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Themed Section: Recent Developments in Research of Melatonin and its Potential Therapeutic Applications

# REVIEW ARTICLE

# Epigenetic regulation of melatonin receptors in neuropsychiatric disorders

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Melatonin, the primary indoleamine hormone of the mammalian pineal gland, is known to have a plethora of neuroregulatory, neuroprotective and other properties. Melatonergic signalling is mediated by its two GPCRs, $MT_1$ and $MT_2$, which are widely expressed in the mammalian CNS. Melatonin levels and receptor expression often show a decrease during normal ageing, and this reduction may be accelerated in some disease states. Depleted melatonergic signalling has been associated with neuropsychiatric dysfunction and impairments in cognition, memory, neurogenesis and neurorestorative processes. The anticonvulsant and mood stabilizer, valproic acid (VPA), up-regulates melatonin $MT_1$ and/or $MT_2$ receptor expression in cultured cells and in the rat brain. VPA is known to affect gene expression through several mechanisms, including the modulation of intracellular kinase pathways and transcription factors, as well as the inhibition of histone deacetylase (HDAC) activity. Interestingly, other HDAC inhibitors, such as trichostatin A, which are structurally distinct from VPA, can also up-regulate melatonin receptor expression, unlike a VPA analogue, valpromide, which lacks HDAC inhibitory activity. Moreover, VPA increases histone H3 acetylation along the length of the $MT_1$ gene promoter in rat C6 cells. These findings indicate that an epigenetic mechanism, linked to histone hyperacetylation/chromatin remodelling and associated changes in gene transcription, is involved in the up-regulation of melatonin receptors by VPA. Epigenetic induction of $MT_1$ and/or $MT_2$ receptor expression, in areas where these receptors are lost because of ageing, injury or disease, may be a promising therapeutic avenue for the management of CNS dysfunction and other disorders.

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## Abbreviations

ALS, amyotrophic lateral sclerosis; BDNF, brain-derived neurotrophic factor; CpG, 5′-C-phosphate-G-3′; CREB, cAMP response element binding protein; DNMT, DNA methyltransferase; GDNF, glial cell line-derived neurotrophic factor; GSK3β, glycogen synthase kinase 3β; HAT, histone acetyltransferase; HDAC, histone deacetylase; HP1, heterochromatin protein 1; Nrf2-ARE, NF-E2-related factor 2-antioxidant responsive element; SCN, suprachiasmatic nucleus; SMC, structural maintenance of chromosomes; VPA, valproic acid



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## Introduction

### *Melatonin receptors*

The mammalian **melatonin** receptor subtypes, **$MT_1$** and **$MT_2$**, are localized on neuronal dendrites and somata in areas that include the cerebral cortex, basal forebrain, hippocampus, basal ganglia, diencephalon and mesencephalon (Lacoste *et al.,* 2015). The anatomical distribution patterns of $MT_1$ and $MT_2$ receptors in the adult rat brain, which are detailed in recent reports (Lacoste *et al.,* 2015; Ng *et al.,* 2017), occur in a complementary fashion, in which relative increases in $MT_1$ receptor expression correspond with relative decreases in $MT_2$ receptors, or *vice versa*. For example, in the hypothalamus, the expression of $MT_1$ receptors is relatively high in the suprachiasmatic nucleus (SCN), whereas that of $MT_2$ receptors is relatively high in the supraoptic nucleus. This property may be indicative of their unique functional specializations (Lacoste *et al.,* 2015).

Functional studies have revealed that both melatonin receptor subtypes are coupled to various pertussis-sensitive and insensitive G proteins. A cornerstone of melatonergic signalling involves the inhibition of adenylate cyclase activity and attenuation of cAMP accumulation, PKA activity and cAMP response element binding protein (CREB) phosphorylation (Brydon *et al.,* 1999); however, the involvement of several other signal transduction pathways has been reported (Dubocovich *et al.,* 2010). Studies of melatonin receptor regulation in the ovine pars tuberalis demonstrated that stimulation of cAMP production, by forskolin or cholera toxin, increases $MT_1$ receptor expression (Barrett *et al.,* 1996). Since cAMP levels regulate the transcription of many genes through the cAMP/PKA/CREB pathway, the transcription factor CREB has been implicated in the regulation of melatonin receptors (Barrett *et al.,* 1996). Cloning of the proximal 1.5 kb region of the rat $MT_1$ promoter (GenBank AY228510) allowed for a more precise analysis of the regulation of $MT_1$ receptor transcription. $MT_1$ promoter activity assays have identified paired-like homeodomain transcription factor 1, a transcription factor widely distributed throughout the rodent and ovine pituitary, as an important regulator of $MT_1$ receptor transcription (Johnston *et al.,* 2003).

### *Protective effects of melatonin*

*Antioxidant effects*. The CNS is highly vulnerable to damage from oxidative stress because of its large composition of lipids, high demands for oxidative metabolism and, relatedly, production of toxic metabolites (Wang and Michaelis, 2010). Melatonin exerts many protective effects in the mammalian CNS. The NF-E2-related factor 2-antioxidant responsive element (Nrf2-ARE) signalling cascade is one of the intracellular pathways that mediates the regulation of antioxidant gene expression by melatonin. Melatonin induces Nrf2 expression, as well as the downstream targets of Nrf2-ARE signalling in multiple models of peripheral (Tripathi and Jena, 2010) and central oxidative stress (Wang *et al.,* 2012). Recent studies in Klotho mutant mice show that the Nrf2-related antioxidant actions of melatonin involve $MT_2$ receptor-mediated activation of the ERK pathway (Shin *et al.,* 2015).

In addition to regulating intracellular antioxidant pathways, melatonin, as well as its metabolites, has direct free radical scavenging capabilities. As such, a single melatonin molecule can forage and neutralize multiple highly toxic reactive oxygen, nitrogen and hydroxyl radicals at any one time (Rosen *et al.,* 2006). Although free radical scavenging and avoidance are widely beneficial properties, they are especially protective of mitochondrial integrity because of the increased susceptibility of this organelle to oxidative damage. Melatonin readily enters the mitochondrial matrix, where it limits electron leakage from the electron transport chain and free radical production (Reiter *et al.,* 2003). While the direct free radical scavenging effects of melatonin do not involve its receptors, other antioxidant effects, such as maintaining glutathione levels and stimulating the activities of catalase and superoxide dismutase, are mediated by melatonin receptors (Rezzani *et al.,* 2006).

*Neurotrophic factor modulation*. Melatonin is thought to promote neuronal development, differentiation and survival in the nervous system by modulating neurotrophic factor expression. Melatonin, as well as its analogues, has been shown to up-regulate neurotrophic factors such as glial cell line-derived neurotrophic factor (GDNF; Armstrong and Niles, 2002; Kong *et al.,* 2008b), nerve growth factor (Pongsa-Asawapaiboon *et al.,* 1998) and brain-derived neurotrophic factor (BDNF; Molteni *et al.,* 2010; Boulle *et al.,* 2014). Reduced expression of these targets has been implicated in the pathogenic mechanisms driving several brain disorders. Therefore, controlling the expression of these and other neurotrophic factors is thought to have therapeutic potential for both neurodegenerative (Allen *et al.,* 2013) and psychiatric disorders (Castrén, 2014).

There is limited information about the mechanisms involved in the modulation of neurotrophic factors by melatonin. The melatonin-induced increase in GDNF mRNA expression in C6 cells, which express both $MT_1$ and $MT_2$ receptors (Armstrong and Niles, 2002), may be mediated by the $MT_1$ subtype, as this effect is not blocked by the $MT_2$ antagonist, **4P-PDOT (4-phenyl-2-propionamidotetraline)** (Niles and Armstrong, 2002). The increase in GDNF protein levels induced by melatonin in primary astrocytes is blocked by the PI3K/Akt antagonist, wortmannin and the non-selective melatonin receptor antagonist, **luzindole**, indicating the involvement of this pathway and melatonin receptors in this effect (Kong *et al.,* 2008a). Recently, melatonin treatment in the presence of the pro-inflammatory cytokine, IL-18, was found to increase the mRNA and protein expression of GDNF and BDNF in neural stem cells, whereas luzindole blocked these effects, indicating that this effect is mediated by stimulation of melatonin receptors (Li *et al.,* 2017). As discussed later, epigenetic regulation of gene transcription, including chromatin remodelling *via* histone modifications, has been linked to synaptic plasticity, neurotrophic factor induction, neuroprotection and related effects (Schmidt *et al.,* 2013; Harrison *et al.,* 2015). Evidence that melatonin can acetylate histone proteins in cultured cells and *in vivo* (Sharma *et al.,* 2008; Niles *et al.,* 2013) suggests that epigenetic induction of gene expression may underlie its effects on neurotrophic factors and other neuroprotective targets.

Moreover, blockade of melatonin-induced histone acetylation by luzindole, in human SH-SY5Y neuroblastoma cells, indicates the involvement of melatonin receptors in these effects (Pan and Niles, 2015).

## Melatonergic signalling in psychiatric and neurodegenerative disease

Several *in vitro* and *in vivo* studies have revealed aberrations in the expression patterns of $MT_1$ and/or $MT_2$ receptors in various models of CNS disorders. *Post-mortem* analyses of human brain tissue from related disorders have also revealed changes in melatonin receptor expression patterns, but these findings are complicated by the fact that the affected patients may have received prolonged treatment with therapeutic drugs, which may induce changes in receptor expression independent of the disease (Hirsch-Rodriguez *et al.,* 2007).

Aberrant melatonergic function has been implicated in various neuropsychiatric conditions. The potential therapeutic use of melatonin in neuropsychiatric disorders has been reviewed recently with a note on the need for selective targeting of receptor subtypes (Mahmood *et al.,* 2016), which exhibit distinct functional specialization. For example, a study of sleep regulation, using receptor knockout mice, revealed that $MT_1$ and $MT_2$ receptor signalling regulate rapid eye movement sleep and non-rapid eye movement sleep respectively (Comai *et al.,* 2013). The sleep-promoting role of $MT_2$ receptors is further supported by the observation that $MT_2$ receptor ligands produce a hypnotic effect, which is eliminated in mice lacking $MT_2$ receptors (Ochoa-Sanchez *et al.,* 2011). $MT_2$ ligands have also been observed to have an antinociceptive effect in rodents, which can be blocked by $MT_2$ receptor antagonists, implying a role for this receptor in pain regulation (Lopez-Canul *et al.,* 2015). Increased $MT_1$ receptor expression, but not $MT_2$, has been observed in the hypothalamic tissue of depressed patients *post-mortem* (Wu *et al.,* 2013). Mice lacking the expression of $MT_1$ receptors exhibit neurobiological variations characteristic of depression, including hyperstress responses, psychomotor disturbances and increased anhedonic and depressive-like behaviours in comparison to their wild-type counterparts (Comai *et al.,* 2015), suggesting a role for the $MT_1$ subtype in the aetiology of depression.

Abnormal melatonin receptor expression profiles have also been noted in various neurodegenerative conditions. For example, rodent models revealed that $MT_1$ receptor expression was reduced in the spinal cords of transgenic amyotrophic lateral sclerosis (ALS) mice (Zhang *et al.,* 2013). Analysis of *post-mortem* brain tissue revealed increased $MT_1$ (Savaskan *et al.,* 2002) and decreased $MT_2$ receptor expression (Savaskan *et al.,* 2005) in the hippocampus of Alzheimer's disease patients. The reason for the different expression of these receptor subtypes in Alzheimer's disease awaits clarification but could involve discrete differences in their localization or other neurodegenerative changes associated with this disorder. Other studies have reported regional decreases in melatonin receptor expression in Alzheimer's disease, including reduced hypothalamic, pineal and cortical $MT_1$ expression (Brunner *et al.,* 2006; Wu *et al.,* 2007), as well as pineal, cortical and retinal $MT_2$ expression (Brunner *et al.,* 2006; Savaskan *et al.,* 2007), relative to healthy controls. Similarly, *post-mortem* analysis of brains from patients with Parkinson's disease revealed decreases in both $MT_1$ and $MT_2$ receptor expression in the substantia nigra as well as the amygdala in comparison to healthy controls (Adi *et al.,* 2010). Consequently, impairments in melatonergic signalling related to aberrant $MT_1$ and/or $MT_2$ expression profiles could contribute to the overall deterioration of the nervous system in these disorders. Evidence that the neuroprotective effect of melatonin in a transgenic mouse model of ALS involves inhibition of receptor interacting protein-2-induced activation of the caspase-1 pathway *via* the $MT_1$ receptor (Zhang *et al.,* 2013) supports the importance of melatonin receptors in brain preservation. Drug-induced up-regulation of melatonin receptors, as proposed in this review, is a potential strategy for restoring depleted melatonin receptor expression in areas where it is lost as a result of normal ageing, injury or disease.

### *Valproic acid and melatonin receptors*

**Valproic acid** (VPA) or 2-propylpentanoic acid is a branched short-chain fatty acid with well-known anticonvulsive and mood-stabilizing effects. A recent study has shown that VPA may be an effective adjunct to antidepressant therapies in treatment-resistant depression (Ghabrash *et al.,* 2016). In addition to the multiple targets and pathways influenced by VPA (Monti *et al.,* 2009), increasing evidence indicates an interaction between this psychotropic agent and the melatonergic system. Treatment with VPA enhances the expression of tryptophan hydroxylase, which contributes to melatonin biosynthesis (Qiu *et al.,* 2015) and reduces the sensitivity of melatonin to light (Hallam *et al.,* 2005). Initial studies on the effects of VPA on melatonin receptor expression were completed in rat C6 glioma cells (Castro *et al.,* 2005; Kim *et al.,* 2008) and in human MCF-7 breast cancer cells (Jawed *et al.,* 2007). This work revealed a dose-dependent relationship in which VPA caused a robust increase in $MT_1$ receptor mRNA and/or protein (Castro *et al.,* 2005; Jawed *et al.,* 2007; Kim *et al.,* 2008). These findings set the groundwork for *in vivo* studies, which showed that chronic VPA administration causes a robust increase in $MT_1$ and $MT_2$ receptor mRNA levels in the rat hippocampus (Niles *et al.,* 2012). Moreover, an *in situ* hybridization study revealed significant increases in $MT_2$ receptor expression in the CA1-3 and dentate gyrus regions of the rat hippocampus, following a similar treatment with VPA (Bahna *et al.,* 2014). As discussed below, this up-regulation of melatonin receptors, especially the $MT_1$ subtype, is thought to involve an epigenetic mechanism.

### *Epigenetic mechanisms*

The term epigenetics refers to heritable changes in gene expression, which do not involve an alteration in DNA sequence. Three major mechanisms including DNA methylation, regulation of transcription by non-coding RNAs and histone modification are involved in the epigenetic-regulation of gene expression. Dysfunction of any of these interacting mechanisms can result in the abnormal expression or silencing of genes and the possible onset of 'epigenetic diseases' (Egger *et al.,* 2004; Hwang *et al.,* 2017)).

*DNA methylation.* The methylation of 5-cytosine residues to 5-methylcytosine, in cytosine-guanine (CpG) dinucleotides, is an established epigenetic mechanism for gene silencing. The methylation of CpG sites within the mammalian genome is maintained by a number of DNA methyltransferases (DNMTs). DNMT3A and DNMT3B are involved in the *de novo* methylation of DNA, while DNMT1 performs a maintenance function by methylating the complementary strand in hemimethylated DNA before its replication (Bayraktar and Kreutz, 2017). It is now apparent that DNA demethylation can occur *via* deaminases, which catalyse the conversion of 5-methylcytosine to thymidine. A second mechanism involves ten-eleven translocation enzymes that reverse the methylation status of DNA by the successive oxidation of 5-methylcytosine to 5-carboxylcytosine, which is subjected to a base-excison-repair process to regenerate cytosine (Nabel and Kohli, 2011).

*Regulation of transcription by non-coding RNAs.* Another category of epigenetic regulators includes non-coding RNAs such as microRNAs (miRNAs), small interfering RNAs and long non-coding RNAs. To date, the most studied are miRNAs, which are thought to target more than 60% of human genes (Friedman *et al.,* 2009) and to regulate gene transcription primarily by suppression of target translation or induction of mRNA decay (Huntzinger and Izaurralde, 2011). Their ability to regulate gene expression in pathways linked to major physiological processes, including neurodevelopment, neurogenesis and neuroprotection, has implicated miRNAs in various central pathological states such as neurodegeneration and psychiatric dysfunction (Kim *et al.,* 2016; Boone *et al.,* 2017).

*Histone modification.* Chromatin consists of nucleosomes, each composed of about 146 base pairs of genomic DNA, which is wrapped around an octamer of core histone proteins consisting of two copies each of H2A, H2B, H3 and H4. Histone proteins have long N-terminal tails that are key targets in multiple post-translational modifications including acetylation, methylation, phosphorylation, sumoylation, ubiquitination and ADP-ribosylation. Acetylation and methylation are the most widely studied of these covalent modifications, which cause conformational changes in chromatin structure and influence the access of transcription factors and other regulatory proteins to their DNA targets (Bannister and Kouzarides, 2011). Enzymes that alter the electrostatic interactions between the negatively charged DNA and the positively charged histone core, *via* histone acetylation or deacetylation, are involved in the epigenetic regulation of gene transcription, as illustrated in Figure 1. Histone acetylation, which is catalysed by histone acetyltransferase (**HAT**) enzymes from various families (Roth *et al.,* 2001), neutralizes the positive charge on histone proteins, causing a reduced affinity between the histone protein and the DNA strand. The associated chromatin assumes a more loosened structure, known as euchromatin, which exposes regulatory genetic sequences and permits the binding of transcription factors to activate transcription (Morse, 2007). The reverse process, deacetylation, is catalysed by histone deacetylase (**HDAC**) enzymes, which cause chromatin condensation leading to the termination of transcriptional activity (Bannister and Kouzarides, 2011).

Based on gene sequence, subcellular localization or functional differences, 11 zinc-dependent HDACs have been assigned to different classes as follows: Class I (HDAC 1, 2, 3, 8), Class IIa (HDAC 4, 5, 7, 9), Class IIb (HDAC 6, 10) and Class IV (HDAC 11). Class III HDACs are a distinct family of nicotinamide adenine dinucleotide-dependent enzymes, termed sirtuins. Several HDAC inhibitors have been examined in studies aimed at identifying clinically relevant epigenetic regulators. Of particular relevance to this review, VPA inhibits the activity of multiple Class I and Class II HDACs (Gurvich *et al.,* 2004), which are involved in critical aspects of CNS physiology, including neurodevelopment and cognition (Morris and Monteggia, 2013).

## *Epigenetic regulation of melatonin receptors by VPA*

VPA can directly inhibit HDAC activity, which results in the conversion of chromatin to an acetylated and transcriptionally active conformation (Phiel *et al.,* 2001). Several factors are involved in the maintenance of chromatin structure in the normal state, such as the structural maintenance of chromosomes (SMC) family of proteins, which are required for the general stabilization of chromosome conformation (Yokomori, 2003), and heterochromatin protein 1 (HP1), which maintains DNA in the heterochromatin state (Maison and Almouzni, 2004). VPA treatments stimulate a conformational change in chromatin structure, from a highly

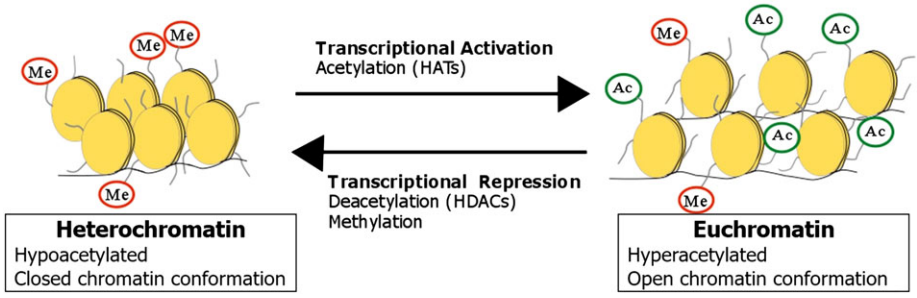


**Figure 1**

Regulation of chromatin conformation by histone acetylation and methylation. Histone acetylation, methylation or other covalent modifications of lysine residues on the N-terminal tail of the histone core can alter gene transcription *via* changes in the accessibility of DNA to regulatory factors.

condensed state to a more loosened structure, which corresponds with a decreased expression of the proteins which promote heterochromatin stability (Marchion *et al.,* 2005; Felisbino *et al.,* 2014).

As noted earlier, VPA treatment up-regulates melatonin receptors in cultured mammalian cells and the rat brain (Castro *et al.,* 2005; Niles *et al.,* 2012), but the mechanism(s) involved awaits clarification. Recent studies indicate that an epigenetic mechanism, specifically that of $MT_1$ promoter histone acetylation due to HDAC inhibition, underlies the transcriptional activation of the $MT_1$ receptor gene by VPA (Bahna and Niles, 2017). This is supported by the observation that structurally diverse drugs, such as the potent HDAC inhibitor, trichostatin A, also increase the expression of $MT_1$ receptors (Kim *et al.,* 2008), whereas valpromide, a VPA analogue without HDAC inhibitory properties, does not (Bahna and Niles, 2017).

VPA and other HDAC inhibitors can alter the demethylation of DNA, either globally or gene-specfically, by enhancing DNA demethylase activity *via* histone acetylation or other mechanisms (Detich *et al.,* 2003). This is important as several CpG islands are located within the promoter regions of many genes, which, when methylated, suppress transcription (Cedar and Bergman, 2009). As noted earlier, diverse epigenetic mechanisms interact in the regulation, amplification and fine-tuning of gene expression (Egger *et al.,* 2004). For example, the inhibition of HDAC activity and the attenuation of DNA methylation induced by VPA are not mutually exclusive events but rather are thought to be in a dynamic correlation and can impose compounded effects on gene transcription (Milutinovic *et al.,* 2007). In view of the foregoing, it is possible that DNA demethylation contributes to the regulation of melatonin receptors by VPA.

Chromatin decondensation surrounding the $MT_1$ promoter region allows transcription factors to access regulatory sequences along the $MT_1$ promoter to initiate transcription of the gene. The ability of VPA to prevent heterochromatin formation following histone acetylation (Marchion *et al.,* 2005), suggests that it can influence the transcriptional kinetics of $MT_1$ receptors. It is interesting to note that histone acetylation is thought to precede the modulation of HP1 and SMC protein expression by VPA (Marchion *et al.,* 2005), suggesting that the conformational changes in chromatin structure induced by VPA can cause prolonged induction of the melatonin receptor (Figure 2).

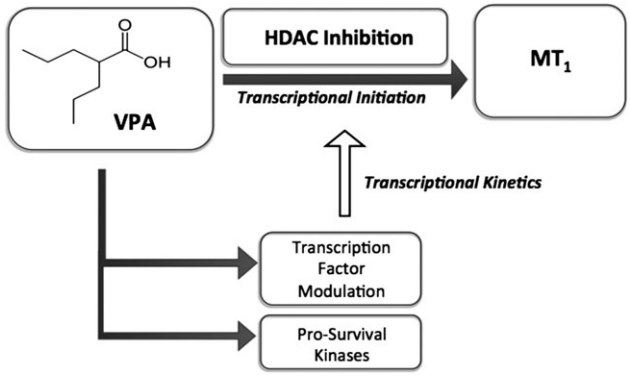


**Figure 2**

Proposed mechanism for the induction of $MT_1$ receptors by VPA. VPA activates the transcription of $MT_1$ receptors by causing chromatin remodelling, mediated by HDAC inhibition and $MT_1$ promoter hyperacetylation. VPA also acts *via* intracellular kinases to regulate the expression of transcription factors that control the activity of the $MT_1$ promoter, suggesting a role for this drug in the transcriptional kinetics of $MT_1$ receptor expression.

## *Molecular regulation of melatonin receptors by VPA*

VPA influences the expression and binding affinities of various transcription factors in cultured cells, as well as in the brain (Monti *et al.,* 2009). These effects may involve its influence on the post-translational modifications of various intracellular kinases, which regulate the activation and subsequent gene expression of transcription factors. VPA is a positive regulator of the AMP-activated protein kinase (Avery and Bumpus, 2014), MAPK, PI3K and Akt (PKB) and PKC signalling pathways, and a negative regulator of glycogen synthase kinase 3β (GSK3β) signalling (Monti *et al.,* 2009). We have reported that pharmacological inhibition of MAPK (Castro *et al.,* 2005), PI3K/Akt, PKC or GSK3β does not affect melatonin $MT_1$ receptor up-regulation (Bahna and Niles, 2017), further supporting our epigenetic hypothesis.

Although the above findings suggest that these kinase pathways are not involved in the up-regulation of $MT_1$ receptors by VPA, caution should be exercised when interpreting the effects of kinase inhibitors, which exhibit limited selectivity (Bain *et al.,* 2007). Moreover, given the multiple cellular and molecular effects of VPA, its administration could affect not just melatonin receptors but also other targets or pathways, which may in turn influence melatonergic function. One such target is tetrahydrobiopterin (BH4), an essential cofactor in the biosynthesis of neurotransmitters including 5-HT and catecholamines, and the enzyme, nitric oxide synthase, which produces nitric oxide (Werner *et al.,* 2011), a modulator of glutamate release (Neitz *et al.,* 2011). Given the functional interaction between melatonin and neurotransmitters such as 5-HT (Matheus *et al.,* 2010), it is possible that melatonergic activity following VPA treatment could also be modulated by other pathways or *in vivo* systems activated by this pleiotropic drug.

Recently, VPA was reported to inhibit internalization of the $MT_1$ receptor and its cAMP signalling by interfering with the association between the receptor and β-arrestin-2 (Hong *et al.,* 2016). If this effect, which was observed in an *in vitro* expression system, translates to the *in vivo* mammalian CNS, it is possible that it could negatively affect the anticipated benefits of melatonin receptor up-regulation. Nonetheless, the novel idea of using epigenetic drugs to restore deficient melatonin receptor expression is worth exploring in various *in vivo* models of neurological or psychiatric disorders, where potential benefits would depend on the net effect of VPA or other HDAC inhibitors on melatonin receptor expression and function. Given the neuroprotective, anti-manic and other effects of VPA treatment, the up-regulation of melatonin receptors by this drug in conjunction with the administration of agonists

(e.g. melatonin, **agomelatine** and **ramelteon**), which are similarly beneficial, could produce enhanced therapeutic outcomes.

## *Clinical relevance of epigenetic up-regulation of melatonin receptor expression*

The protective effects of increasing melatonin receptor expression have been demonstrated using several *in vitro* models of glutamate toxicity. Melatonin treatments in cultured embryonic rat VSC4.1 motor neurons transfected with $MT_1$ and/or $MT_2$ receptors were found to have a large increase in cell survival markers and a concurrent suppression in apoptotic and inflammatory markers (Das *et al.,* 2013). Melatonin treatments in untransfected cells did not have the same effect, emphasizing the importance of receptor density in the neuroprotective effects of this hormone (Das *et al.,* 2013). The beneficial effects of melatonin receptor overexpression have also been explored in the periphery. In human MCF-7 breast cancer cells, melatonin was found to have a greater antiproliferative effect in $MT_1$-transfected cells than in untransfected cells (Yuan *et al.,* 2002).

A limitation of these studies is how melatonin receptor overexpression is achieved. Gene therapy is a challenging approach and may not be a clinically suitable treatment option for a large number of patients (Gonin *et al.,* 2005). There is increasing evidence that dysregulated epigenetic processes underlie several neurological dysfunctions, including Parkinson's and Alzheimer's disease (Urdinguio *et al.,* 2009; Masliah *et al.,* 2013) and especially psychiatric disorders such as depression and schizophrenia (Houtepen *et al.,* 2016; Pishva *et al.,* 2017). Diverse psychopharmacological agents including antidepressants, antipsychotics and mood stabilizers can influence epigenomic profiles, as reflected by histone protein modifications and DNA methylation (Boks *et al.,* 2012). While the mechanisms driving melatonin receptor depletion are not yet known, accruing evidence proposes a role for dysregulated epigenetic processes in the pathogenesis of many neurological and neurodegenerative diseases (Saha and Pahan, 2006; Coppedè, 2014). This implies that the reduction in melatonin receptor expression in the diseased state may be a maladaptive response caused by an imbalance in epigenetic activities associated with disease progression. Given the central role of GPCRs in mediating the effects of diverse therapeutics, evidence that GPCR expression can be regulated by epigenetic mechanisms suggests that this approach of receptor regulation may be beneficial for a wide range of CNS disorders (Dogra *et al.,* 2016). As illustrated in Figure 3, epigenetic reprogramming of gene expression by VPA (or other HDAC inhibitors) to regain transcriptional control of the melatonin receptor gene can be a therapeutic strategy used to offset imbalances in receptor expression and, relatedly, melatonergic signalling. Some examples of neuropsychiatric disorders, which may benefit from the epigenetic manipulation of melatonin receptor expression, are discussed below.

## *Psychiatric disorders*

It is well known that disruptions in the circadian rhythms of biological processes such as sleep and the secretion of hormones including melatonin are linked to mood disorders (Germain and Kupfer, 2008; Srinivasan *et al.,* 2009). Melatonin has shown efficacy in alleviating insomnia and sleep abnormalities in depressed patients (Ferracioli-Oda *et al.,* 2013). These beneficial effects are thought to involve the modulation of circadian function *via* melatonin receptors in the SCN, where the $MT_1$ subtype can inhibit neuronal activity while the $MT_2$ receptor plays a role in phase-shifting rhythms (Liu *et al.,* 1997). Adjunctive administration of slow-release melatonin decreased insomnia in patients with treatment-resistant depression, but it did not augment the effect of antidepressant treatment (Dalton *et al.,* 2000).

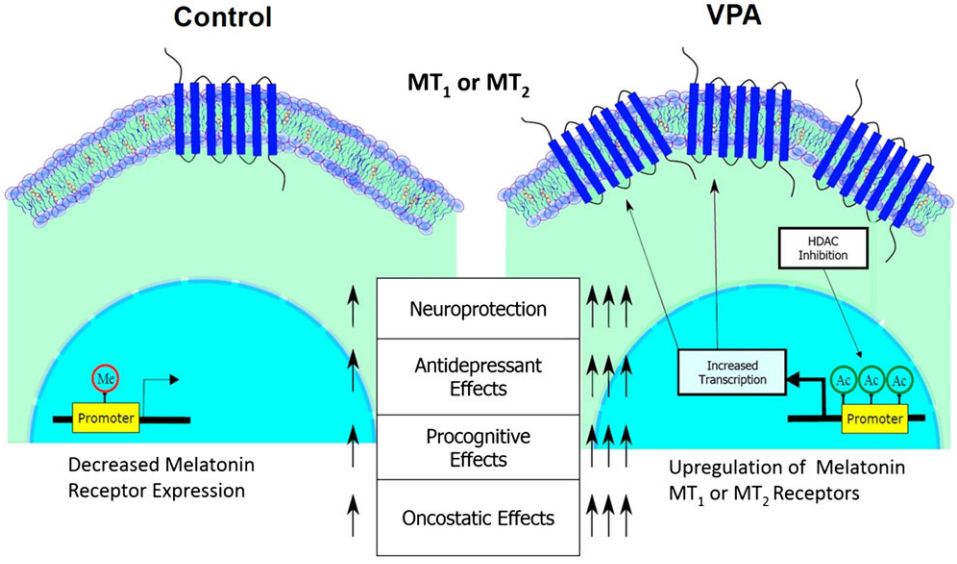


**Figure 3**

An overview of the potential therapeutic benefits of epigenetic up-regulation of melatonin receptors. While VPA is shown in this illustration, a similar epigentic up-regulation of melatonin receptors can be induced by other HDAC inhibitors.

Other studies suggest that melatonin may be beneficial in reversing symptoms associated with anxiety and depression (Hansen *et al.,* 2014), among other psychiatric conditions (Mahmood *et al.,* 2016). The melatonin analogue, agomelatine, which couples agonistic activity at $MT_1$ and $MT_2$ receptors with antagonistic action at the **5-HT$_{2c}$** receptor, is the first melatonergic agent approved for psychiatric treatment. Agomelatine has shown promise in the management of depression and other psychiatric conditions (Bourin and Prica, 2009; Spadoni *et al.,* 2011; Laudon and Frydman-Marom, 2014). Another melatonin $MT_1/MT_2$ agonist, ramelteon, improves sleep quality and duration across age groups including older insomniacs (Pandi-Perumal *et al.,* 2007; Schroeck *et al.,* 2016). These findings suggest that the melatonergic system is a worthwhile target for future psychiatric treatment strategies (Catena-Dell'Osso *et al.,* 2012).

Increasing evidence indicates that epigenetic dysfunction may contribute to psychiatric conditions, such as major depressive disorder and schizophrenia (Dogra *et al.,* 2016; Hoffmann *et al.,* 2017). As noted earlier, melatonin itself can induce histone acetylation in cultured brain-derived cells or in the mammalian brain by a mechanism that appears to involve $MT_1$ receptors and HAT (Pan and Niles, 2015). This raises the interesting possibility that combination therapy with VPA (or other HDAC inhibitors) and melatonin agonists could normalize epigenetic function with associated stabilization of brain physiology and related therapeutic effects.

### *Ageing and neurodegenerative disease*

It is well-known that melatonin acts *via* its receptors to confer an assortment of neuroprotective and neurorestorative effects in the aged and neurodegenerative brain. Melatonin receptors are depleted during physiological ageing (Sánchez-Hidalgo *et al.,* 2009), as well as in various neurodegenerative states, as mentioned previously. This reduction may contribute to the overall deterioration of the CNS in neurodegenerative disorders. Melatonin treatments have been associated with delayed disease onset and progression, as well as decreased mortality. For example, melatonin attenuates dopaminergic cell loss in the nigrostriatal pathway in the Parkinson's disease brain of animal models and restores the associated motor function deficits (Capitelli *et al.,* 2008; Ma *et al.,* 2009; Carriere *et al.,* 2015). Other studies show that melatonin administration reverses impairments in cognition by augmenting adult hippocampal neurogenesis (Chern *et al.,* 2012; Liu *et al.,* 2013). Additional support for this notion comes from the observation that chronic melatonin treatment causes enhanced dendritic maturation and arborization in the mouse hippocampus (Ramirez-Rodriguez *et al.,* 2011). This is important because increasing adult hippocampal neurogenesis has been found to be capable of improving cognition (Sahay *et al.,* 2011).

In keeping with the above, low melatonin levels and disrupted circadian rhythmicity have been found in patients with Alzheimer's disease (Wu *et al.,* 2003). Melatonin has been reported to protect against memory impairment, synaptic dysfunction and neurodegeneration, by activating the PI3K/Akt/GSK3β pathway, in a mouse model of Alzheimer's disease (Ali and Kim, 2015). The protective effects of melatonin on the aged and neurodegenerative brain further include the modulation of energy metabolism, circadian function (Jenwitheesuk *et al.,* 2014) and suppression of the accumulation of toxic-free radicals and other metabolic by-products in the CNS (Reiter *et al.,* 2003). The ability to optimize melatonergic function by restoring depleted melatonin receptor populations in the CNS holds clear therapeutic promise in ageing and neurodegeneration.

## Conclusions

GPCRs act as molecular sensors that convert extracellular stimuli into intracellular responses. The expression of melatonin receptors, as well as other GPCRs, is often depleted in the aged and/or diseased state. Although the mechanisms driving these receptor abnormalities are not yet characterized, increasing evidence suggests that epigenetic processes influence the development of several CNS diseases, which may also involve aberrant GPCR expression. VPA, as well as other HDAC inhibitors, up-regulates the expression of melatonin receptors *via* a mechanism that involves histone acetylation/chromatin remodelling with associated gene transcription. The epigenetic control of melatonin receptor expression provides a novel therapeutic strategy for offsetting the negative trajectory of melatonergic impairment associated with ageing and/or disease. However, the successful utilization of this strategy will require clarification of important issues, including which of the multiple HDAC isoforms inhibited by VPA or other HDAC inhibitors are specifically linked to the regulation of the $MT_1$ or $MT_2$ receptors, in order to permit selective targeting of melatonin receptor subtypes in future therapeutic approaches.

### *Nomenclature of targets and ligands*

Key protein targets and ligands in this article 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 (Alexander *et al.,* 2015a,b).

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## Conflict of interest

The authors declare no conflicts of interest.

## References

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.

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

Ali T, Kim MO (2015). Melatonin ameliorates amyloid beta-induced memory deficits, tau hyperphosphorylation and neurodegeneration via PI3/Akt/GSk3β pathway in the mouse hippocampus. J Pineal Res 59: 47–59.

Allen SJ, Watson JJ, Shoemark DK, Barua NU, Patel NK (2013). GDNF, NGF and BDNF as therapeutic options for neurodegeneration. Pharmacol Ther 138: 155–175.

Armstrong KJ, Niles LP (2002). Induction of GDNF mRNA expression by melatonin in rat C6 glioma cells. Neuroreport 13: 473–475.

Avery LB, Bumpus NN (2014). Valproic acid is a novel activator of AMP-activated protein kinase and decreases liver mass, hepatic fat accumulation, and serum glucose in obese mice. Mol Pharmacol 85: 1–10.

Bahna SG, Niles LP (2017). Epigenetic induction of melatonin MT 1 receptors by valproate: neurotherapeutic implications. Eur Neuropsychopharmacol 27: 828–832.

Bahna SG, Sathiyapalan A, Foster JA, Niles LP (2014). Regional upregulation of hippocampal melatonin MT receptors by valproic acid: therapeutic implications for Alzheimer's disease. Neurosci Lett 576C: 84–87.

Bain J, Plater L, Elliott M, Shpiro N, Hastie CJ, McLauchlan H *et al*. (2007). The selectivity of protein kinase inhibitors: a further update. Biochem J 408: 297–315.

Bannister AJ, Kouzarides T (2011). Regulation of chromatin by histone modifications. Cell Res 21: 381–395.

Barrett P, MacLean A, Davidson G, Morgan PJ (1996). Regulation of the Mel 1a melatonin receptor mRNA and protein levels in the ovine pars tuberalis: evidence for a cyclic adenosine 3′,5′-monophosphate-independent Mel 1a receptor coupling and an autoregulatory mechanism of expression. Mol Endocrinol 10: 892–902.

Bayraktar G, Kreutz MR (2017). Neuronal DNA methyltransferases: epigenetic mediators between synaptic activity and gene expression? Neuroscientist. https://doi.org/10.1177/1073858417707457.

Boks MP, de Jong NM, Kas MJH, Vinkers CH, Fernandes C, Kahn RS *et al*. (2012). Current status and future prospects for epigenetic psychopharmacology. Epigenetics 7: 20–28.

Boone DK, Weisz HA, Bi M, Falduto MT, Torres KEO, Willey HE *et al*. (2017). Evidence linking microRNA suppression of essential prosurvival genes with hippocampal cell death after traumatic brain injury. Sci Rep 7: 6645.

Boulle F, Massart R, Stragier E, Païzanis E, Zaidan L, Marday S *et al*. (2014). Hippocampal and behavioral dysfunctions in a mouse model of environmental stress: normalization by agomelatine. Transl Psychiatry 4: e485.

Bourin M, Prica C (2009). Melatonin receptor agonist agomelatine: a new drug for treating unipolar depression. Curr Pharm Des 15: 1675–1682.

Brunner P, Sözer-Topcular N, Jockers R, Ravid R, Angeloni D, Fraschini F *et al*. (2006). Pineal and cortical melatonin receptors MT1 and MT2 are decreased in Alzheimer's disease. Eur J Histochem 50: 311–316.

Brydon L, Roka F, Petit L, de Coppet P, Tissot M, Barrett P *et al*. (1999). Dual signaling of human mel1a melatonin receptors via G i2, G i3, and G q/11 proteins. Mol Endocrinol 13: 2025–2038.

Capitelli C, Sereniki A, Lima MM, Reksidler AB, Tufik S, Vital MA (2008). Melatonin attenuates tyrosine hydroxylase loss and hypolocomotion in MPTP-lesioned rats. Eur J Pharmacol 594: 101–108.

Carriere C, Kang N, Niles L (2015). Chronic low-dose melatonin treatment maintains nigrostriatal integrity in an intrastriatal rotenone model of Parkinson's disease. Brain Res 1633: 115–125.

Castrén E (2014). Neurotrophins and psychiatric disorders. In: Lewin G., Carter B. (eds) Neurotrophic Factors. Handbook of Experimental Pharmacology, vol 220. Springer: Berlin, Heidelberg, pp. 461–479.

Castro LMR, Gallant M, Niles LP (2005). Novel targets for valproic acid: up-regulation of melatonin receptors and neurotrophic factors in C6 glioma cells. J Neurochem 95: 1227–1236.

Catena-Dell'Osso M, Marazziti D, Rotella F, Bellantuono C (2012). Emerging targets for the pharmacological treatment of depression: focus on melatonergic system. Curr Med Chem 19: 428–437.

Cedar H, Bergman Y (2009). Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet 10: 295–304.

Chern C-M, Liao J-F, Wang Y-H, Shen Y-C (2012). Melatonin ameliorates neural function by promoting endogenous neurogenesis through the MT2 melatonin receptor in ischemic-stroke mice. Free Radic Biol Med 52: 1634–1647.

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

Comai S, Ochoa-Sanchez R, Dominguez-Lopez S, Bambico FR, Gobbi G (2015). Melancholic-Like behaviors and circadian neurobiological abnormalities in melatonin MT1 receptor knockout mice. Int J Neuropsychopharmacol 18: pyu075–pyu075.

Coppedè F (2014). The potential of epigenetic therapies in neurodegenerative diseases. Front Genet 5: 220.

Dalton EJ, Rotondi D, Levitan RD, Kennedy SH, Brown GM (2000). Use of slow-release melatonin in treatment-resistant depression. J Psychiatry Neurosci 25: 48–52.

Das A, Wallace G, Reiter RJ, Varma AK, Ray SK, Banik NL (2013). Overexpression of melatonin membrane receptors increases calcium-binding proteins and protects VSC4.1 motoneurons from glutamate toxicity through multiple mechanisms. J Pineal Res 54: 58–68.

Detich N, Bovenzi V, Szyf M (2003). Valproate induces replication-independent active DNA demethylation. J Biol Chem 278: 27586–27592.

Dogra S, Sona C, Kumar A, Yadav PN (2016). Epigenetic regulation of G protein coupled receptor signaling and its implications in psychiatric disorders. Int J Biochem Cell Biol 77: 226–239.

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.

Egger G, Liang G, Aparicio A, Jones PA (2004). Epigenetics in human disease and prospects for epigenetic therapy. Nature 429: 457–463.

Felisbino MB, Gatti MSV, Mello MLS (2014). Changes in chromatin structure in NIH 3T3 cells induced by valproic acid and trichostatin A. J Cell Biochem 115: 1937–1947.

Ferracioli-Oda E, Qawasmi A, Bloch MH (2013). Meta-analysis: melatonin for the treatment of primary sleep disorders. PLoS One 8: e63773.

Friedman RC, Farh KK-H, Burge CB, Bartel DP (2009). Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 19: 92–105.

Germain A, Kupfer DJ (2008). Circadian rhythm disturbances in depression. Hum Psychopharmacol 23: 571–585.

Ghabrash MF, Comai S, Tabaka J, Saint-Laurent M, Booij L, Gobbi G (2016). Valproate augmentation in a subgroup of patients with treatment-resistant unipolar depression. World J Biol Psychiatry 17: 165–170.

Gonin P, Buchholz CJ, Pallardy M, Mezzina M (2005). Gene therapy bio-safety: scientific and regulatory issues. Gene Ther 12 (Suppl 1): S146–S152.

Gurvich N, Tsygankova OM, Meinkoth JL, Klein PS (2004). Histone deacetylase is a target of valproic acid-mediated cellular differentiation. Cancer Res 64: 1079–1086.

Hallam KT, Olver JS, Norman TR (2005). Effect of sodium valproate on nocturnal melatonin sensitivity to light in healthy volunteers. Neuropsychopharmacology 30: 1400.

Hansen MV, Andersen LT, Madsen MT, Hageman I, Rasmussen LS, Bokmand S *et al*. (2014). Effect of melatonin on depressive symptoms and anxiety in patients undergoing breast cancer surgery: a randomized, double-blind, placebo-controlled trial. Breast Cancer Res Treat 145: 683–695.

Harrison IF, Crum WR, Vernon AC, Dexter DT (2015). Neurorestoration induced by the HDAC inhibitor sodium valproate in the lactacystin model of Parkinson's is associated with histone acetylation and up-regulation of neurotrophic factors. Br J Pharmacol 172: 4200–4215.

Hirsch-Rodriguez E, Imbesi M, Manev R, Uz T, Manev H (2007). The pattern of melatonin receptor expression in the brain may influence antidepressant treatment. Med Hypotheses 69: 120–124.

Hoffmann A, Sportelli V, Ziller M, Spengler D (2017). Epigenomics of major depressive disorders and schizophrenia: early life decides. Int J Mol Sci 18: E1711.

Hong LJ, Jiang Q, Long S, Wang H, Zhang LD, Tian Y *et al*. (2016). Valproic acid influences mtnr1a intracellular trafficking and signaling in a beta-arrestin 2-dependent manner. Mol Neurobiol 53: 1237–1246.

Houtepen LC, van Bergen AH, Vinkers CH, Boks MP (2016). DNA methylation signatures of mood stabilizers and antipsychotics in bipolar disorder. Epigenomics 8: 208–216.

Huntzinger E, Izaurralde E (2011). Gene silencing by microRNAs: contributions of translational repression and mRNA decay. Nat Rev Genet 12: 99–110.

Hwang J-Y, Aromolaran KA, Zukin RS (2017). The emerging field of epigenetics in neurodegeneration and neuroprotection. Nat Rev Neurosci 18: 347–361.

Jawed S, Kim B, Ottenhof T, Brown GM, Werstiuk ES, Niles LP (2007). Human melatonin MT1 receptor induction by valproic acid and its effects in combination with melatonin on MCF-7 breast cancer cell proliferation. Eur J Pharmacol 560: 17–22.

Jenwitheesuk A, Nopparat C, Mukda S, Wongchitrat P, Govitrapong P (2014). Melatonin regulates aging and neurodegeneration through energy metabolism, epigenetics, autophagy and circadian rhythm pathways. Int J Mol Sci 15: 16848–16884.

Johnston JD, Messager S, Ebling FJ, Williams LM, Barrett P, Hazlerigg DG (2003). Gonadotrophin-releasing hormone drives melatonin receptor down-regulation in the developing pituitary gland. Proc Natl Acad Sci U S A 100: 2831–2835.

Kim B, Rincon Castro LM, Jawed S, Niles LP (2008). Clinically relevant concentrations of valproic acid modulate melatonin MT(1) receptor, HDAC and MeCP2 mRNA expression in C6 glioma cells. Eur J Pharmacol 589: 45–48.

Kim Y, Zhang Y, Pang K, Kang H, Park H, Lee Y *et al*. (2016). Bipolar disorder associated microRNA, miR-1908-5p, regulates the expression of genes functioning in neuronal glutamatergic synapses. Exp Neurobiol 25: 296.

Kong P-J, Byun J-S, Lim S-Y, Lee J-J, Hong S-J, Kwon K-J *et al*. (2008a). Melatonin Induces Akt phosphorylation through melatonin receptor- and PI3K-dependent pathways in primary astrocytes. Korean J Physiol Pharmacol 12: 37–41.

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

Lacoste B, Angeloni D, Dominguez-Lopez S, Calderoni S, Mauro A, Fraschini F *et al*. (2015). Anatomical and cellular localization of melatonin MT and MT receptors in the adult rat brain. J Pineal Res 58: 397–417.

Laudon M, Frydman-Marom A (2014). Therapeutic effects of melatonin receptor agonists on sleep and comorbid disorders. Int J Mol Sci 15: 15924–15950.

Li Z, Li X, Chan MTV, Wu WKK, Tan D, Shen J (2017). Melatonin antagonizes interleukin-18-mediated inhibition on neural stem cell proliferation and differentiation. J Cell Mol Med. https://doi.org/10.1111/jcmm.13140.

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 Y, Ni C, Tang Y, Tian X, Zhou Y, Qian M *et al*. (2013). Melatonin attenuates isoflurane-induced acute memory impairments in aged rats. Basic Clin Pharmacol Toxicol 113: 215–220.

Lopez-Canul M, Palazzo E, Dominguez-Lopez S, Luongo L, Lacoste B, Comai S *et al*. (2015). Selective melatonin MT2 receptor ligands relieve neuropathic pain through modulation of brainstem descending antinociceptive pathways. Pain 156: 305–317.

Ma J, Shaw VE, Mitrofanis J (2009). Does melatonin help save dopaminergic cells in MPTP-treated mice? Parkinsonism Relat Disord 15: 307–314.

Mahmood D, Muhammad BY, Alghani M, Anwar J, el- Lebban N, Haider M (2016). Advancing role of melatonin in the treatment of neuropsychiatric disorders. Egypt J Basic Appl Sci 3: 203–218.

Maison C, Almouzni G (2004). HP1 and the dynamics of heterochromatin maintenance. Nat Rev Mol Cell Biol 5: 296–304.

Marchion DC, Bicaku E, Daud AI, Sullivan DM, Munster PN (2005). Valproic acid alters chromatin structure by regulation of chromatin modulation proteins. Cancer Res 65: 3815–3822.

Masliah E, Dumaop W, Galasko D, Desplats P (2013). Distinctive patterns of DNA methylation associated with Parkinson disease: identification of concordant epigenetic changes in brain and peripheral blood leukocytes. Epigenetics 8: 1030–1038.

Matheus N, Mendoza C, Iceta R, Mesonero JE, Alcalde AI (2010). Melatonin inhibits serotonin transporter activity in intestinal epithelial cells. J Pineal Res 48: 332–339.

Milutinovic S, D'Alessio AC, Detich N, Szyf M (2007). Valproate induces widespread epigenetic reprogramming which involves demethylation of specific genes. Carcinogenesis 28: 560–571.

Molteni R, Calabrese F, Pisoni S, Gabriel C, Mocaer E, Racagni G *et al*. (2010). Synergistic mechanisms in the modulation of the neurotrophin BDNF in the rat prefrontal cortex following acute agomelatine administration. World J Biol Psychiatry 11: 148–153.

Monti B, Polazzi E, Contestabile A (2009). Biochemical, molecular and epigenetic mechanisms of valproic acid neuroprotection. Curr Mol Pharmacol 2: 95–109.

Morris MJ, Monteggia LM (2013). Unique functional roles for class I and class II histone deacetylases in central nervous system development and function. Int J Dev Neurosci 31: 370–381.

Morse RH (2007). Transcription factor access to promoter elements. J Cell Biochem 102: 560–570.

Nabel CS, Kohli RM (2011). Molecular biology. Demystifying DNA demethylation. Science 333: 1229–1230.

Neitz A, Mergia E, Eysel UT, Koesling D, Mittmann T (2011). Presynaptic nitric oxide/cGMP facilitates glutamate release via hyperpolarization-activated cyclic nucleotide-gated channels in the hippocampus. Eur J Neurosci 33: 1611–1621.

Ng KY, Leong MK, Liang H, Paxinos G (2017). Melatonin receptors: distribution in mammalian brain and their respective putative functions. Brain Struct Funct. https://doi.org/10.1007/s00429-017-1439-6.

Niles LP, Armstrong KJ (2002). Modulation of GDNF expression by melatonin. Program No. 691.2. *2002. Abstract Viewer/Itinerary Planner*. Washington DC: Society for Neuroscience. Online.

Niles LP, Sathiyapalan A, Bahna S, Kang NH, Pan Y (2012). Valproic acid up-regulates melatonin MT1 and MT2 receptors and neurotrophic factors CDNF and MANF in the rat brain. Int J Neuropsychopharmacol 15: 1343–1350.

Niles LP, Pan Y, Kang S, Lacoul A (2013). Melatonin induces histone hyperacetylation in the rat brain. Neurosci Lett 541: 49–53.

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.

Pan Y, Niles LP (2015). Epigenetic mechanisms of melatonin action in human SH-SY5Y neuroblastoma cells. Mol Cell Endocrinol 402: 57–63.

Pandi-Perumal SR, Srinivasan V, Poeggeler B, Hardeland R, Cardinali DP (2007). Drug Insight: the use of melatonergic agonists for the treatment of insomnia-focus on ramelteon. Nat Clin Pract 3: 221–228.

Phiel CJ, Zhang F, Huang EY, Guenther MG, Lazar MA, Klein PS (2001). Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer, and teratogen. J Biol Chem 276: 36734–36741.

Pishva E, Rutten BPF, van den Hove D (2017). DNA methylation in major depressive disorder. Adv Exp Med Biol 978: 185–196.

Pongsa-Asawapaiboon A, Asavaritikrai P, Withyachumnarnkul B, Sumridthong A (1998). Melatonin increases nerve growth factor in mouse submandibular gland. J Pineal Res 24: 73–77.

Qiu H-M, Yang J-X, Jiang X-H, Hu X-Y, Liu D, Zhou Q-X (2015). Enhancing tyrosine hydroxylase and tryptophan hydroxylase expression and improving oxidative stress involved in the antidepressant effect of sodium valproate on rats undergoing chronic unpredicted stress. Neuroreport 26: 1145–1150.

Ramirez-Rodriguez G, Ortiz-Lopez L, Dominguez-Alonso A, Benitez-King GA, Kempermann G (2011). Chronic treatment with melatonin stimulates dendrite maturation and complexity in adult hippocampal neurogenesis of mice. J Pineal Res 50: 29–37.

Reiter RJ, Tan D-X, Mayo JC, Sainz RM, Leon J, Czarnocki Z (2003). Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. Acta Biochim Pol 50: 1129–1146.

Rezzani R, Rodella LF, Bonomini F, Tengattini S, Bianchi R, Reiter RJ (2006). Beneficial effects of melatonin in protecting against cyclosporine A-induced cardiotoxicity are receptor mediated. J Pineal Res 41: 288–295.

Rosen J, Than NN, Koch D, Poeggeler B, Laatsch H, Hardeland R (2006). Interactions of melatonin and its metabolites with the ABTS cation radical: extension of the radical scavenger cascade and formation of a novel class of oxidation products, C2-substituted 3-indolinones. J Pineal Res 41: 374–381.

Roth SY, Denu JM, Allis CD (2001). Histone acetyltransferases. Annu Rev Biochem 70: 81–120.

Saha RN, Pahan K (2006). HATs and HDACs in neurodegeneration: a tale of disconcerted acetylation homeostasis. Cell Death Differ 13: 539–550.

Sahay A, Scobie KN, Hill AS, O'Carroll CM, Kheirbek MA, Burghardt NS *et al*. (2011). Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature 472: 466–470.

Sánchez-Hidalgo M, Guerrero Montávez JM, Carrascosa-Salmoral MDP, Naranjo Gutierrez MDC, Lardone PJ, de la Lastra Romero CA (2009). Decreased MT1 and MT2 melatonin receptor expression in extrapineal tissues of the rat during physiological aging. J Pineal Res 46: 29–35.

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, Ayoub MA, Ravid R, Angeloni D, Fraschini F, Meier F *et al*. (2005). Reduced hippocampal MT2 melatonin receptor expression in Alzheimer's disease. J Pineal Res 38: 10–16.

Savaskan E, Jockers R, Ayoub M, Angeloni D, Fraschini F, Flammer J *et al*. (2007). The MT2 melatonin receptor subtype is present in human retina and decreases in Alzheimer's disease. Curr Alzheimer Res 4: 47–51.

Schmidt HD, McGinty JF, West AE, Sadri-Vakili G (2013). Epigenetics and psychostimulant addiction. Cold Spring Harb Perspect Med 3: a012047–a012047.

Schroeck JL, Ford J, Conway EL, Kurtzhalts KE, Gee ME, Vollmer KA *et al*. (2016). Review of safety and efficacy of sleep medicines in older adults. Clin Ther 38: 2340–2372.

Sharma R, Ottenhof T, Rzeczkowska PA, Niles LP (2008). Epigenetic targets for melatonin: induction of histone H3 hyperacetylation and gene expression in C17.2 neural stem cells. J Pineal Res 45: 277–284.

Shin E-J, Chung YH, Le H-LT, Jeong JH, Dang D-K, Nam Y *et al*. (2015). Melatonin attenuates memory impairment induced by Klotho gene deficiency via interactive signaling between MT2 receptor, ERK, and Nrf2-related antioxidant potential. Int J Neuropsychopharmacol. https://doi.org/10.1093/ijnp/pyu105.

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: D1054–D1068.

Spadoni G, Bedini A, Rivara S, Mor M (2011). Melatonin receptor agonists: new options for insomnia and depression treatment. CNS Neurosci Ther 17: 733–741.

Srinivasan V, Pandi-Perumal SR, Trakht I, Spence DW, Hardeland R, Poeggeler B *et al*. (2009). Pathophysiology of depression: role of sleep and the melatonergic system. Psychiatry Res 165: 201–214.

Tripathi DN, Jena GB (2010). Effect of melatonin on the expression of Nrf2 and NF-kappaB during cyclophosphamide-induced urinary bladder injury in rat. J Pineal Res 48: 324–331.

Urdinguio RG, Sanchez-Mut JV, Esteller M (2009). Epigenetic mechanisms in neurological diseases: genes, syndromes, and therapies. Lancet Neurol 8: 1056–1072.

Wang X, Michaelis EK (2010). Selective neuronal vulnerability to oxidative stress in the brain. Front Aging Neurosci 2: 12.

Wang Z, Ma C, Meng C-J, Zhu G-Q, Sun X-B, Huo L *et al*. (2012). Melatonin activates the Nrf2-ARE pathway when it protects against early brain injury in a subarachnoid hemorrhage model. J Pineal Res 53: 129–137.

Werner ER, Blau N, Thöny B (2011). Tetrahydrobiopterin: biochemistry and pathophysiology. Biochem J 438: 397–414.

Wu YH, Feenstra MG, Zhou JN, Liu RY, Torano JS, Kan HJV *et al*. (2003). Molecular changes underlying reduced pineal melatonin levels in Alzheimer disease: alterations in preclinical and clinical stages. J Clin Endocrinol Metab 88: 5898–5906.

Wu YH, Ursinus J, Zhou JN, Scheer FA, Ai-Min B, Jockers R *et al*. (2013). Alterations of melatonin receptors MT1 and MT2 in the hypothalamic suprachiasmatic nucleus during depression. J Affect Disord 148: 357–367.

Wu Y-H, Zhou J-N, Heerikhuize JV, 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.

Yokomori K (2003). SMC protein complexes and the maintenance of chromosome integrity. Curr Top Microbiol Immunol 274: 79–112.

Yuan L, Collins AR, Dai J, Dubocovich ML, Hill SM (2002). MT(1) melatonin receptor overexpression enhances the growth suppressive effect of melatonin in human breast cancer cells. Mol Cell Endocrinol 192: 147–156.

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.