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INTERNATIONAL UNION OF BASIC AND CLINICAL PHARMACOLOGY REVIEW

Update on melatonin receptors: IUPHAR Review 20

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This article is an NC-IUPHAR review contributed by members of the International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification (NC-IUPHAR) subcommittee for the melatonin receptors.

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Melatonin receptors are seven transmembrane-spanning proteins belonging to the GPCR superfamily. In mammals, two melatonin receptor subtypes exist - 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 subcommittee 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 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 their functions in the immune system and the CNS. Finally, antioxidant and free radical scavenger properties of melatonin and its relation to melatonin receptors will be critically addressed.

Abbreviations

AD, Alzheimer's disease; ADHD, attention-deficit/hyperactivity disorder; ASDs, autism spectrum disorders; fMLP, *N*-formyl-l-methionyl-l-leucyl-l-phenylalanine; FPG, fasting plasma glucose; HD, Huntington's disease; IOP, intraocular pressure; MS, multiple sclerosis; MTR, melatonin receptor; NREM, non-rapid eye movement; PD, Parkinson's disease; QR2, quinone reductase 2; REM, rapid eye movement; SCN, suprachiasmatic nucleus; T2D, type 2 diabetes

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These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016) and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 ([a,b,c,d]Alexander *et al.*, 2015a,b,c,d).

Introduction

The hormone melatonin is mainly produced by the pineal gland following a circadian rhythm, with high levels during the subjective night. Melatonin can also be produced by extra-pineal sites like the retina, the gastrointestinal tract and the innate immune system. This hormone regulates a variety of physiological and neuroendocrine functions in mammals through activation of two GPCRs, the MT_1 and MT_2 receptors. Both receptors are typically coupled to $G_{i/o}$-type proteins and the MT_1 receptor is coupled, 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.

This article will review and discuss recent updates by the melatonin receptor subcommittee 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 MT receptor ligands, radioligands and structural perspectives of the MT receptors. The discovery of MT receptor dimers with physiological function *in vivo* as well as genetic variants and mutants of MT receptors will be discussed as they provide a new dimension to understand MT receptor pharmacology and function. An update on the latest results obtained with MT receptor knockout (KO) mouse models will be provided. Among the many physiological effects of MT receptors, we chose to focus on those of the immune system and the CNS. At the end of the review, MT receptor-independent effects, including antioxidant and free radical scavenger properties of melatonin, will be critically addressed.

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

MT receptor ligands

MT_1 and MT_2 receptors share a high degree of sequence homology and bind both the natural ligand, melatonin, with high affinity. Important progress has been made in the development of synthetic MT receptor antagonists and agonists and subtype-selective ligands by diversifying the chemical scaffolds. Indeed, MT receptor ligands from different structural classes show distinct structure–activity relationships on native and recombinant MT receptors (Dubocovich *et al.*, 1997; Dubocovich *et al.*, 2010; Zlotos, 2012; Zlotos *et al.*, 2014). The methoxy group and the acetamido side chain of melatonin determine the intrinsic activity and the binding affinity, respectively, at both hMT_1 and hMT_2 receptors (Dubocovich *et al.*, 1997; Browning *et al.*, 2000; Audinot *et al.*, 2003). Replacement of the amide methyl group by ethyl and propyl 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 two-position with a halogen or a phenyl group generate agonists with ~10-fold increased binding affinity. The majority of non-selective MT_1–MT_2 receptor ligands, including drugs used in humans, for example,

ramelteon [Rozerem®, (Kato *et al.,* 2005; Mini *et al.,* 2007; Rawashdeh *et al.,* 2011)], agomelatine [Valdoxan®, (de Bodinat *et al.,* 2010)] and tasimelteon [Hetlioz®, (Rajaratnam *et al.,* 2009; Lavedan *et al.,* 2015)], are agonists (Figure 1). Ramelteon and tasimelteon are MT receptor 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 receptors as agonists were recently reviewed (Liu *et al.,* 2016). Other non-selective MT receptor agonists include 6-chloromelatonin, 6-hydroxymelatonin, 2-iodomelatonin, GR 196429 (Dubocovich *et al.,* 1997; Browning *et al.,* 2000; Audinot *et al.,* 2003), 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 ~3500-fold greater potency than melatonin in the melanophore aggregation assay. TIK 301 (Mulchahey *et al.,* 2004) acts also as an antagonist at the 5-HT_{2C} and 5-HT_{2B} receptors (Landolt and Wehrle, 2009). 5-HEAT has a unique pharmacological profile acting as a full agonist at the hMT_1 receptor and antagonist at the hMT_2 receptor (Nonno *et al.,* 2000). EFPPEA, a high-affinity hMT_1 (K_i = 0.062 nM) and hMT_2 receptor (K_i = 0.420 nM) agonist, decreases the percentage of wakefulness and increases the percentage of slow wave

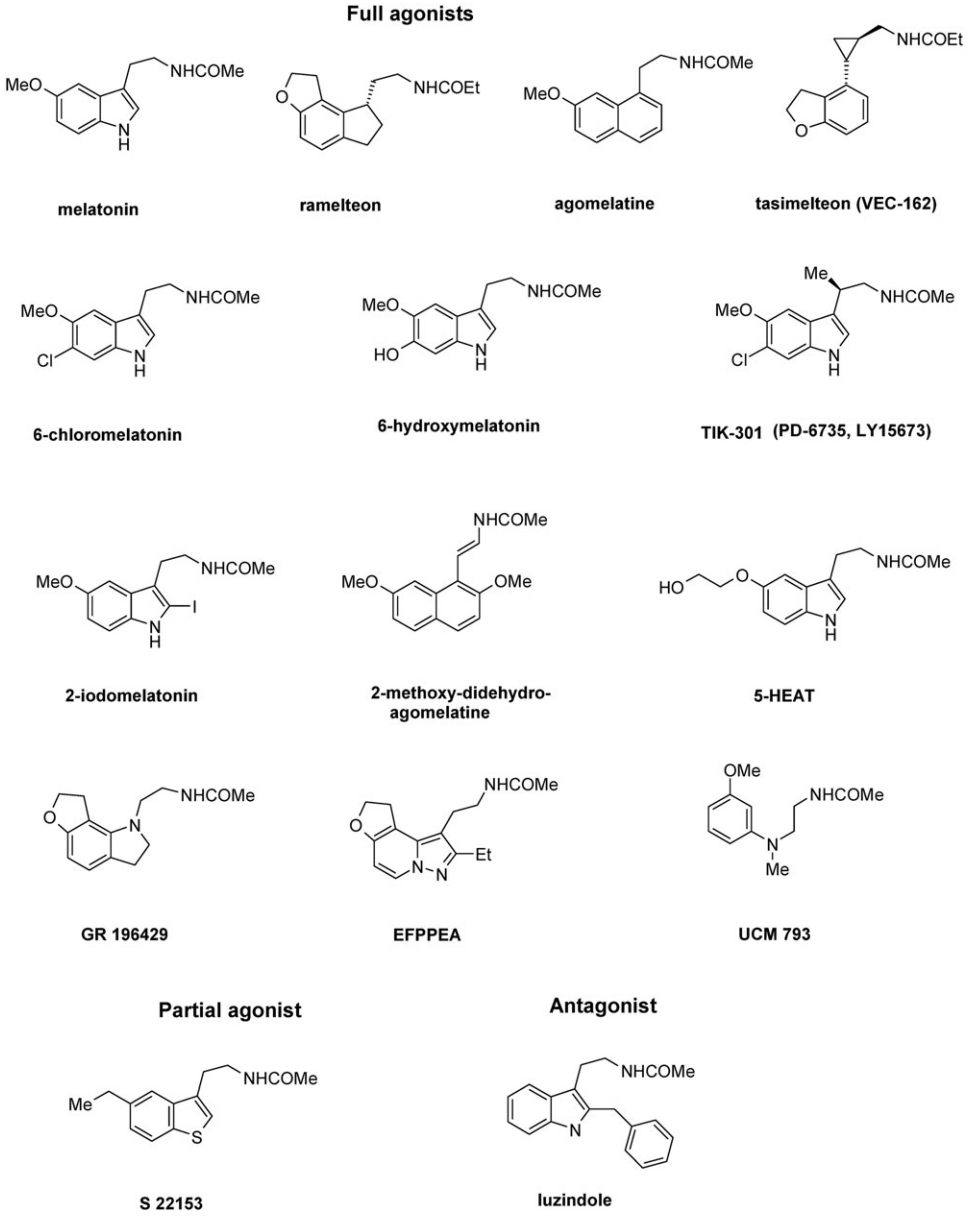

Figure 1

Structures of non-selective MT_1/MT_2 receptor ligands. 5-HEAT, 5-hydroxyethoxy-*N*-acetyltryptamine; EFPPEA, ethyl-furo-pyrazolo-pyridine-ethyl-acetamide.

sleep in cats (Koike *et al.,* 2011). The competitive MT receptor 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 receptors, is widely used for pharmacological characterization of functional MT receptors (Dubocovich *et al.,* 1997; Dubocovich *et al.,* 1998; Browning *et al.,* 2000; Dubocovich *et al.,* 2010).

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 both receptors (e.g. MT_1 and MT_2 receptors). This is particularly of concern for melatonin and synthetic MT receptor ligands activating receptors at picomolar concentrations (Dubocovich *et al.,* 1997; Browning *et al.,* 2000; Audinot *et al.,* 2003). Therefore, caution should be taken when interpreting selective MT receptor activation *in vivo* using MT receptor-selective ligands, unless pharmacological selectivity or lack of it, is confirmed by KO models with deletion of each receptor type.

Numerous ligands with high selectivity for hMT_2 over hMT_1 receptors have been identified (Zlotos *et al.,* 2014). MT_2 receptors possess a lipophilic pocket close to the N1–C2 binding region of melatonin, which is absent in MT_1 receptors (Rivara *et al.,* 2005). Accordingly, most MT_2 receptor-selective ligands bear a flexible bulky hydrophobic substituent in a position equivalent to C2 or N1 of melatonin (Figure 2). The tetrahydroquinoline analogue UCM1014 is the most potent MT_2 receptor-selective ligand reported to date. It shows picomolar binding affinity (K_i = 0.001 nM) at hMT_2 receptors, >10 000-fold selectivity over hMT_1 receptors and full agonist profile in the GTPγS test (Spadoni *et al.,* 2015). Other agonists with approximately 800-fold hMT_2 receptor selectivity are BOMPPA (Hu *et al.,* 2010; Heckman *et al.,* 2011; Chan *et al.,* 2013; Hu *et al.,* 2013) and CIFEA (Koike *et al.,* 2011). In imprinting control region mice, CIFEA reentrainment effects to a new light/dark cycle indicate the involvement of MT receptors in the regulation of chronobiotic activity (Koike *et al.,* 2011). The dose of CIFEAA used in this study most likely reached micromolar concentrations, which would activate both MT_1 and MT_2 receptors, precluding any conclusion about the specific receptor type involved in the regulation of chronobiotic processes. Similarly, doses of the MT_2 receptor-selective antagonist 4P-PDOT (90 μg/mouse s.c.) used to block the melatonin-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 receptors.

Two moderately selective MT_2 receptor 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 MT receptor type in the modulation of sleep architecture. UCM 765 promoted non-rapid eye movement (NREM) sleep in rodents, and this effect was blocked by the MT_2 receptor antagonist 4P-PDOT (Ochoa-Sanchez *et al.,* 2011). In contrast, the non-selective MT_1–MT_2 receptor agonist UCM793 decreased sleep onset without having an effect on NREM sleep maintenance suggesting that dual MT_1 and MT_2 receptor agonistic activity accounts for the effect on sleep onset, whereas selectivity for MT_2 receptors 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 rapid eye movement (REM) sleep latency or the amount of REM sleep (Fisher and Sugden, 2009).

Among the hMT_2 receptor-selective partial agonists GR 128107, 5-methoxyluzindole, S 24014, S 24773 (Dubocovich *et al.,* 1997; Audinot *et al.,* 2003) and isoamyl agomelatine, the latter shows the highest affinity (K_i = 0.01 nM) and selectivity (7200-fold) (Ettaoussi *et al.,* 2012). 4P-PDOT, an hMT_2 receptor-selective antagonist with 300- to 1500-fold higher affinity for hMT_2 receptors, is still considered the gold standard for pharmacological characterization of MT receptors (Dubocovich *et al.,* 1997). Other MT_2 receptor-selective antagonists, such as K185 (Sugden *et al.,* 1999; Faust *et al.,* 2000), UCM 454 (Rivara *et al.,* 2005) and 2-(indolin-1yl) melatonin (Zlotos *et al.,* 2009), display ~100-fold higher affinity for hMT_2 receptors. For (hydroxymethyl)phenyl agomelatine, the affinity for hMT_2 receptors is 750-times higher than for hMT_1 receptors (Poissonnier-Durieux *et al.,* 2008).

Discovery of MT_1 receptor-selective ligands remains a challenge, and only few compounds with preference for hMT_1 receptors have been reported (Zlotos *et al.,* 2014). Ligands preferentially binding to these receptors 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 receptor selectivity is a bulky, hydrophobic ether replacing the methoxy group. The first hMT_1 receptor-selective agents were obtained by connecting two agomelatine units via their ether oxygen 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 receptor selectivity and partial agonist activity (Spadoni *et al.,* 2011). Monomeric ligands, such as CBOBNEA (Mesangeau *et al.,* 2010), and AAE M PBP amine (Rivara *et al.,* 2012) are partial agonists showing similar ~100-fold selectivity for hMT_1 receptors. *N*-acetyl-*O*-phenoxypropyl serotonin is a full agonist obtained by exchange of the methoxy group of melatonin with an O $(CH_2)_3$OPh moiety. Although it shows only 10-fold binding preference toward hMT_1 receptors, its MT_1–MT_2 receptor binding ratio and hMT_1 receptor affinity were higher than that for the MT_1 receptor-selective reference compound S 26131 that was retested under the same experimental conditions (Markl *et al.,* 2011). A 140-fold MT_1 receptor selectivity could be attained by introduction of two fluorine atoms into the *N*-acetyl group of agomelatine. The resulting difluoroagomelatine shows high hMT_1 receptor binding (K_i = 0.03 nM) and is a non-selective MT_1–MT_2 receptor 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 receptors than the parent ligand (Zlotos *et al.,* 2015).

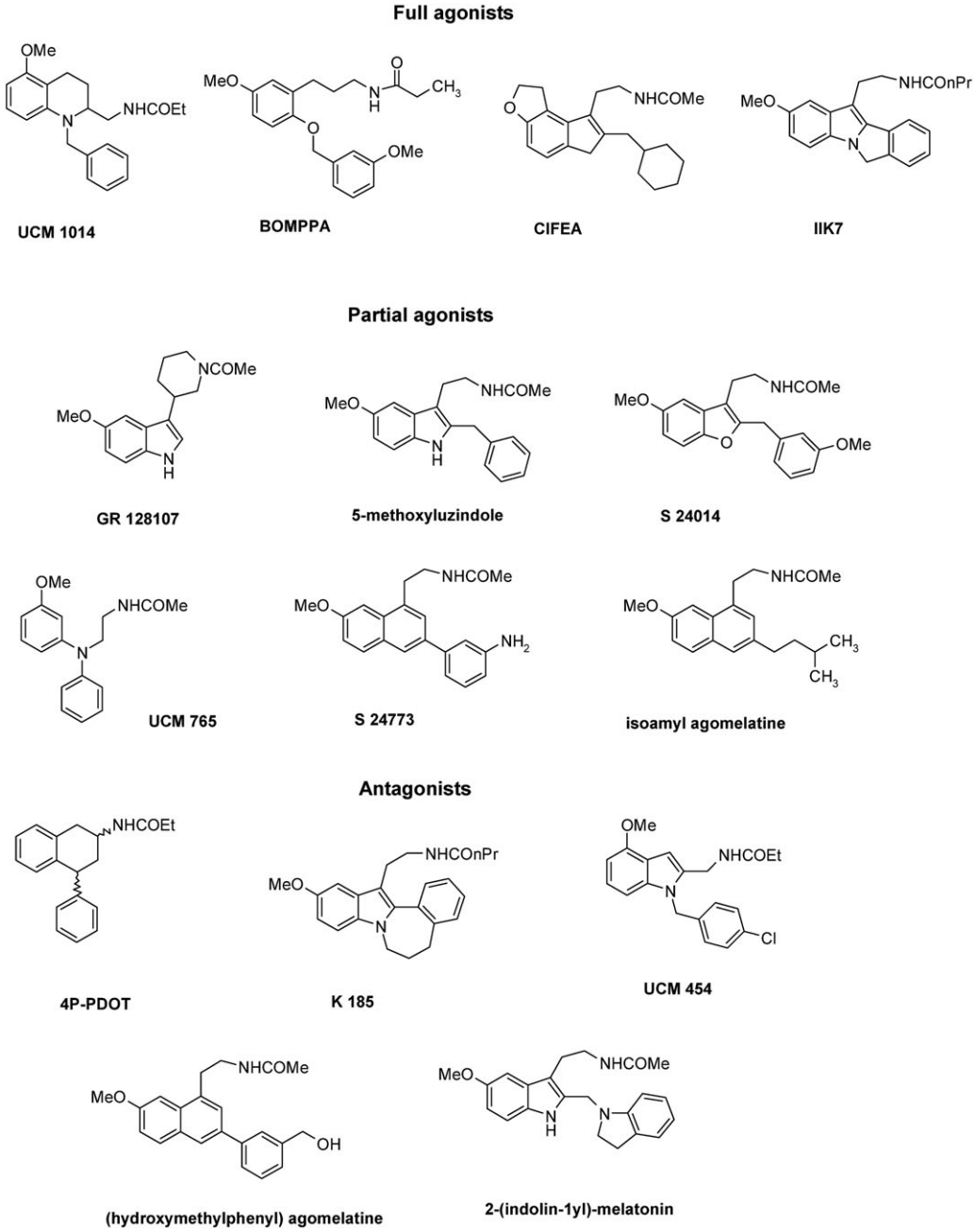

Figure 2

Structures of MT_2 receptor-selective ligands. BOMPPA, benzyloxy-methoxyphenyl-propylamide; CIFEA, cyclohexylmethyl-indenofurane-ethylacetamide; 4P-PDOT, 4-phenyl-2-propionamidotetralin.

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

Radioligands – update

Radioactive- and fluorescent-labelled ligands are indispensable tools for the pharmacological characterization of GPCRs. A major breakthrough in the field of MT receptor 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 MT receptors in tissues (Dubocovich and Takahashi, 1987). The radioligand, 2-[^{125}I]iodomelatonin (2-[^{125}I]-IMLT), has

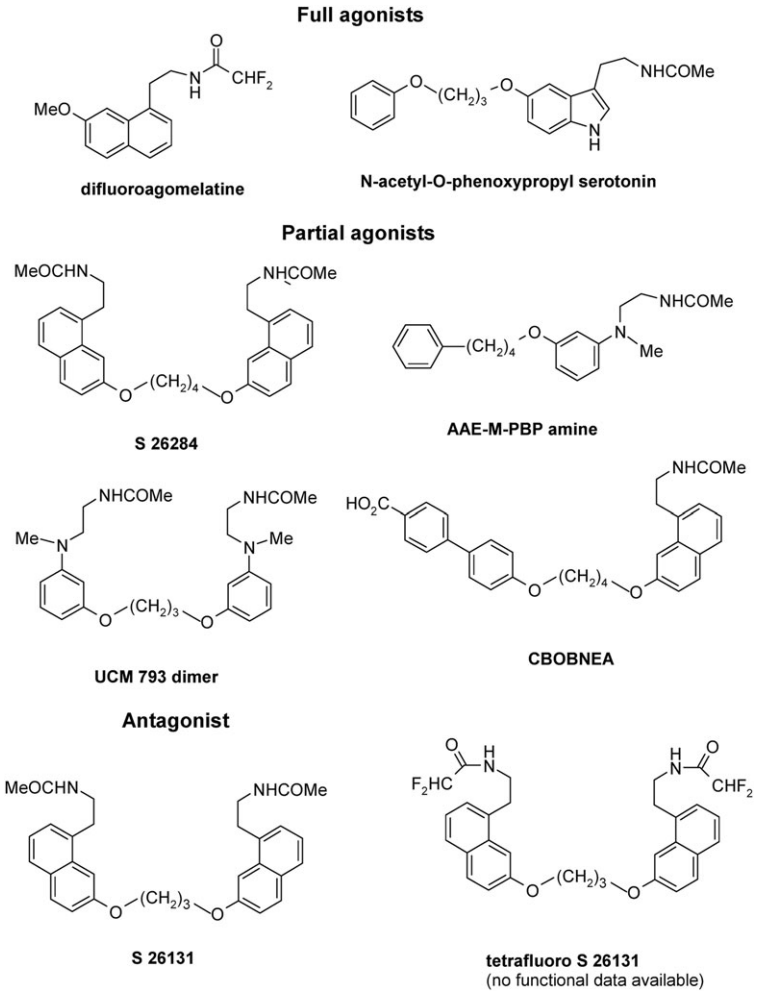

Figure 3

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

been extensively used as a high-affinity radioligand for both MT_1 and MT_2 receptors, and was until recently the only available radioligand for the characterization and localization of melatonin 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 receptors, as being identical to that established using 2-[^{125}I]-IMLT as a radioligand. However, due to the rather low specific activity of this [^{3}H]-MLT, its use to characterize and/or localize melatonin sites in tissues with low MT receptor density is limited (Browning *et al.*, 2000).

Three new iodinated radioligands have been recently characterized for use in the pharmacological characterization and localization of MT receptors (Figure 4). These radioligands are as follows: 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.*, 2013; Legros *et al.*, 2016). [^{125}I]-SD6 has a similar pharmacological profile to that of 2-[^{125}I]-IMLT with the same affinity for MT_1 and MT_2 receptors. On the contrary, the two other radioligands [^{125}I]-S70254 and [^{125}I]-DIV880 show selectivity for MT_2 receptors with pK_d values of 9.6 and 9.7, respectively, in the absence of any specific binding to MT_1 receptors. All radioligands are agonists, either partial agonists ([^{125}I]-S70254, [^{125}I]-DIV880) or full agonists (2-[^{125}I]-IMLT, [^{125}I]-SD6, [^{3}H]-MLT), which means that their K_d values depend not only 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]-IMLT and [^{3}H]-MLT on membrane preparations from CHO-K1 cell lines stably expressing hMT_1 or hMT_2 receptors showed that [^{125}I]-S70254 and [^{125}I]-DIV880 mainly differ from 2-[^{125}I]-IMLT in their dissociation kinetics, which are faster for [^{125}I]-S70254 and [^{125}I]-DIV880 than for 2-[^{125}I]-IMLT (Legros *et al.*, 2016). Interestingly, [^{125}I]-SD6 labelled only approximately half of the binding sites detected with 2-[^{125}I]-IMLT in cells expressing hMT_1 receptors while comparable amounts were detected in cells expressing hMT_2

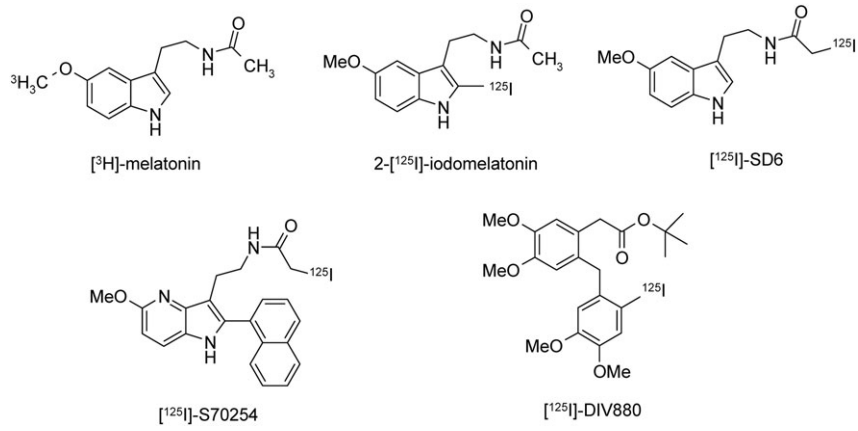

Figure 4

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

receptors (Legros *et al.,* 2013). This suggests the existence of different receptor subpopulations for hMT_1 receptors of which [^{125}I]-SD6 labels a more restricted number than 2-[^{125}I]-IMLT. In contrast, for hMT_2 receptors, similar subpopulations would be detectable by both radioligands. The nature of these receptor subpopulations is currently unknown but could be related to the differential engagement of hMT_1 receptors into complexes with different G proteins or ß-arrestins following the binding of these agonistic radioligands. [^{125}I]-SD6 detected as 2-[^{125}I]-IMLT binding sites in sheep retinal membranes, while the MT_2 receptor-specific ligands [^{125}I]-S70254 and [^{125}I]-DIV880 failed to do so.

The MT_2 receptor-specific [^{125}I]-S70254 was successfully used for autoradiography studies in rat and sheep brain and retina slices (Legros *et al.,* 2016). A similar labelling pattern to 2-[^{125}I]-IMLT (detecting MT_1 and MT_2 receptors) was observed in several areas but also distinct labelling in others. Absence of labelling by [^{125}I]-S70254 in regions that are labelled by 2-[^{125}I]-IMLT can be explained by low (undetectable) MT_2 receptor levels. Absence of 2-[^{125}I]-IMLT labelling in regions labelled 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 MT receptors with the development of MT_2 receptor-specific radioligands and radioligands detecting distinct receptor populations revealing a previously unrecognized diversity. The availability of a radiolabelled antagonist would largely contribute in a better characterization of these different populations. Further advances can be expected from the development of fluorescent-labelled ligands.

Structural perspectives for MT receptors

Currently, crystal structures of MT_1 and MT_2 receptors are not available. Despite a sequence identity lower than 30% between MT receptors and the closest crystallized GPCRs, several three-dimensional (3D) models have produced some structural hypotheses for binding of (non)selective MT_1 and/or MT_2 receptor agonists (Table 1). 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 receptors. Although His5.46 seems to be an anchoring residue for polar interactions with the methoxy or amide group of melatonin, only a few models of MT_1 receptors display a direct participation of serine residues in melatonin 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 MT receptors, E2 and I3 loops are known to be key features for ligand accessibility and G protein binding of GPCRs. Moreover, amino acid sequences of MT receptors 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 affect receptor activation and/or signalling specificity of MT receptors rather than the ligand binding process.

Whereas 3D models of MT receptors were up to now dedicated to the discovery and optimization of new efficient drugs, the next generation of 3D models should be expanded toward 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 and Filizola, 2014). Depicting free energy landscapes of MT receptors 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 (Provasi *et al.,* 2011; Dror *et al.,* 2013), *in silico* optimization of new efficient ligand structures could be explored by predicting its functional selectivity through arrestin or G_i-mediated pathways. These approaches also open the way for the exploration of homodimers and heterodimers, particularly MT_1/MT_2 and MT_1/GPR50 receptor complexes, as discussed in the following section.

Table 1

Summary of reported MT receptor homology models

Homology model	Crystal template	Binding amino acid	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
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.,* 2011a,b)	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 β_2-adrenoceptor (Rasmussen *et al.,* 2011a,b)	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.

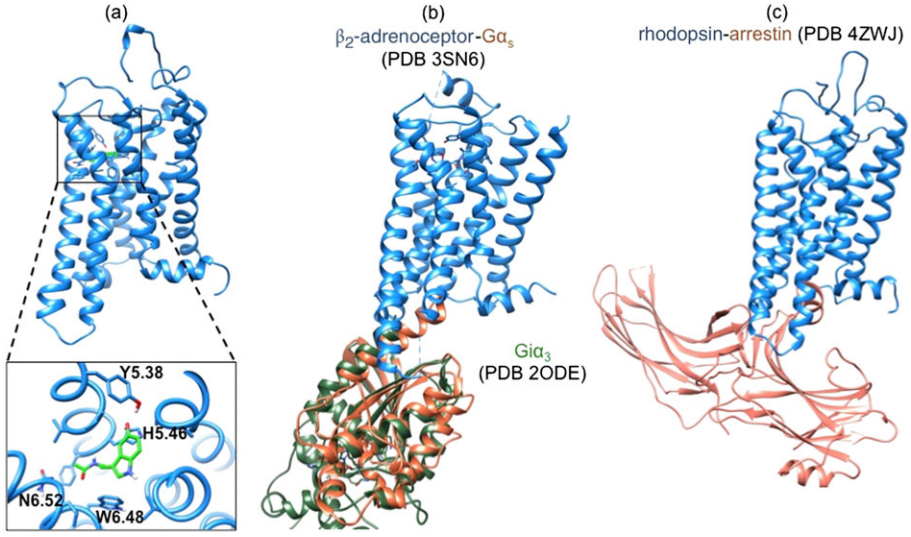

Figure 5

Structures useful for the study of MT_1 receptor structure–function relationships. MT_1*-MLT (A) was derived from active forms of rhodopsin, β_2-adrenoceptor and A_{2A} adenosine receptors (unpublished data, N.R.). Docking of melatonin (MLT) 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 modelled 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 modelled based on the crystallized rhodopsin-arrestin complex (Kang *et al.,* 2015) (C).

MT receptor dimers

MT receptors are part of dynamic signalling complexes that contain proteins involved in receptor biosynthesis, export, signalling, 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 Maurice *et al.,* 2011 and Ferre *et al.,* 2014). Initial observations have been made in 2002 in transfected HEK293 cells demonstrating the capacity of MT_1 and MT_2 receptors to form homodimers 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 receptors were reported to form heterodimers with the orphan GPR50, which completely abolished the function of MT_1 receptors in MT_1/GPR50 heterodimers (Levoye *et al.,* 2006a). Sporadic reports on Western blots of endogenously expressed MT receptors in chicken astrocyte cultures (Adachi *et al.,* 2002) and *Xenopus* tectal cells (Prada *et al.,* 2005) further indicated the possible existence of MT receptor 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, melatonin enhances the light sensitivity during the night. The phenotype of MT_1 receptor KO ($MT_1^{-/-}$) and MT_2 receptor KOs ($MT_2^{-/-}$) mice, the use of type-selective ligands and overexpression of a dominant negative form of MT_2 receptors in photoreceptor cells of transgenic mice indicated the exclusive involvement of MT_1/MT_2 heterodimers in this physiological effect of melatonin (Baba *et al.,* 2013). Interestingly, this effect was dependent on the activation of the G_q/PLC pathway by melatonin, an observation that could be confirmed *in vitro* in cells co-expressing MT_1 and MT_2 receptors.

Whether MT receptor 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 receptors and an antagonist with moderate affinity for 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 MT receptors and their binding properties have been determined (Audinot *et al.,* 2003; Descamps-Francois *et al.,* 2003; Mesangeau *et al.,* 2010; Spadoni *et al.,* 2011; Journe *et al.,* 2014). 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 BRET approach (Journe *et al.,* 2014). Compounds linked through 22–24 atom spacers were able to bind to MT_1 and MT_2 receptor protomers in pre-existing homodimers 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 MT receptor dimers are increasingly recognized, but its functional role and pharmacological exploitation are still ongoing.

Genetic variants and mutants of MT receptors

The existence of many rare variants in the human population was discovered in recent genome sequencing programmes. The 1000 human genome project detected 38 million variants (Abecasis *et al.,* 2012) and 172 variants, including 46 non-synonymous variants, has been identified on average per GPCR in a population of 14 002 individuals (Nelson *et al.,* 2012; Karamitri and Jockers, 2014). 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) that were threefold and twofold more frequent in people with non-24 h sleep–wake syndrome (Table 2) (Ebisawa *et al.,* 1999). Due to the small sample size (N = 22), statistical significance was not reached.

Alteration of melatonin synthesis has been reported in autism spectrum disorders (ASDs) 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 10 for *MTNR1B* (Tables 2 and 3). The majority of these mutants showed altered receptor function. Particularly deleterious mutants were MT_1-I49N, which is devoid of any melatonin binding and cell surface expression, and MT_1-G166E and MT_1-I212T, which showed severely impaired cell surface expression and biased behaviour toward the ERK1/2 pathway. No significant difference in the prevalence of these mutations was found indicating that they do not represent major risk factors 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) (Tables 2 and 3); however, none of them was enriched in ADHD individuals as compared with the general population (Chaste *et al.,* 2011). The MT_1-Y170X nonsense mutation was only detected in one ADHD patient and introduced a premature STOP codon resulting in complete loss of receptor function.

MT receptor variants have been most extensively sought in studies focused on type 2 diabetes (T2D) based on the discovery of several frequent polymorphisms associated with

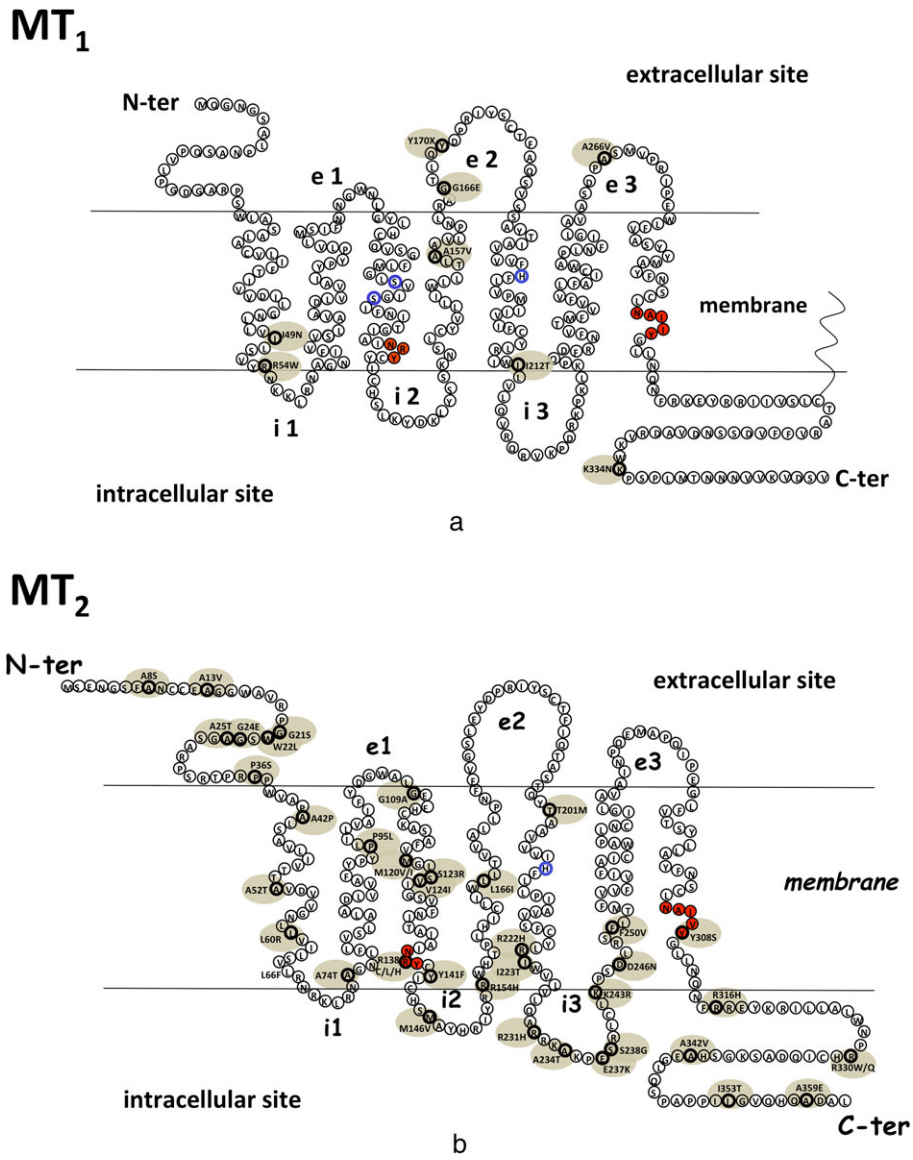

Figure 6

Distribution of non-synonymous MT_1 (A) and MT_2 (B) receptor variants identified in various human populations. Positions of variants are highlighted in light brown. Typical signatures of MT receptors such as 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 melatonin binding (S3.35 and S3.39 in MT_1 and H5.46 in both MT_1 and MT_2 receptors) are highlighted with a blue circle. The putative palmitoylation site at C314 is indicated in MT_1 receptors.

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 and 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* (Tables 2–4) (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. There was complete loss of melatonin binding in four very rare cases (A42P, L60R, P95L and Y308S) and partially and severely blunted signalling (Gαqi9 chimera and ERK1/2 activation) in one rare case (R138C) and nine very rare cases (W22L, A52T, A74T, R138H, R138L, L166I, R222H, R330W and I353T). Carriers of the 13 very rare loss-of-function variants showed increased T2D risk establishing a functional link between *MTNR1B* and T2D (see Karamitri *et al.,* 2013).

In conclusion, the genetic variability of the *MTNR1B* gene in terms of non-synonymous variants has now been well

Table 2

Biologically important MT_1 receptor variants

Amino acid change	Type of variant	Description	Reference
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

Common [minor allelic frequency (MAF) >1%], rare (MAF 0.1–1%) variants.

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.

MT receptor mouse models – update

$MT_1^{-/-}$ mice were created in the late 1990s followed by the generation of $MT_2^{-/-}$ mice in 2003 (Liu *et al.*, 1997; Jin *et al.*, 2003). Studies using these mice have provided important insights on the role that MT receptors play in the modulation of many different biological functions. In $MT_1^{-/-}$ mice, but not in $MT_2^{-/-}$ mice, the inhibitory effect of melatonin on neuronal activity in the suprachiasmatic nucleus (SCN) is impaired suggesting the involvement of MT_1 receptors. In contrast, in SCN slices from $MT_1^{-/-}$ mice melatonin (1–10 pM) phase shifts the peak of circadian rhythms of neuronal firing by approximately 3 h suggesting the involvement of MT_2 receptors (Liu *et al.*, 1997; Dubocovich *et al.*, 2005). Blockade of this effect using the MT_2 receptor-selective 4P-PDOT antagonist confirmed the latter conclusion shaping a pathway where MT_2 phase shifts the peak of neuronal firing through PLC-PKC signalling pathway (Mc Arthur *et al.*, 1997; Hunt *et al.*, 2001; Dubocovich *et al.*, 2005). 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 wild type (WT) mice suggesting a role for the MT_1 receptors in this response (see detailed discussion in Dubocovich, 2007). Together, these findings suggest a potential role for both MT_1 and MT_2 receptors in the phase shift of circadian rhythms of neuronal firing in the SCN slice *in vitro*. The use of $MT_1^{-/-}$ mice demonstrated that the MT_1 receptor is required for the melatonin-meditated phase shift of the onset of overt circadian rhythm of locomotor activity (Dubocovich *et al.*, 2005). An independent study demonstrated that C3H/HeN mice (melatonin-proficient) entrained faster to a phase advance of dark onset than the C57BL/6J mice (melatonin-deficient), suggesting a facilitating role of endogenous melatonin 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 mouse 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 receptors. This apparent contradiction could be explained by the activation of MT_2 and MT_1 receptors by endogenous and exogenous melatonin, respectively, at different periods of sensitivity (subjective night vs. subjective day, respectively). Changes in efficacy could also result from desensitization and/or internalization of MT receptors in response to exposure to physiological and supraphysiological melatonin concentrations as demonstrated by the phase shift of the peak of neuronal firing in the SCN by physiological levels of melatonin, which involved the desensitization of MT_2 receptors (Gerdin *et al.*, 2004).

MT receptor 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 receptor signalling is implicated in the modulation of the daily rhythm of REM sleep (Ochoa-Sanchez *et al.*, 2011). An additional study in which

Table 3

Biologically important MT_2 receptor variants

Amino acid change	Type of variant	Description	Reference
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.*, 1999
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.*, 1999
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
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
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

(Continues)

Table 3 (Continued)

Amino acid change	Type of variant	Description	Reference
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
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

Common [minor allelic frequency (MAF) >1%], rare (MAF 0.1–1%) and very rare (MAF < 0.1%) variants.

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

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

As mentioned before, recent studies have also implicated MT receptors in the pathogenesis of T2D in humans (Bouatia-Naji *et al.*, 2009; Lyssenko *et al.*, 2009; Bonnefond *et al.*, 2012). Thus, a few studies used MT receptor KO mice to determine the mechanisms by which these receptors contribute to regulation of glucose homeostasis and insulin sensitivity (Stumpf *et al.*, 2008; Muhlbauer *et al.*, 2009; Contreras-Alcantara *et al.*, 2010). Mice lacking MT_1 receptors 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

Table 4

Mutations in the *MTNR1B* gene associated with susceptibility to type 2 diabetes

Amino acid change	Type of variant	Description	Reference
A42P	Missense mutation	Very rare variant identified in type 2 diabetes patients, associated with type 2 diabetes risk, no melatonin binding and signalling	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 signalling	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 signalling	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 signalling	Bonnefond *et al.,* 2012

Very rare (minor allelic frequency <0.1%) variants.

(Contreras-Alcantara *et al.,* 2010). Furthermore, removal of MT_1 or MT_2 receptors abolishes the daily rhythm in blood glucose levels (Owino *et al.,* 2016).

Finally, it is important to mention that although the reproductive system of mice is not sensitive to photoperiod, the development of MT receptor KO mice provided an important tool for dissecting the mechanisms by which melatonin regulates reproduction in photoperiodic species. For example, MT_1 receptor signalling 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 receptor signalling as well (Jilg *et al.,* 2005). MT_1 receptor signalling 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 and Korf, 2006; Yasuo *et al.,* 2009). Finally, we should mention that a recent study reported that MT_1 receptor signalling plays a key role in photoperiodic programming of serotonergic neurons as well as depression- and anxiety-related behaviours in mice (Green *et al.,* 2015).

In conclusion, studies in the last 20 years using MT receptor 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 melatonin signalling affects these functions.

Functional role of MT receptors in physiology and pathophysiology

MT receptors 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; Tosini *et al.,* 2012; Karamitri *et al.,* 2013; Tosini *et al.,* 2014; Johnston and Skene, 2015). Here, we have focused our attention on two major processes, the immune system and the CNS. 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 melatonin, such as its binding to additional binding sites and the intrinsic antioxidant and free radical scavenger properties of this hormone.

MT receptors in the immune system

The role of melatonin as a player in immunity, first proposed by Berman in 1926, is now well accepted (Carrillo-Vico *et al.,* 2013). Several reports have demonstrated that melatonin produced by the either pineal gland or immune cells can regulate the activation of an immune response. Melatonin derived from activated human lymphocytes induces the synthesis of 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 melatonin. Interestingly, daily rhythms of plasma melatonin 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 melatonin rhythm (Pontes *et al.,* 2007). In addition, the daily and seasonal variation of melatonin production contributes to the seasonality of some diseases. In multiple sclerosis (MS), melatonin blocks the differentiation of Th17 cells and boosts the generation of protective type 1 regulatory T-cells by an MT_1 receptor-dependent mechanism, resulting in the seasonal variation of MS symptoms (Farez *et al.,* 2015). Seasonality of regular immunity is also related to changes in the melatonin system (Weil *et al.,* 2015). In the spleen of several species, extended light exposure decreases MT_1 receptor expression (Maestroni, 1993; Lahiri and Haldar, 2009; Yadav and Haldar, 2013). In healthy conditions, rolling and adhesion of neutrophils to the endothelial cell layer are inhibited by activation of MT_2 receptors and ligands binding to the putative *MT3* binding site, respectively (Lotufo *et al.,* 2001). In contrast, other effects of melatonin such as the inhibition of transcription factors that mediate acute inflammation induced by LPS (Tamura *et al.,* 2010) or *N*-formyl-l-methionyl-l-leucyl-l-phenylalanine (fMLP) (Cernysiov *et al.,* 2015) were not

blocked by luzindole suggesting a mode of action independent of MT receptors.

MT receptors also play an important role in promoting engulfing of bacteria, fungi and parasites. Melatonin 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 polymorphonuclear and mononuclear 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 melatonin. Luzindole and 4P-PDOT blocked the expression of dectin-1, a protein that is important for phagocytosis, suggesting the participation of MT_2 receptors in this effect (Muxel *et al.,* 2012; Pires-Lapa *et al.,* 2013; Muxel *et al.,* 2016). Thus, the evaluation of binding parameters and functional states of MT receptors in immune-competent cells needs to consider the masking effect of on-demand synthesized melatonin.

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

MT receptors in the CNS

MT receptors 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 melatonin on circadian rhythms (see above). In addition to its chronobiotic effect, melatonin participates in the modulation of neuronal functions, 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 (Wang *et al.,* 2005; Savaskan *et al.,* 2006) and reward (Hutchinson *et al.,* 2012; Clough *et al.,* 2014). MT receptors mediate the melatonin-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, melatonin-induced differentiation and maturation of adult neural stem cells were almost abolished in the presence of luzindole (de la Fuente Revenga *et al.,* 2015). A recent study using $MT_2^{-/-}$ mice showed that MT_2 receptors were essential for axogenesis and for the formation of functional synapses (Liu *et al.,* 2015). MT_2 receptors were also involved in melatonin-induced protection against oxidative stress and memory impairment in a mouse model of ageing (Shin *et al.,* 2015). Recent advances in the understanding of presynaptic MT receptors and their role in neurodegenerative diseases are discussed in the following sections.

Presynaptic MT receptors. The role of melatonin on the regulation of calcium-dependent dopamine release from axon terminals in brain and amacrine cells in the retina was shown in the early 1980s (Zisapel and Laudon, 1982; Dubocovich, 1983). However, more direct and global proof for the presence of presynaptic melatonin 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 has established that MT_1, but not MT_2 receptors, are expressed on presynaptic axon terminal membranes in the hypothalamus, striatum, cortex and hippocampus, where they are part of the presynaptic protein network (Benleulmi-Chaachoua *et al.,* 2016). Notably, this study shows a strong physical association between MT_1 receptors and presynaptic proteins such as synapsin, SNAP25, Munc-18 and voltage-gated $Ca_v2.2$ channels. Interaction with the latter was responsible for constitutive inhibition of calcium entry by MT_1 receptors in a Gβγ-dependent manner (Benleulmi-Chaachoua *et al.,* 2016).

These recent findings provide strong support for the involvement of MT receptors in synaptic functions, particularly in neurotransmitter release as indicated by previous studies. Indeed, activation of MT receptors has been implicated in the inhibition of ^{3}H-dopamine release from the ventral hippocampus, medulla pons, preoptic area and hypothalamus (median and posterior) (Zisapel and Laudon, 1982; Dubocovich, 1983). 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 noradrenaline turnover via activation of presynaptic melatonin heteroreceptors was demonstrated in hypothalamus (Fang and Dubocovich, 1990). In this model, luzindole, applied during the night when melatonin levels are high, accelerated noradrenaline turnover suggesting the involvement of MT receptors stimulated by endogenous melatonin (Fang and Dubocovich, 1990). The presence of presynaptic MT heteroreceptors on retino-hypothalamic fibres innervating superficial retinorecipient layers of the avian optic tectum has been inferred by the presence of 2-[^{125}I]-MLT binding sites and its decrease following transsection of the retinotectal pathway (Krause *et al.,* 1992; Krause *et al.,* 1994). The function of these presynaptic MT receptors is currently unknown, but a modulatory role of these receptors on the light input pathway to visual and circadian target responses is likely. Recent electrophysiological evidence suggests that melatonin acting through presynaptic MT receptors increases glutamatergic neurotransmission in the habenula, an effect blocked by luzindole (Evely *et al.,* 2016). Finally, it is worth mentioning in this context that MT receptors were 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, 1983; Dubocovich, 1985). These mammalian functional presynaptic heteroreceptors were used to establish the first structure–activity relationship for MT receptor ligands, which correlated with the pharmacological profile of MT_2 receptors (Dubocovich *et al.,* 1997), and to identify and pharmacologically characterize the first competitive MT receptor ligands, luzindole and 4P-PDOT (Dubocovich, 1988).

In summary, proteomic studies of the MT_1 receptor interactome has revived interest in the function of presynaptic MT receptors and reinforced previous functional studies

indicating the role of presynaptic MT receptors in neurotransmitter release. Use of MT receptor KO mouse models will be particularly instrumental in this context, as they will clarify the respective roles of MT_1 and MT_2 receptors. Based on current data, a predominant role of MT_1 receptors in presynaptic functions, such as neurotransmitter release, and a potential role of MT_2 receptors in axogenesis and synapse formation can be postulated.

MT receptors in neurodegenerative diseases. Altered expression of MT receptors has been frequently reported in neurodegenerative diseases and psychiatric disorders, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and ASD. In AD patients, MT_1 receptor expression in the SCN and MT_2 receptor expression in the hippocampus are reduced compared to control subjects in *post-mortem* brains (Wu *et al.,* 2007). Intriguingly, higher expression of MT_1 receptors 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 melatonin in these patients (Zhou *et al.,* 2003). These observations suggest that the expression of MT receptors under pathological conditions can be differentially regulated depending on the brain area. In PD patients, down-regulation of MT_1 and MT_2 receptor 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 receptor expression 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 receptors, was detected in the striatum (Wang *et al.,* 2011). Interestingly, the progressive loss of MT_1 receptors correlates with HD severity, also confirmed in a mouse model of HD (Wang *et al.,* 2011). In ASD patients, no information on MT_1 and MT_2 receptor expression is available, but several MT_1 and MT_2 receptor mutants with strongly reduced function have been identified (Chaste *et al.,* 2010).

Additional evidence supports the emerging concept of MT receptor dysfunction as a permissive condition favouring the development and/or progression of neurodegenerative diseases. The neuroprotective effect of endogenous and exogenous melatonin has been demonstrated in different systems (see Escribano *et al.,* 2014). In a neuroinflammatory model induced by LPS administration, cerebellar neuronal death was observed only in animals pretreated with luzindole (Pinato *et al.,* 2015). Similarly, depletion of endogenous melatonin 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 MT receptors for the neuroprotective action of melatonin has also been elegantly demonstrated in a series of *in vitro* studies in which luzindole treatment or siRNA-mediated knockdown of MT_1 receptors 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 receptors. Accordingly, the AD-related neurotoxic amyloid β peptide (Aβ) impairs the function of MT receptors (Cecon *et al.,* 2015), implying that these receptors and melatonin signalling are among the primary molecular targets affected in the course of AD.

Insights in the effects of MT receptors on cognitive functions are also obtained from MT receptor 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 from WT mice in memory test performances and show increased long-term potentiation responses, even though the deletion of MT receptors 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 MT receptors for cognitive performance was clearly shown with an AD mouse model lacking MT_1 and MT_2 receptors, in which melatonin treatment failed to improve performance on hippocampal-dependent spatial learning tasks, as observed in the AD mouse model in the presence of MT_1 and MT_2 receptors. Impressively, the lack of MT receptors *per se* markedly increased the mortality in young AD mice (O'Neal-Moffitt *et al.,* 2015). Finally, the therapeutic use of melatonin has been proposed and tested in a number of murine models and clinical trials in several neurodegenerative conditions, including AD (Olcese *et al.*, 2009; Cardinali *et al.*, 2010; 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 melatonin 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 MT receptors 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 MT receptor expression and function might provide the most efficient result.

Taken together, the subcellular localization and role of MT receptors in neuronal functions and their participation in neurodegererative diseases are now starting to be understood and suggest a broad modulatory role of melatonin 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 melatonin persist even in the absence of MT_1 and MT_2 receptors or upon complete pharmacological blockade of MT receptors, indicating the existence of MT receptor-independent mechanisms, which are still not fully understood. In addition, MT receptor-dependent and -independent mechanisms can participate simultaneously, as demonstrated by O'Neal-Moffitt *et al.* (2015) regarding the antioxidant and pro-cognitive effects of melatonin on AD mice models, for example. Two main mechanisms have been put forward to explain the antioxidant and free radical properties of melatonin: these are melatonin binding to the *MT3* binding site (Nosjean *et al.*, 2000; Dubocovich *et al.*, 2003) and to the

cytosolic enzyme quinone reductase 2 (QR2) (Nosjean *et al.,* 2000; Dubocovich *et al.,* 2003), and melatonin scavenging of free radicals, as this hormone has been suggested to be an electron donor (see Tan *et al.,* 2015). Binding of melatonin to intracellular targets is readily achieved, due to the hydrophilic nature of this indolamine. Melatonin binds with nanomolar affinity to *MT3*/*QR2* binding sites but shows a pharmacological profile distinct from MT_1 and MT_2 receptors. The order of affinities for the *MT3* binding site is 2-iodomelatonin > *N*-acetyl-serotonin > melatonin (Dubocovich, 1995; Nosjean *et al.,* 2000), the order for MT_1 and MT_2 receptors is 2-iodomelatonin > melatonin >>>>*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 and Williams-Ashman, 1961), thus acting as a detoxifying enzyme to increase the antioxidant defence (Jockers *et al.,* 2008). There are still open questions as to whether the melatonin binding site on QR2 corresponds to the *MT3* binding site, in particular regarding those sites that are membrane-associated.

Several physiological effects of melatonin such as inhibition of leukocyte adhesion to rat endothelial cell layers were mimicked 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 receptors at submicromolar concentrations suggesting the possibility that some of the effects of MCA-NAT might be mediated by MT_1 and/or MT_2 receptors (Vincent *et al.,* 2010).

Melatonin and its metabolites, with or without open ring structures, have been described as potent electron donors. Cyclic-3-hydroxymelatonin, *N*1-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 melatonin molecule and its associated metabolites could scavenge a large number of reactive species, and thus, the overall antioxidant capacity of melatonin is believed to be greater than that of other well-known antioxidants, such as vitamin C and vitamin E, under *in vitro* or *in vivo* conditions (Gitto *et al.,* 2001; Sharma and Haldar, 2006; Ortiz *et al.,* 2013). However, the ability of melatonin in reducing oxidative stress does not only rely on donating electrons. Indeed, by acting on MT_1 and MT_2 receptors, low pM and low nM concentrations of melatonin increased the expression or activity of enzymes such as superoxide dismutase, catalase and glutathione peroxidase, which are involved in oxygen detoxification (Rosen *et al.,* 2009). Thus, depending on the dose of exogenous or endogenous melatonin, receptor-dependent or -independent mechanisms may be involved. A further complexity in the interplay between receptor-dependent and -independent processes could arise from the fact that melatonin, 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 MT receptors has to be addressed in future studies. Although endogenous melatonin 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 melatonin, it is surprising that opposing opinions are still in the literature. Overall, the antioxidant effects of melatonin appear to be complex, relying on a mixture of MT receptor-dependent and -independent processes.

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Conflict of interests

The authors declare no conflicts of interest.

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: 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: 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: BR61–BR67.

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: 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: 61–64.

Alexander SPH, Davenport AP, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015a). The Concise Guide to PHARMACOLOGY 2015/16: G Protein-Coupled Receptors. Br J Pharmacol 172: 5744–5869.

Alexander SPH, Catterall WA, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015b). The Concise Guide to PHARMACOLOGY 2015/16: Voltage-gated ion channels. Br J Pharmacol 172: 5904–5941.

Alexander SPH, Kelly E, Marrion N, Peters JA, Benson HE, Faccenda E *et al.* (2015c). The Concise Guide to PHARMACOLOGY 2015/16: Transporters. Br J Pharmacol 172: 6110–6202.

Alexander SPH, Fabbro D, Kelly E, Marrion N, Peters JA, Benson HE *et al.* (2015d). The Concise Guide to PHARMACOLOGY 2015/16: Enzymes. Br J Pharmacol 172: 6024–6109.

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: 1539–1548.

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: 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: 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: 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: ra89.

Baba K, Davidson AJ, Tosini G (2015). Melatonin Entrains PER2::LUC Bioluminescence Circadian Rhythm in the Mouse Cornea. Invest Ophthalmol Vis Sci 56: 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: 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: 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: 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: 89–94.

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

Cardinali DP, Furio AM, Brusco LI (2010). Clinical aspects of melatonin intervention in Alzheimer's disease progression. Curr Neuropharmacol 8: 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 J 18: 537–539.

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

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: 2566–2582.

Cernysiov V, Mauricas M, Girkontaite I (2015). Melatonin inhibits granulocyte adhesion to ICAM via MT3/QR2 and MT2 receptors. Int Immunol 27: 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: 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: e11495.

Chen X, Li X, Du Z, Shi W, Yao Y, Wang C *et al.* (2014). Melatonin promotes the acquisition of neural identity through extracellular-signal-regulated kinases $^{1}/_{2}$ activation. J Pineal Res 57: 168–176.

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: 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: 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: 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: 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: 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: 628–642.

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: 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: 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: 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: 295–299.

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

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

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

Dubocovich ML (1995). Melatonin receptors: Are there multiple subtypes? Trends Pharmacol Sci 16: 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: 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: 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: 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–d1108.

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: 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: 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: 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: 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: 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: 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: 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: 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: 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: 413–434.

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

Gerdin MJ, Masana MI, Rivera-Bermudez MA, Hudson RL, Earnest DJ, Gillette MU *et al.* (2004). Melatonin desensitizes endogenous MT2 melatonin receptors in the rat suprachiasmatic nucleus: relevance for defining the periods of sensitivity of the mammalian circadian clock to melatonin. FASEB J 18: 1646–1656.

Gerdin MJ, Mseeh F, Dubocovich ML (2003). Mutagenesis studies of the human MT2 melatonin receptor. Biochem Pharmacol 66: 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: 94–104.

Gitto E, Karbownik M, Reiter RJ, Tan DX, Cuzzocrea S, Chiurazzi P *et al.* (2001). Effects of melatonin treatment in septic newborns. Pediatr Res 50: 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: 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: 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: 466–468.

Hu Y, Ho MKC, Chan KH, New DC, Wong YH (2010). Synthesis of substituted N-[3-(3-methoxyphenyl)propyl] amides as highly potent MT2-selective melatonin ligands. Bioorg Med Chem Lett 20: 2582–2585.

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: 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. Am J Physiol Cell Physiol 280: 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: 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: 2845–2854.

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

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

Johnston JD, Skene DJ (2015). 60 YEARS OF NEUROENDOCRINOLOGY: Regulation of mammalian neuroendocrine physiology and rhythms by melatonin. J Endocrinol 226: T187–T198.

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: 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: 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: 561–567.

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

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: 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: 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: 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: 1463–1471.

Kong X, Li X, Cai Z, Yang N, Liu Y, Shu J *et al.* (2008). Melatonin regulates the viability and differentiation of rat midbrain neural stem cells. Cell Mol Neurobiol 28: 569–579.

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

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: 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: 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: 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: 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: 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: 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: 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: 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: 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: 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: 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: 1094–1098.

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: 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: 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: 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: 82–88.

Maestroni G (1993). Th immunoneuroendocrine role of melatonin. J Pineal Res 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: 666–674.

Markus RP, Cecon E, Pires-Lapa MA (2013). Immune-Pineal Axis: Nuclear Factor Œ∫B (NF-kB) Mediates the Shift in the Melatonin Source from Pinealocytes to Immune Competent Cells. Int J Mol Sci 14: 10979–10997 %! Immune-Pineal Axis: Nuclear Factor Œ∫B (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: 1556–1569.

Maurice P, Kamal M, Jockers R (2011). Asymmetry of GPCR oligomers supports their functional relevance. Trends Pharmacol Sci 32: 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: 836–842.

McArthur 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: 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: 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: 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: 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: 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: 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: 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: 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: 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 60: 394–404.

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: e52010.

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: 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: 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: 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: 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: 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: 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: 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: 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: 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: e0148214.

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: 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: 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: 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: 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: 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: 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: 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: 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: 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: 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: 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 12: 574–587.

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: 482–491.

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: 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: 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: 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: 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: 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: 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: 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: 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: 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: 497–502.

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: 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: 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: 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: 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: 6457–6462.

Southan C, Sharman JL, Benson HE, Faccenda E, Pawson AJ, Alexander SPH *et al*. (2016). The IUPHAR/BPS Guide to PHARMACOLOGY in 2016: towards curated quantitative interactions between 1300 protein targets and 6000 ligands. Nucl Acids Res 44 (Database Issue): D1054–D1068.

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: 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: 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: 318–327.

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

Sugden D, Yeh LK, Teh MT (1999). Design of subtype selective melatonin receptor agonists and antagonists. Reprod Nutr Dev 39: 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: 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: 18886–18906.

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: 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: 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: 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: 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: 549–559.

Vanecek J (1988). Melatonin binding sites. J Neurochem 51: 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: 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. Nat Neurosci 5: 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.

Wang LM, Suthana NA, Chaudhury D, Weaver DR, Colwell CS (2005). Melatonin inhibits hippocampal long-term potentiation. Eur J Neurosci 22: 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: 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: 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: 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: 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: 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: 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: 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: 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: 102–108.

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: 1610–1616.

Zlotos DP (2012). Recent progress in the development of agonists and antagonists for melatonin receptors. Curr Med Chem 19: 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: 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: 3161–3185.

Zlotos DP, Riad NM, Osman MB, 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.