514-520.

Mazna, P, Obsilova, V, Jelinkova, I, Balik, A, Berka, K, Sovova, Z, *et al.* (2004) Molecular modeling of human MT2 melatonin receptor: the role of Val204, Leu272 and Tyr298 in ligand binding. *J Neurochem* **91**(4)**:** 836-842.

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Mc Arthur, A, Hunt, A, Gillette, M (1997) Melatonin action and signal transduction in the rat suprachiasmatic circadian clock: Activation of protein kinase C at dusk and dawn. *Endocrinology* **138**(2)**:** 627-634.

Medeiros, CA, Carvalhedo de Bruin, PF, Lopes, LA, Magalhaes, MC, de Lourdes Seabra, M, de Bruin, VM (2007) Effect of exogenous melatonin on sleep and motor dysfunction in Parkinson's disease. A randomized, double blind, placebo-controlled study. *J Neurol* **254**(4)**:** 459-464.

Mesangeau, C, Peres, B, Descamps-Francois, C, Chavatte, P, Audinot, V, Coumailleau, S, *et al.* (2010) Design, synthesis and pharmacological evaluation of novel naphthalenic derivatives as selective MT(1) melatoninergic ligands. *Bioorg Med Chem* **18**(10)**:** 3426-3436.

Millan, MJ, Gobert, A, Lejeune, F, Dekeyne, A, Newman-Tancredi, A, Pasteau, V, *et al.* (2003) The novel melatonin agonist agomelatine (S20098) is an antagonist at 5-hydroxytryptamine2C receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways. *J Pharmacol Exp Ther* **306**(3)**:** 954-964.

Mini, LJ, Wang-Weigand, S, Zhang, J (2007) Self-reported efficacy and tolerability of ramelteon 8 mg in older adults experiencing severe sleep-onset difficulty. *Am J Geriatr Pharmacother* **5**(3)**:** 177-184.

Molinari, EJ, North, PC, Dubocovich, ML (1996) 2-[I-125]iodo-5-methoxycarbonylamino-N-acetyltryptamine: A selective radioligand for the characterization of melatonin ML(2) binding sites. *Eur J Pharmacol* **301**(1-3)**:** 159-168.

Morellato, L, Lefas-Le Gall, M, Langlois, M, Caignard, DH, Renard, P, Delagrange, P, *et al.* (2013) Synthesis of new N-(arylcyclopropyl)acetamides and N-(arylvinyl)acetamides as conformationally-restricted ligands for melatonin receptors. *Bioorg Med Chem Lett* **23**(2)**:** 430-434.

Muhlbauer, E, Gross, E, Labucay, K, Wolgast, S, Peschke, E (2009) Loss of melatonin signalling and its impact on circadian rhythms in mouse organs regulating blood glucose. *Eur J Pharmacol* **606**(1-3)**:** 61-71.

Mulchahey, JJ, Goldwater, DR, Zemlan, FP (2004) A single blind, placebo controlled, across groups dose escalation study of the safety, tolerability, pharmacokinetics and pharmacodynamics of the melatonin analog beta-methyl-6-chloromelatonin. *Life Sci* **75**(15)**:** 1843-1856.

Muxel, SM, Laranjeira-Silva, MF, de Carvalho Sousa, CE, Floeter-Winter, LM, Markus, RP (2016) The RelA/cRel nuclear factor-kappaB (NF-kappaB) dimer, crucial for inflammation resolution, mediates the transcription of the key enzyme in melatonin synthesis in RAW 264.7 macrophages. *J Pineal Res.*

Muxel, SM, Pires-Lapa, MA, Monteiro, AW, Cecon, E, Tamura, EK, Floeter-Winter, LM, *et al.* (2012) NF-kappaB drives the synthesis of melatonin in RAW 264.7 macrophages by inducing the transcription of the arylalkylamine-N-acetyltransferase (AA-NAT) gene. *PLoS One* **7**(12)**:** e52010.

This article is protected by copyright. All rights reserved.

Naskar, A, Prabhakar, V, Singh, R, Dutta, D, Mohanakumar, KP (2015) Melatonin enhances L-DOPA therapeutic effects, helps to reduce its dose, and protects dopaminergic neurons in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinsonism in mice. *J Pineal Res* **58**(3)**:** 262-274.

Nelson, MR, Wegmann, D, Ehm, MG, Kessner, D, St Jean, P, Verzilli, C, *et al.* (2012) An abundance of rare functional variants in 202 drug target genes sequenced in 14,002 people. *Science* **337**(6090)**:** 100-104.

Nonno, R, Lucini, V, Spadoni, G, Pannacci, M, Croce, A, Esposti, D, *et al.* (2000) A new melatonin receptor ligand with mt(1)-agonist and MT2-antagonist properties. *J Pineal Res* **29**(4)**:** 234-240.

Nosjean, O, Ferro, M, Cogé, F, Beauverger, P, Henlin, JM, Lefoulon, F, *et al.* (2000) Identification of the melatonin-binding site MT3 as the quinone reductase 2. *J Biol Chem* **275**(40)**:** 31311-31317.

O'Neal-Moffitt, G, Delic, V, Bradshaw, PC, Olcese, J (2015) Prophylactic melatonin significantly reduces Alzheimer's neuropathology and associated cognitive deficits independent of antioxidant pathways in AbetaPP(swe)/PS1 mice. *Mol Neurodegener* **10:** 27.

O'Neal-Moffitt, G, Pilli, J, Kumar, SS, Olcese, J (2014) Genetic deletion of MT1/MT2 melatonin receptors enhances murine cognitive and motor performance. *Neuroscience* **277:** 506-521.

Ochoa-Sanchez, R, Comai, S, Lacoste, B, Bambico, FR, Dominguez-Lopez, S, Spadoni, G, *et al.* (2011) Promotion of non-rapid eye movement sleep and activation of reticular thalamic neurons by a novel MT2 melatonin receptor ligand. *J Neurosci* **31**(50)**:** 18439-18452.

Okada, T, Fujiyoshi, Y, Silow, M, Navarro, J, Landau, EM, Shichida, Y (2002) Functional role of internal water molecules in rhodopsin revealed by X-ray crystallography. *Proc Natl Acad Sci U S A* **99**(9)**:** 5982-5987.

Okada, T, Sugihara, M, Bondar, AN, Elstner, M, Entel, P, Buss, V (2004) The retinal conformation and its environment in rhodopsin in light of a new 2.2 A crystal structure. *J Mol Biol* **342**(2)**:** 571-583.

Olcese, JM, Cao, C, Mori, T, Mamcarz, MB, Maxwell, A, Runfeldt, MJ, *et al.* (2009) Protection against cognitive deficits and markers of neurodegeneration by long-term oral administration of melatonin in a transgenic model of Alzheimer disease. *J Pineal Res* **47**(1)**:** 82-96.

Ortiz, GG, Pacheco-Moises, FP, Gomez-Rodriguez, VM, Gonzalez-Renovato, ED, Torres-Sanchez, ED, Ramirez-Anguiano, AC (2013) Fish oil, melatonin and vitamin E attenuates midbrain cyclooxygenase-2 activity and oxidative stress after the administration of 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine. *Metab Brain Dis* **28**(4)**:** 705-709.

Owino, S, Contreras-Alcantara, S, Baba, K, Tosini, G (2016) Melatonin Signaling Controls the Daily Rhythm in Blood Glucose Levels Independent of Peripheral Clocks. *PLoS One* **11**(1)**:** e0148214.

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Accepted Article

Pala, D, Beuming, T, Sherman, W, Lodola, A, Rivara, S, Mor, M (2013) Structure-based virtual screening of MT2 melatonin receptor: influence of template choice and structural refinement. *J Chem Inf Model* **53**(4)**:** 821-835.

Palczewski, K, Kumasaka, T, Hori, T, Behnke, CA, Motoshima, H, Fox, BA, *et al.* (2000) Crystal structure of rhodopsin: A G protein-coupled receptor. *Science* **289**(5480)**:** 739-745.

Peng, CX, Hu, J, Liu, D, Hong, XP, Wu, YY, Zhu, LQ, *et al.* (2013) Disease-modified glycogen synthase kinase-3beta intervention by melatonin arrests the pathology and memory deficits in an Alzheimer's animal model. *Neurobiol Aging* **34**(6)**:** 1555-1563.

Pfeffer, M, Rauch, A, Korf, HW, von Gall, C (2012) The endogenous melatonin (MT) signal facilitates reentrainment of the circadian system to light-induced phase advances by acting upon MT2 receptors. *Chronobiol Int* **29**(4)**:** 415-429.

Pinato, L, da Silveira Cruz-Machado, S, Franco, DG, Campos, LM, Cecon, E, Fernandes, PA, *et al.* (2015) Selective protection of the cerebellum against intracerebroventricular LPS is mediated by local melatonin synthesis. *Brain Struct Funct* **220**(2)**:** 827-840.

Pires-Lapa, MA, Tamura, EK, Salustiano, EM, Markus, RP (2013) Melatonin synthesis in human colostrum mononuclear cells enhances dectin-1-mediated phagocytosis by mononuclear cells. *J Pineal Res* **55**(3)**:** 240-246.

Poissonnier-Durieux, S, Ettaoussi, M, Peres, B, Boutin, JA, Audinot, V, Bennejean, C, *et al.* (2008) Synthesis of 3-phenylnaphthalenic derivatives as new selective MT(2) melatoninergic ligands. *Bioorg Med Chem* **16**(18)**:** 8339-8348.

Pontes, GN, Cardoso, EC, Carneiro-Sampaio, MM, Markus, RP (2007) Pineal melatonin and the innate immune response: the TNF-alpha increase after cesarean section suppresses nocturnal melatonin production. *J Pineal Res* **43**(4)**:** 365-371.

Prada, C, Udin, SB, Wiechmann, AF, Zhdanova, IV (2005) Stimulation of melatonin receptors decreases calcium levels in xenopus tectal cells by activating GABA(C) receptors. *J Neurophysiol* **94**(2)**:** 968-978.

Prokopenko, I, Langenberg, C, Florez, JC, Saxena, R, Soranzo, N, Thorleifsson, G, *et al.* (2009) Variants in MTNR1B influence fasting glucose levels. *Nat Genet* **41**(1)**:** 77-81.

Provasi, D, Artacho, MC, Negri, A, Mobarec, JC, Filizola, M (2011) Ligand-induced modulation of the free-energy landscape of G protein-coupled receptors explored by adaptive biasing techniques. *PLoS Comput Biol* **7**(10)**:** e1002193.

Racagni, G, Riva, MA, Molteni, R, Musazzi, L, Calabrese, F, Popoli, M, *et al.* (2011) Mode of action of agomelatine: Synergy between melatonergic and 5-HT(2C) receptors. *World J Biol Psychiatry*.

Rajaratnam, SM, Polymeropoulos, MH, Fisher, DM, Roth, T, Scott, C, Birznieks, G, *et al.* (2009) Melatonin agonist tasimelteon (VEC-162) for transient insomnia after sleep-time shift: two randomised controlled multicentre trials. *Lancet* **373**(9662)**:** 482-491.

This article is protected by copyright. All rights reserved.

Rasmussen, SG, Choi, HJ, Fung, JJ, Pardon, E, Casarosa, P, Chae, PS, *et al.* (2011a) Structure of a nanobody-stabilized active state of the beta(2) adrenoceptor. *Nature* **469**(7329)**:** 175-180.

Rasmussen, SG, DeVree, BT, Zou, Y, Kruse, AC, Chung, KY, Kobilka, TS, *et al.* (2011b) Crystal structure of the beta2 adrenergic receptor-Gs protein complex. *Nature* **477**(7366)**:** 549-555.

Rawashdeh, O, Hudson, RL, Stepien, I, Dubocovich, ML (2011) Circadian periods of sensitivity for ramelteon on the onset of running-wheel activity and the peak of suprachiasmatic nucleus neuronal firing rhythms in C3H/HeN mice. *Chronobiol Int* **28**(1)**:** 31-38.

Ressmeyer, AR, Mayo, JC, Zelosko, V, Sainz, RM, Tan, DX, Poeggeler, B, *et al.* (2003) Antioxidant properties of the melatonin metabolite N1-acetyl-5-methoxykynuramine (AMK): scavenging of free radicals and prevention of protein destruction. *Redox Rep* **8**(4)**:** 205-213.

Rivara, S, Lodola, A, Mor, M, Bedini, A, Spadoni, G, Lucini, V, *et al.* (2007) N-(substituted-anilinoethyl)amides: design, synthesis, and pharmacological characterization of a new class of melatonin receptor ligands. *J Med Chem* **50**(26)**:** 6618-6626.

Rivara, S, Lorenzi, S, Mor, M, Plazzi, PV, Spadoni, G, Bedini, A, *et al.* (2005) Analysis of structure-activity relationships for MT2 selective antagonists by melatonin MT1 and MT2 receptor models. *J Med Chem* **48**(12)**:** 4049-4060.

Rivara, S, Pala, D, Lodola, A, Mor, M, Lucini, V, Dugnani, S, *et al.* (2012) MT1-selective melatonin receptor ligands: synthesis, pharmacological evaluation, and molecular dynamics investigation of N-{[(3-O-substituted)anilino]alkyl}amides. *ChemMedChem* **7**(11)**:** 1954-1964.

Rosen, R, Hu, DN, Perez, V, Tai, K, Yu, GP, Chen, M, *et al.* (2009) Urinary 6-sulfatoxymelatonin level in age-related macular degeneration patients. *Mol Vis* **15:** 1673-1679.

Sampaio Lde, F, Mesquita, FP, de Sousa, PR, Silva, JL, Alves, CN (2014) The melatonin analog 5-MCA-NAT increases endogenous dopamine levels by binding NRH:quinone reductase enzyme in the developing chick retina. *Int J Dev Neurosci* **38:** 119-126.

Savaskan, E, Olivieri, G, Meier, F, Brydon, L, Jockers, R, Ravid, R, *et al.* (2002) Increased melatonin 1a-receptor immunoreactivity in the hippocampus of Alzheimer's disease patients. *J Pineal Res* **32**(1)**:** 59-62.

Savaskan, E, Schnitzler, C, Schroder, C, Cajochen, C, Muller-Spahn, F, Wirz-Justice, A (2006) Treatment of behavioural, cognitive and circadian rest-activity cycle disturbances in Alzheimer's disease: haloperidol vs. quetiapine. *Int J Neuropsychopharmacol* **9**(5)**:** 507-516.

Scheerer, P, Park, JH, Hildebrand, PW, Kim, YJ, Krauss, N, Choe, HW, *et al.* (2008) Crystal structure of opsin in its G-protein-interacting conformation. *Nature* **455**(7212)**:** 497-502.

This article is protected by copyright. All rights reserved.

Sengupta, A, Baba, K, Mazzoni, F, Pozdeyev, NV, Strettoi, E, Iuvone, PM, *et al.* (2011) Localization of melatonin receptor 1 in mouse retina and its role in the circadian regulation of the electroretinogram and dopamine levels. *PLoS One* **6**(9)**:** e24483.

Sharma, S, Haldar, C (2006) Melatonin prevents X-ray irradiation induced oxidative damagein peripheral blood and spleen of the seasonally breeding rodent, Funambulus pennanti during reproductively active phase. *Int J Radiat Biol* **82**(6)**:** 411-419.

Sheynzon, P, Korf, HW (2006) Targeted deletions of Mel1a and Mel1b melatonin receptors affect pCREB levels in lactotroph and pars intermedia cells of mice. *Neurosci Lett* **407**(1)**:** 48-52.

Shin, IS, Shin, NR, Park, JW, Jeon, CM, Hong, JM, Kwon, OK, *et al.* (2015) Melatonin attenuates neutrophil inflammation and mucus secretion in cigarette smoke-induced chronic obstructive pulmonary diseases via the suppression of Erk-Sp1 signaling. *J Pineal Res* **58**(1)**:** 50-60.

Soundararajan, M, Willard, FS, Kimple, AJ, Turnbull, AP, Ball, LJ, Schoch, GA, *et al.* (2008) Structural diversity in the RGS domain and its interaction with heterotrimeric G protein alpha-subunits. *Proc Natl Acad Sci U S A* **105**(17)**:** 6457-6462.

Spadoni, G, Bedini, A, Lucarini, S, Mari, M, Caignard, DH, Boutin, JA, *et al.* (2015) Highly Potent and Selective MT2 Melatonin Receptor Full Agonists from Conformational Analysis of 1-Benzyl-2-acylaminomethyl-tetrahydroquinolines. *J Med Chem* **58**(18)**:** 7512-7525.

Spadoni, G, Bedini, A, Orlando, P, Lucarini, S, Tarzia, G, Mor, M, *et al.* (2011) Bivalent ligand approach on N-{2-[(3-methoxyphenyl)methylamino]ethyl}acetamide: synthesis, binding affinity and intrinsic activity for MT(1) and MT(2) melatonin receptors. *Bioorg Med Chem* **19**(16)**:** 4910-4916.

Stumpf, I, Muhlbauer, E, Peschke, E (2008) Involvement of the cGMP pathway in mediating the insulin-inhibitory effect of melatonin in pancreatic beta-cells. *J Pineal Res* **45**(3)**:** 318-327.

Sugden, D, Chong, NW, Lewis, DF (1995) Structural requirements at the melatonin receptor. *Br J Pharmacol* **114**(3)**:** 618-623.

Sugden, D, Yeh, LK, Teh, MT (1999) Design of subtype selective melatonin receptor agonists and antagonists. *Reprod Nutr Develop* **39**(3)**:** 335-344.

Tamura, EK, Fernandes, PA, Marcola, M, da Silveira Cruz-Machado, S, Markus, RP (2010) Long-lasting priming of endothelial cells by plasma melatonin levels. *PLoS One* **5**(11)**:** e13958.

Tan, DX, Manchester, LC, Esteban-Zubero, E, Zhou, Z, Reiter, RJ (2015) Melatonin as a Potent and Inducible Endogenous Antioxidant: Synthesis and Metabolism. *Molecules* **20**(10)**:** 18886-18906.

Accepted Article

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Tan, DX, Manchester, LC, Terron, MP, Flores, LJ, Reiter, RJ (2007) One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? *J Pineal Res* **42**(1)**:** 28-42.

Tchekalarova, J, Nenchovska, Z, Atanasova, D, Atanasova, M, Kortenska, L, Stefanova, M, *et al.* (2016) Consequences of long-term treatment with agomelatine on depressive-like behavior and neurobiological abnormalities in pinealectomized rats. *Behav Brain Res* **302:** 11-28.

Tosini, G, Baba, K, Hwang, CK, Iuvone, PM (2012) Melatonin: an underappreciated player in retinal physiology and pathophysiology. *Exp Eye Res* **103:** 82-89.

Tosini, G, Owino, S, Guillaume, JL, Jockers, R (2014) Understanding melatonin receptor pharmacology: Latest insights from mouse models, and their relevance to human disease. *Bioessays* **36**(8)**:** 778-787.

Uchikawa, O, Fukatsu, K, Tokunoh, R, Kawada, M, Matsumoto, K, Imai, Y, *et al.* (2002) Synthesis of a novel series of tricyclic indan derivatives as melatonin receptor agonists. *J Med Chem* **45**(19)**:** 4222-4239.

Vakkuri, O, Leppaluoto, J, Vuolteenaho, O (1984) Development and validation of a melatonin radioimmunoassay using radioiodinated melatonin as a tracer. *Acta Endocrinol.* **106:** 152-157.

van Wamelen, DJ, Aziz, NA, Anink, JJ, van Steenhoven, R, Angeloni, D, Fraschini, F, *et al.* (2013) Suprachiasmatic nucleus neuropeptide expression in patients with Huntington's Disease. *Sleep* **36**(1)**:** 117-125.

van Wamelen, DJ, Roos, RA, Aziz, NA (2015) Therapeutic strategies for circadian rhythm and sleep disturbances in Huntington disease. *Neurodegener Dis Manag* **5**(6)**:** 549-559.

Vanecek, J (1988) Melatonin binding sites. *J Neurochem* **51**(5)**:** 1436-1440.

Vincent, L, Cohen, W, Delagrange, P, Boutin, JA, Nosjean, O (2010) Molecular and cellular pharmacological properties of 5-methoxycarbonylamino-N-acetyltryptamine (MCA-NAT): a nonspecific MT3 ligand. *J Pineal Res* **48**(3)**:** 222-229.

von Gall, C, Garabette, ML, Kell, CA, Frenzel, S, Dehghani, F, Schumm, DP, *et al.* (2002) Rhythmic gene expression in pituitary depends on heterologous sensitization by the neurohormone melatonin. *Nature Neuroscience* **5**(3)**:** 234-238.

Voronkov, AE, Ivanov, AA, Baskin, II, Palyulin, VA, Zefirov, NS (2005) Molecular modeling study of the mechanism of ligand binding to human melatonin receptors. *Dokl Biochem Biophys* **403:** 284-288.

Wade, AG, Farmer, M, Harari, G, Fund, N, Laudon, M, Nir, T, *et al.* (2014) Add-on prolonged-release melatonin for cognitive function and sleep in mild to moderate Alzheimer's disease: a 6-month, randomized, placebo-controlled, multicenter trial. *Clin Interv Aging* **9:** 947-961.

This article is protected by copyright. All rights reserved.

Wang, LM, Suthana, NA, Chaudhury, D, Weaver, DR, Colwell, CS (2005) Melatonin inhibits hippocampal long-term potentiation. *Eur J Neurosci* **22**(9)**:** 2231-2237.

Wang, X, Sirianni, A, Pei, Z, Cormier, K, Smith, K, Jiang, J, *et al.* (2011) The melatonin MT1 receptor axis modulates mutant Huntingtin-mediated toxicity. *J Neurosci* **31**(41)**:** 14496-14507.

Weil, ZM, Borniger, JC, Cisse, YM, Abi Salloum, BA, Nelson, RJ (2015) Neuroendocrine control of photoperiodic changes in immune function. *Front Neuroendocrinol* **37:** 108-118.

Weishaupt, JH, Bartels, C, Polking, E, Dietrich, J, Rohde, G, Poeggeler, B, *et al.* (2006) Reduced oxidative damage in ALS by high-dose enteral melatonin treatment. *J Pineal Res* **41**(4)**:** 313-323.

Wu, YH, Zhou, JN, Van Heerikhuize, J, Jockers, R, Swaab, DF (2007) Decreased MT1 melatonin receptor expression in the suprachiasmatic nucleus in aging and Alzheimer's disease. *Neurobiol Aging* **28**(8)**:** 1239-1247.

Yadav, SK, Haldar, C (2013) Reciprocal interaction between melatonin receptors (Mel(1a), Mel(1b), and Mel(1c)) and androgen receptor (AR) expression in immunoregulation of a seasonally breeding bird, Perdicula asiatica: role of photoperiod. *J Photochem Photobiol B* **122:** 52-60.

Yasuo, S, Yoshimura, T, Ebihara, S, Korf, HW (2009) Melatonin transmits photoperiodic signals through the MT1 melatonin receptor. *J Neurosci* **29**(9)**:** 2885-2889.

Zavala-Oseguera, C, Galano, A, Merino, G (2014) Computational study on the kinetics and mechanism of the carbaryl + OH reaction. *J Phys Chem A* **118**(36)**:** 7776-7781.

Zefirova, ON, Baranova, TY, Ivanova, AA, Ivanov, AA, Zefirov, NS (2011) Application of the bridgehead fragments for the design of conformationally restricted melatonin analogues. *Bioorg Chem* **39**(2)**:** 67-72.

Zhang, W, Chen, XY, Su, SW, Jia, QZ, Ding, T, Zhu, ZN, *et al.* (2016) Exogenous melatonin for sleep disorders in neurodegenerative diseases: a meta-analysis of randomized clinical trials. *Neurol Sci* **37**(1)**:** 57-65.

Zhang, Y, Cook, A, Kim, J, Baranov, SV, Jiang, J, Smith, K, *et al.* (2013) Melatonin inhibits the caspase-1/cytochrome c/caspase-3 cell death pathway, inhibits MT1 receptor loss and delays disease progression in a mouse model of amyotrophic lateral sclerosis. *Neurobiol Dis* **55:** 26-35.

Zhou, JN, Liu, RY, Kamphorst, W, Hofman, MA, Swaab, DF (2003) Early neuropathological Alzheimer's changes in aged individuals are accompanied by decreased cerebrospinal fluid melatonin levels. *J Pineal Res* **35**(2)**:** 125-130.

Zisapel, N, Egozi, Y, Laudon, M (1985) Circadian variations in the inhibition of dopamine release from adult and newborn rat hypothalamus by melatonin. *Neuroendocrinology* **40**(2)**:** 102-108.

This article is protected by copyright. All rights reserved.

Zisapel, N, Laudon, M (1982) Dopamine release induced by electrical field stimulation of rat hypothalamus in vitro: inhibition by melatonin. *Biochem Biophys Res Commun* **104**(4)**:** 1610-1616.

Zlotos, DP (2012) Recent progress in the development of agonists and antagonists for melatonin receptors. *Curr Med Chem* **19**(21)**:** 3532-3549.

Zlotos, DP, Attia, MI, Julius, J, Sethi, S, Witt-Enderby, PA (2009) 2-[(2,3-dihydro-1H-indol-1-yl)methyl]melatonin analogues: a novel class of MT2-selective melatonin receptor antagonists. *J Med Chem* **52**(3)**:** 826-833.

Zlotos, DP, Jockers, R, Cecon, E, Rivara, S, Witt-Enderby, PA (2014) MT1 and MT2 Melatonin Receptors: Ligands, Models, Oligomers, and Therapeutic Potential. *J Med Chem* **57**(8)**:** 3161-3185.

Zlotos, DP, Riad, NM, M.B., O, Dodda, BR, Witt-Enderby, PA (2015) Novel difluoroacetamide analogues of agomelatine and melatonin: probing the melatonin receptors for MT1 selectivity. *Med Chem Commun* **6:** 1340-1344.

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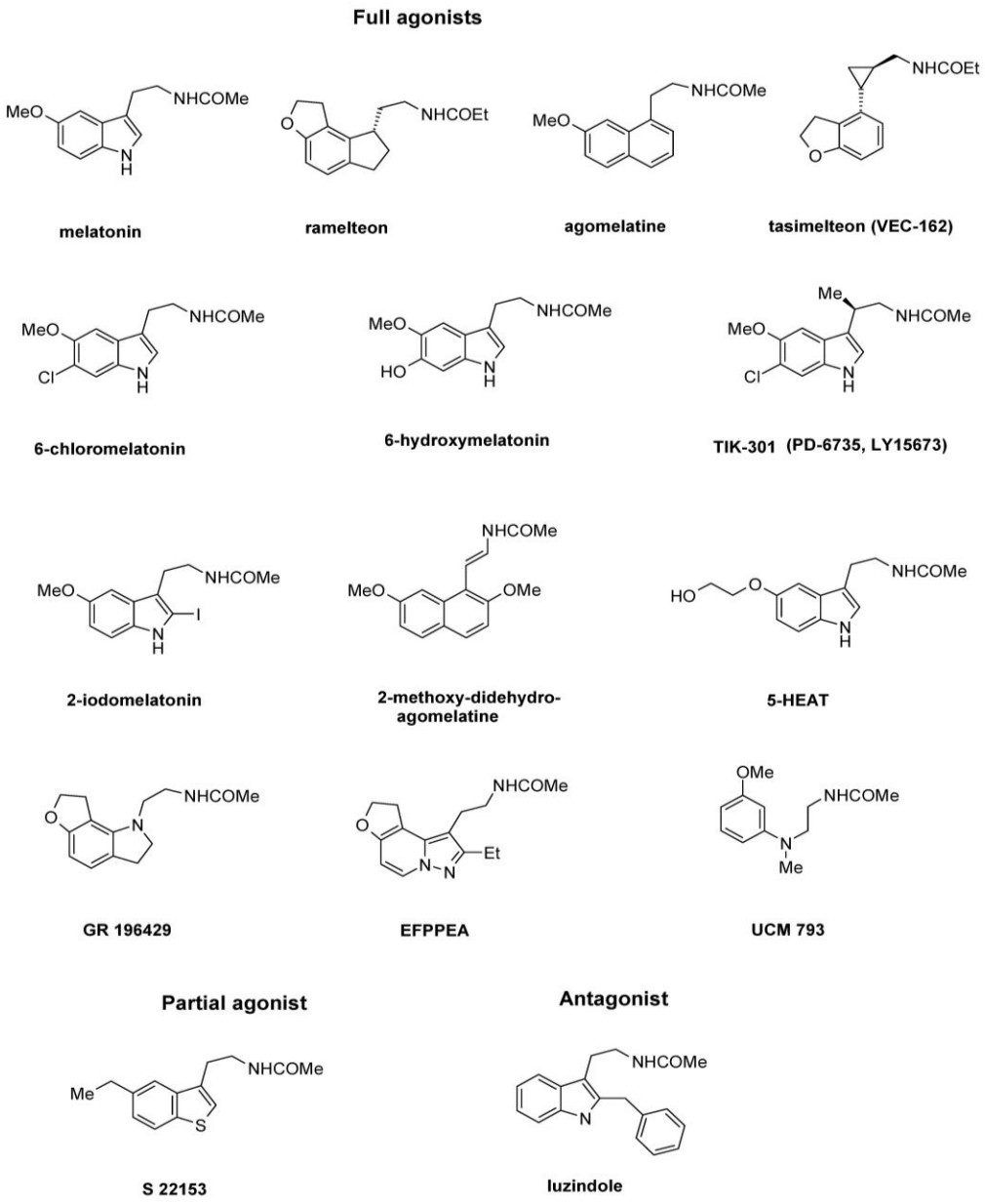


Figure 1. Structures of nonselective MT_1/MT_2 ligands. 5-HEAT, 5-hydroxyethoxy-N-acetyltryptamine; EFPPEA, ethyl-furo-pyrazolo-pyridine-ethyl-acetamide.

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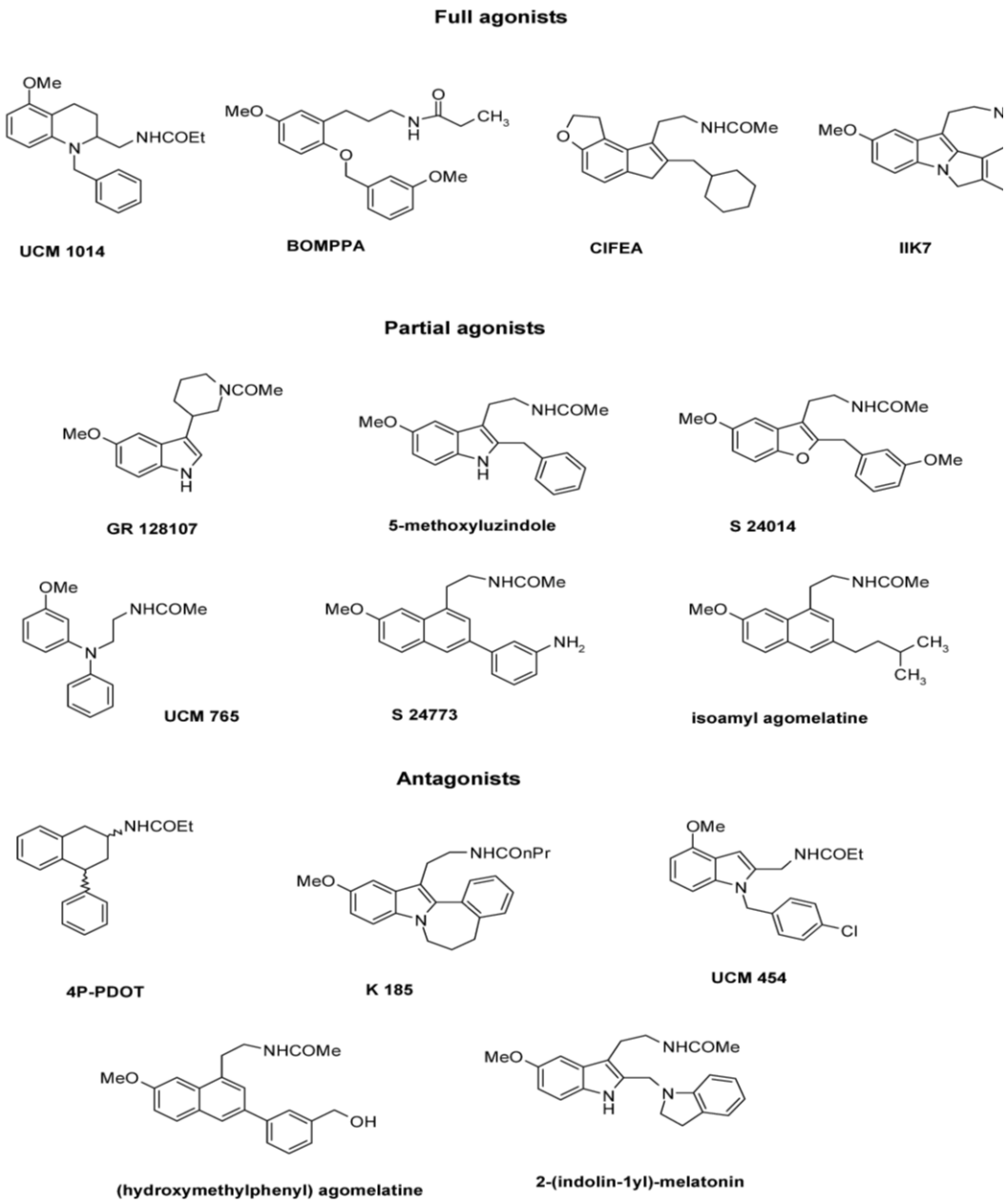


Figure 2. Structures of MT2-selective ligands. BOMPPA, benzyloxy-methoxyphenyl-propylamide; CIFEA, cyclohexylmethyl-indenofurane-ethylacetamide; 4P-PDOT, 4-phenyl-2-propionamidotetralin.

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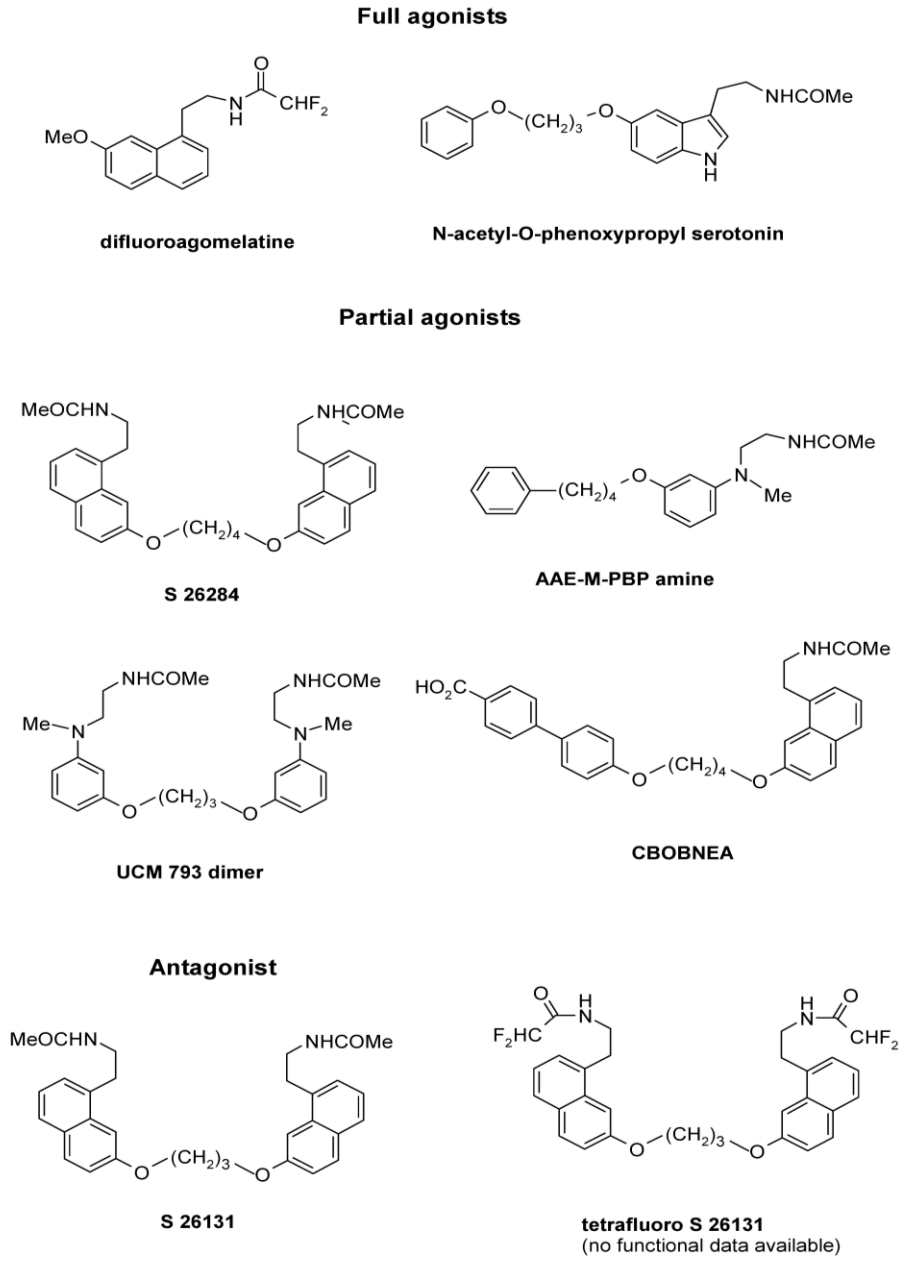


Figure 3. Structures of MT_1-selective ligands. CBOBNEA, carboxybiphenyloxy-butoxy-naphthalene-ethylacetamide; AAE M PBP amine, acetylaminoethyl-methyl-phenylbutoxyphenyl- amine.

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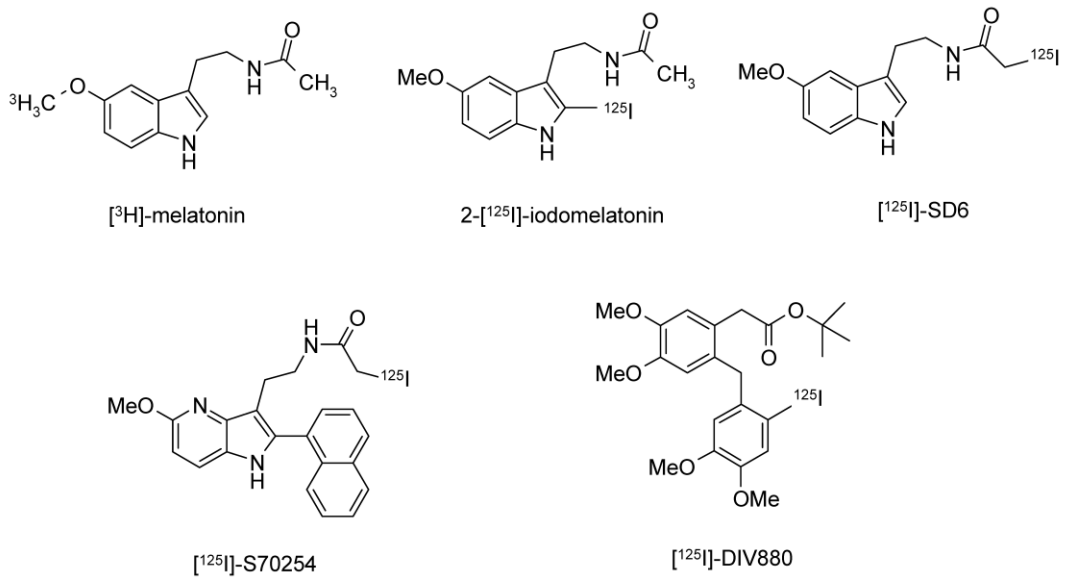


Figure 4. Structures of radioligands used to determine binding affinity for MT_1 and MT_2.

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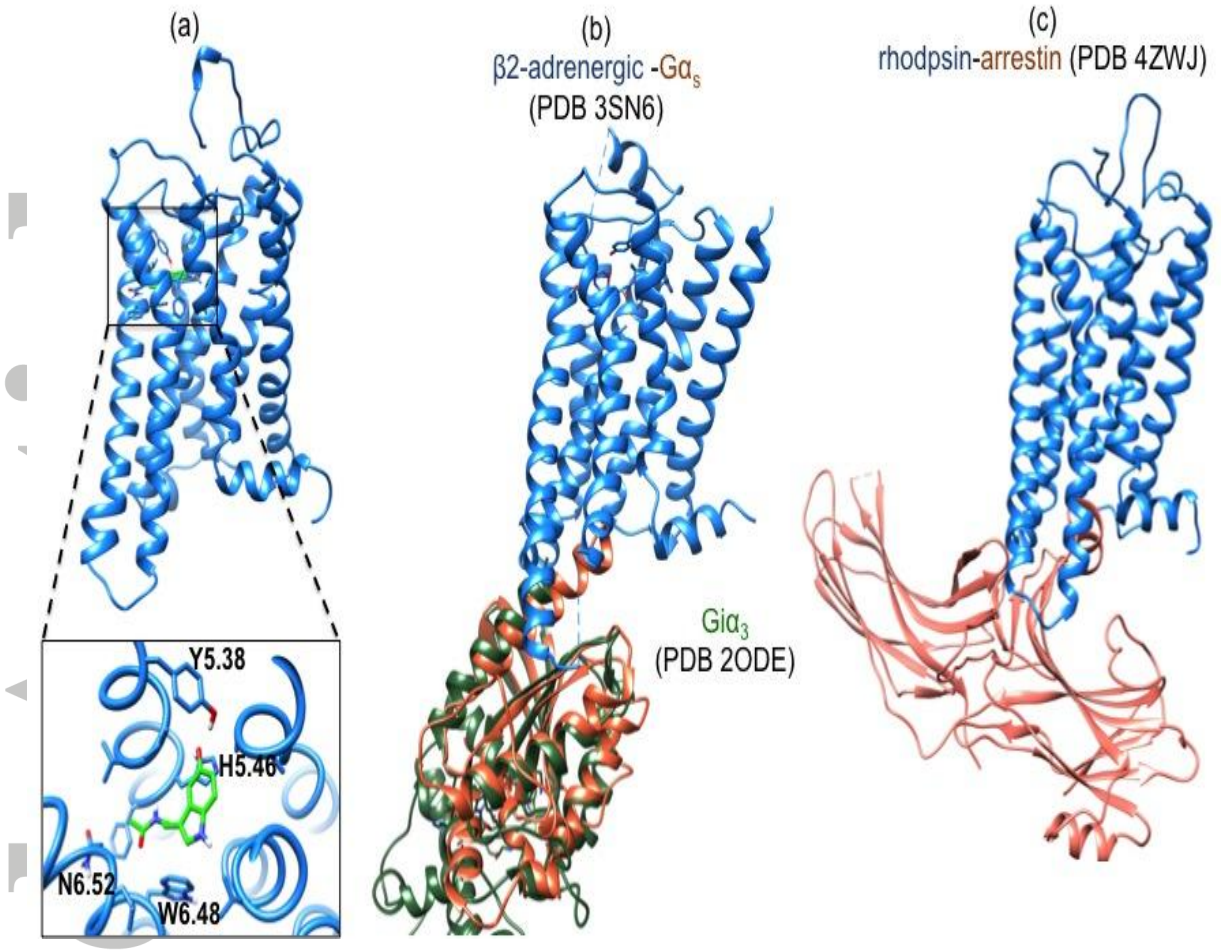
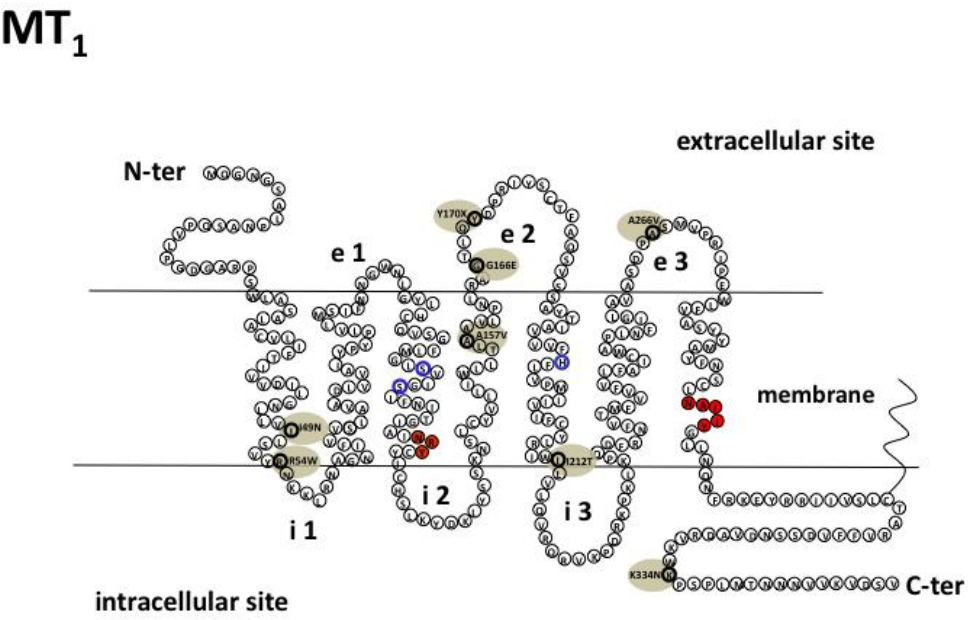


Figure 5. Structures useful for the study of MT_1 receptor structure-function relationships. MT_1*-MLT (a) was derived from active forms of rhodopsin, β2-adrenergic and A_{2A} adenosine receptors (unpublished data, N.R.). Docking of melatonin in the solvent-accessible cavity was achieved by energy relaxation by 300 ns molecular dynamics simulations. The structure of the MT_1*-MLT-$Gi\alpha_3$ complex could be modeled on the basis of the sequence homology with the β2-adrenoceptor-$G\alpha_s$ structure (Rasmussen *et al.*, 2011b), and the homology between $G\alpha_s$ and $Gi\alpha_3$ (Soundararajan *et al.*, 2008) (b) whereas the structure of the MT_1*-MLT-arrestin complex could be modeled based on the crystalized rhodopsin-arrestin complex (Kang *et al.*, 2015) (c).

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a)



b)

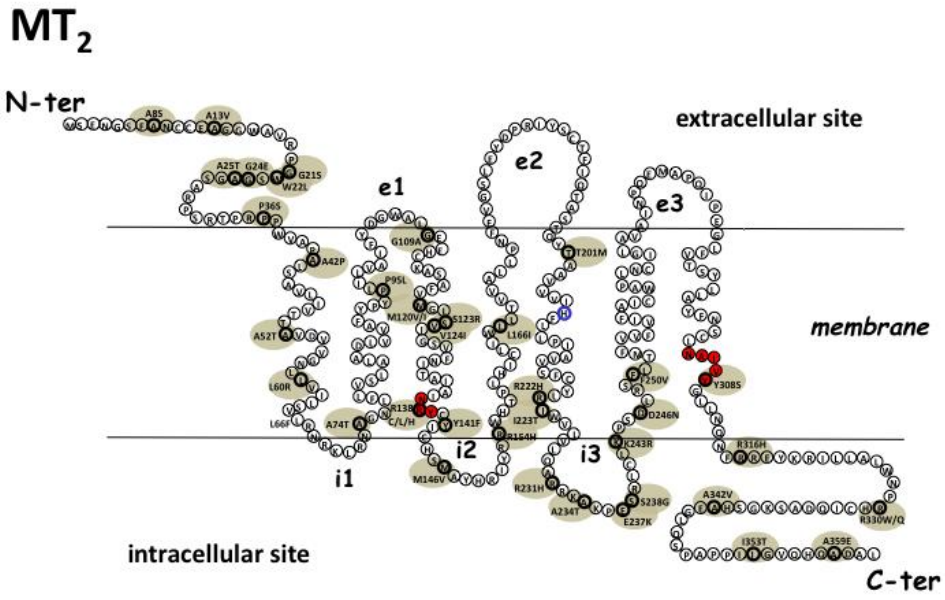


Figure 6: Distribution of non-synonymous MT_1 (a) and MT_2 (b) variants identified in various human populations. Position of variants are highlighted in light brown. Typical signatures of MTRs like the $^{3.49}$NRY$^{3.51}$ motif and the $^{7.49}$NAXXY$^{7.53}$ motif are highlighted in red. Residues suspected to be directly involved in MLT binding (S3.35 and S3.39 in MT_1 and H5.46 in both MT_1 and MT_2) are highlighted with a blue circle. The putative palmitoylation site at C314 is indicated in MT_1.

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Table I: Summary of reported MT receptor homology models.

Homology models	Crystal templates	Binding amino acids	Reference
MT_1-ramelteon (non-selective agonist)	Inactive rhodopsin (Palczewski et al., 2000)	Y175(E2), S182(E2), V5.43, ***H5.46***	Uchikawa et al., 2002[2]
MT_1-agomelatine (non-selective agonist)	Inactive rhodopsin (Okada et al., 2002)	L2.46, M3.32, ***S3.35***, ***S3.39***, ***H5.46***, F5.47, P5.50	Voronkov et al., 2005
MT_1-melatonin (non-selective agonist)	Inactive rhodopsin (Okada et al., 2002)	L2.46, M3.32, ***S3.35***, V3.36, ***S3.39***, ***H5.46***, F5.47, F6.44, W6.48, L6.51, N6.52, N7.49	Farce et al., 2008
MT_1-2phenylmelatonin (selective agonist)	Active β2-adrenergic (Rasmussen et al., 2011)	M3.32, G3.33, Y5.38, ***H5.46***, W6.48, L6.51, N6.52, Y7.39, A7.42, Y7.43	Rivara et al., 2012
MT_2-2iodomelatonin (non-selective agonist)	Inactive rhodopsin (Okada et al., 2002)	***V5.42***, ***H5.46***, N6.52, L6.56, ***Y7.43***	Mazna et al., 2004
MT_2-UCM454 (selective antagonist)	Inactive rhodopsin (Okada et al., 2004)	V3.36, I3.37, V3.40, Y183(E2), ***H5.46***, F5.47, P5.50, I5.51, F6.44, W6.48	Rivara et al., 2005
MT_2-melatonin	Inactive rhodopsin (Okada et al., 2004)	L2.46, A2.49, S3.35, I3.37, S3.39, ***V5.42***, V5.43, ***H5.46***, F5.47	Voronkov et al., 2005
MT_2-melatonin	Inactive rhodopsin (Okada et al., 2002)	S3.35, V3.36, S3.39, ***V5.42***, ***H5.46***, W6.48, ***Y7.43***	Chugunov et al., 2006
MT_2-melatonin	Inactive rhodopsin (Okada et al., 2002)	G3.33, V3.36, I3.37, ***N4.60***, L4.57, T191(E2), Y5.38, ***H5.46***	Farce et al., 2008
MT_2-melatonin	Active rhodopsin (Scheerer et al., 2008)	A3.29, V3.36, ***N4.60***, ***H5.46***, W6.48, L6.51	Zefirova et al. 2011
MT_2-acylaminoethyl tetralin (selective partial agonist)	Active β2-adrenergic (Rasmussen et al., 2011)	M3.32, V3.36, ***N4.60***, ***H5.46***, W6.48, N6.52, ***Y7.43***	Pala et al., 2013

According to Ballesteros numbering, amino acids critical for ligand binding based on site-directed mutagenesis data (Conway et al., 2001; Gerdin et al., 2003; Kokkola et al., 2003) are displayed in italic and bold.

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Table II: Biologically Important MT_1 Variants.
Common (minor allelic frequency (MAF) >1%), rare (MAF 0.1%–1%) variants.

Amino acid change	Type of variant	Description	References
I49N	Missense mutation	Rare variant identified in autism spectrum disorder patients, impaired cell surface expression, melatonin binding, cAMP inhibition and ERK1/1 activation	Chaste et al., 2010
R54W	Missense mutation	Common variant identified in control population without obvious functional defect	Ebisawa et al., 1999
A157V	Missense mutation	Common variant identified in control population without obvious functional defect	Chaste et al., 2010; Ebisawa et al., 1999
G166E	Missense mutation	Common variant identified in control population, impaired cell surface expression, reduced cAMP inhibition and ERK1/2 activation	Chaste et al., 2010
Y170X	Nonsense mutation	Rare variant identified in attention-deficit hyperactivity disorder (ADHD) patient, premature STOP codon with impaired cell surface expression and cAMP inhibition	Chaste et al., 2011
I212T	Missense mutation	Common variant identified in control population, impaired cell surface expression, cAMP inhibition and reduced ERK1/2 activation	Chaste et al., 2010
A266V	Missense mutation	Common variant identified in control population with reduced ERK1/2 activation	Chaste et al., 2010
K334N	Missense mutation	Rare variant identified in control population with reduced cAMP inhibition	Chaste et al., 2010

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Table III: Biologically Important MT_2 Variants. Common (minor allelic frequency (MAF) >1%), rare (MAF 0.1%–1%) and very rare (MAF< 0.1%) variants

Amino acid change	Type of variant	Description	References
A8S	Missense mutation	Very rare variant identified in control population without obvious functional defect	Bonnefond et al., 2012
A13V	Missense mutation	Very rare variant identified in control population without obvious functional defect	Bonnefond et al., 2012
G21S	Missense mutation	Very rare variant identified in control population without obvious functional defect	Bonnefond et al., 2012
W22L	Missense mutation	Very rare variant identified in type 2 diabetes patients, associated with type 2 diabetes risk, impaired Gi protein activation	Bonnefond et al., 2012
G24E	Missense mutation	Common variant, not associated with type 2 diabetes risk but associated with prevalence of obesity and increased BMI shown in one study but not in another	Andersson et al., 2010; Bonnefond et al., 2012; Chaste et al., 2010; Ebisawa et al., 2000
A25T	Missense mutation	Very rare variant identified in control population without obvious functional defect	Bonnefond et al., 2012
P36S	Missense mutation	Very rare variant identified in type 2 diabetes patients, without obvious functional defect	Bonnefond et al., 2012
A52T	Missense mutation	Very rare variant identified in type 2 diabetes patients, associated with type 2 diabetes risk, impaired G_i protein activation	Bonnefond et al., 2012
L66F	Missense mutation	Very rare variant identified in control population without obvious functional defect	Ebisawa et al., 2000
A74T	Missense mutation	Very rare variant identified in control population and type 2 diabetes patients, associated with type 2 diabetes risk, impaired G_i protein activation	Bonnefond et al., 2012

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Amino acid change	Type of variant	Description	References
G109A	Missense mutation	Very rare variant identified in control population without obvious functional defect	Bonnefond et al., 2012
M120V	Missense mutation	Very rare variant identified in control population without obvious functional defect	Bonnefond et al., 2012
M120I	Missense mutation	Very rare variant identified in population with impaired fasting glucose and control population without obvious functional defect	Bonnefond et al., 2012
S123R	Missense mutation	Very rare variant identified in population with impaired fasting glucose and control population without obvious functional defect	Bonnefond et al., 2012
V124I	Missense mutation	Very rare variant identified in several populations including type 2 diabetes and ADSD without obvious functional defect in one study and impaired ERK1/2 activation in another	Andersson et al., 2010; Bonnefond et al., 2012; Chaste et al., 2010
R138C	Missense mutation	Rare variant, not associated with type 2 diabetes risk, no Gi and ERK1/2 activation	Andersson et al., 2010; Bonnefond et al., 2012; Chaste et al., 2010
R138L	Missense mutation	Very rare variant identified in control population, associated with type 2 diabetes risk, impaired G_i protein activation	Bonnefond et al., 2012
R138H	Missense mutation	Very rare variant identified in control population, associated with type 2 diabetes risk, impaired Gi protein activation	Bonnefond et al., 2012
Y141F	Missense mutation	Very rare variant identified in type 2 diabetes patients, without obvious functional defect	Bonnefond et al., 2012

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Amino acid change	Type of variant	Description	References
M146V	Missense mutation	Very rare variant identified in control population without obvious functional defect	Bonnefond et al., 2012
R154H	Missense mutation	Very rare variant identified in control population and type 2 diabetes patients without obvious functional defect	Bonnefond et al., 2012
L166I	Missense mutation	Very rare variant identified in control population, associated with type 2 diabetes risk, impaired G_i protein activation	Bonnefond et al., 2012
T201M	Missense mutation	Very rare variant identified in type 2 diabetes patients, without obvious functional defect	Bonnefond et al., 2012
R222H	Missense mutation	Very rare variant identified in type 2 diabetes patients, associated with type 2 diabetes risk, impaired G_i protein activation	Bonnefond et al., 2012
I223T	Missense mutation	Very rare variant identified in type 2 diabetes patients, without obvious functional defect	Bonnefond et al., 2012
R231H	Missense mutation	Rare variant, not associated with type 2 diabetes risk	Andersson et al., 2010; Bonnefond et al., 2012; Chaste et al., 2010
A234T	Missense mutation	Very rare variant identified in control population without obvious functional defect	Bonnefond et al., 2012
E237K	Missense mutation	Very rare variant identified in control population without obvious functional defect	Bonnefond et al., 2012

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Amino acid change	Type of variant	Description	References
S238G	Missense mutation	Very rare variant identified in type 2 diabetes patients, without obvious functional defect	Bonnefond et al., 2012
K243R	Missense mutation	Common variant, not associated with type 2 diabetes risk	Bonnefond et al., 2012
D246N	Missense mutation	Very rare variant identified in type 2 diabetes patients, without obvious functional defect	Bonnefond et al., 2012
F250V	Missense mutation	Very rare variant identified in type 2 diabetes patients with impaired ERK1/2 activation	Bonnefond et al., 2012
R316H	Missense mutation	Very rare variant identified in control population without obvious functional defect	Bonnefond et al., 2012
R330W	Missense mutation	Very rare variant identified in type 2 diabetes patients, associated with type 2 diabetes risk, impaired G_i protein activation	Bonnefond et al., 2012
R330Q	Missense mutation	Very rare variant identified in control population without obvious functional defect	Chaste et al., 2010
A342V	Missense mutation	Very rare variant identified in type 2 diabetes patients, without obvious functional defect	Bonnefond et al., 2012
I353T	Missense mutation	Very rare variant identified in type 2 diabetes patients and control population, associated with type 2 diabetes risk, impaired G_i protein activation	Bonnefond et al., 2012
A359E	Missense mutation	Very rare variant identified in control population without obvious functional defect	Bonnefond et al., 2012

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Table IV: Mutations in the *MTNR1B* gene associated with susceptibility to type II diabetes.
Very rare (minor allelic frequency (MAF) <0.1%) variants.

Amino acid change	Type of variant	Description	References
A42P	Missense mutation	Very rare variant identified in type 2 diabetes patients, associated with type 2 diabetes risk, no melatonin binding and signaling	Bonnefond et al., 2012
L60R	Missense mutation	Very rare variant identified in control population and type 2 diabetes patients, associated with type 2 diabetes risk, no melatonin binding and signaling	Andersson et al., 2010; Bonnefond et al., 2012
P95L	Missense mutation	Very rare variant identified in type 2 diabetes patients, associated with type 2 diabetes risk, no melatonin binding and signaling	Bonnefond et al., 2012
Y308S	Missense mutation	Very rare variant identified in type 2 diabetes patients, associated with type 2 diabetes risk, no melatonin binding and signaling	Bonnefond et al., 2012

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