

## Melatonin Antioxidative Defense: Therapeutical Implications for Aging and Neurodegenerative Processes

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**Abstract** The pineal product melatonin has remarkable antioxidant properties. It is secreted during darkness and plays a key role in various physiological responses including regulation of circadian rhythms, sleep homeostasis, retinal neuromodulation, and vasomotor responses. It scavenges hydroxyl, carbonate, and various organic radicals as well as a number of reactive nitrogen species. Melatonin also enhances the antioxidant potential of the cell by stimulating the synthesis of antioxidant enzymes including superoxide dismutase, glutathione peroxidase, and glutathione reductase, and by augmenting glutathione levels. Melatonin preserves mitochondrial homeostasis, reduces free radical generation and protects mitochondrial ATP synthesis by stimulating Complexes I and IV

activities. The decline in melatonin production in aged individuals has been suggested as one of the primary contributing factors for the development of age-associated neurodegenerative diseases. The efficacy of melatonin in preventing oxidative damage in either cultured neuronal cells or in the brains of animals treated with various neurotoxic agents, suggests that melatonin has a potential therapeutic value as a neuroprotective drug in treatment of Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), stroke, and brain trauma. Therapeutic trials with melatonin indicate that it has a potential therapeutic value as a neuroprotective drug in treatment of AD, ALS, and HD. In the case of other neurological conditions, like PD,

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the evidence is less compelling. Melatonin's efficacy in combating free radical damage in the brain suggests that it can be a valuable therapeutic agent in the treatment of cerebral edema following traumatic brain injury or stroke. Clinical trials employing melatonin doses in the range of 50–100 mg/day are warranted before its relative merits as a neuroprotective agent is definitively established.

**Keywords** Melatonin · Mitochondria · Free radicals · Oxidative stress · Aging · Parkinson's disease · Alzheimer's disease · Huntington's disease · Amyotrophic lateral sclerosis · Stroke

### Abbreviations

3xTg-AD	Triple-Tg mouse model of Alzheimer's disease
6-OHDA	6-Hydroxydopamine
AANAT	Arylalkylamine <i>N</i> -acetyltransferase
AD	Alzheimer's disease
AFMK	<i>N</i> <sup>1</sup> -acetyl- <i>N</i> <sup>2</sup> -formyl-5-methoxykynuramine
AMK	<i>N</i> <sup>1</sup> -acetyl-5-methoxykynuramine
ALS	Amyotrophic lateral sclerosis
apoE4	Apolipoprotein E4
APP	Amyloid protein precursor
ASMT	Acetylserotonin <i>O</i> -methyltransferase
AVP	Arginine vasopressin
A $\beta$	Amyloid beta
BBB	Blood brain barrier
Bcl-2	B cell lymphoma proto-oncogene protein
c3OHM	Cyclic 3-hydroxymelatonin
CaM	Calmodulin
CSF	Cerebrospinal fluid
DA	Dopamine
ETC	Electron transport chain
GABA	$\gamma$ -Aminobutyric acid
GH	Growth hormone
GPx	Glutathione peroxidase
GR	Glutathione reductase
GSH	Glutathione
GSK-3	Glycogen synthase kinase 3
HD	Huntington's disease
HIOMT	Hydroxyindole- <i>O</i> -methyl transferase
IL-1 $\beta$	Interleukin-1 $\beta$
IL-R <sub>1</sub>	Interleukin-1 receptor 1
iNOS	Inducible nitric oxide synthase
KA	Kainic acid
MAO	Monoamine oxidase
MAP	Microtubule-associated protein
MCI	Mild cognitive impairment
mHtt	Mutated huntingtin gene
MPTP	1-Methyl-4-phenyl-1,2,3,6 tetrahydropyridine
MT <sub>1</sub>	Melatonin receptor 1
MT <sub>2</sub>	Melatonin receptor 2

mtNOS	Mitochondrial nitric oxide synthase
mtPTP	Mitochondrial permeability transition pore
NMDA	<i>N</i> -methyl-D-aspartate
nNOS	Neuronal nitric oxide synthase
NOS	Nitric oxide synthase
PD	Parkinson's disease
PP	Protein phosphatase
PS1	Presenilin 1
QR2	Quinone reductase
RBD	Rapid eye movement-associated sleep behavior disorder
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SCN	Suprachiasmatic nuclei
SOD	Superoxide dismutase
Tg	Transgenic
TNF-R <sub>1</sub>	Tumor necrosis factor receptor 1
TNF- $\alpha$	Tumor necrosis factor- $\alpha$
VEGF	Vascular endothelial growth factor
VIP	Vasoactive intestinal polypeptide

### Basic Melatonin Physiology

Melatonin (*N*-acetyl-5-methoxytryptamine) is a ubiquitous substance secreted by the pineal gland of all mammals, including man. Additionally, its presence has been confirmed in many plants (Dubbels et al. 1995), Chinese herbs (Chen et al. 2003), and unicellular organisms (Balzer and Hardeland 1991; Hardeland et al. 1995). Melatonin participates in diverse functions of the body including sleep and circadian rhythm regulation, immunoregulation and may have anti-cancer actions (Pandi-Perumal et al. 2006). Melatonin is a potent free radical scavenger and regulator of redox-active enzymes (for a recent review see Galano et al. 2011).

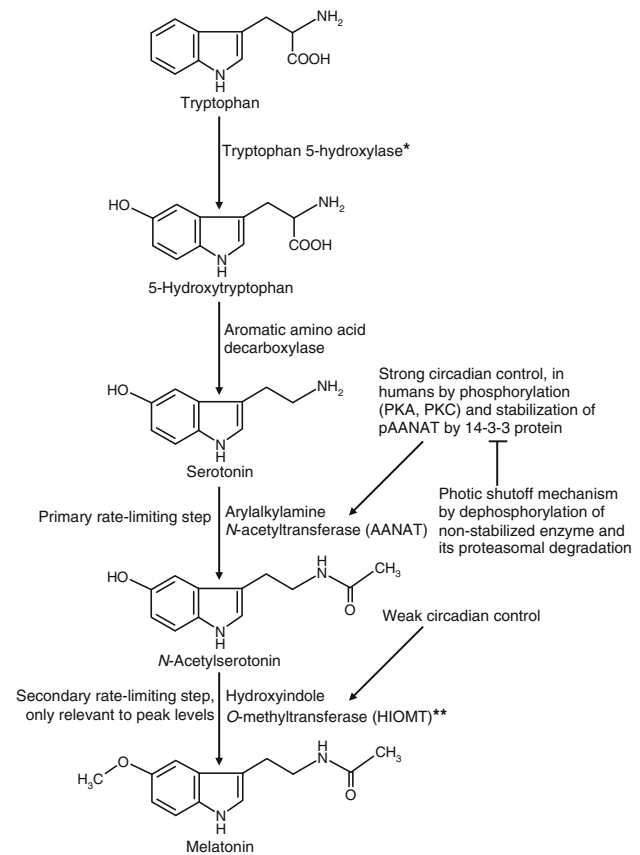
Besides the pineal gland or related structures, such as the retina, a quite a number of different organs or cells have the capability to synthesize melatonin. These include the gastrointestinal tract, bone marrow, leukocytes, membranous cochlea, Harderian gland, and, perhaps, also skin and other brain areas (Hardeland 2008, 2009a; Hardeland and Poeggeler 2007, 2008; Jimenez-Jorge et al. 2007; Tan et al. 2003). From these other sites of formation, melatonin is either poorly released or only in response to specific stimuli, e.g., as a post-prandial surge from the gastrointestinal tract (Bubenik 2002; Hardeland and Pandi-Perumal 2005; Huether et al. 1992; Huether 1993, 1994). Relative to the amounts present in the pineal gland and the circulation, the quantities of melatonin in extrapineal tissues are by no means negligible (Bubenik 2002; Huether 1993).

Melatonin is synthesized from serotonin through two enzymatic steps. A first step is the *N*-acetylation by

arylalkylamine *N*-acetyltransferase (AANAT) to yield *N*-acetylserotonin. The physiological regulation of AANAT, with its sharp increase in activity at night and very rapid decrease at light onset, has received considerable attention as the major regulatory phenomenon controlling the onset and offset of melatonin synthesis (Klein 2007). The second step in melatonin synthesis is the transfer of a methyl group from *S*-adenosylmethionine to the 5-hydroxy group of *N*-acetylserotonin to yield melatonin. This reaction is catalyzed by the enzyme hydroxyindole-*O*-methyl transferase (HIOMT), more recently called acetylserotonin *O*-methyltransferase (ASMT) in human genetic databases. Although the day/night changes of HIOMT are less prominent (Cardinali and Pévet 1998; Claustrat et al. 2005), it is now known to be responsible for the amplitude of peak levels of melatonin reached during darkness (Liu and Borjigin 2005; Ribelayga et al. 2000). Other details of melatonin biosynthetic pathway are discussed in the legend of Fig. 1.

Environmental lighting, acting through the eye in adult mammals and in part directly on the pineal in lower vertebrates and birds, has profound effects on rhythms in pineal melatonin biosynthesis. Exposure of animals to light at night rapidly depresses pineal melatonin synthesis. Based on denervation or nerve stimulation studies, a simple model of pineal regulation was envisioned, comprising two premises: (i) the neural route for environmental lighting control of melatonin secretion is the neuronal circuit “retina–retinohypothalamic tract–suprachiasmatic nuclei (SCN)–periventricular hypothalamus–intermediolateral column of the thoracic cord gray–superior cervical ganglion–internal carotid nerves–pineal gland”; (ii) norepinephrine released from sympathetic terminals at night activates postsynaptic  $\beta$ -adrenoceptors coupled to the adenylate cyclase–cAMP system, with a contribution of  $\alpha_{1B}$ -adrenergic activation of phospholipase *C* $\beta$  that leads to rises in  $Ca^{2+}$ , protein kinase C, and calmodulin (CaM) kinases (Cardinali 1983; Cardinali and Pévet 1998; Maronde and Stehle 2007). These processes jointly stimulate melatonin synthesis and release. The additional presence of central peptidergic pinealopetal pathways indicates that regulation of melatonin biosynthesis is more complex and multifactorial than commonly inferred (Cardinali 1983; Cardinali and Pévet 1998; Moller and Baeres 2002; Mukda et al. 2009; Simonneaux and Ribelayga 2003).

Once formed melatonin is not stored within the pineal gland but diffuses out into capillary blood and cerebrospinal fluid (CSF) (Tan et al. 2010). The delicate connective tissue capsule of the pineal gland does not prevent diffusion of melatonin into CSF. Inasmuch as melatonin arrives early in CSF at the 3rd ventricle as compared to the lateral ventricles. As melatonin passes through all biological membranes with ease, brain tissue may have higher melatonin levels than other tissues in the body (Tan et al.



**Fig. 1** Biosynthetic pathway of melatonin in the pineal gland.

\* In various non-mammalian species, tryptophan 5-hydroxylase can act as a rate-limiting enzyme. This may be also discussed for some extrapineal sources of melatonin. At some extrapineal sites, AANAT seems to be replaced by other, less specific *N*-acetyltransferases (NATs). In rodents, the circadian increase of AANAT activity is instead caused by strong upregulation of gene expression. The complex of a pAANAT dimer with 14-3-3 proteins is only moderately stable. Upon its dissociation, pAANAT is readily dephosphorylated. The photic shutoff is initiated by decreases in cAMP and  $Ca^{2+}$ . In humans, this leads to a lack of re-phosphorylation of AANAT subunits, in rodents it decreases pCREB (cAMP/ $Ca^{2+}$  response element-binding protein)-dependent AANAT transcription. \*\* Alternate name (also denomination of gene): acetylserotonin methyltransferase (ASMT). Again, the possibility of *O*-methylation by other, less specific *O*-methyltransferases has been discussed for some extrapineal sites

2010). Indeed melatonin levels in the CSF entering the 3rd ventricle from the pineal recess have been found to be 5–10 times higher than simultaneous blood levels (Tricoire et al. 2002). However, in most parts of the ventricular system and in the spinal canal, melatonin concentrations are much lower. Whether melatonin is taken up by the brain tissue and/or rapidly metabolized, is unknown, since determinations of melatonin levels in the CNS and brain areas have yielded highly divergent results (Hardeland 2010b).

Melatonin is involved in the control of various physiological functions in the body such as seasonal reproduction (Dardente 2012; Reiter 1980), sleep regulation (Cardinali

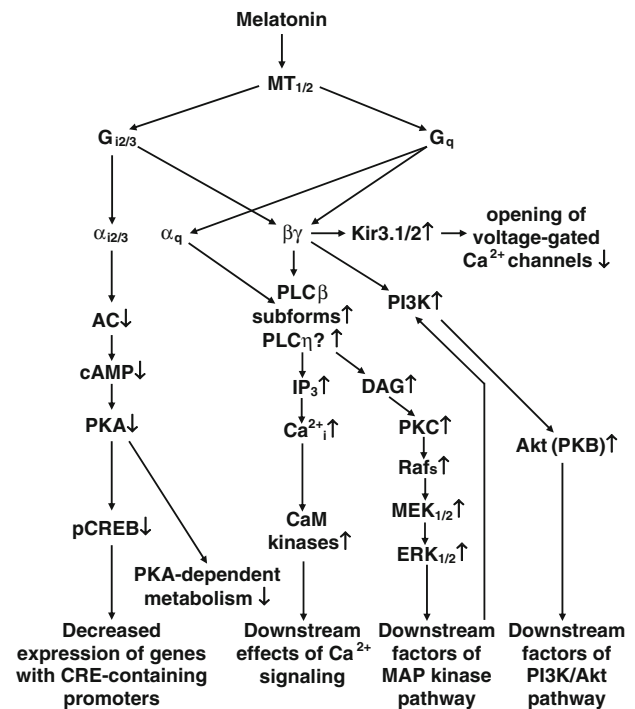
et al. 2012; Monti and Cardinali 2000; Wurtman and Zhdanova 1995), immune function (Carrillo-Vico et al. 2006; Esquifino et al. 2004; Radogna et al. 2010), inhibition of tumor growth (Blask et al. 2011; Mediavilla et al. 2010), blood pressure regulation (Domínguez-Rodríguez et al. 2010; Scheer et al. 2004), retinal physiology (Guido et al. 2010; Rosenstein et al. 2010; Scher et al. 2003), control of circadian rhythms (Dawson and Armstrong 1996), modulation of human mood and behavior (Brown et al. 2010), and free radical scavenging (Galano et al. 2011; Hardeland et al. 2011).

Melatonin participates in many of these mechanisms by acting through two G-protein-coupled membrane receptors (MT<sub>1</sub> and MT<sub>2</sub>) (Dubocovich et al. 2010). MT<sub>1</sub> and MT<sub>2</sub> receptors are found in the cell membrane as dimers and heterodimers. GPR50, a G-protein coupled melatonin receptor ortholog that does not bind melatonin itself, dimerizes with MT<sub>1</sub> receptors and can block melatonin binding (Levoye et al. 2006). As representatives of the G-protein coupled receptor family, MT<sub>1</sub> and MT<sub>2</sub> melatonin receptors act through a number of signal transduction mechanisms that ultimately result in specific physiological responses (Dubocovich et al. 2010). Figure 2 depicts an overview of major signaling pathways of the melatonin membrane receptors MT<sub>1</sub> and MT<sub>2</sub>.

In addition to binding to MT<sub>1</sub> and MT<sub>2</sub> receptors, melatonin has been shown to display affinity for another binding site, originally considered to represent a membrane-bound receptor (MT<sub>3</sub>), but ultimately confirmed to be an enzyme, quinone reductase 2 (QR2 or NQO2) (Nosjean et al. 2000). Quinone reductases are generally believed to protect against oxidative stress resulting from electron transfer reactions of quinones, but the specific role of subform QR2 is still poorly understood. Polymorphisms in the promoter of the human QR2 gene are associated with Parkinson's disease (PD) (Harada et al. 2001) and a decline in cognitive ability over time (Payton et al. 2010).

Melatonin also binds to transcription factors belonging to the retinoic acid receptor superfamily, in particular, splice variants of ROR $\alpha$  (ROR $\alpha$ 1, ROR $\alpha$ 2, and ROR $\alpha$  isoform d) and RZR $\beta$  (Lardone et al. 2011; Wiesenber et al. 1995). Retinoic acid receptor subforms are ubiquitously expressed in mammalian tissues and relatively high levels were detected especially in T and B lymphocytes, neutrophils and monocytes (Lardone et al. 2011). Although the activity of these nuclear binding proteins has for quite some time been a matter of debate and although their affinity to melatonin is lower, compared to MT<sub>1</sub>, their classification as nuclear receptors now seems to be justified (Hardeland et al. 2011).

Melatonin may also act directly on cells through its binding to CaM (Benítez-King et al. 1996), tubulin (Cardinali and Freire 1975), calreticulin and, perhaps, other



**Fig. 2** Overview of major signaling pathways of the melatonin membrane receptors MT<sub>1</sub> and MT<sub>2</sub>. The figure combines various pathways, which are not collectively present in every target cell. Moreover, MT<sub>1</sub>- and MT<sub>2</sub>-dependent signaling pathways, which are only partially identical, are combined, because of possible heterodimerization and uncertainties concerning several cell types. Additional routes or interconnections have been reported. Up- and downregulations of cGMP, by decreases of phosphodiesterase and decreased NO, respectively, require further elucidation. AC adenylyl cyclase, Akt homolog of kinase from retrovirus AKT8, Ca<sup>2+</sup> intracellular calcium, CaM calmodulin, cAMP cyclic adenosine 3',5'-monophosphate, DAG diacyl glycerol, ERK extracellular signal-regulated kinase, IP<sub>3</sub> inositol 1,4,5-tris-phosphate, Kir3.1/2 subtypes 3.1/2 of inward rectifier K<sup>+</sup> channels, MAP mitogen-activated protein kinase, MEK MAP ERK kinase, pCREB phosphorylated cAMP/Ca<sup>2+</sup> response element-binding protein, PI3K phosphoinositide 3-kinase, PLC phospholipase C, PK protein kinase, Raf homolog of retroviral kinase, the product of oncogene *v-raf*, ↑ upregulation or rise, ↓ downregulation or decrease

Ca<sup>2+</sup>-binding proteins (Macias et al. 2003). The relevance of these findings to mammalian cells largely depends on the affinity of these binding sites to melatonin, which seems to be modulated, in the case of CaM, by its interaction with CaM-activated enzymes (Landau and Zisapel 2007). Activations of protein kinase C and CaM kinases can now be explained by G $\alpha_q$ - and G $\beta\gamma$ -dependent activations of phospholipase C $\beta$  subforms and, perhaps, phospholipase C $\eta$  (Hardeland 2009a).

Based on what is known at present, melatonin's action as an antioxidant agent appears to be independent of the above mentioned receptors (León-Blanco et al. 2004). However, upregulation of the antioxidant enzyme  $\gamma$ -glutamylcysteine synthase involves nuclear transcription;

electrophoretic mobility shift assay analysis has shown melatonin-dependent increases in DNA binding of AP-1 and RZR/ROR $\alpha$  (Urata et al. 1999), indicating the involvement of a nuclear receptor. In addition findings on protection of liver and heart by the MT<sub>1</sub>/MT<sub>2</sub>-selective melatonergic agonist ramelteon under conditions leading to oxidative stress points to the inference that these membrane receptors may have antioxidant activity, mainly because ramelteon has no substantial radical scavenging properties (Mathes et al. 2008).

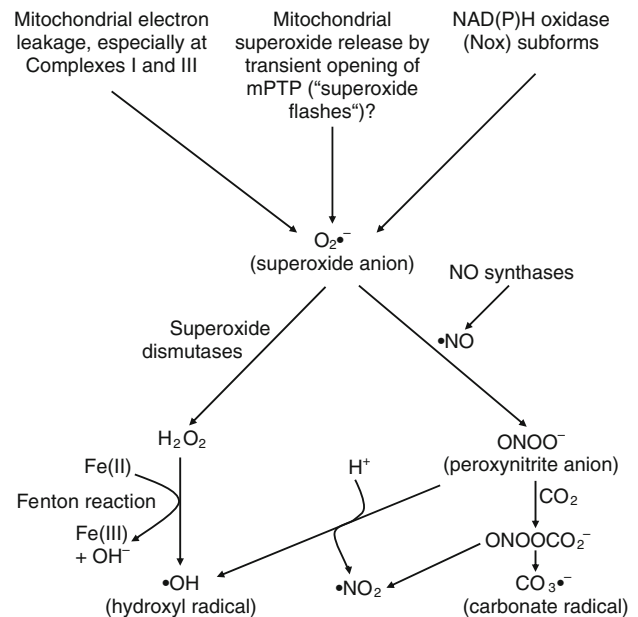
## Melatonin Effects on Mitochondria

### Free Radical Generation

The synthesis of ATP via the mitochondrial respiratory chain is the result of a proton potential generated by the electron transport chain (ETC) (for rev. see Acuña-Castroviejo et al. 2011). Although ideally all the oxygen should be reduced to water via a 4-electron reduction reaction driven by Complex IV, under normal conditions a certain, but relevant percentage of oxygen can be reduced by dissipating single electrons yielding free radicals. Approximately 3–5 % of oxygen is converted to reactive oxygen species (ROS).

The primary and quantitatively most abundant ROS produced by the ETC is the superoxide anion radical ( $O_2^{\bullet-}$ ), which is formed by electron leakage from molecular oxygen ( $O_2$ ) (Fig. 3) (Acuña-Castroviejo et al. 2011; Boveris and Boveris 2007). Multiple electron transfer leading to other ROS is negligible, but, secondarily, peroxyntrite anion ( $ONOO^-$ , formed from  $O_2^{\bullet-}$  and nitric oxide  $\bullet NO$ ), hydroxyl radical ( $\bullet OH$ , formed via  $H_2O_2$  from superoxide dismutation, or from homolysis of peroxyntrous acid,  $ONOOH$ ), carbonate radical ( $CO_3^{\bullet-}$ , formed from  $\bullet OH$  and  $HCO_3^-$ , or from  $ONOOCO_2^-$  decomposition), and nitrogen dioxide ( $\bullet NO_2$ , formed from  $ONOOH$  or  $ONOOCO_2^-$ ) are generated (Fig. 3). These other ROS and RNS (reactive nitrogen species) have a much higher reactivity than  $O_2^{\bullet-}$ . Hence, the rates at which single-electron transfer reactions generate the low-reactivity superoxide anion radical as a precursor, and, additionally, the rates at which the secondary toxic reactants are produced, are crucial to mitochondrial and cellular activity (Acuña-Castroviejo et al. 2011; Boveris and Boveris 2007; Genova et al. 2004).

Mitochondria are not only the primary site of ROS generation but also the primary target of attack for ROS and RNS (Acuña-Castroviejo et al. 2011). Damage to the mitochondrial ETC can cause breakdown of the proton potential, apoptosis or lead to further generation of free radicals maintaining a vicious cycle, which may also



**Fig. 3** Formation of most damaging free radicals: overview of the main routes leading from the primary, low-reactivity radical, the superoxide anion, to hydroxyl and peroxyntrite-derived radicals. *mPTP* mitochondrial permeability transition pore. The question mark in this field indicates a debate on the existence of superoxide flashes. Various additional routes of uncertain quantitative relevance have been omitted, e.g., on formation and reactions of the peroxyntrite radical and on the formation of other nitrosating intermediates. Moreover, hydroxyl and carbonate radicals can lead, by interaction with organic compounds, to numerous organic radicals. Well-known examples are peroxy and alkyl radicals formed by reactions with hydroxyl radicals

ultimately result in cell death either of the necrotic or the apoptotic type.

$O_2^{\bullet-}$ , once formed, quickly undergoes dismutation to  $H_2O_2$  and  $O_2$  by superoxide dismutases (SODs) in mitochondrial matrix by a subform carrying manganese in the active center (Mn-SOD). However, the affinity of  $O_2^{\bullet-}$  to  $\bullet NO$  is similarly high as that to SODs. Therefore, the availability of  $\bullet NO$  determines the rates at which the adduct, peroxyntrite, and the decomposition products from  $ONOOH$  or  $ONOOCO_2^-$ , i.e.,  $\bullet NO_2$ ,  $CO_3^{\bullet-}$  and/or  $\bullet OH$  are generated. High rates of  $\bullet NO$  synthesis, which are typical for calcium-dependent excited states of neurons and for inflammatory conditions, can contribute to oxidative and nitrosative stress.  $H_2O_2$  is converted into  $\bullet OH$  in the presence of transition metals such as  $Fe^{2+}$  and is disposed of by peroxidases, such as hemoperoxidase ("catalase") and glutathione (GSH) peroxidase (GPx) (Chance et al. 1979).

In the brain, GPx plays the dominant role in metabolizing  $H_2O_2$ . GPx uses  $H_2O_2$  and other hydroxyperoxides as substrates in the process of conversion of GSH into its oxidized form GSSG. Because GSH is required in sufficient quantities for GPx activity, auxiliary enzymes providing the reduced cosubstrate are also important,



including GSH reductase (GR) and glucose-6-phosphate dehydrogenase (which synthesize reducing equivalents for the action of GR) as well as the rate-limiting enzyme of GSH formation,  $\gamma$ -glutamylcysteine synthase (Ghanta and Chattopadhyay 2011). All these enzymes are upregulated by melatonin, either through direct or indirect mechanisms (Hardeland 2005).

Among other sources of ROS, formation of  $\text{H}_2\text{O}_2$ ,  $\text{O}_2^{\bullet-}$ , and  $\text{OCl}^-$  by microglia or invading myeloperoxidase-expressing leukocytes becomes relevant in inflammatory situations. This is not restricted to acute brain inflammation, but also plays a role in some neurodegenerative diseases (Cunningham 2011). Alzheimer's disease (AD) is an example of an atypical, lingering form of inflammation, in which some classical hallmarks such as neutrophil infiltration and edema are usually absent, whereas other characteristics including acute-phase proteins and cytokines are clearly demonstrable (Cunningham 2011). In the brain, inflammatory ROS production is intertwined with secondary excitotoxicity and RNS formation that is, e.g., demonstrable by enhanced protein nitration (for discussion see Hardeland 2009b). Various flavin enzymes also generate ROS, usually  $\text{H}_2\text{O}_2$ , sometimes accompanied by  $\text{O}_2^{\bullet-}$  production. In dopaminergic neurons, the oxidation of dopamine (DA) by monoamine oxidase (MAO) generates hydrogen peroxide. The increased destruction of DA by MAO has been postulated to be the reason for degeneration of dopaminergic neurons causing PD (Olanow 1990).

The free radical  $\bullet\text{NO}$  is produced by several subforms of nitric oxide synthase (NOS) (Pacher et al. 2007). Since  $\bullet\text{NO}$  is a gaseous compound, it can cross membranes with ease and, therefore, it enters mitochondria, regardless of its neuronal, glial or vascular origin.  $\bullet\text{NO}$  strongly interferes with components of the respiratory chain, in particular cytochrome c oxidase (Pacher et al. 2007). Moreover, its metabolite  $\text{ONOO}^-$  and radicals derived from this can damage proteins of the respiratory complexes. The existence of a separate mitochondrial NOS (mtNOS) has been described but many of the published data are debatable (Chuang 2010). In the adult mouse brain, no mtNOS was demonstrated under carefully controlled conditions (Lacza et al. 2004).  $\bullet\text{NO}$  entering neuronal mitochondria and peroxynitrite formed there by combination with  $\text{O}_2^{\bullet-}$  from electron leakage do not only interfere with the respiratory chain, at already moderate concentrations, but, at elevated levels, lead to free radical-mediated chain reactions that destroy protein, lipid, and DNA molecules.

#### Melatonin and Mitochondrial Homeostasis

Several studies have suggested that the neuroprotective role of melatonin in aging and in many neurodegenerative conditions such as AD and PD can be due to its direct antioxidant

role in mitochondrial homeostasis (Acuña-Castroviejo et al. 2011; Srinivasan et al. 2011). When acutely added in vitro, melatonin diminished the increase in respiration caused by addition of Krebs' cycle substrates and ADP to mitochondrial preparations (Reyes Toso et al. 2003). This finding demonstrates melatonin's ability to interact with the ETC (Hardeland 2005), but the possibility of pharmacological effects of electron interception by elevated melatonin levels has to be considered, which may partially block the electron flux by alternate electron acceptance. Melatonin does not affect basal mitochondrial respiration (Reyes Toso et al. 2003).

In one study the daily administration of physiological amounts of melatonin to senescence-accelerated or senescence-resistant mice over a period of 5 months was reported to increase state three respiration and respiratory control indexes in liver mitochondria (Okatani et al. 2002). These results were taken as evidence of melatonin's ability to normalize cellular respiration, to attenuate oxidant formation and, thus, to protect cells against oxidative damage.

Although mild uncoupling is usually expected to reduce electron leakage, additionally observed rises in dinitrophenol-dependent uncoupled respiration may reflect melatonin-induced changes in respiratory capacity. A long-term improvement of mitochondrial function in vivo can be explained in different ways, either in terms of interactions with the electron flux or by induction of antioxidant enzymes which prevent damage to the respiratory chain (Acuña-Castroviejo et al. 2011). Moreover, increases in gene expression of components of Complexes I and IV were observed, which may have also contributed to normalized respiration.

A low-affinity mitochondrial binding site for melatonin ( $\text{IC}_{50} = 0.8 \mu\text{M}$ ) has been associated with an inhibition of the mitochondrial permeability transition pore (mtPTP) and reported to mediate an anti-apoptotic action of melatonin at pharmacological concentrations (Andrabi et al. 2004). Under physiological conditions, this binding site would be only relevant if melatonin accumulated in sufficiently high concentrations, a fact suggested by the intramitochondrial concentrations of melatonin observed in some experiments (Venegas et al. 2012). Indeed melatonin, which possesses both hydrophilic and lipophilic properties, crosses cell membranes with ease and is capable of concentrating within subcellular compartments (Menéndez-Pelaez and Reiter 1993) including mitochondria (Martin et al. 2000). Melatonin at millimolar concentrations has been reported to stabilize the fluidity of mitochondrial inner membranes (García et al. 1999).

The binding of melatonin to mitochondrial membranes was first reported in studies using 2-[ $^{125}\text{I}$ ]-iodomelatonin (Yuan and Pang 1991). Administration of melatonin increases the activities of mitochondrial respiratory

Complexes I and IV in a time-dependent manner in brain and liver tissues (Martin et al. 2002). It has been suggested that melatonin interacts with complexes of the ETC and may donate electrons and, as an oxidized intermediate, may also accept electrons, thereby increasing the electron flow. This property, which is not shared by most antioxidants previously investigated (with the exception of ubiquinone), can enable melatonin both to participate in electron transport and to act as an antioxidant (Acuña-Castroviejo et al. 2011).

The phenomenon of electron donation by melatonin and electron acceptance by the melatonyl cation radical was used as the basis of a model proposing that melatonin could act at low, quasi-catalytic concentrations to reduce electron leakage (Hardeland 2005). Similar effects were predicted for the melatonin metabolite *N*<sup>1</sup>-acetyl-5-methoxykynuramine (AMK), with regard to its single-electron transfer reactions, reactivity and amphiphilicity (Hardeland 2005). In fact, mitochondrial protection by AMK was confirmed by the findings of Acuña-Castroviejo et al. (2002). However, melatonin-mediated modulation of electron flux can be explained by other mechanisms, including direct flux control at Complex I, prevention of ROS- and RNS-induced interruptions of electron transport, and de novo synthesis of respirasomal proteins (Acuña-Castroviejo et al. 2011).

Melatonin increases the mitochondrial GSH pool and reduces hydroxyperoxide levels. The latter effect can be regarded as the sum of indirect antioxidant effects via GSH levels and of reduction of electron leakage. By safeguarding electron flow through the transport chain melatonin increases ATP production (Acuña-Castroviejo et al. 2011). Melatonin prevents membrane lipid peroxidation and mitochondrial and nuclear DNA oxidation induced by enhanced oxidative stress and has been shown to be anti-apoptotic in several experimental model systems (Sainz et al. 2003).

Melatonin administration has been found to effectively counteract oxidative mitochondrial DNA damage and to restore the mitochondrial respiratory control system, by preventing the decrease in Complexes I and IV activities (Zhang et al. 2009). Since reduced Complex I activity is associated with and is a sign of enhanced electron leakage, the resulting increase in oxidative stress is sufficient to induce apoptosis. Melatonin's action in restoring Complex I activity back to normal levels thus assumes significance for overall health in its prevention of age-associated degenerative changes (Acuña-Castroviejo et al. 2011).

### Melatonin and Neuronal Damage

Many acute conditions, e.g., hypoxia, stroke, physical trauma, hypoglycemia, drug neurotoxicity, viruses,

radiation or noxious stimuli, are sufficient to produce neuronal damage. Similar mechanisms are also likely to be involved in neurodegenerative disorders, a group of chronic and progressive diseases that are characterized by selective and symmetric losses of neurons in motor, sensory, or cognitive systems. Clinically relevant examples of these disorders are AD, PD, amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) (Aliev et al. 2011; Green et al. 2011).

Although the origin of neurodegenerative diseases has remained mostly undefined, three major and frequently interrelated processes, i.e., glutamate excitotoxicity, free radical-mediated damage and mitochondrial dysfunction, have been identified as common pathophysiological mechanisms leading to neuronal death (Reiter 1998). Because melatonin is a direct and indirect antioxidant it has been proposed as a neuroprotective agent (Reiter et al. 2010). Melatonin's neuroprotective properties, as well as regulatory effects on circadian disturbances, validate melatonin's benefits as a therapeutic substance in the symptomatic treatment of neurodegenerative diseases. Moreover, melatonin exerts anti-excitatory, and at sufficient dosage, sedating effects (Caumo et al. 2009; Golombek et al. 1996) so that a second neuroprotective mode of action may exist involving the  $\gamma$ -aminobutyric acid (GABA)-ergic system as a mediator. This view is supported by studies indicating that melatonin protects neurons from the toxicity of the amyloid- $\beta$  (A $\beta$ ) peptide (the main neurotoxin involved in AD) via activation of GABA receptors (Louzada et al. 2004).

Melatonin also has anti-excitotoxic actions. Early studies in this regard employed kainate, an agonist of ionotropic glutamate receptors, and gave support to the hypothesis that melatonin prevents neuronal death induced by glutamate (Giusti et al. 1996b; Manev et al. 1996). It has also been reported that administration of melatonin reduces the injury of hippocampal CA1 neurons caused by transient forebrain ischemia (Cho et al. 1997; Kilic et al. 1999) or high glucocorticoid doses (Furio et al. 2008). Following a hypoxic injury in rats, melatonin administration reduced glutamate levels and structural damage caused by hypoxia to neurons, axons, and dendrites in the brainstem suggesting that it is capable of ameliorating excitotoxic damage (Kaur et al. 2011). A further demonstration that melatonin deficiency can potentiate neuronal damage is the finding that more severe brain damage and neurodegeneration occurs after stroke or excitotoxic seizures in melatonin-deficient rats (Manev et al. 1996). Pineal melatonin concentration decreased after hypoxic injuries in rats (Kaur et al. 2007).

Melatonin has often demonstrated superiority to vitamins C and E in protection against oxidative damage and in scavenging free radicals (Galano et al. 2011). Additionally, melatonin potentiates effects by other antioxidants, such as

vitamin C, Trolox (a water soluble vitamin E analog) and NADH. The antioxidative efficiency of melatonin is high because the metabolites formed by free radical scavenging also act as free radical scavengers. This holds, in particular, for cyclic 3-hydroxymelatonin, *N*<sup>1</sup>-acetyl-*N*<sup>2</sup>-formyl-5-methoxykynuramine (AFMK) (Tan et al. 2003) and, with highest potency, AMK (Behrends et al. 2004; Silva et al. 2004). Thus, the interaction of melatonin with free radicals initiates an antioxidant cascade, which may allow the elimination of, in the extreme, up to ten oxidizing free radicals.

Nevertheless, these effects are not sufficient for explaining melatonin's protective potency. Various secondary antioxidant effects have been described, which are based on upregulation of antioxidant and downregulation of prooxidant enzymes. In several studies some of these effects were highly variable, depending on the tissue and species involved, such as induction of SODs and catalase (for details see Hardeland 2005). Other effects were consistently observed; in particular, this holds for GPx and for GR, presumably in response to GPx-dependent increases in GSSG, the oxidized form of GSH. Notably, enzyme inductions were more pronounced in the CNS of avian and other non-mammalian species.

Melatonin contributes to maintain normal GSH levels (Subramanian et al. 2007) by stimulating GSH biosynthesis via  $\gamma$ -glutamylcysteine synthase and glucose-6-phosphate dehydrogenase (Kilanczyk and Bryszewska 2003; Rodríguez et al. 2004). Melatonin treatment enhanced brain GSH levels which were depressed following a hypoxic injury of developing rats (Kaur et al. 2010).

Antioxidative signaling is of particular significance because blood concentrations of melatonin are low, even at night, as compared to other antioxidants such as vitamin C or GSH (Galano et al. 2011). Although melatonin levels are several times higher in certain body fluids and tissues than in blood this may not be sufficient to fully explain the protective effects observed. Nevertheless, the much higher melatonin concentrations, e.g., in the bile (Tan et al. 1999), gastrointestinal tract (Bubenik 2002; Kvetnoy et al. 2002), or bone marrow (Conti et al. 2000) should be of significance. According to Reiter and Tan (2003) melatonin concentrations in the blood should not be taken as an index to judge its concentration in other body fluids and intracellular compartments of the cell. Inasmuch as many cells of the body have the potential to synthesize melatonin, local melatonin production could increase under high free radical generating conditions. Assuming these conditions are met, melatonin could fulfill all the requirements for designation as an effective physiological antioxidant (Galano et al. 2011).

In addition to stroke, the efficacy of melatonin in inhibiting oxidative damage has also been tested in a variety of neurological disease models where free radicals

have been implicated as being at least partial causal agents of the condition. Thus, melatonin has been shown to reduce A $\beta$  protein toxicity in AD animal models (Dragicevic et al. 2011; Matsubara et al. 2003; Olcese et al. 2009; Pappolla et al. 1997), to reduce oxidative damage in several models of PD (Acuña-Castroviejo et al. 1997; Chuang and Chen 2004; Dabbeni-Sala et al. 2001; Jin et al. 1998; Saravanan et al. 2007; Singhal et al. 2011), to protect against glutamate excitotoxicity (Das et al. 2010; Giusti et al. 1996a) and to lower neural damage due to cadmium toxicity (Jiménez-Ortega et al. 2011; Poliandri et al. 2006),  $\delta$ -aminolevulinic acid toxicity (porphyria) (Carneiro and Reiter 1998; Onuki et al. 2005; Princ et al. 1997), hyperbaric hyperoxia (Pablos et al. 1997; Shaikh et al. 1997), brain trauma (Beni et al. 2004; Kabadi and Maher 2010; Tsai et al. 2011),  $\gamma$  radiation (Erol et al. 2004; Shirazi et al. 2011; Taysi et al. 2008), focal ischemia (Kilic et al. 2011; Koh 2012; Lee et al. 2004; Tai et al. 2011), and a variety of neural toxins (Reiter et al. 2010).

The various types of toxicities listed above can result in cell death by necrosis or apoptosis. Apoptotic neuronal death requires RNA and protein synthesis, and depletion of trophic factors. Apoptosis also involves single-strand breaks of DNA. Neurotrophic factors have been found to rescue neurons from this type of death (Green et al. 2011). They may act via cellular anti-apoptotic components, such as the B cell lymphoma proto-oncogene protein (Bcl-2). The neuroprotective function of Bcl-2 is particularly well demonstrated in naturally occurring or experimentally induced neuronal death, which can be prevented by overexpression of Bcl-2 (Chuang 2010; Green et al. 2011). In the highly complex context of different roles of members of the Bcl family, Bcl-2 is capable of blocking the apoptotic pathway by preventing the formation of a functional mtPTP and, thus, the release of the mitochondrial enzyme cytochrome c, which represents the final and no-return signal of the apoptotic program (Green et al. 2011; Khandelwal et al. 2011; Kluck et al. 1997). Studies in vitro indicate that melatonin enhances expression of Bcl-2 and prevents apoptosis (Jiao et al. 2004; Koh 2011; Radogna et al. 2010; Wang 2009). More recent studies have gone beyond the functions of Bcl. Melatonin was shown to directly inhibit the opening of the mtPTP, thereby rescuing cells (Jou 2011; Peng et al. 2012). In addition to its antioxidant actions, melatonin directly diminished mtPTP currents, with an IC<sub>50</sub> of 0.8  $\mu$ M (Andrabi et al. 2004), a concentration which seems physiologically possible if mitochondrial accumulation of melatonin occurs.

Besides glutamate toxicity and oxidative stress, inflammation has been reported as another mechanism leading to damage/death of neurons in several brain pathologies including hypoxic-ischemic injuries. Activated microglia and endothelial cells under such conditions release



proinflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ), which cause damage to neurons (Floden et al. 2005). TNF- $\alpha$  has been shown to mediate neuronal death through binding with its receptor TNF-R<sub>1</sub> which has an intracellular death domain and its activation results in mitochondrial dysfunction, oxidative damage and silencing of survival signals (Nakazawa et al. 2006). IL-1 $\beta$  damages neurons by binding to its receptor IL-R<sub>1</sub> and by initiating mechanisms such as excitotoxicity and increased inducible NOS (iNOS) production through IL-R<sub>1</sub> signaling. Melatonin is known to exert anti-inflammatory actions and has been shown to reverse the inflammatory response in several brain pathologies by suppressing the production of inflammatory cytokines. Experimental studies have shown that melatonin is neuroprotective in ischemia/reperfusion injury as it inhibits the inflammatory response (Pei and Cheung 2004). Melatonin treatment has also been demonstrated to reduce the levels of proinflammatory cytokines TNF- $\alpha$  and IL-1 $\beta$  induced by A $\beta$  in rat brain (Rosales-Corral et al. 2003) and to suppress the release of these cytokines by microglia (Kaur et al., unpublished data).

Therefore, data have accumulated indicating that melatonin may curtail all major processes in neuronal damage, i.e., glutamate excitotoxicity, free radical-mediated injury, neuroinflammation and apoptosis. In addition, melatonin, in acting as an endocrine arm of the circadian clock, promotes the restorative phases of sleep, a situation associated with neurotrophic effects. Thus melatonin may function as a unique chronobiotic–cytoprotective agent (Cardinali and Scacchi 2010).

Neurodegenerative diseases have become a major health problem and a growing recognition exists that efforts to prevent these diseases must be undertaken by both governmental and non-governmental organizations. Regular intake of antioxidants by the elderly has been recommended for prevention of age-associated neurodegenerative diseases, although the efficacy of this treatment is still discussed (Hausman et al. 2011). In this context, the pineal product melatonin may have major significance since one of the features of advancing age is the gradual decrease in the endogenous synthesis of this important antioxidant (Bubenik and Konturek 2011).

## Melatonin and Aging

In humans pineal melatonin production is higher in younger age groups (18–54 years) as compared to older individuals (Karasek and Reiter 2002; Sack et al. 1986; Skene and Swaab 2003). The highest peak secretion of melatonin is found in children at 3–5 years of age (Cavallo 1993). With some exceptions (Fourtillan et al. 2001;

Zeitzer et al. 1999) the decline of melatonin production with age has been consistently reported (Brown et al. 1979; Dori et al. 1994; Ferrari et al. 2008; Girotti et al. 2000; Iguchi et al. 1982; Lieverse et al. 2011; Luboshitzky et al. 2001; Mazzoccoli et al. 2010b; Mishima et al. 2000, 2001; Rosen et al. 2009; Siegrist et al. 2001; Waldhauser and Steger 1986).

Age-associated changes in the day/night rhythm of melatonin production have been found, with phase advances being encountered more frequently in the elderly as compared to young women (Skene and Swaab 2003). It has also been shown that SCN function declines with age, particularly in patients with aging-associated neurodegenerative disorders, a major cause of dementia and other poor health condition in the elderly population (Pandi-Perumal et al. 2002; Wu and Swaab 2007). As shown in non-human primates, in addition to the age-associated attenuation of hormone levels and reduction of humoral circadian signaling, there are also significant age-related changes in intracrine processing enzymes and hormone receptors which may further affect the functional efficacy of these hormones (Urbanski and Sorwell 2011). In addition to degenerative processes in the SCN or its input and output pathways, pineal calcification can be another reason for age-related declines in melatonin secretion (Mahlberg et al. 2009).

The decline in melatonin production and altered melatonin rhythms can be major contributing factors to the increased levels of oxidative stress and the associated degenerative changes that are seen in the elderly. Nevertheless, individuals of the same chronological age can exhibit dissimilar degrees of senescence-associated functional impairment, differences which may be attributable to the well documented inter-individual variations in melatonin levels (Bergiannaki et al. 1995; Ferrari et al. 2008; Grof et al. 1985; Travis et al. 2003). Variations in the degenerative changes of cells and tissues have been attributed to variations in melatonin production, changes which are more often determined by the physiological age of an individual rather than his chronological age (Barzilai et al. 2010). Recently it has been shown that there are genetic variations in the enzyme ASMT (HIOMT), a metabolic step that determines the amount of melatonin produced. These variations have been linked to autism spectrum disorder (Jonsson et al. 2010; Melke et al. 2008), recurrent depression (Galecki et al. 2010), and intellectual disability (Pagan et al. 2011).

In terms of aging, the immunostimulatory actions of melatonin are of particularly high relevance (Cardinali et al. 2008; Guerrero and Reiter 2002; Mazzoccoli et al. 2010a). Although the importance of the age-dependent decreases in melatonin production as contributors to immunosenescence is not yet fully established, this issue

merits further research attention. Age-associated changes in the average melatonin concentration, as well as in the amplitude of its circadian rhythm, can have profound effects on the entire circadian system. These changes can be expected to produce a host of multiple, pleiotropic effects and, presumably, dysfunctions that could be efficiently antagonized by melatonin (Cardinali et al. 2008).

### Melatonin in AD

AD is an age-associated neurodegenerative disease that is characterized by progressive loss of cognitive function, loss of memory, and other neurobehavioral manifestations. In spite of the number of studies undertaken, its etiology remains an enigma. Many mechanisms such as genetic factors, chronic inflammation associated with cytokine release, oxidative stress, and trace element neurotoxicity have been suggested as possible underlying causes (Ehrnhoefer et al. 2011; Jucker and Walker 2011). The pathological manifestations of AD include amyloid plaques and neurofibrillary tangles. The free  $A\beta$  molecule is Fenton-reactive due to bound copper and, therefore, leads to cell death through induction of oxidative stress. Additionally,  $A\beta$  initiates flavoenzyme-dependent increases in intracellular  $H_2O_2$  and lipid peroxides, which also promote free radical generation.

AD is related to mitochondrial dysfunction and can now be viewed as having characteristics reminiscent of other mitochondrial diseases associated with pathological oxidant formation (Swerdlow 2011). Attenuation or prevention by administration of suitable antioxidants should be the basis for a strategic treatment of AD. Though vitamins E and C have been used for treatment of patients (Devore et al. 2010), the neurohormone melatonin has assumed a significant role in view of the fact that it has been shown to be an effective antioxidant in a number of transgenic mouse models of AD, as discussed below. In one of these models, 4-month-old transgenic mice exhibited increases in levels of brain thiobarbituric acid-reactive substances, and reductions in SOD and GSH content, changes that were attenuated by administration of melatonin (Feng et al. 2006). Moreover, melatonin has been shown to exhibit antifibrillogenic activities, even when fibrillogenesis was enhanced by apolipoprotein E4 (apoE4), effects which were not seen to this extent when antioxidant vitamins were applied (Poeggeler et al. 2001).

Experimentally it has been shown that suppression of serum melatonin levels in rats through exposure to constant illumination results in a number of AD-like behavioral effects and neurochemical changes (Ling et al. 2009). These included spatial memory deficits, tau hyperphosphorylation at multiple sites, activation of glycogen synthase

kinase-3 and protein kinase A, as well as suppression of protein phosphatase-1. An increased expression of endoplasmic reticulum stress-related proteins including BiP/GRP78 and CHOP/GADD153 was also taken as evidence of prominent oxidative damage and organelle lesions. All these impairments were partially attenuated by the simultaneous administration of melatonin (Ling et al. 2009). Endogenous melatonin deficiency can, therefore, contribute to the disease progression, and the beneficial effects of replacement therapy may well be warranted in AD.

Melatonin prevents the death of neuroblastoma cells exposed to  $A\beta$  polypeptide (Pappolla et al. 1997, 1999, 2000). Using murine neuroblastoma cells (N2a) (Pappolla et al. 1997) demonstrated that co-incubation of neuroblastoma cells with  $A\beta$  polypeptide and melatonin significantly reduced several features of apoptosis such as cellular shrinkage or formation of membrane blebs. Melatonin also reduced the levels of lipid peroxidation in the cultured neuroblastoma cells by scavenging free radicals released by  $A\beta$  (He et al. 2010). The neurofibrillary tangles of AD patients are composed of abnormally bundled cytoskeletal fibers, due to hyperphosphorylation of tau, a microtubule-associated protein, and of neurofilament H/M subunits, processes which lead to misfolding and accumulation of these proteins, along with disruption of microtubules. Thus, inhibition or reversal of hyperphosphorylation may be effective in preventing tauopathies.

Okadaic acid, a potent protein phosphatase inhibitor, induced cell death in neuroblastoma cells, accompanied by a striking decrease in mitochondrial metabolic activity (Benítez-King et al. 2003). Okadaic acid induces physiological and biochemical changes similar to those seen in AD. It increased the levels of 4-hydroxynonenal and decreased antioxidant enzyme activities in cultured neuronal cells (Perez et al. 2002). Either melatonin or vitamin C administration prevented the effects of okadaic acid in NIE 115 cells (Benítez-King et al. 2003). While both vitamin C and melatonin reduced free radical damage, the reduction was greater with melatonin. Although vitamin C failed to increase the levels of glutathione S-transferase and GR, melatonin increased the levels of both enzymes significantly. Melatonin also inhibited the phosphorylation and accumulation of neurofilaments.

Similar results were obtained, in neuroblastoma N2a cells, with calyculin A, an inhibitor of protein phosphatases 2A and 1, but in this study an additional activation of glycogen synthase kinase 3 (GSK-3, a redox-controlled enzyme involved in various cell regulatory mechanisms) was observed (Li et al. 2005). Apart from hyperphosphorylation and lethal oxidative stress, including decreases in SOD, melatonin also reversed GSK-3 activation, thus supporting the conclusion that the methoxyindole action was not only based on its antioxidant properties, but also on

interference with protein phosphorylation, especially by stress kinases.

Inhibition of protein phosphatase (PP)-2A and PP-1 by calyculin A-induced AD-like hyperphosphorylation of tau and spatial memory retention impairment. The administration of melatonin before injection of calyculin A could prevent calyculin A-induced synaptophysin loss, memory retention deficits, as well as hyperphosphorylation of tau and neurofilaments. Furthermore, melatonin partially reversed the phosphorylation of the catalytic subunit of PP-2A at Tyrosine 307 (Y307), a crucial site negatively regulating the activity of PP-2A, and reduced the levels of malondialdehyde, a marker of oxidative stress, induced by calyculin A (Yang et al. 2011). Melatonin's antioxidant action may be of additional importance with regard to inflammatory responses of chronically activated microglia in AD (Kitazawa et al. 2004). For a recent review on the effect of melatonin on several neurological diseases with inflammatory components, see Esposito and Cuzzocrea (2010).

In several experiments transgenic (Tg) murine models of AD have been used to assess a possible therapeutic beneficial effect of melatonin (Bedrosian et al. 2011; Dragicevic et al. 2011; Feng et al. 2006; Feng and Zhang 2004; García et al. 2010; García-Mesa et al. 2012; Matsubara et al. 2003; Olcese et al. 2009; Spuch et al. 2010). The administration of melatonin partially inhibited the expected time-dependent elevation of A $\beta$ , reduced abnormal nitration of proteins, and increased survival in the treated amyloid protein precursor (APP) Tg mice (Matsubara et al. 2003). Melatonin was found to be effective in inhibiting A $\beta$  deposition in APP 695 Tg mice, a model in which senile plaques appear in the cortex as early as 8 months of age (Feng et al. 2006; Feng and Zhang 2004). These mice display behavioral impairments and memory deficits, deficiencies that were alleviated following long-term administration of melatonin at a daily dose of 10 mg/kg. The treatment also reduced the number of apoptotic neurons (Feng et al. 2006; Feng and Zhang 2004). AAP Tg mice were also used to explore a “sundowning”-like behavior and the efficacy of melatonin to treat it (Bedrosian et al. 2011). A temporal pattern of anxiety-like behavior emerged with elevated locomotor activity relative to adult mice near the end of the dark phase which was refractory to melatonin treatment (Bedrosian et al. 2011).

In other experiments APP + presenilin 1 (PS1) double Tg mice were used. In one of these experiments APP/PS1 Tg mice receiving melatonin from 2 to 2.5 months of age to their killing at age 7.5 months exhibited reduced cognitive impairments. Decreased A $\beta$  deposition and inflammatory cytokines in hippocampus and entorhinal cortex were also found. Cortical mRNA expression of three antioxidant enzymes (SOD-1, GPx, catalase) was also

significantly reduced (Olcese et al. 2009). In a similar group of animals treated for 1 month with melatonin the analysis of isolated brain mitochondria indicated that melatonin treatment decreased mitochondrial A $\beta$  levels by two- to fourfold in different brain regions (Dragicevic et al. 2011). This was accompanied by a near complete restoration of mitochondrial respiratory rates, membrane potential, and ATP levels in isolated mitochondria. In APP-expressing neuroblastoma cells in culture, mitochondrial function was restored by melatonin or by the structurally related compounds indole-3-propionic acid or AFMK, an effect blocked by melatonin receptor antagonists indicating melatonin receptor signaling is required for the full effect (Dragicevic et al. 2011). APP/PS1 Tg mice were also used to assess the efficacy of a tacrine-melatonin hybrid to inhibit amyloid-induced cell death and amyloid burden in brain parenchyma. The efficacy of the tacrine-melatonin hybrid to reduce A $\beta$  toxicity suggested the possibility for a new potential therapeutic strategy in AD (Spuch et al. 2010).

In AAPP Tg 2576 mice fed with aluminum lactate melatonin co-administration prevented the prooxidant effect of the toxic in the hippocampus (García et al. 2010) but not its behavioral effects (García et al. 2009). The triple-Tg mouse model of AD (3xTg-AD) is the only model to exhibit both A $\beta$  and tau pathology that is characteristic of the human form (Sterniczuk et al. 2010). Melatonin was highly effective against the immunosenescence and cognitive loss that 3xTg-AD mice show (García-Mesa et al. 2012).

From all these studies it is clear that melatonin treatment gives neuroprotection against oxidative injury by maintaining the survival of both neuronal cells and glial cells. Studies carried out on cultured neuroblastoma cells in transgenic models of AD reveal that melatonin can attenuate the oxidative damage induced by A $\beta$ . Overall, they explain why clinical studies on melatonin efficacy at the early stages of AD showed significant regression of disease.

#### Melatonin Secretion in AD Patients

Circulating melatonin levels are lower in AD patients than in age-matched controls (Ferrari et al. 2000; Liu et al. 1999; Mishima et al. 1999; Ohashi et al. 1999; Skene et al. 1990; Uchida et al. 1996). Decreased CSF melatonin levels observed in AD patients have been linked to a reduced pineal melatonin synthesis rather than to dilution of the CSF (Tan et al. 2010). It is interesting to note that CSF melatonin levels are decreased even in the preclinical stages when the patients have no cognitive impairment (Braak stages I–II), thus suggesting that reduced melatonin

levels may be an early marker for the very first stages of the disease (Wu et al. 2003; Zhou et al. 2003). The decreased nocturnal melatonin levels with loss of melatonin diurnal rhythmicity may be the consequences of SCN clock gene dysfunction with altered noradrenergic regulation and depletion of the melatonin precursor serotonin by increased MAO A activity (Wu et al. 2006b). According to one study (Skene and Swaab 2003), reduced light transmission through the ocular lens or a defective retina-retinohypothalamic tract-SCN pathway are possible causes of disturbed circadian rhythmicity in AD patients.

#### Treatment of AD Patients with Melatonin

There are two reasons why it is quite convenient the use of melatonin or melatonin analogs in AD patients. AD patients show a greater breakdown of the circadian sleep/wake cycle compared to similarly aged, non demented controls. Demented patients spend their nights in a state of frequent restlessness and their days in a state of frequent sleepiness. These sleep/wake disturbances become increasingly more marked with progression of the disease (Zhong et al. 2011). Hence, replacement of melatonin levels in brain can be highly convenient in these patients. On the other hand, the bulk of information on the neuroprotective properties of melatonin derived from experimental studies turns highly desirable to employ pharmacological doses in AD patients with the aim of arresting or slowing disease's progression.

In AD patients with disturbed sleep/wake rhythms there is a greater degree of irregularities in melatonin secretion than in patients who do not exhibit such disturbances (Mishima et al. 1999). Impairments in melatonin secretion are also related to both age and severity of mental dysfunction. Other factors that are related to mental impairment are suppressed levels of nocturnal growth hormone (GH) and increases in both the mean levels and nadir values of plasma cortisol (Magri et al. 2004; Mazzoccoli et al. 2010b). Interestingly, melatonin augments GH secretion as well as prevents in animal models of glucocorticoid-induced neuronal loss in the hippocampus (Furio et al. 2008).

In several studies loss or damage of neurons in the hypothalamic SCN and other parts of the circadian timing system have been implicated in the circadian disturbances of demented patients (Hu et al. 2009; Skene and Swaab 2003; Swabb et al. 1985; van Someren 2000). The SCN of AD patients have tangles (Stopa et al. 1999). Wu et al. (2007) have shown by immunocytochemistry that both MT<sub>1</sub>-expressing neurons and arginine vasopressin (AVP)/vasoactive intestinal polypeptide (VIP)-expressing neurons in the SCN are strongly diminished in the advanced neuropathological stages of AD.

A chronobiological approach using melatonin, bright light therapy, restricted time in bed and diurnal activity has been proposed as a therapeutic alternative for the management of sleep/wake disorders in AD patients. The aim of these therapies is to improve sleep and diurnal activity and consequently to increase the quality of life in patients. The safety of melatonin treatment is high, with very few or no adverse effects. In the case of bright light therapy, however, there is a very significant risk of retinal damage from repeated exposure to the high intensities of visible light (Beatty et al. 2000; Hall and Gale 2002; Wu et al. 2006a).

Table 1 summarizes the clinical studies in AD patients published so far. An initial, preliminary examination of the sleep-promoting action of melatonin (3 mg p.o. for 21 days) was carried out in a small non-homogenous group of elderly patients with primary insomnia and with insomnia associated with dementia or depression. The investigation found that 7 out of 10 dementia patients who had sleep disorders and who were treated with melatonin (3 mg p.o. at bed time) showed decreased sundowning and reduced variability of sleep onset time (Fainstein et al. 1997). In another study, 10 individuals with mild cognitive impairment (MCI) were given 6 mg of melatonin before bedtime. Improvement was found in sleep, mood, and memory (Jean-Louis et al. 1998b).

Another study in this line of investigation evaluated the effect on AD patients with sleep disorders and sundowning agitation of daily administration of 6–9 mg melatonin for longer periods of time (2–3 years). The retrospective account of 14 AD patients after the extended period of therapy with melatonin indicated that all experienced improvements in sleep quality (Brusco et al. 2000). Sundowning, diagnosed clinically in all patients examined, was no longer detectable in 12 of them, with attenuated symptoms in the other 2 patients. Another significant observation in this study was the arrest of the cognitive and amnesic alterations expected in comparable populations of patients not receiving melatonin. This should be contrasted with the significant deterioration of clinical conditions of the disease found in patients after 1–3 years of progression of the disease. Further support for the hypothesis that melatonin could be useful to slow cognitive decay in AD was provided by a case report study which included two 79-year-old male monozygotic twins diagnosed 8 years earlier. One of them was treated with melatonin whereas his brother did not receive melatonin treatment (Brusco et al. 1998).

The potential efficacy of melatonin for treatment of AD patients is also supported by other studies. Mishima et al. (2000) administered a 6-mg dose of melatonin for 4 weeks to 7 inpatients with AD who exhibited an irregular sleep-waking cycle. Melatonin significantly reduced the

**Table 1** Studies including treatment of AD patients with melatonin

Design	Subjects	Treatment	Study's duration	Measured	Results	References
Open-label study	10 demented patients	3 mg melatonin p.o./daily at bed time	3 weeks	Daily logs of sleep and wake quality completed by caretakers	7 out of 10 dementia patients having sleep disorders treated with melatonin showed a significant decrease in sundowning and reduced variability of sleep onset time	Fainstein et al. (1997)
Open-label study	14 AD patients	9 mg melatonin p.o./daily at bed time	22–35 months	Daily logs of sleep and wake quality completed by caretakers. Neuropsychological assessment	Sundowning was not longer detectable in 12 patients and persisted, although attenuated in 2 patients. A significant improvement of sleep quality was found. Lack of progression of the cognitive and behavioral signs of the disease during the time they received melatonin	Brusco et al. (2000)
Case report	Monozygotic twins with AD of 8 years duration	One of the patients was treated with melatonin 9 mg p.o./daily at bed time	36 months	Neuropsychological assessment. Neuroimaging	Sleep and cognitive function severely impaired in the twin not receiving melatonin as compared to the melatonin-treated twin	Brusco et al. (1998)
Open-label, placebo-controlled trial	14 AD patients	6 mg melatonin p.o./daily at bed time or placebo	4 weeks	Daily logs of sleep and wake quality completed by caretakers. Actigraphy	AD patients receiving melatonin showed a significantly reduced percentage of nighttime activity compared to a placebo group	Mishima et al. (2000)
Open-label study	11 AD patients	3 mg melatonin p.o./daily at bed time	3 weeks	Daily logs of sleep and wake quality completed by the nurses	Significant decrease in agitated behaviors in all three shifts; significant decrease in daytime sleepiness	Cohen-Mansfield et al. (2000)
Open-label study	45 AD patients	6–9 mg melatonin p.o./daily at bed time	4 months	Daily logs of sleep and wake quality completed by caretakers. Neuropsychological assessment	Melatonin improved sleep and suppressed sundowning, an effect seen regardless of the concomitant medication employed	Cardinali et al. (2002)
Randomized double-blind placebo-controlled cross over study	25 AD patients	6 mg of slow-release melatonin p.o. or placebo at bed time	7 weeks	Actigraphy	Melatonin had no effect on median total time asleep, number of awakenings or sleep efficiency	Serfaty et al. (2002)
Double-blind, placebo-controlled study	20 AD patients	Placebo or 3 mg melatonin p.o./daily at bed time	4 weeks	Actigraphy. Neuropsychological assessment	Melatonin significantly prolonged the sleep time and decreased activity in the night. Cognitive function was improved by melatonin	Asayama et al. (2003)



Table 1 continued

Design	Subjects	Treatment	Study's duration	Measured	Results	References
Randomized, placebo-controlled clinical trial	157 AD patients	2.5-mg slow-release melatonin, or 10-mg melatonin or placebo at bed time	2 months	Actigraphy. Caregiver ratings of sleep quality	Non significant trends for increased nocturnal total sleep time and decreased wake after sleep onset were observed in the melatonin groups relative to placebo. On subjective measures, caregiver ratings of sleep quality showed a significant improvement in the 2.5-mg sustained-release melatonin group relative to placebo	Singer et al. (2003)
Open-label study	7 AD patients	3 mg melatonin p.o./daily at bed time	3 weeks	Actigraphy. Neuropsychological assessment	Complete remission of day/night rhythm disturbances or sundowning was seen in 4 patients, with partial remission in other 2	Mahlberg et al. (2004)
Randomized, placebo-controlled study	17 AD patients	3 mg melatonin p.o./daily at bed time (7 patients). Placebo (10 patients)	2 weeks	Actigraphy. Neuropsychological assessment	In melatonin-treated group, autographic nocturnal activity and agitation showed significant reductions compared to baseline	Mahlberg and Walther (2007)
Randomized, placebo-controlled study	50 AD patients	Morning light exposure (2,500 lux, 1 h) and 5 mg melatonin ( $n = 16$ ) or placebo ( $n = 17$ ) in the evening. Control subjects ( $n = 17$ ) received usual indoor light (150–200 lux)	10 weeks	Nighttime sleep variables, day sleep time, day activity, day: night sleep ratio, and rest-activity parameters were determined using actigraphy	Light treatment alone did not improve nighttime sleep, daytime wake, or rest-activity rhythm. Light treatment plus melatonin increased daytime wake time and activity levels and strengthened the rest-activity rhythm	Dowling et al. (2008)
Case report	68-year-old man with AD who developed rapid eye movement (REM) sleep behavior disorder	5–10 mg melatonin p.o./daily at bed time	20 months	Polysomnography	Melatonin was effective to suppress REM sleep behavior disorder	Anderson et al. (2008)
Randomized, placebo-controlled study	41 AD patients	Melatonin (8.5 mg immediate release and 1.5 mg sustained release) ( $N = 24$ ) or placebo ( $N = 17$ ) administered at 10:00 P.M.	10 days	Actigraphy	There were no significant effects of melatonin, compared with placebo, on sleep, circadian rhythms, or agitation	Gehrman et al. (2009)

percentage of nighttime activity compared to placebo. Cohen-Mansfield et al. (2000) reported the efficacy of melatonin (3 mg/day at bed time) for improving sleep and alleviating sundowning in 11 elderly AD patients. Analysis revealed a significant decrease in agitated behavior and a significant decrease in daytime sleepiness. A remarkable effect of a 3-mg dose of melatonin to reduce sundowning was reported in an open study using actigraphy to define sleep patterns in 7 AD patients (Mahlberg et al. 2004). In another study including 45 AD patients having sleep disturbances and treated for 4 months with 6–9 mg melatonin/day, a positive effect of melatonin was documented when used as an adjuvant medication to treat cognitive or behavioral consequences of AD (Cardinali et al. 2002). In a double blind study 3 mg of melatonin given for 4 weeks significantly prolonged actigraphically evaluated sleep time, decreased activity at night, and improved cognitive function in AD type of dementia (Asayama et al. 2003). In a multicenter, randomized, placebo-controlled clinical trial of two dose formulations of oral melatonin, 157 subjects with AD and nighttime sleep disturbance were randomly assigned to 1 of 3 treatment groups: placebo, 2.5-mg slow-release melatonin, or 10-mg melatonin given daily for 2 months (Singer et al. 2003). When sleep was defined by actigraphy, trends for increased nocturnal total sleep time and a decreased number of awakenings after sleep onset in the melatonin groups were observed. On subjective measures, caregiver ratings of sleep quality showed significant improvements in the 2.5-mg sustained-release melatonin group relative to placebo (Singer et al. 2003).

In a recent publication, published data concerning melatonin treatment in AD were analyzed (Cardinali et al. 2010). As summarized in Table 1, seven reports (5 open-label studies, 2 case reports) ( $N = 89$  patients) supported a possible efficacy of melatonin: sleep quality improved and in patients with AD sundowning was reduced and the progression of cognitive decay was slowed. In six double blind, randomized placebo-controlled trials 210 AD patients were examined. Sleep was objectively measured by wrist actigraphy ( $N = 5$ ) and additionally neuropsychological assessment and sleep quality were subjectively evaluated ( $N = 6$ ). In four studies ( $N = 143$ ) sleep quality increased, sundowning decreased significantly and cognitive performance improved whereas there was absence of effects in two studies ( $N = 67$ ) (Cardinali et al. 2010).

In another paper de Jonghe et al. (2010) performed a systematic search of studies published between 1985 and April 2009 on melatonin and sundowning in AD patients. All papers on melatonin treatment in dementia were retrieved. The effects of melatonin on circadian rhythm disturbances were scored by means of scoring sundowning/agitated behavior, sleep quality, and daytime functioning. A total of nine papers, including four randomized

controlled trials ( $n = 243$ ), and five case series ( $n = 87$ ) were reviewed. Two of the randomized controlled trials found a significant improvement in sundowning/agitated behavior. All five case series found an improvement. The results on sleep quality and daytime functioning were inconclusive.

Therefore, the question whether melatonin has any value in preventing or treating AD, affecting disease progression of the neuropathology and the driving mechanisms, remains unanswered. One of the problems with AD patients in fully developed pathology is the heterogeneity of the group examined. Moreover, a reduced hippocampal expression of  $MT_2$  melatonin receptors has been reported in AD patients, heralding a possible lack of response to exogenous melatonin (Savaskan et al. 2005). In addition, the diminution of  $MT_1$  receptors in the circadian apparatus at late stages the disease may explain why melatonin treatment is less effective at this stage (Wu and Swaab 2007).

Thus, early initiation of treatment can be decisive for therapeutic success (Quinn et al. 2005). MCI is an etiologically heterogeneous syndrome characterized by cognitive impairment shown by objective measures adjusted for age and education in advance of dementia (Gauthier et al. 2006). Some of these patients develop AD. As shown in Table 2, five double blind, randomized placebo-controlled trials and one open-label retrospective study ( $N = 651$ ) consistently show that the administration of daily evening melatonin improves sleep quality and cognitive performance in MCI (Cardinali et al. 2010; Furio et al. 2007; Jean-Louis et al. 1998b; Peck et al. 2004; Riemersma-van der Lek et al. 2008). Therefore, melatonin treatment could be very effective at early stages of the disease.

In any event, double-blind multicenter studies are needed to further explore and investigate the potential and usefulness of melatonin as an antidementia drug. Its apparent usefulness in symptomatic treatment, enhancing sleep quality, reducing sundowning, etc., even in a progressed state, further underlines the need for such decisive studies.

The mechanisms accounting for the therapeutic effect of melatonin in AD patients and MCI remain unknown. Melatonin treatment mainly promotes slow wave sleep in the elderly (Monti et al. 1999), and can be beneficial in AD by augmenting the restorative phases of sleep, including the augmented secretion of GH and neurotrophins. Melatonin was found to protect against the circadian changes produced by  $\beta$ -amyloid<sub>25–35</sub> microinjection in SCN of golden hamsters (Furio et al. 2002). Demonstration of the utility of melatonin treatment in correcting the biochemical pathology of AD was established in the Tg murine models, as discussed above.

**Table 2** Studies including treatment of MCI patients with melatonin

Design	Subjects	Treatment	Study's duration	Measured	Results	References
Double-blind, placebo-controlled, crossover study	10 patients with MCI	6 mg melatonin p.o./daily at bed time	10 days	Actigraphy. Neuropsychological assessment	Melatonin enhanced the rest-activity rhythm and improved sleep quality. Total sleep time unaffected. The ability to remember previously learned items improved along with a significant reduction in depressed mood	Jean-Louis et al. (1998a)
Double-blind, placebo-controlled pilot study	26 individuals with age-related MCI	1 mg melatonin p.o. or placebo at bed time	4 weeks	Sleep questionnaire and a battery of cognitive tests at baseline and at 4 weeks	Melatonin administration improved reported morning "restedness" and sleep latency after nocturnal awakening. It also improved scores on the California Verbal Learning Test-interference subtest	Peck et al. (2004)
Open-label, retrospective study	50 MCI outpatients	25 had received daily 3–9 mg of a fast-release melatonin preparation p.o. at bedtime. Melatonin was given in addition to the standard medication	9–18 months	Daily logs of sleep and wake quality. Initial and final neuropsychological assessment	Patients treated with melatonin showed significantly better performance in neuropsychological assessment. Abnormally high Beck Depression Inventory scores decreased in melatonin-treated patients, concomitantly with an improvement in wakefulness and sleep quality	Furio et al. (2007)
Randomized, double-blind, placebo-controlled study	354 individuals with age-related MCI	Prolonged release melatonin (Circadin, 2 mg) or placebo, 2 h before bedtime	3 weeks	Leeds Sleep Evaluation and Pittsburgh Sleep Questionnaires, Clinical Global Improvement scale score and quality of life	PR-melatonin resulted in significant improvements in sleep quality, morning alertness, sleep onset latency and quality of life	Wade et al. (2007)
Long-term, double-blind, placebo-controlled, 2 × 2 factorial randomized study	189 individuals with age-related cognitive decay	Long-term daily treatment with whole-day bright (1,000 lux) or dim (300 lux) light. Evening melatonin (2.5 mg) or placebo administration	1–3.5 years	Standardized scales for cognitive and noncognitive symptoms, limitations of activities of daily living, and adverse effects assessed every 6 months	Light attenuated cognitive deterioration and ameliorated depressive symptoms. Melatonin shortened sleep onset latency and increased sleep duration but adversely affected scores for depression. The combined treatment of bright light plus melatonin showed the best effects	Riemersma-van der Lek et al. (2008)
Prospective, randomized, double-blind, placebo-controlled, study	22 individuals with age-related cognitive decay	Participants received 2 months of melatonin (5 mg p.o./day) and 2 months of placebo	2 months	Sleep disorders were evaluated with the Northside Hospital Sleep Medicine Institute (NHSMI) test. Behavioral disorders were evaluated with the Yesavage Geriatric Depression Scale and Goldberg Anxiety Scale	Melatonin treatment significantly improved sleep quality scores. Depression also improved significantly after melatonin administration	Garzon et al. (2009)

Table 2 continued

Design	Subjects	Treatment	Study's duration	Measured	Results	References
Open-label, retrospective study	60 MCI outpatients	35 had received daily 3–9 mg of a fast-release melatonin preparation p.o. at bedtime. Melatonin was given in addition to the standard medication	9–24 months	Daily logs of sleep and wake quality. Initial and final neuropsychological assessment	Abnormally high Beck Depression Inventory scores decreased in melatonin-treated patients, concomitantly with an improvement in wakefulness and sleep quality. Patients treated with melatonin showed significantly better performance in neuropsychological assessment	Cardinali et al. (2010)

## Melatonin in PD

PD is a major neurodegenerative disease characterized, in its clinically relevant stages, by the progressive degeneration of DA-containing neurons in the substantia nigra pars compacta. However, PD does not start in the nigrostriatum, but rather in the brainstem or even the spinal cord of subjects who remain asymptomatic for a long period of time (Braak et al. 2004; Grinberg et al. 2010). Although the appearance of Lewy bodies (clumps of  $\alpha$ -synuclein and ubiquitin proteins in neurons which are detectable in post-mortem brain histology) can be traced back to the earlier stages, the primary etiology remains unknown.

Key symptoms, such as tremor, rigidity, bradykinesia and postural instability, develop when about 3/4 of dopaminergic cells are lost in the substantia nigra pars compacta, and consequently the smooth, coordinated regulation of striatal motor circuits is lost (Maguire-Zeiss and Federoff 2010; Tansey et al. 2007). Other, non-motor symptoms are seen in PD, and some of them, such as hyposmia, depression or rapid eye movement (REM)-associated sleep behavior disorder (RBD), can precede the onset of disease. Non-motor symptoms are often misdiagnosed and untreated although their appearance is an index of a worse prognosis and lower quality of life.

At this time, there is no treatment that will delay or stop the progression of PD, and medications currently available are mostly symptomatic. The increased incidence of age-associated neurodegenerative diseases such as PD has been attributed to the augmented generation of free radicals and the associated oxidative stress, which is enhanced in certain regions of the aging brain (Fahn and Cohen 1992; Gibson et al. 2010; Olanow 1992). Increased lipid peroxidation, decreased levels of GSH and increased iron levels occur in the brains of PD patients (Dexter et al. 1989). As the increased iron levels can promote the Fenton reaction, it seems feasible that an increased hydroxyl radical formation induces free radical damage. Free radical damage of lipids, proteins and nucleic acids have all been reported in the substantia nigra of Parkinsonian patients (Alam et al. 1997). Oxidative stress has been suggested to be the major cause of dopaminergic neuronal cell death. Exposure to high concentrations of  $H_2O_2$  that are formed during oxidation of DA by MAO may also be a major cause for destruction of dopaminergic neurons in PD (Fahn and Cohen 1992).

What is the role of melatonin in prevention and treatment of PD? The studies designed to answer this question have produced controversial findings. Some studies suggest that melatonin has beneficial effects in that it arrests the neurodegenerative changes induced in the experimental models of Parkinsonism. Others have reported adverse effects, demonstrating in experimental models of PD that melatonin exacerbates motor deficits.

Acuña-Castroviejo et al. (1997) used the neurotoxin 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP) model of PD in rats to show that melatonin could counteract MPTP-induced lipid peroxidation in striatum, hippocampal, and midbrain regions. Mayo et al. (1998) showed that when added to incubation medium containing the dopaminergic neurotoxin 6-hydroxydopamine (6-OHDA), melatonin significantly prevented the increased lipid peroxidation which normally would have occurred in cultured DA PC 12 cells. Melatonin also increased the levels of antioxidant enzymes (Mayo et al. 1998). Additionally, melatonin reduced pyramidal cell loss in the hippocampus, a cellular area which undergoes degeneration in the brains of PD patients, and which presumably causes memory deficits in patients. Thomas and Mohanakumar (2004) similarly demonstrated *in vitro* and *ex vivo* models, as well as in an *in vivo* MPTP rodent model, that melatonin had potent hydroxyl radical scavenger activity in the mouse striatum and in isolated mitochondria. In addition to these primary effects the investigators also found secondary increases in SOD activity.

The attenuation of MPTP-induced superoxide formation indicates an additional neuroprotective mechanism by melatonin. Intra-median forebrain bundle infusion of a ferrous-ascorbate-DA hydroxyl radical generating system, which causes significant depletion of striatal DA, could be significantly attenuated by melatonin administration (Borah and Mohanakumar 2009). In another study, Antolin et al. (2002) used the MPTP model and found that melatonin was effective in preventing neuronal cell death in the nigrostriatal pathway as indicated by the number of preserved DA cells, of tyrosine hydroxylase levels, and other ultrastructural features. These findings thus demonstrated that melatonin clearly prevents nigral dopaminergic cell death induced by chronic treatment with MPTP.

MPTP elicits its neurotoxic effects by increasing the amount of  $\bullet\text{NO}$  derived from iNOS. This action mainly affects DA neurons while  $\bullet\text{NO}$  derived from neuronal NOS (nNOS) has a damaging effect on dopaminergic fibers and terminals in the striatum. A future therapy for PD may require agents that inhibit the degenerative effects of iNOS in the substantia nigra pars compacta (Zhang et al. 2000). Since melatonin can effectively downregulate iNOS and prevent  $\bullet\text{NO}$  formation in the brain (Cuzzocrea et al. 1997; Escames et al. 2004) it should be regarded as a drug of potential choice for arresting the neuronal degeneration associated with PD.

MPTP, through its metabolite 1-methyl-4-phenylpyridinium, causes direct inhibition of Complex I of the mitochondrial ETC. Such an inhibition of Complex I has been reported in the substantia nigra of patients suffering from PD. By increasing Complexes I and IV activities of the mitochondrial ETC, melatonin may exert a beneficial effect

on MPTP-induced lesions (Acuña-Castroviejo et al. 2011). Melatonin also stimulates the gene expression of three antioxidant enzymes Cu/Zn-SOD, Mn-SOD, and GPx in cultured dopaminergic cells (Mayo et al. 1998).

It has been said that an abnormal assembly of the cytoskeleton is involved in the pathogenesis of neurodegenerative diseases. Lewy bodies, which are considered to be cytopathologic markers of Parkinsonism, comprise abnormal arrangements of tubulin, ubiquitin, microtubule-associated protein (MAP) 1 and MAP 2 (Vekrellis et al. 2011). Melatonin is very effective in promoting cytoskeletal rearrangements and thus may have a potential therapeutic value in the treatment of neurodegenerative diseases including Parkinsonism (Benítez-King et al. 2004).

However, other studies do not support the hypothesis that melatonin is of therapeutic benefit in Parkinsonism. For instance, reduction of melatonin by pinealectomy, or by exposure of rats to bright light, have been found to enhance recovery from experimental Parkinsonism in rats, i.e., spontaneous remission of symptoms following 6-OHDA or MPTP have been observed, whereas melatonin administration aggravated them (Willis and Armstrong 1999). The mechanism by which melatonin exacerbated motor impairments appeared to be similar to that seen caused by excess DA production and release, processes which occur in degenerating indoleamine and catecholamine containing neurons in experimental PD. Tapias et al. (2010), using a rotenone model of PD in rats, found that melatonin administration led to striatal catecholamine depletion, striatal terminal loss, and nigral DA cell loss, thus aggravating the disease.

Based on this, Willis and Armstrong (1999) suggested that a possible treatment for PD could involve the reduction of melatonin production or the pharmacological blockade of melatonin action on its receptors. Indeed, reduction of rigidity and bradykinesia through inhibition of melatonin synthesis (through the strategic application of bright light) has been found in pilot studies to reduce the incidence of insomnia as well as fragmented sleep (Willis and Armstrong 1999). These observations thus contradict published reports of melatonin's beneficial effects on Parkinsonian symptoms, and the use of melatonin as an adjunct therapy to either halt progressive degeneration or for providing symptomatic relief in PD patients has been questioned (Willis and Robertson 2004). In a study in rats using the 6-OHDA model of Parkinsonism, the administration of the melatonin receptor antagonist ML-23 totally abolished 6-OHDA-induced mortality, thus suggesting that blockade of melatonin's functions or decreasing its bioavailability may offer a novel approach for the treatment of Parkinsonism (Willis and Robertson 2004).

A possible hampering role of melatonin in PD is also suggested by epidemiological studies. Because of the lower



rates of cancer mortality/incidence in patients with PD, speculations about risk or preventive factors common to both diseases, including life-style factors (such as smoking) and genetic susceptibility have been entertained (Rod et al. 2010). Longer intervals of working night shifts is associated with reduced melatonin levels and reduced risk of PD among whereas longer hours of sleep appear to increase their risk (Schernhammer et al. 2006). While lower melatonin concentrations may predict a higher cancer risk (Stevens et al. 2011), there is also some evidence that they may be associated with a lower risk of PD.

The study of melatonin secretion in PD has revealed some interesting findings. A phase advance in nocturnal melatonin levels in levodopa-treated Parkinsonian patients was noted but this was not observed in untreated patients when compared to control subjects (Fertl et al. 1993). Similarly a phase advance of about 2 h in plasma melatonin secretion was seen in PD patients receiving dopaminergic treatment when compared to untreated patients (Bordet et al. 2003). An increase in daytime melatonin secretion was also noted in levodopa-treated patients and was suggested to be an adaptive response to neurodegeneration that could play a neuroprotective role through an antioxidant effect (Bordet et al. 2003).

The occurrence of motor fluctuations in PD was related to fluctuations in serum melatonin levels, a finding that was attributed to interactions of monoamines with melatonin in the striatal complex (Escames et al. 1996). Melatonin may exert direct motor effects through its interactions with DA and serotonin. Changes in levodopa-related motor complications may be related to changes in melatonin secretion pattern. Levodopa-related motor complications occur in nearly half of the patients with PD on completion of first 5 years of treatment (Koller 1996).

The hypothesis that melatonin has an inhibitory motor effect which is probably involved in wearing-off episode (i.e., the progressively shorter intervals during which symptoms remain adequately controlled as if the effects of medication would start to “wear off”) has been supported by some therapeutic studies. Stimulation of globus pallidus inhibited an increase in daytime plasma melatonin levels seen in PD patients as compared to healthy subjects (Catala et al. 1997) and was also reported to improve motor symptoms and complications in patients with PD (Olanow et al. 2000).

The finding that a reduced expression of melatonin  $MT_1$  and  $MT_2$  receptors occurs in amygdala and substantia nigra in patients with PD (Adi et al. 2010) suggests the involvement of the melatonergic system in the abnormal sleep mechanisms seen in PD. Indeed, melatonin has been used for treating sleep problems, insomnia, and daytime sleepiness in PD patients. The published data are summarized in Table 3.

In a study undertaken on 40 patients (11 women, 29 men; range 43–76 years) melatonin was administered for a treatment period of 2 weeks, in doses ranging from 5 mg to 50 mg/day (Dowling et al. 2005). To avoid the possibility of producing a circadian shift melatonin was administered 30 min before bedtime (circadian shifts can occur if administered melatonin is administered at any other time). All subjects were taking stable doses of antiparkinsonian medications during the course of the study. Relative to placebo, treatment with 50 mg of melatonin significantly increased night time sleep, as revealed by actigraphy. As compared to 50 mg or placebo, administration of 5 mg of melatonin was associated with significant improvement of sleep in the subjective reports. The study also found that the high dose of melatonin (50 mg) was well tolerated (Dowling et al. 2005).

In another study 18 PD patients were randomized after performing a basal polysomnography to receive melatonin (3 mg) or placebo 1 h before bedtime for 4 weeks (Medeiros et al. 2007). Subjective sleep quality was assessed by the Pittsburgh Sleep Quality Index and daytime somnolence by the Epworth Sleepiness Scale. All measures were repeated at the end of treatment. On initial assessment, 14 patients (70 %) showed poor quality sleep and eight (40 %) excessive diurnal somnolence. Increased sleep latency (50 %), REM sleep without atonia (66 %), and reduced sleep efficiency (72 %) were found in polysomnography (PSG). Sleep fragmentation tended to be more severe in patients on lower doses of levodopa although melatonin significantly improved subjective quality of sleep. The objective abnormalities remained unchanged. Motor dysfunction was not improved by the use of melatonin (Medeiros et al. 2007).

Administration of melatonin 3–12 mg at bedtime has been shown to be effective in the treatment of RBD (Table 3). This benefit has been reported in one case report (Kunz and Bes 1997), two open-label prospective case series of patients with RBD (Kunz and Bes 1999; Takeuchi et al. 2001), and two retrospective case series (Anderson and Shneerson 2009; Boeve et al. 2003). Taken together, these reports include a total of 38 patients. Thirty-one were noted to experience improvement with melatonin, two more experienced transient improvements and one seemed to worsen. Follow-up as far as 25 months was reported. PSG showed statistically significant decreases in number of R epochs without atonia and in movement time in R. This contrasted with the persistence of tonic muscle tone in R sleep seen with patients treated with clonazepam. Because of these data a recent clinical consensus recommended melatonin use in RBD at Level B, i.e., “assessment supported by sparse high grade data or a substantial amount of low-grade data and/or clinical consensus by the task force” (Aurora et al. 2010).

**Table 3** Studies including treatment of PD and RBD patients with melatonin

Design	Subjects	Treatment	Study's duration	Measured	Results	References
Open-label, placebo-controlled trial	40 PD patients	5–50 mg melatonin p.o./daily at bed time. All subjects were taking stable doses of antiparkinsonian medications	2 weeks	Actigraphy	Relative to placebo, treatment with 50 mg of melatonin significantly increased night time sleep, as revealed by actigraphy. As compared to 50 mg or placebo, administration of 5 mg of melatonin was associated with significant improvement of sleep in the subjective reports	Dowling et al. (2005)
Open-label, placebo-controlled trial	18 PD patients	3 mg melatonin p.o./daily at bed time	4 weeks	Polysomnography (PSG). Subjective evaluation by the Pittsburgh Sleep Quality Index and Epworth Sleepiness Scale	On initial assessment, 14 patients showed poor quality sleep EDS. Increased sleep latency (50 %), REM sleep without atonia (66 %), and reduced sleep efficiency (72 %) were found in PSG. Melatonin significantly improved subjective quality of sleep. Motor dysfunction was not improved by the use of melatonin	Medeiros et al. (2007)
Case report	1 RBD patient	3 mg melatonin p.o./daily at bed time	5 months	Actigraphy, PSG	Significant reduction of motor activity during sleep, as measured by actigraphy. After 2 months' treatment, PSG showed no major changes except an increase of REM sleep.	Kunz and Bes (1997)
Open-label prospective case series	6 consecutive RBD patients	3 mg melatonin p.o./daily at bed time	6 weeks	PSG	Significant PSG improvement in 5 patients within a week which extended beyond the end of treatment for weeks or months	Kunz and Bes (1999)
Open-label prospective case series	14 RBD patients	3–9 mg melatonin p.o./daily at bed time	Variable	PSG	Thirteen patients and their partners noticed a suppressing effect on problem sleep behaviors after melatonin administration. % tonic REM activity in PSG findings was decreased after melatonin administration. Melatonin concentrations in 10 RBD patients were under 30 pg/mL at maximal values, their mean 33.5 pg/mL. RBD patients with low melatonin secretion tended to respond to melatonin therapy	Takeuchi et al. (2001)
Retrospective case series	39 RBD	All initially treated with clonazepam. When melatonin was used, it was given at a 10 mg p.o./daily at bed time			21 patients continued to take clonazepam, 8 used another medication, and 4 required a combination of medications to control symptoms adequately. Zopiclone was used in 11 patients either alone or in combination. Two patients used melatonin (10 mg) and both found it effective. Combination therapy (lonazepam/gabapentin/melatonin) was used in one patient	Anderson and Shneerson (2009)
Retrospective case series	14 RBD patients	3–12 mg melatonin p.o./daily at bed time	14 months	PSG	8 patients experienced continued benefit with melatonin beyond 12 months of therapy	Boeve et al. (2003)

As mentioned above, not all the clinical studies are in the vein to support a beneficial role of melatonin in PD. Exposure to light of 1,000–1,500 lux intensity for 1–1.5 h, 1 h prior to bedtime for 2–5 weeks has been found to improve the bradykinesia and rigidity observed in 12 PD patients (Willis and Turner 2007). A reduction in agitation and psychiatric side effects were also reported in this study. The authors suggested that activation of the circadian system by antagonizing melatonin secretion with bright light has a therapeutic value for treating the symptoms of PD (Willis 2008).

It must be noted, however, that bright light has been employed in a number of studies for treating depressive symptoms (Rosenthal et al. 1984) on the basis that it could reset the phase of abnormal circadian rhythms seen in depressed patients (Lewy et al. 1984). Indeed, although evening bright light exposure produces a momentary suppression of melatonin, it actually causes a rebound increase in melatonin secretion late in the night (Beck-Friis et al. 1985). Hence, the bright light exposure may ultimately facilitate melatonin secretion rather than suppress it. The conclusion that PD is a “melatonin hyperplasia” disorder (Willis 2008) awaits further demonstration. Indeed, the decrease in the amplitude of circulating melatonin rhythm (Bordet et al. 2003) and the reduced melatonin receptor expression in the substantia nigra reported in PD patients (Adi et al. 2010) indicate a weakened melatonin signaling in PD.

Bright light effects may be indicative of circadian changes in PD. This is supported by the reduced expression of the circadian clock gene *Bmal1* in leukocytes (Cai et al. 2010b), although effects on peripheral oscillators do not necessarily allow conclusions on changes in the hypothalamic master clock. The recent finding that the mouse striatal DA receptors D1R and D2R are under circadian control (Cai et al. 2010a), can be seen as an interesting facet in this context, although circadian variations in receptor expression are by no means exceptional features.

### Melatonin in HD

Sleep disturbances are very prevalent in HD patients and can substantially impair their quality of life. They coexist with abnormal melatonin secretion as shown by examining the 24 h secretion profiles in early stage HD patients and normal controls (Aziz et al. 2009). Although mean diurnal melatonin levels were not different between the two groups, the timing of the evening rise in melatonin levels was significantly delayed by about 90 min in HD patients. Moreover, diurnal melatonin levels strongly correlated with both motor and functional impairment, indicating that melatonin levels in HD may progressively decline with advancing disease (Alders et al. 2009; Aziz et al. 2009).

Mitochondrial dysfunction occurs in HD (Chen 2011). However, although current evidence from genetic models of HD indicated the link between mutation of the huntingtin gene (mHtt) and mitochondrial dysfunction, impairment of ETC appears to be a late secondary event in disease’s evolution (Oliveira 2010). Upstream events include defective mitochondrial calcium handling and impaired ATP production. Also, transcription abnormalities affecting mitochondria composition, reduced mitochondria trafficking to synapses, and direct interference with mitochondrial structures enriched in striatal neurons, are possible mechanisms by which mHtt amplifies striatal vulnerability (Oliveira 2010).

An animal model was developed by using 3-nitropropionic acid, an inhibitor of mitochondrial Complex II. In this model, that replicated the neurochemical, histological and clinical features of HD, melatonin administration was reported to defer the clinical signs of the disease (Tunez et al. 2004). The data confirmed an early observation in rat brain homogenate treated with quinolinic acid (Southgate and Daya 1999).

Recently, a detailed examination of the neuroprotective properties of melatonin in the genetic model of HD was published (Wang et al. 2011). Melatonin delayed disease onset and prolongs lifespan in the R6/2 transgenic mouse HD model. In addition, melatonin MT<sub>1</sub> receptor levels decrease in cultured striatal cells, mouse brain, and human striatum associated with mHtt-mediated toxicity, receptor depletion becoming greater as the disease progresses. Further, MT<sub>1</sub> receptor knockdown made cells more vulnerable to cell death, whereas MT<sub>1</sub> receptor overexpression increases resistance to cell death (Wang et al. 2011). The administration of melatonin counteracted the MT<sub>1</sub> receptor depletion attributable to mHtt in vitro and in vivo. The authors concluded that functional MT<sub>1</sub> receptor depletion contributed to cell death in the genetic model of HD (Wang et al. 2011). Melatonin had little or no inhibitory effect on huntingtin fibrillogenesis (Heiser et al. 2000).

### Melatonin in ALS

ALS is a neurodegenerative disease in which motoneurons in the anterior horn of the spinal cord and motor neurons of cerebral cortex gradually degenerate. Dysfunction and premature death of these neurons causes spasticity, hyperreflexia, muscular atrophy, and generalized paralysis (Blackhall 2012). The disorder produces muscle weakness and muscular atrophy as both the upper and lower motoneurons deteriorate. In contrast, mental function remains normal. Respiratory failure is the common cause of death, occurring within 2–5 years after diagnosis. Most ALS cases occur sporadic. Among those individuals with the

familial form, roughly 20 % have a mutation in the gene for the antioxidant enzyme SOD (Synofzik et al. 2012).

Different pathological mechanisms have been suggested to contribute to cell death in ALS, independent of the underlying molecular/genetic defect. These include impaired axonal transport, mitochondrial dysfunction, neurofilament disorganization, protein aggregation, and impaired proteasome function. Also excitotoxic mechanisms contribute to ALS. Indeed, the only therapeutic drug with a marginal effect on patient survival is riluzole, an antiexcitotoxin (Cifra et al. 2012).

Since there is no known cure for ALS a number of strategies have been tested. Melatonin is one of them. However, a limited number of studies are available. Given that oxidative stress has been associated with ALS, Weishaupt et al. (2006) examined the ability of melatonin to attenuate neural damage in a genetic mouse model of ALS (SOD1G93A-transgenic mouse) and in 31 patients with sporadic ALS. In the mouse model, orally administered melatonin delayed disease progression and extended survival. In this report, disease progression was defined as the time span between the onset of hind limb tremor and premature death; this was delayed by 25 % in the melatonin-treated mice. In the ALS patients, melatonin (5 mg/kg) was applied nightly as a suppository; duration of treatment varied among the patients and ranged from 2 to 24 months. Melatonin was well tolerated with some patients reporting improved sleep quality (Weishaupt et al. 2006). Circulating protein carbonyls were reduced in the blood after more than 4 months of treatment compared to levels before treatment onset. Therefore, combining melatonin with the conventional drugs used for treatment, due to their presumed synergistic actions, may improve the outcome of ALS patients (Weishaupt et al. 2006). Additional trials with melatonin, alone and in combination with other drugs, are needed to clarify the potential benefit of melatonin in subjects with ALS.

### Melatonin and Brain Trauma

The neuropathological changes observed during neuronal injury, trauma, or stroke, have all been ascribed to an enhancement of oxidative stress which in turn induces lipid peroxidation, protein and DNA oxidation (Hall et al. 2010; Hall 2011; Lin and Lee 2009). When administered to rats kainic acid (KA), an agonist of the ionotropic glutamate receptor, causes excessive release of glutamate and consequently produces severe brain damage via *N*-methyl-D-aspartate (NMDA) receptor activation. The KA model of excitotoxicity is used as a model for studying oxidative stress during neuronal degeneration induced by brain trauma. KA ionotropic glutamate receptor activation results in

increased  $\text{Ca}^{2+}$  influx via NMDA-controlled channels causing  $\bullet\text{NO}$  production through activation of nNOS. Melatonin inhibits either glutamate- or NMDA-induced excitation (Tan et al. 1998). Melatonin iontophores have been found to counteract excitation induced by tris(2-carboxyethyl)phosphine hydrochloride, showing that the redox site of the NMDA receptor may be target of melatonin action (Escames et al. 2004). Both in vivo and in vitro studies have shown that melatonin prevents the neurodegeneration induced by kainate. Melatonin greatly reduces kainate induced oxidative damage in homogenates of cerebral cortex, cerebellum, hippocampus, hypothalamus, and striatum (Melchiorri et al. 1996).

Oxidative apoptotic cell death induced directly by glutamate in cultured hippocampal cells (HT-22) and hippocampal brain cells have been found to be reduced after melatonin administration (Lezoualc'h et al. 1996).  $\text{MT}_2$  receptors are present in the human hippocampus and presumably are involved in the mediation of these effects of melatonin (Savaskan et al. 2005). Bouslama et al. (2007) reported that melatonin administration could prevent learning disorders in brain-lesioned newborn mice. Injections of the glutamate analog ibotenate into the brains of 5-day-old mice were used to produce excitotoxic lesions resembling those of white-matter lesions that are seen in hypoxic preterm newborns. Behavioral conditioning and learning responses were also abolished by the ibotenate treatment, the administration of melatonin protecting against the development of these deficits. Melatonin also reduced ibotenate-induced lesions in white matter as confirmed by histological analysis (Bouslama et al. 2007). A recent study has shown that melatonin treatment reduced the apoptosis of oligodendrocytes, axon degeneration and vascular endothelial damage in the white matter of neonatal rats following a hypoxic injury (Kaur et al. 2010).

The neuroprotective effect of melatonin in cerebral edema has also been investigated. Edema plays a major contributing role in neurotrauma that follows traumatic brain injury (Fraser 2011). Trauma causes neuronal death by abnormal production of ROS such as superoxide radical, hydroxyl radical, and hydrogen peroxide leading to excessive lipid peroxidation. Pinealectomy or melatonin deficiency has been found to aggravate brain damage caused by stroke (Manev et al. 1996) or focal ischemia (Kilic et al. 1999). In a study of vasogenic brain edema caused by cold-induced lesions in rats, Gorgulu et al. (2001) evaluated the effect of melatonin in the development of edema and the destruction of blood brain barrier (BBB) that become manifest 24 h after injury. Melatonin was found to be effective in decreasing brain edema and restoring BBB permeability at the penumbra zone of cold injury, and in reducing the infarct area (Kabadi and Maher 2010). Ultrastructural studies showed that melatonin treatment

decreased nuclear and mitochondrial damage, axonal and myelin changes. Recently evidence that this effect was independent on membrane melatonin receptors was provided in MT<sub>1</sub>/MT<sub>2</sub> knockout mice (Kilic et al. 2011).

The pathophysiology of traumatic cerebral injury and cerebral edema has been attributed to excessive •NO release (Wada et al. 1998), a process which can induce the formation of peroxynitrite anions by reacting with superoxide anions. Melatonin prevents the toxic effects of peroxynitrite both by inhibiting •NO synthesis as well as by scavenging peroxynitrite (Cuzzocrea et al. 1997; Lin and Lee 2009; Mesenge et al. 1998). In a study of children with epilepsy under carbamazepine therapy, administration of melatonin (6–9 mg/day for 14 days) increased the activity of the antioxidant enzyme GPx in plasma, thus suggesting that melatonin exerts antioxidant activity in these patients (Gupta et al. 2004).

Besides •NO, vascular endothelial growth factor (VEGF) has been suggested as a factor underlying disruption of the BBB and subsequent edema formation (Nag et al. 2011). Following a hypoxic injury, disruption of the BBB was evidenced in many parts of the brain by an increased permeability of the blood vessels which resulted in swelling of the astrocyte processes in close association with the blood vessels. This was attributed to increased VEGF and •NO and production following the hypoxic injury (Kaur et al. 2006). The swelling of astrocytes or their processes was reduced significantly following melatonin administration. The concomitant reduction in VEGF production was thought to be responsible for a decrease in edema formation through decreased vascular permeability and reduced astrocytic swelling (Kaur et al. 2006).

A number of animal models are being used to investigate the effects of melatonin on brain injury caused by ischemia followed by reperfusion (Lin and Lee 2009). One of these models is the Mongolian gerbil which has an incomplete circle of Willis and in which bilateral ligation of the carotid arteries totally interrupts the blood supply to the forebrain. By using this model it was demonstrated that a 10-min ischemic period caused by occlusion of carotid arteries and followed by 5 min reperfusion resulted in significant increases in nitrite/nitrate levels an effect prevented by the administration of melatonin prior to occlusion inhibited •NO production (Guerrero et al. 1997). In another study melatonin reduced the cortical and striatal infarct volume by 60 and 30 %, respectively ((Borlongan et al. 2000). In addition to reducing infarct volume, melatonin also decreased DNA double and single-strand breaks and enhanced cell viability in the penumbral area of the infarct (Cheung 2003). In a meta-analysis of 14 studies involving 432 animals examined in different models of focal cerebral ischemia, melatonin was found equally effective in permanent or temporary ischemia, thus

suggesting that melatonin should be considered as a candidate neuroprotective drug for the treatment of human stroke (Macleod et al. 2005). Indeed, a cross-sectional matched case–control analysis indicated an impaired nocturnal melatonin secretion in the acute phase of ischemic stroke in humans (Atanassova et al. 2009).

### Melatonin Versus Melatonin Analogs

As melatonin exhibits both hypnotic and chronobiotic properties, it has been used for treatment of age-related insomnia as well as of other primary and secondary insomnia. A recent consensus of the British Association for Psychopharmacology on evidence-based treatment of insomnia, parasomnia and circadian rhythm sleep disorders concluded that melatonin is the first choice treatment when a hypnotic is indicated in patients over 55 years (Wilson et al. 2010). Melatonin has also been successfully used for treatment of sleep problems related to perturbations of the circadian time keeping system like those caused by jet-lag, shift-work disorder, or delayed sleep phase syndrome (Arendt et al. 1997; Srinivasan et al. 2010).

Since melatonin has a short half-life (less than 30 min) its efficacy in promoting and maintaining sleep has not been uniform in the studies undertaken so far. Thus the need for the development of prolonged release preparations of melatonin or of melatonin agonists with a longer duration of action on sleep regulatory structures in the brain arose (Turek and Gillette 2004). Slow-release forms of melatonin (e.g., Circadin<sup>®</sup>, a 2-mg preparation developed by Neurim, Tel Aviv, Israel, and approved by the European Medicines Agency in 2007) and the melatonin analogs ramelteon, agomelatine, tasimelteon and TK-301 are examples of this strategy.

Ramelteon (Rozerem<sup>®</sup>, Takeda Pharmaceuticals, Japan) is a melatonergic hypnotic analog approved by the FDA for treatment of insomnia in 2005. It is a selective agonist for MT<sub>1</sub>/MT<sub>2</sub> receptors without significant affinity for other receptor sites (Miyamoto 2009). In vitro binding studies have shown that ramelteon affinity for MT<sub>1</sub> and MT<sub>2</sub> receptors is 3–16 times higher than that of melatonin.

Agomelatine (Valdoxan<sup>®</sup>, Servier, France) is a recently introduced melatonergic antidepressant, acts on both MT<sub>1</sub> and MT<sub>2</sub> melatonergic receptors with a similar affinity to that of melatonin (IC<sub>50</sub> 1.3 × 10<sup>-10</sup> and 4.7 × 10<sup>-10</sup> M, respectively) and also acts as an antagonist to 5-HT<sub>2C</sub> receptors at three orders of magnitude greater concentration (IC<sub>50</sub> 2.7 × 10<sup>-7</sup> M) (Millan et al. 2003). Agomelatine has been licensed by EMEA for treatment of major depressive disorder at doses of 25–50 mg/day.

Tasimelteon (VES-162) is a MT<sub>1</sub>/MT<sub>2</sub> agonist developed by Vanda Pharmaceuticals that completed phase III



trial in 2010. In animal studies, tasimelteon exhibited the circadian phase shifting properties of melatonin. In clinical studies involving healthy human subjects, tasimelteon was administered at doses of 10–100 mg/day (Rajaratnam et al. 2009). The FDA granted tasimelteon orphan drug designation status for blind individuals without light perception with non-24-h sleep–wake disorder in 2010.

TIK-301 (formerly LY-156,735) has been in a phase II clinical trial in the USA since 2002. Originally it was developed by Eli Lilly and Company and called LY-156,735. In 2007 Tikvah Pharmaceuticals took over the development and named it TIK-301. It is a chlorinated derivative of melatonin with MT<sub>1</sub>/MT<sub>2</sub> agonist activity and 5HT<sub>2C</sub> antagonist activity. TIK-301 pharmacokinetics, pharmacodynamics and safety have been examined in a placebo-controlled study using 20–100 mg/day doses in healthy volunteers (Mulchahey et al. 2004). The FDA granted TIK-301 orphan drug designation in 2004, to use as a treatment for circadian rhythm sleep disorder in blind individuals without light perception and individuals with tardive dyskinesia.

As shown by the binding affinities, half-life and relative potencies of the different melatonin agonists it is clear that studies using 2–5 mg melatonin/day are probably unsuitable to give appropriate comparison with the effect of ramelteon, agomelatine, tasimelteon or TIK-301, which in addition to being generally more potent than the native molecule are employed in considerably higher amounts (Cardinali et al. 2012).

Several other compounds are being investigated with relatively more selective activity on melatonin receptor subtypes, therefore heralding an interesting future era for melatonin agonist research (Hardeland 2010a; Spadoni et al. 2011).

## Conclusions

Oxidative stress has been implicated in the pathogenesis of a number of neurodegenerative diseases such as AD, PD, HD, and ALS, and of pathologic neurological conditions such as stroke and brain trauma. A high oxygen consumption rate coupled with a low anti-oxidant potential of the brain are the main causes for enhanced oxidative stress of the brain in aged individuals.

Melatonin is an effective antioxidant both in vitro and in vivo in experimental models of neurodegenerative diseases. Melatonin has been shown to arrest neuropathological changes not only by scavenging free radicals but also by increasing the antioxidant enzymes and by attenuating free radical formation by neuronal, astrocyte, and microglial cells. At both physiological and pharmacological concentrations, melatonin increases gene expression

and the activities of antioxidant enzymes, e.g. GPx, GR, and SODs. Melatonin also increases the efficiency of mitochondrial ETC by enhancing Complex I and IV activity in brain tissues. Through all of these mechanisms melatonin prevents nuclear and mitochondrial DNA cleavage and apoptosis induced by neurotoxic agents such as A $\beta$ , kainate or quinolinic acid. Melatonin also inhibits NMDA-induced neuronal excitation by inhibiting nNOS and by redox site modulation.

The efficacy of melatonin in preventing oxidative damage in either cultured neuronal cells or in the brains of animals treated with various neurotoxic agents, suggests that melatonin has a potential therapeutic value as a neuroprotective drug in treatment of AD, HD, ALS, and brain trauma. In the case of other neurological conditions, like PD, the evidence is less compelling. Inasmuch as melatonin deficiency seems to be one of the major causes of the development of neurodegenerative diseases, intake of melatonin or its synthetic analogs (selected according to their chronobiotic and/or antioxidant actions) can be recommended not only for arresting the progress of this disease but also for preventing its occurrence as well.

It must be noted that the doses of melatonin employed in humans may be unnecessarily low when one takes into consideration the binding affinities, half-life and relative potencies of the different melatonin agonists on the market. In addition to being generally more potent than the native molecule these analogs are employed in considerably higher amounts (Cardinali et al. 2011). Licensed doses of ramelteon vary from 8 to 32 mg/day while agomelatine has been licensed for treatment of major depressive disorder at doses of 25–50 mg/day. In clinical studies involving healthy human subjects, tasimelteon was administered at doses of 10–100 mg/day (Rajaratnam et al. 2009) and TIK-301 pharmacokinetics, pharmacodynamics and safety have been examined in a placebo-controlled study using 20–100 mg/day (Mulchahey et al. 2004).

Melatonin has a high safety profile and it is usually remarkably well tolerated. In some studies melatonin has been administered at large doses to patients (Chahbouni et al. 2010; Voordouw et al. 1992; Waldhauser et al. 1984; Weishaupt et al. 2006). Therefore, further studies employing melatonin doses in the 100 mg/day are needed to clarify its potential therapeutical implications in humans. From animal studies it is clear that a number of preventive effects of melatonin, like those in neurodegenerative disorders, need high doses of melatonin to become apparent. If one expects melatonin to be an effective neuroprotector it is likely that the low doses of melatonin employed so far (2–5 mg/day) are not very beneficial.

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Corporation. He declares that he has no competing interests that might be perceived to influence the content of this article. All remaining authors declare that they have no proprietary, financial, professional, nor any other personal interest of any nature or kind in any product or services and/or company that could be construed or considered to be a potential conflict of interest that might have influenced the views expressed in this manuscript.

## References

- Acuña-Castroviejo D, Coto-Montes A, Gaia MM, Ortiz GG, Reiter RJ (1997) Melatonin is protective against MPTP-induced striatal and hippocampal lesions. *Life Sci* 60:L23–L29
- Acuña-Castroviejo CD, Escames G, Carazo A, León J, Khaldy H, Reiter RJ (2002) Melatonin, mitochondrial homeostasis and mitochondrial-related diseases. *Curr Top Med Chem* 2:133–151
- Acuña-Castroviejo CD, Lopez LC, Escames G, Lopez A, García JA, Reiter RJ (2011) Melatonin-mitochondria interplay in health and disease. *Curr Top Med Chem* 11:221–240
- 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* 16:61–67
- Alam ZI, Jenner A, Daniel SE, Lees AJ, Cairns N, Marsden CD, Jenner P, Halliwell B (1997) Oxidative DNA damage in the parkinsonian brain: an apparent selective increase in 8-hydroxyguanine levels in substantia nigra. *J Neurochem* 69:1196–1203
- Alders J, Smits M, Kremer B, Naarding P (2009) The role of melatonin in sleep disturbances in end-stage Huntington's disease. *J Neuropsychiatry Clin Neurosci* 21:226–227
- Aliev G, Li Y, Palacios HH, Obrenovich ME (2011) Oxidative stress induced mitochondrial DNA deletion as a hallmark for the drug development in the context of the cerebrovascular diseases. *Recent Pat Cardiovasc Drug Discov* 6:222–241
- Anderson KN, Shneerson JM (2009) Drug treatment of REM sleep behavior disorder: the use of drug therapies other than clonazepam. *J Clin Sleep Med* 5:235–239
- Anderson KN, Jamieson S, Graham AJ, Shneerson JM (2008) REM sleep behaviour disorder treated with melatonin in a patient with Alzheimer's disease. *Clin Neurol Neurosurg* 110:492–495
- Andrabi SA, Sayeed I, Siemen D, Wolf G, Horn TF (2004) Direct inhibition of the mitochondrial permeability transition pore: a possible mechanism responsible for anti-apoptotic effects of melatonin. *FASEB J* 18:869–871
- Antolin I, Mayo JC, Sainz RM, del Brio ML, Herrera F, Martín V, Rodríguez C (2002) Protective effect of melatonin in a chronic experimental model of Parkinson's disease. *Brain Res* 943:163–173
- Arendt J, Skene DJ, Middleton B, Lockley SW, Deacon S (1997) Efficacy of melatonin treatment in jet lag, shift work, and blindness. *J Biol Rhythms* 12:604–617
- Asayama K, Yamadera H, Ito T, Suzuki H, Kudo Y, Endo S (2003) Double blind study of melatonin effects on the sleep-wake rhythm, cognitive and non-cognitive functions in Alzheimer type dementia. *J Nippon Med Sch* 70:334–341
- Atanassova PA, Terzieva DD, Dimitrov BD (2009) Impaired nocturnal melatonin in acute phase of ischaemic stroke: cross-sectional matched case-control analysis. *J Neuroendocrinol* 21:657–663
- Aurora RN, Zak RS, Maganti RK, Auerbach SH, Casey KR, Chowdhuri S, Karippot A, Ramar K, Kristo DA, Morgenthaler TI (2010) Best practice guide for the treatment of REM sleep behavior disorder (RBD). *J Clin Sleep Med* 6:85–95
- Aziz NA, Pijl H, Frolich M, Schroder-van der Elst JP, van der BC, Roelfsema F, Roos RA (2009) Delayed onset of the diurnal melatonin rise in patients with Huntington's disease. *J Neurol* 256:1961–1965
- Balzer I, Hardeland R (1991) Photoperiodism and effects of indoleamines in a unicellular alga, *Gonyaulax polyedra*. *Science* 253:795–797
- Barzilai N, Gabriely I, Atzmon G, Suh Y, Rothenberg D, Bergman A (2010) Genetic studies reveal the role of the endocrine and metabolic systems in aging. *J Clin Endocrinol Metab* 95:4493–4500
- Beatty S, Koh H, Phil M, Henson D, Boulton M (2000) The role of oxidative stress in the pathogenesis of age-related macular degeneration. *Surv Ophthalmol* 45:115–134
- Beck-Friis J, Kjellman BF, Aperia B, Unden F, von Rosen D, Ljunggren JG, Wetterberg L (1985) Serum melatonin in relation to clinical variables in patients with major depressive disorder and a hypothesis of a low melatonin syndrome. *Acta Psychiatr Scand* 71:319–330
- Bedrosian TA, Herring KL, Weil ZM, Nelson RJ (2011) Altered temporal patterns of anxiety in aged and amyloid precursor protein (APP) transgenic mice. *Proc Natl Acad Sci USA* 108:11686–11691
- Behrends A, Hardeland R, Ness H, Grube S, Poeggeler B, Haldar C (2004) Photocatalytic actions of the pesticide metabolite 2-hydroxyquinoxaline: destruction of antioxidant vitamins and biogenic amines—implications of organic redox cycling. *Redox Rep* 9:279–288
- Beni SM, Kohen R, Reiter RJ, Tan DX, Shohami E (2004) Melatonin-induced neuroprotection after closed head injury is associated with increased brain antioxidants and attenuated late-phase activation of NF-kappaB and AP-1. *FASEB J* 18:149–151
- Benítez-King G, Ríos A, Martínez A, Anton-Tay F (1996) In vitro inhibition of Ca<sup>2+</sup>/calmodulin-dependent kinase II activity by melatonin. *Biochim Biophys Acta* 1290:191–196
- Benítez-King G, Tunez I, Bellon A, Ortiz GG, Anton-Tay F (2003) Melatonin prevents cytoskeletal alterations and oxidative stress induced by okadaic acid in N1E-115 cells. *Exp Neurol* 182:151–159
- Benítez-King G, Ramirez-Rodríguez G, Ortiz L, Meza I (2004) The neuronal cytoskeleton as a potential therapeutic target in neurodegenerative diseases and schizophrenia. *Curr Drug Targets CNS Neurol Disord* 3:515–533
- Bergiannaki JD, Soldatos CR, Paparrigopoulos TJ, Syrengelas M, Stefanis CN (1995) Low and high melatonin excretors among healthy individuals. *J Pineal Res* 18:159–164
- Blackhall LJ (2012) Amyotrophic lateral sclerosis and palliative care: where we are, and the road ahead. *Muscle Nerve* 45:311–318
- Blask DE, Hill SM, Dauchy RT, Xiang S, Yuan L, Duplessis T, Mao L, Dauchy E, Sauer LA (2011) Circadian regulation of molecular, dietary, and metabolic signaling mechanisms of human breast cancer growth by the nocturnal melatonin signal and the consequences of its disruption by light at night. *J Pineal Res* 51:259–269
- Boeve BF, Silber MH, Ferman TJ (2003) Melatonin for treatment of REM sleep behavior disorder in neurologic disorders: results in 14 patients. *Sleep Med* 4:281–284
- Borah A, Mohanakumar KP (2009) Melatonin inhibits 6-hydroxydopamine production in the brain to protect against experimental parkinsonism in rodents. *J Pineal Res* 47:293–300
- Bordet R, Devos D, Brique S, Touitou Y, Guieu JD, Libersa C, Destee A (2003) Study of circadian melatonin secretion pattern at different stages of Parkinson's disease. *Clin Neuropharmacol* 26:65–72
- Borlongan CV, Yamamoto M, Takei N, Kumazaki M, Ungsuparkorn C, Hida H, Sanberg PR, Nishino H (2000) Glial cell survival is enhanced during melatonin-induced neuroprotection against cerebral ischemia. *FASEB J* 14:1307–1317

- Bouslama M, Renaud J, Olivier P, Fontaine RH, Matrot B, Gressens P, Gallego J (2007) Melatonin prevents learning disorders in brain-lesioned newborn mice. *Neuroscience* 150:712–719
- Boveris DL, Boveris A (2007) Oxygen delivery to the tissues and mitochondrial respiration. *Front Biosci* 12:1014–1023
- Braak H, Ghebremedhin E, Rub U, Bratzke H, Del Tredici K (2004) Stages in the development of Parkinson's disease-related pathology. *Cell Tissue Res* 318:121–134
- Brown GM, Young SN, Gauthier S, Tsui H, Grota LJ (1979) Melatonin in human cerebrospinal fluid in daytime; its origin and variation with age. *Life Sci* 25:929–936
- Brown GM, Cardinali DP, Pandi-Perumal SR (2010) Melatonin and mental illness. In: Pandi-Perumal SR, Kramer M (eds) *Sleep and Mental Illness*. Cambridge University Press, Cambridge, pp 119–129
- Brusco LI, Marquez M, Cardinali DP (1998) Monozygotic twins with Alzheimer's disease treated with melatonin: case report. *J Pineal Res* 25:260–263
- Brusco LI, Marquez M, Cardinali DP (2000) Melatonin treatment stabilizes chronobiologic and cognitive symptoms in Alzheimer's disease. *Neuroendocrinol Lett* 21:39–42
- Bubenik GA (2002) Gastrointestinal melatonin: localization, function, and clinical relevance. *Dig Dis Sci* 47:2336–2348
- Bubenik GA, Konturek SJ (2011) Melatonin and aging: prospects for human treatment. *J Physiol Pharmacol* 62:13–19
- Cai Y, Ding H, Li N, Chai Y, Zhang Y, Chan P (2010a) Oscillation development for neurotransmitter-related genes in the mouse striatum. *NeuroReport* 21:79–83
- Cai Y, Liu S, Sothorn RB, Xu S, Chan P (2010b) Expression of clock genes *Per1* and *Bmal1* in total leukocytes in health and Parkinson's disease. *Eur J Neurol* 17:550–554
- Cardinali DP (1983) Molecular mechanisms of neuroendocrine integration in the central nervous system: an approach through the study of the pineal gland and its innervating sympathetic pathway. *Psychoneuroendocrinology* 8:3–30
- Cardinali DP, Freire F (1975) Melatonin effects on brain. Interaction with microtubule protein, inhibition of fast axoplasmic flow and induction of crystalloid and tubular formations in the hypothalamus. *Mol Cell Endocrinol* 2:317–330
- Cardinali DP, Pévet P (1998) Basic aspects of melatonin action. *Sleep Med Rev* 2:175–190
- Cardinali DP, Scacchi PA (2010) Chronophysiology of melatonin: therapeutical implications. *Open Neuroendocrinol J* 3:72–84
- Cardinali DP, Brusco LI, Liberzuck C, Furio AM (2002) The use of melatonin in Alzheimer's disease. *Neuroendocrinol Lett* 23(suppl. 1):20–23
- Cardinali DP, Esquifino AI, Srinivasan V, Pandi-Perumal SR (2008) Melatonin and the immune system in aging. *NeuroImmunoModulation* 15:272–278
- Cardinali DP, Furio AM, Brusco LI (2010) Clinical aspects of melatonin intervention in Alzheimer's disease progression. *Curr Neuropharmacol* 8:218–227
- Cardinali DP, Cano P, Jimenez-Ortega V, Esquifino AI (2011) Melatonin and the metabolic syndrome: physiopathologic and therapeutical implications. *Neuroendocrinology* 93:133–142
- Cardinali DP, Srinivasan V, Brzezinski A, Brown GM (2012) Melatonin and its analogs in insomnia and depression. *J Pineal Res* 52:365–375
- Carneiro RC, Reiter RJ (1998) Melatonin protects against lipid peroxidation induced by delta-aminolevulinic acid in rat cerebellum, cortex and hippocampus. *Neuroscience* 82:293–299
- Carrillo-Vico A, Reiter RJ, Lardone PJ, Herrera JL, Fernandez-Montesinos R, Guerrero JM, Pozo D (2006) The modulatory role of melatonin on immune responsiveness. *Curr Opin Investig Drugs* 7:423–431
- Catala MD, Canete-Nicolas C, Iradi A, Tarazona PJ, Tormos JM, Pascual-León A (1997) Melatonin levels in Parkinson's disease: drug therapy versus electrical stimulation of the internal globus pallidus. *Exp Gerontol* 32:553–558
- Caumo W, Levandovski R, Hidalgo MP (2009) Preoperative anxiolytic effect of melatonin and clonidine on postoperative pain and morphine consumption in patients undergoing abdominal hysterectomy: a double-blind, randomized, placebo-controlled study. *J Pain* 10:100–108
- Cavallo A (1993) Melatonin and human puberty: current perspectives. *J Pineal Res* 15:115–121
- Chahbouni M, Escames G, Venegas C, Sevilla B, García JA, Lopez LC, Munoz-Hoyos A, Molina-Carballo A, Acuña-Castroviejo D (2010) Melatonin treatment normalizes plasma pro-inflammatory cytokines and nitrosative/oxidative stress in patients suffering from Duchenne muscular dystrophy. *J Pineal Res* 48:282–289
- Chance B, Sies H, Boveris A (1979) Hydroperoxide metabolism in mammalian organs. *Physiol Rev* 59:527–605
- Chen CM (2011) Mitochondrial dysfunction, metabolic deficits, and increased oxidative stress in Huntington's disease. *Chang Gung Med J* 34:135–152
- Chen G, Huo Y, Tan DX, Liang Z, Zhang W, Zhang Y (2003) Melatonin in Chinese medicinal herbs. *Life Sci* 73:19–26
- Cheung RT (2003) The utility of melatonin in reducing cerebral damage resulting from ischemia and reperfusion. *J Pineal Res* 34:153–160
- Cho S, Joh TH, Baik HH, Dibinis C, Volpe BT (1997) Melatonin administration protects CA1 hippocampal neurons after transient forebrain ischemia in rats. *Brain Res* 755:335–338
- Chuang YC (2010) Mitochondrial dysfunction and oxidative stress in seizure-induced neuronal cell death. *Acta Neurol Taiwan* 19:3–15
- Chuang JI, Chen TH (2004) Effect of melatonin on temporal changes of reactive oxygen species and glutathione after MPP<sup>+</sup> treatment in human astrocytoma U373MG cells. *J Pineal Res* 36:117–125
- Cifra A, Mazzone GL, Nistri A (2012) Riluzole: What It Does to Spinal and Brainstem Neurons and How It Does It. *Neuroscientist*. <http://dx.doi.org/10.1177/1073858412444932>
- Claustrat B, Brun J, Chazot G (2005) The basic physiology and pathophysiology of melatonin. *Sleep Med Rev* 9:11–24
- Cohen-Mansfield J, Garfinkel D, Lipson S (2000) Melatonin for treatment of sundowning in elderly persons with dementia—a preliminary study. *Arch Gerontol Geriatr* 31:65–76
- 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
- Cunningham C (2011) Systemic inflammation and delirium: important co-factors in the progression of dementia. *Biochem Soc Trans* 39:945–953
- Cuzzocrea S, Zingarelli B, Gilad E, Hake P, Salzman AL, Szabo C (1997) Protective effect of melatonin in carrageenan-induced models of local inflammation: relationship to its inhibitory effect on nitric oxide production and its peroxynitrite scavenging activity. *J Pineal Res* 23:106–116
- Dabbeni-Sala F, Di Santo S, Franceschini D, Skaper SD, Giusti P (2001) Melatonin protects against 6-OHDA-induced neurotoxicity in rats: a role for mitochondrial complex I activity. *FASEB J* 15:164–170
- Dardente H (2012) Melatonin-dependent timing of seasonal reproduction by the pars tuberalis: pivotal roles for long daylengths and thyroid hormones. *J Neuroendocrinol* 24:249–266
- Das A, McDowell M, Pava MJ, Smith JA, Reiter RJ, Woodward JJ, Varma AK, Ray SK, Banik NL (2010) The inhibition of apoptosis by melatonin in VSC4.1 motoneurons exposed to oxidative stress, glutamate excitotoxicity, or TNF-alpha toxicity

- involves membrane melatonin receptors. *J Pineal Res* 48:157–169
- Dawson D, Armstrong SM (1996) Chronobiotics—drugs that shift rhythms. *Pharmacol Ther* 69:15–36
- de Jonghe A, Korevaar JC, van Munster BC, de Rooij SE (2010) Effectiveness of melatonin treatment on circadian rhythm disturbances in dementia. Are there implications for delirium? A systematic review. *Int J Geriatr Psychiatry* 25:1201–1208
- Devore EE, Grodstein F, van Rooij FJ, Hofman A, Stampfer MJ, Witteman JC, Breteler MM (2010) Dietary antioxidants and long-term risk of dementia. *Arch Neurol* 67:819–825
- Dexter DT, Carter CJ, Wells FR, Javoy-Agid F, Agid Y, Lees A, Jenner P, Marsden CD (1989) Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J Neurochem* 52:381–389
- Domínguez-Rodríguez A, Abreu-Gonzalez P, Sanchez-Sanchez JJ, Kaski JC, Reiter RJ (2010) Melatonin and circadian biology in human cardiovascular disease. *J Pineal Res* 49:14–22
- Dori D, Casale G, Solerte SB, Fioravanti M, Migliorati G, Cuzzoni G, Ferrari E (1994) Chrono-neuroendocrinological aspects of physiological aging and senile dementia. *Chronobiologia* 21:121–126
- Dowling GA, Mastick J, Colling E, Carter JH, Singer CM, Aminoff MJ (2005) Melatonin for sleep disturbances in Parkinson's disease. *Sleep Med* 6:459–466
- Dowling GA, Burr RL, Van Someren EJ, Hubbard EM, Luxenberg JS, Mastick J, Cooper BA (2008) Melatonin and bright-light treatment for rest-activity disruption in institutionalized patients with Alzheimer's disease. *J Am Geriatr Soc* 56:239–246
- Dragicvic N, Copes N, O'Neal-Moffitt G, Jin J, Buzzeo R, Mamcarz M, Tan J, Cao C, Olcese JM, Arendash GW, Bradshaw PC (2011) Melatonin treatment restores mitochondrial function in Alzheimer's mice: a mitochondrial protective role of melatonin membrane receptor signaling. *J Pineal Res* 51:75–86
- Dubbels R, Reiter RJ, Klenke E, Goebel A, Schnakenberg E, Ehlers C, Schiwarwa HW, Schloot W (1995) Melatonin in edible plants identified by radioimmunoassay and by high performance liquid chromatography-mass spectrometry. *J Pineal Res* 18:28–31
- 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
- Ehrnhoefer DE, Wong BK, Hayden MR (2011) Convergent pathogenic pathways in Alzheimer's and Huntington's diseases: shared targets for drug development. *Nat Rev Drug Discov* 10:853–867
- Erol FS, Topsakal C, Ozveren MF, Kaplan M, Ilhan N, Ozercan IH, Yildiz OG (2004) Protective effects of melatonin and vitamin E in brain damage due to gamma radiation: an experimental study. *Neurosurg Rev* 27:65–69
- Escames G, Acuña-Castroviejo CD, Vives F (1996) Melatonin-dopamine interaction in the striatal projection area of sensorimotor cortex in the rat. *NeuroReport* 7:597–600
- Escames G, León J, Lopez LC, Acuña-Castroviejo D (2004) Mechanisms of *N*-methyl-D-aspartate receptor inhibition by melatonin in the rat striatum. *J Neuroendocrinol* 16:929–935
- Esposito E, Cuzzocrea S (2010) Antiinflammatory activity of melatonin in central nervous system. *Curr Neuropharmacol* 8:228–242
- Esquifino AI, Pandi-Perumal SR, Cardinali DP (2004) Circadian organization of the immune response: a role for melatonin. *Clin Appl Immunol Rev* 4:423–433
- Fahn S, Cohen G (1992) The oxidant stress hypothesis in Parkinson's disease: evidence supporting it. *Ann Neurol* 32:804–812
- Fainstein I, Bonetto A, Brusco LI, Cardinali DP (1997) Effects of melatonin in elderly patients with sleep disturbance. A pilot study. *Curr Ther Res* 58:990–1000
- Feng Z, Zhang JT (2004) Melatonin reduces amyloid beta-induced apoptosis in pheochromocytoma (PC12) cells. *J Pineal Res* 37:257–266
- Feng Z, Qin C, Chang Y, Zhang JT (2006) Early melatonin supplementation alleviates oxidative stress in a transgenic mouse model of Alzheimer's disease. *Free Radic Biol Med* 40:101–109
- Ferrari E, Fioravanti M, Magri F, Solerte SB (2000) Variability of interactions between neuroendocrine and immunological functions in physiological aging and dementia of the Alzheimer's type. *Ann N Y Acad Sci* 917:582–596
- Ferrari E, Cravello L, Falvo F, Barili L, Solerte SB, Fioravanti M, Magri F (2008) Neuroendocrine features in extreme longevity. *Exp Gerontol* 43:88–94
- Fertl E, Auff E, Doppelbauer A, Waldhauser F (1993) Circadian secretion pattern of melatonin in de novo parkinsonian patients: evidence for phase-shifting properties of L-dopa. *J Neural Transm Park Dis Dement Sect* 5:227–234
- Floden AM, Li S, Combs CK (2005) Beta-amyloid-stimulated microglia induce neuron death via synergistic stimulation of tumor necrosis factor alpha and NMDA receptors. *J Neurosci* 25:2566–2575
- Fourtillan JB, Brisson AM, Fourtillan M, Ingrand I, Decourt JP, Girault J (2001) Melatonin secretion occurs at a constant rate in both young and older men and women. *Am J Physiol Endocrinol Metab* 280:E11–E22
- Fraser PA (2011) The role of free radical generation in increasing cerebrovascular permeability. *Free Radic Biol Med* 51:967–977
- Furio AM, Cutrera RA, Castillo TV, Perez LS, Riccio P, Caccuri RL, Brusco LL, Cardinali DP (2002) Effect of melatonin on changes in locomotor activity rhythm of Syrian hamsters injected with beta amyloid peptide 25–35 in the suprachiasmatic nuclei. *Cell Mol Neurobiol* 22:699–709
- Furio AM, Brusco LI, Cardinali DP (2007) Possible therapeutic value of melatonin in mild cognitive impairment: a retrospective study. *J Pineal Res* 43:404–409
- Furio AM, Fontao R, Falco N, Ruiz JI, Caccuri RL, Cardinali DP (2008) Neuroprotective effect of melatonin on glucocorticoid toxicity in the rat hippocampus. *Open Physiol J* 1:23–27
- Galano A, Tan DX, Reiter RJ (2011) Melatonin as a natural ally against oxidative stress: a physicochemical examination. *J Pineal Res* 51:1–16
- Galecki P, Szmraj J, Bartosz G, Bienkiewicz M, Galecka E, Florkowski A, Lewinski A, Karbownik-Lewinska M (2010) Single-nucleotide polymorphisms and mRNA expression for melatonin synthesis rate-limiting enzyme in recurrent depressive disorder. *J Pineal Res* 48:311–317
- García JJ, Reiter RJ, Pie J, Ortiz GG, Cabrera J, Sainz RM, Acuña-Castroviejo D (1999) Role of pinoline and melatonin in stabilizing hepatic microsomal membranes against oxidative stress. *J Bioenerg Biomembr* 31:609–616
- García T, Ribes D, Colomina MT, Cabre M, Domingo JL, Gomez M (2009) Evaluation of the protective role of melatonin on the behavioral effects of aluminum in a mouse model of Alzheimer's disease. *Toxicology* 265:49–55
- García T, Esparza JL, Nogues MR, Romeu M, Domingo JL, Gomez M (2010) Oxidative stress status and RNA expression in hippocampus of an animal model of Alzheimer's disease after chronic exposure to aluminum. *Hippocampus* 20:218–225
- García-Mesa Y, Gimenez-Llort L, Lopez LC, Venegas C, Cristofol R, Escames G, Acuña-Castroviejo D, Sanfeliu C (2012) Melatonin plus physical exercise are highly neuroprotective in the 3xTg-AD mouse. *Neurobiol Aging* 33:1124–1129

- Garzon C, Guerrero JM, Aramburu O, Guzman T (2009) Effect of melatonin administration on sleep, behavioral disorders and hypnotic drug discontinuation in the elderly: a randomized, double-blind, placebo-controlled study. *Aging Clin Exp Res* 21:38–42
- Gauthier S, Reisberg B, Zaudig M, Petersen RC, Ritchie K, Broich K, Belleville S, Brodaty H, Bennett D, Chertkow H, Cummings JL, de León M, Feldman H, Ganguli M, Hampel H, Scheltens P, Tierney MC, Whitehouse P, Winblad B (2006) Mild cognitive impairment. *Lancet* 367:1262–1270
- Gehrman PR, Connor DJ, Martin JL, Shochat T, Corey-Bloom J, Ancoli-Israel S (2009) Melatonin fails to improve sleep or agitation in double-blind randomized placebo-controlled trial of institutionalized patients with Alzheimer disease. *Am J Geriatr Psychiatry* 17:166–169
- Genova ML, Pich MM, Bernacchia A, Bianchi C, Biondi A, Bovina C, Falasca AI, Formiggin G, Castelli GP, Lenaz G (2004) The mitochondrial production of reactive oxygen species in relation to aging and pathology. *Ann N Y Acad Sci* 1011:86–100
- Ghanta S, Chattopadhyay S (2011) Glutathione as a signaling molecule: another challenge to pathogens. *Plant Signal Behav* 6:783–788
- Gibson GE, Starkov A, Blass JP, Ratan RR, Beal MF (2010) Cause and consequence: mitochondrial dysfunction initiates and propagates neuronal dysfunction, neuronal death and behavioral abnormalities in age-associated neurodegenerative diseases. *Biochim Biophys Acta* 1802:122–134
- Girotti L, Lago M, Ivanovsky O, Carbajales J, Elizari MV, Brusco LI, Cardinali DP (2000) Low urinary 6-sulphatoxymelatonin levels in patients with coronary artery disease. *J Pineal Res* 29:138–142
- Giusti P, Franceschini D, Petrone M, Manev H, Floreani M (1996a) In vitro and in vivo protection against kainate-induced excitotoxicity by melatonin. *J Pineal Res* 20:226–231
- Giusti P, Lipartiti M, Franceschini D, Schiavo N, Floreani M, Manev H (1996b) Neuroprotection by melatonin from kainate-induced excitotoxicity in rats. *FASEB J* 10:891–896
- Golombek DA, Pévet P, Cardinali DP (1996) Melatonin effects on behavior: possible mediation by the central GABAergic system. *Neurosci Biobehav Rev* 20:403–412
- Gorgulu A, Palaoglu S, Ismailoglu O, Tuncel M, Surucu MT, Erbil M, Kilinc K (2001) Effect of melatonin on cerebral edema in rats. *Neurosurgery* 49:1434–1441
- Green DR, Galluzzi L, Kroemer G (2011) Mitochondria and the autophagy-inflammation-cell death axis in organismal aging. *Science* 333:1109–1112
- Grinberg LT, Rueb U, Alho AT, Heinsen H (2010) Brainstem pathology and non-motor symptoms in PD. *J Neurol Sci* 289:81–88
- Grof E, Grof P, Brown GM, Arato M, Lane J (1985) Investigations of melatonin secretion in man. *Prog Neuropsychopharmacol Biol Psychiatry* 9:609–612
- Guerrero JM, Reiter RJ (2002) Melatonin-immune system relationships. *Curr Top Med Chem* 2:167–179
- Guerrero JM, Reiter RJ, Ortiz GG, Pablos MI, Sewerynek E, Chuang JI (1997) Melatonin prevents increases in neural nitric oxide and cyclic GMP production after transient brain ischemia and reperfusion in the Mongolian gerbil (*Meriones unguiculatus*). *J Pineal Res* 23:24–31
- Guido ME, Garbarino-Pico E, Contin MA, Valdez DJ, Nieto PS, Verra DM, Acosta-Rodríguez VA, de Zavalía N, Rosenstein RE (2010) Inner retinal circadian clocks and non-visual photoreceptors: novel players in the circadian system. *Prog Neurobiol* 92:484–504
- Gupta M, Gupta YK, Agarwal S, Aneja S, Kalaivani M, Kohli K (2004) Effects of add-on melatonin administration on antioxidant enzymes in children with epilepsy taking carbamazepine monotherapy: a randomized, double-blind, placebo-controlled trial. *Epilepsia* 45:1636–1639
- Hall ED (2011) Antioxidant therapies for acute spinal cord injury. *Neurotherapeutics* 8:152–167
- Hall NF, Gale CR (2002) Prevention of age related macular degeneration. *BMJ* 325:1–2
- Hall ED, Vaishnav RA, Mustafa AG (2010) Antioxidant therapies for traumatic brain injury. *Neurotherapeutics* 7:51–61
- Harada S, Fujii C, Hayashi A, Ohkoshi N (2001) An association between idiopathic Parkinson's disease and polymorphisms of phase II detoxification enzymes: glutathione S-transferase M1 and quinone oxidoreductase 1 and 2. *Biochem Biophys Res Commun* 288:887–892
- Hardeland R (2005) Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. *Endocrine* 27:119–130
- Hardeland R (2008) Melatonin, hormone of darkness and more—occurrence, control mechanisms, actions and bioactive metabolites. *Cell Mol Life Sci* 65:2001–2018
- Hardeland R (2009a) Melatonin: signaling mechanisms of a pleiotropic agent. *BioFactors* 35:183–192
- Hardeland R (2009b) Neuroprotection by radical avoidance: search for suitable agents. *Molecules* 14:5054–5102
- Hardeland R (2010a) Investigational melatonin receptor agonists. *Expert Opin Investig Drugs* 19:747–764
- Hardeland R (2010b) Melatonin metabolism in the central nervous system. *Curr Neuropharmacol* 8:168–181
- Hardeland R, Pandi-Perumal SR (2005) Melatonin, a potent agent in antioxidative defense: actions as a natural food constituent, gastrointestinal factor, drug and prodrug. *Nutr Metab (Lond)* 2:22
- Hardeland R, Poeggeler B (2007) Actions of melatonin, its structural and functional analogs in the central nervous system and the significance of metabolism. *Cent Nerv Syst Agents Med Chem* 7:303
- Hardeland R, Poeggeler B (2008) Melatonin beyond its classical functions. *Open Physiol J* 1:1–23
- Hardeland R, Balzer I, Poeggeler B, Fuhrberg B, Uria H, Behrmann G, Wolf R, Meyer TJ, Reiter RJ (1995) On the primary functions of melatonin in evolution: mediation of photoperiodic signals in a unicell, photooxidation, and scavenging of free radicals. *J Pineal Res* 18:104–111
- Hardeland R, Cardinali DP, Srinivasan V, Spence DW, Brown GM, Pandi-Perumal SR (2011) Melatonin-A pleiotropic, orchestrating regulator molecule. *Prog Neurobiol* 93:350–384
- Hausman DB, Fischer JG, Johnson MA (2011) Nutrition in centenarians. *Maturitas* 68:203–209
- He H, Dong W, Huang F (2010) Anti-amyloidogenic and anti-apoptotic role of melatonin in Alzheimer disease. *Curr Neuropharmacol* 8:211–217
- Heiser V, Scherzinger E, Boeddrich A, Nordhoff E, Lurz R, Schugardt N, Lehrach H, Wanker EE (2000) Inhibition of huntingtin fibrillogenesis by specific antibodies and small molecules: implications for Huntington's disease therapy. *Proc Natl Acad Sci USA* 97:6739–6744
- Hu K, Van Someren EJ, Shea SA, Scheer FA (2009) Reduction of scale invariance of activity fluctuations with aging and Alzheimer's disease: involvement of the circadian pacemaker. *Proc Natl Acad Sci USA* 106:2490–2494
- Huether G (1993) The contribution of extrapineal sites of melatonin synthesis to circulating melatonin levels in higher vertebrates. *Experientia* 49:665–670
- Huether G (1994) Melatonin synthesis in the gastrointestinal tract and the impact of nutritional factors on circulating melatonin. *Ann N Y Acad Sci* 719:146–158
- Huether G, Poeggeler B, Reimer A, George A (1992) Effect of tryptophan administration on circulating melatonin levels in



- chicks and rats: evidence for stimulation of melatonin synthesis and release in the gastrointestinal tract. *Life Sci* 51:945–953
- Iguchi H, Kato KI, Ibayashi H (1982) Melatonin serum levels and metabolic clearance rate in patients with liver cirrhosis. *J Clin Endocrinol Metab* 54:1025–1027
- Jean-Louis G, von Gizycki H, Zizi F (1998a) Melatonin effects on sleep, mood, and cognition in elderly with mild cognitive impairment. *J Pineal Res* 25:177–183
- Jean-Louis G, Zizi F, von Gizycki H, Taub H (1998b) Effects of melatonin in two individuals with Alzheimer's disease. *Percept Mot Skills* 87:331–339
- Jiao S, Wu MM, Hu CL, Zhang ZH, Mei YA (2004) Melatonin receptor agonist 2-iodomelatonin prevents apoptosis of cerebellar granule neurons via K(+) current inhibition. *J Pineal Res* 36:109–116
- Jimenez-Jorge S, Guerrero JM, Jimenez-Caliani AJ, Naranjo MC, Lardone PJ, Carrillo-Vico A, Osuna C, Molinero P (2007) Evidence for melatonin synthesis in the rat brain during development. *J Pineal Res* 42:240–246
- Jiménez-Ortega V, Cano P, Scacchi PA, Cardinali DP, Esquifino AI (2011) Cadmium-induced disruption in 24-h expression of clock and redox enzyme genes in rat medial basal hypothalamus. Prevention by melatonin. *Front Neurol* 2:13
- Jin BK, Shin DY, Jeong MY, Gwag MR, Baik HW, Yoon KS, Cho YH, Joo WS, Kim YS, Baik HH (1998) Melatonin protects nigral dopaminergic neurons from 1-methyl-4-phenylpyridinium (MPP+) neurotoxicity in rats. *Neurosci Lett* 245:61–64
- Jonsson L, Ljunggren E, Bremer A, Pedersen C, Landen M, Thuresson K, Giacobini M, Melke J (2010) Mutation screening of melatonin-related genes in patients with autism spectrum disorders. *BMC Med Genomics* 3:10
- Jou MJ (2011) Melatonin preserves the transient mitochondrial permeability transition for protection during mitochondrial Ca<sup>2+</sup> stress in astrocyte. *J Pineal Res* 50:427–435
- Jucker M, Walker LC (2011) Pathogenic protein seeding in Alzheimer disease and other neurodegenerative disorders. *Ann Neurol* 70:532–540
- Kabadi SV, Maher TJ (2010) Posttreatment with uridine and melatonin following traumatic brain injury reduces edema in various brain regions in rats. *Ann N Y Acad Sci* 1199:105–113
- Karasek M, Reiter RJ (2002) Melatonin and aging. *Neuroendocrinol Lett* 23(Suppl 1):14–16
- Kaur C, Sivakumar V, Zhang Y, Ling EA (2006) Hypoxia-induced astrocytic reaction and increased vascular permeability in the rat cerebellum. *Glia* 54:826–839
- Kaur C, Sivakumar V, Ling EA (2007) Expression of transferrin receptors in the pineal gland of postnatal and adult rats and its alteration in hypoxia and melatonin treatment. *Glia* 55:263–273
- Kaur C, Sivakumar V, Ling EA (2010) Melatonin protects periventricular white matter from damage due to hypoxia. *J Pineal Res* 48:185–193
- Kaur C, Viswanathan S, Ling EA (2011) Hypoxia-induced cellular and vascular changes in the nucleus tractus solitarius and ventrolateral medulla. *J Neuropathol Exp Neurol* 70:201–217
- Khandelwal PJ, Herman AM, Moussa CE (2011) Inflammation in the early stages of neurodegenerative pathology. *J Neuroimmunol* 238:1–11
- Kilanczyk E, Bryszewska M (2003) The effect of melatonin on antioxidant enzymes in human diabetic skin fibroblasts. *Cell Mol Biol Lett* 8:333–336
- Kilic E, Ozdemir YG, Bolay H, Kelestimur H, Dalkara T (1999) Pinelectomy aggravates and melatonin administration attenuates brain damage in focal ischemia. *J Cereb Blood Flow Metab* 19:511–516
- Kilic U, Yilmaz B, Ugur M, Yuksel A, Reiter RJ, Hermann DM, Kilic E (2011) Evidence that membrane-bound G protein-coupled melatonin receptors MT1 and MT2 are not involved in the neuroprotective effects of melatonin in focal cerebral ischemia. *J Pineal Res* 52:228–235
- Kitazawa M, Yamasaki TR, Laferla FM (2004) Microglia as a potential bridge between the amyloid {beta}-peptide and tau. *Ann N Y Acad Sci* 1035:85–103
- Klein DC (2007) Arylalkylamine N-acetyltransferase: “the Timezyme”. *J Biol Chem* 282:4233–4237
- Kluck RM, Bossy-Wetzel E, Green DR, Newmeyer DD (1997) The release of cytochrome c from mitochondria: a primary site for Bcl-2 regulation of apoptosis. *Science* 275:1132–1136
- Koh PO (2011) Melatonin prevents down-regulation of astrocytic phosphoprotein PEA-15 in ischemic brain injury. *J Pineal Res* 51:381–386
- Koh PO (2012) Melatonin attenuates decrease of protein phosphatase 2A subunit B in ischemic brain injury. *J Pineal Res* 52:57–61
- Koller WC (1996) Management of motor fluctuations in Parkinson's disease. *Eur Neurol* 36(Suppl 1):43–48
- Kunz D, Bes F (1997) Melatonin effects in a patient with severe REM sleep behavior disorder: case report and theoretical considerations. *Neuropsychobiology* 36:211–214
- Kunz D, Bes F (1999) Melatonin as a therapy in REM sleep behavior disorder patients: an open-labeled pilot study on the possible influence of melatonin on REM-sleep regulation. *Mov Disord* 14:507–511
- Kvetnoy IM, Ingel IE, Kvetnaia TV, Malinovskaya NK, Rapoport SI, Raikhlin NT, Trofimov AV, Yuzhakov VV (2002) Gastrointestinal melatonin: cellular identification and biological role. *Neuro Endocrinol Lett* 23:121–132
- Lacza Z, Horn TF, Snipes JA, Zhang J, Roychowdhury S, Horvath EM, Figueroa JP, Kollai M, Szabo C, Busija DW (2004) Lack of mitochondrial nitric oxide production in the mouse brain. *J Neurochem* 90:942–951
- Landau M, Zisapel N (2007) The low affinity binding of melatonin to calmodulin: use of computational methods to explain its physiological relevance. In: Cardinali DP, Pandi-Perumal SR (eds) *Melatonin - From Molecules to Therapy*. Nova Science, New York, pp 69–79
- Lardone PJ, Guerrero JM, Fernandez-Santos JM, Rubio A, Martin-Lacave I, Carrillo-Vico A (2011) Melatonin synthesized by T lymphocytes as a ligand of the retinoic acid-related orphan receptor. *J Pineal Res* 51:454–462
- Lee EJ, Wu TS, Lee MY, Chen TY, Tsai YY, Chuang JI, Chang GL (2004) Delayed treatment with melatonin enhances electrophysiological recovery following transient focal cerebral ischemia in rats. *J Pineal Res* 36:33–42
- León-Blanco MM, Guerrero JM, Reiter RJ, Pozo D (2004) RNA expression of human telomerase subunits TR and TERT is differentially affected by melatonin receptor agonists in the MCF-7 tumor cell line. *Cancer Lett* 216:73–80
- Levoye A, Dam J, Ayoub MA, Guillaume JL, Couturier C, Delagrance P, Jockers R (2006) The orphan GPR50 receptor specifically inhibits MT1 melatonin receptor function through heterodimerization. *EMBO J* 25:3012–3023
- Lewy AJ, Sack RA, Singer CL (1984) Assessment and treatment of chronobiologic disorders using plasma melatonin levels and bright light exposure: the clock-gate model and the phase response curve. *Psychopharmacol Bull* 20:561–565
- Lezoualc'h F, Skutella T, Widmann M, Behl C (1996) Melatonin prevents oxidative stress-induced cell death in hippocampal cells. *NeuroReport* 7:2071–2077
- Li XC, Wang ZF, Zhang JX, Wang Q, Wang JZ (2005) Effect of melatonin on calyculin A-induced tau hyperphosphorylation. *Eur J Pharmacol* 510:25–30
- Lieverse R, Van Someren EJ, Nielen MM, Uitdehaag BM, Smit JH, Hoogendijk WJ (2011) Bright light treatment in elderly patients

- with nonseasonal major depressive disorder: a randomized placebo-controlled trial. *Arch Gen Psychiatry* 68:61–70
- Lin HW, Lee EJ (2009) Effects of melatonin in experimental stroke models in acute, sub-acute, and chronic stages. *Neuropsychiatr Dis Treat* 5:157–162
- Ling ZQ, Tian Q, Wang L, Fu ZQ, Wang XC, Wang Q, Wang JZ (2009) Constant illumination induces Alzheimer-like damages with endoplasmic reticulum involvement and the protection of melatonin. *J Alzheimers Dis* 16:287–300
- Liu T, Borjigin J (2005) *N*-acetyltransferase is not the rate-limiting enzyme of melatonin synthesis at night. *J Pineal Res* 39:91–96
- Liu RY, Zhou JN, Van Heerikhuizen J, Hofman MA, Swaab DF (1999) Decreased melatonin levels in postmortem cerebrospinal fluid in relation to aging, Alzheimer's disease, and apolipoprotein E-ε4/4 genotype. *J Clin Endocrinol Metab* 84:323–327
- Louzada PR, Paula Lima AC, Mendonca-Silva DL, Noel F, De Mello FG, Ferreira ST (2004) Taurine prevents the neurotoxicity of beta-amyloid and glutamate receptor agonists: activation of GABA receptors and possible implications for Alzheimer's disease and other neurological disorders. *FASEB J* 18:511–518
- Luboshitzky R, Shen-Orr Z, Tzischichinsky O, Maldonado M, Herer P, Lavie P (2001) Actigraphic sleep-wake patterns and urinary 6-sulfatoxymelatonin excretion in patients with Alzheimer's disease. *Chronobiol Int* 18:513–524
- Macias M, Escames G, León J, Coto A, Sbihi Y, Osuna A, Acuña-Castroviejo D (2003) Calreticulin–melatonin. An unexpected relationship. *Eur J Biochem* 270:832–840
- Macleod MR, O'Collins T, Horky LL, Howells DW, Donnan GA (2005) Systematic review and meta-analysis of the efficacy of melatonin in experimental stroke. *J Pineal Res* 38:35–41
- Magri F, Sarra S, Cinchetti W, Guazzoni V, Fioravanti M, Cravello L, Ferrari E (2004) Qualitative and quantitative changes of melatonin levels in physiological and pathological aging and in centenarians. *J Pineal Res* 36:256–261
- Maguire-Zeiss KA, Federoff HJ (2010) Future directions for immune modulation in neurodegenerative disorders: focus on Parkinson's disease. *J Neural Transm* 117:1019–1025
- Mahlberg R, Walther S (2007) Actigraphy in agitated patients with dementia: monitoring treatment outcomes. *Z Gerontol Geriatr* 40:178–184
- Mahlberg R, Kunz D, Sutej I, Kuhl KP, Hellweg R (2004) Melatonin treatment of day–night rhythm disturbances and sundowning in Alzheimer disease: an open-label pilot study using actigraphy. *J Clin Psychopharmacol* 24:456–459
- Mahlberg R, Kienast T, Hadel S, Heidenreich JO, Schmitz S, Kunz D (2009) Degree of pineal calcification (DOC) is associated with polysomnographic sleep measures in primary insomnia patients. *Sleep Med* 10:439–445
- Manev H, Uz T, Kharlamov A, Joo JY (1996) Increased brain damage after stroke or excitotoxic seizures in melatonin-deficient rats. *FASEB J* 10:1546–1551
- Maronde E, Stehle JH (2007) The mammalian pineal gland: known facts, unknown facets. *Trends Endocrinol Metab* 18:142–149
- Martin M, Macias M, Escames G, Reiter RJ, Agapito MT, Ortiz GG, Acuña-Castroviejo D (2000) Melatonin-induced increased activity of the respiratory chain complexes I and IV can prevent mitochondrial damage induced by ruthenium red in vivo. *J Pineal Res* 28:242–248
- Martin M, Macias M, León J, Escames G, Khaldy H, Acuña-Castroviejo D (2002) Melatonin increases the activity of the oxidative phosphorylation enzymes and the production of ATP in rat brain and liver mitochondria. *Int J Biochem Cell Biol* 34:348–357
- Mathes AM, Kubulus D, Weiler J, Bentley A, Waibel L, Wolf B, Bauer I, Rensing H (2008) Melatonin receptors mediate improvements of liver function but not of hepatic perfusion and integrity after hemorrhagic shock in rats. *Crit Care Med* 36:24–29
- Matsubara E, Bryant-Thomas T, Pacheco QJ, Henry TL, Poeggeler B, Herbert D, Cruz-Sanchez F, Chyan YJ, Smith MA, Perry G, Shoji M, Abe K, León A, Grundke-Ikbal I, Wilson GL, Ghiso J, Williams C, Refolo LM, Pappolla MA, Chain DG, Neria E (2003) Melatonin increases survival and inhibits oxidative and amyloid pathology in a transgenic model of Alzheimer's disease. *J Neurochem* 85:1101–1108
- Mayo JC, Sainz RM, Uria H, Antolin I, Esteban MM, Rodríguez C (1998) Melatonin prevents apoptosis induced by 6-hydroxydopamine in neuronal cells: implications for Parkinson's disease. *J Pineal Res* 24:179–192
- Mazzoccoli G, De Cata A, Carughi S, Greco A, Inglese M, Perfetto F, Tarquini R (2010a) A possible mechanism for altered immune response in the elderly. *In Vivo (Athens, Greece)* 24:471–487
- Mazzoccoli G, Vendemiale G, Inglese M, De Cata A, Piepoli A, Paziienza V, Muscarella LA, Tarquini R (2010b) Neuroendocrine axes function in healthy aging: Evaluation of predictive and manipulable blood serum indexes. *Biomed Pharmacother*. <http://dx.doi.org/10.1016/j.biopha.2010.09.001>
- Medeiros CA, Carvalhede de Bruin PF, Lopes LA, Magalhaes MC, de Lourdes SM, Sales dBV (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
- Mediavilla MD, Sanchez-Barcelo EJ, Tan DX, Manchester L, Reiter RJ (2010) Basic mechanisms involved in the anti-cancer effects of melatonin. *Curr Med Chem* 17:4462–4481
- Melchiorri D, Reiter RJ, Chen LD, Sewerynek E, Nistico G (1996) Melatonin affords protection against kainate-induced in vitro lipid peroxidation in brain. *Eur J Pharmacol* 305:239–242
- Melke J, Goubran BH, Chaste P, Betancur C, Nygren G, Anckarsater H, Rastam M, Stahlberg O, Gillberg IC, Delorme R, Chabane N, Mouren-Simeoni MC, Fauchereau F, Durand CM, Chevalier F, Drouot X, Collet C, Launay JM, Leboyer M, Gillberg C, Bourgeron T (2008) Abnormal melatonin synthesis in autism spectrum disorders. *Mol Psychiatry* 13:90–98
- Menéndez-Pelaez A, Reiter RJ (1993) Distribution of melatonin in mammalian tissues: the relative importance of nuclear versus cytosolic localization. *J Pineal Res* 15:59–69
- Mesenge C, Margail I, Verrecchia C, Allix M, Boulu RG, Plotkine M (1998) Protective effect of melatonin in a model of traumatic brain injury in mice. *J Pineal Res* 25:41–46
- Millan MJ, Gobert A, Lejeune F, Dekeyne A, Newman-Tancredi A, Pasteau V, Rivet JM, Cussac D (2003) The novel melatonin agonist agomelatine (S20098) is an antagonist at 5-hydroxytryptamine<sub>2C</sub> receptors, blockade of which enhances the activity of frontocortical dopaminergic and adrenergic pathways. *J Pharmacol Exp Ther* 306:954–964
- Mishima K, Tozawa T, Satoh K, Matsumoto Y, Hishikawa Y, Okawa M (1999) Melatonin secretion rhythm disorders in patients with senile dementia of Alzheimer's type with disturbed sleep-waking. *Biol Psychiatry* 45:417–421
- Mishima K, Okawa M, Hozumi S, Hishikawa Y (2000) Supplementary administration of artificial bright light and melatonin as potent treatment for disorganized circadian rest-activity and dysfunctional autonomic and neuroendocrine systems in institutionalized demented elderly persons. *Chronobiol Int* 17:419–432
- Mishima K, Okawa M, Shimizu T, Hishikawa Y (2001) Diminished melatonin secretion in the elderly caused by insufficient environmental illumination. *J Clin Endocrinol Metab* 86:129–134
- Miyamoto M (2009) Pharmacology of ramelteon, a selective MT<sub>1</sub>/MT<sub>2</sub> receptor agonist: a novel therapeutic drug for sleep disorders. *CNS Neurosci Ther* 15:32–51

- Moller M, Baeres FM (2002) The anatomy and innervation of the mammalian pineal gland. *Cell Tissue Res* 309:139–150
- Monti JM, Cardinali DP (2000) A critical assessment of the melatonin effect on sleep in humans. *Biol Signals Recept* 9:328–339
- Monti JM, Alvarino F, Cardinali DP, Savio I, Pintos A (1999) Polysomnographic study of the effect of melatonin on sleep in elderly patients with chronic primary insomnia. *Arch Gerontol Geriatr* 28:85–98
- Mukda S, Moller M, Ebadi M, Govitrapong P (2009) The modulatory effect of substance P on rat pineal norepinephrine release and melatonin secretion. *Neurosci Lett* 461:258–261
- 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
- Nag S, Kapadia A, Stewart DJ (2011) Review: molecular pathogenesis of blood-brain barrier breakdown in acute brain injury. *Neuropathol Appl Neurobiol* 37:3–23
- Nakazawa T, Nakazawa C, Matsubara A, Noda K, Hisatomi T, She H, Michaud N, Hafezi-Moghadam A, Miller JW, Benowitz LI (2006) Tumor necrosis factor- $\alpha$  mediates oligodendrocyte death and delayed retinal ganglion cell loss in a mouse model of glaucoma. *J Neurosci* 26:12633–12641
- Nosjean O, Ferro M, Coge F, Beauverger P, Henlin JM, Lefoulon F, Fauchere JL, Delagrance P, Canet E, Boutin JA (2000) Identification of the melatonin-binding site MT3 as the quinone reductase 2. *J Biol Chem* 275:31311–31317
- Ohashi Y, Okamoto N, Uchida K, Iyo M, Mori N, Morita Y (1999) Daily rhythm of serum melatonin levels and effect of light exposure in patients with dementia of the Alzheimer's type. *Biol Psychiatry* 45:1646–1652
- Okatani Y, Wakatsuki A, Reiter RJ, Miyahara Y (2002) Hepatic mitochondrial dysfunction in senescence-accelerated mice: correction by long-term, orally administered physiological levels of melatonin. *J Pineal Res* 33:127–133
- Olanow CW (1990) Oxidation reactions in Parkinson's disease. *Neurology* 40(Suppl):32–37
- Olanow CW (1992) An introduction to the free radical hypothesis in Parkinson's disease. *Ann Neurol* 32(Suppl):S2–S9
- Olanow CW, Brin MF, Obeso JA (2000) The role of deep brain stimulation as a surgical treatment for Parkinson's disease. *Neurology* 55:S60–S66
- Olcese JM, Cao C, Mori T, Mamcarz MB, Maxwell A, Runfeldt MJ, Wang L, Zhang C, Lin X, Zhang G, Arendash GW (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
- Oliveira JM (2010) Nature and cause of mitochondrial dysfunction in Huntington's disease: focusing on huntingtin and the striatum. *J Neurochem* 114:1–12
- Onuki J, Almeida EA, Medeiros MH, Di Mascio P (2005) Inhibition of 5-aminolevulinic acid-induced DNA damage by melatonin, N1-acetyl-N2-formyl-5-methoxykynuramine, quercetin or resveratrol. *J Pineal Res* 38:107–115
- Pablos MI, Reiter RJ, Chuang JJ, Ortiz GG, Guerrero JM, Sewerynek E, Agapito MT, Melchiorri D, Lawrence R, Deneke SM (1997) Acutely administered melatonin reduces oxidative damage in lung and brain induced by hyperbaric oxygen. *J Appl Physiol* 83:354–358
- Pacher P, Beckman JS, Liaudet L (2007) Nitric oxide and peroxynitrite in health and disease. *Physiol Rev* 87:315–424
- Pagan C, Goubran-Botros H, Poirier K, Dumaine A, Jamain S, Moreno S, de Brouwer A, Van Esch H, Delorme R, Launay JM, Tzschach A, Kalscheuer VM, Lacombe D, Briault S, Laumonier F, Raynaud M, van Bon BW, Willemsen MH, Leboyer M, Chelly J, Bourgeron T (2011) Mutation screening of ASMT, the last enzyme of the melatonin pathway, in a large sample of patients with Intellectual Disability. *BMC Med Genet* 12:17
- Pandi-Perumal SR, Seils LK, Kayumov L, Ralph MR, Lowe A, Moller H, Swaab DF (2002) Senescence, sleep, and circadian rhythms. *Ageing Res Rev* 1:559–604
- Pandi-Perumal SR, Srinivasan V, Maestroni GJM, Cardinali DP, Poeggeler B, Hardeland R (2006) Melatonin: nature's most versatile biological signal? *FEBS J* 273(13):2813–2838
- Pappolla MA, Sos M, Omar RA, Bick RJ, Hickson-Bick DL, Reiter RJ, Efthimiopoulos S, Robakis NK (1997) Melatonin prevents death of neuroblastoma cells exposed to the Alzheimer amyloid peptide. *J Neurosci* 17:1683–1690
- Pappolla MA, Chyan YJ, Poeggeler B, Bozner P, Ghiso J, LeDoux SP, Wilson GL (1999) Alzheimer beta protein mediated oxidative damage of mitochondrial DNA: prevention by melatonin. *J Pineal Res* 27:226–229
- Pappolla MA, Chyan YJ, Poeggeler B, Frangione B, Wilson G, Ghiso J, Reiter RJ (2000) An assessment of the antioxidant and the anti-amyloidogenic properties of melatonin: implications for Alzheimer's disease. *J Neural Transm* 107:203–231
- Payton A, Miyajima F, Ollier W, Rabbitt P, Pickles A, Weiss V, Pendleton N, Horan M (2010) Investigation of a functional quinone oxidoreductase (NQO2) polymorphism and cognitive decline. *Neurobiol Aging* 31:351–352
- Peck JS, LeGoff DB, Ahmed I, Goebert D (2004) Cognitive effects of exogenous melatonin administration in elderly persons: a pilot study. *Am J Geriatr Psychiatry* 12:432–436
- Pei Z, Cheung RT (2004) Pretreatment with melatonin exerts anti-inflammatory effects against ischemia/reperfusion injury in a rat middle cerebral artery occlusion stroke model. *J Pineal Res* 37:85–91
- Peng TI, Hsiao CW, Reiter RJ, Tanaka M, Lai YK, Jou MJ (2012) mtDNA T8993G mutation-induced mitochondrial complex V inhibition augments cardiolipin-dependent alterations in mitochondrial dynamics during oxidative,  $Ca^{2+}$ , and lipid insults in NARP cybrids: a potential therapeutic target for melatonin. *J Pineal Res* 52:93–106
- Perez M, Hernandez F, Gomez-Ramos A, Smith M, Perry G, Avila J (2002) Formation of aberrant phosphotau fibrillar polymers in neural cultured cells. *Eur J Biochem* 269:1484–1489
- Poeggeler B, Miravalle L, Zagorski MG, Wisniewski T, Chyan YJ, Zhang Y, Shao H, Bryant-Thomas T, Vidal R, Frangione B, Ghiso J, Pappolla MA (2001) Melatonin reverses the profibrillogenic activity of apolipoprotein E4 on the Alzheimer amyloid A $\beta$  peptide. *Biochemistry* 40:14995–15001
- Poliandri AH, Esquifino AI, Cano P, Jimenez V, Lafuente A, Cardinali DP, Duvilanski BH (2006) In vivo protective effect of melatonin on cadmium-induced changes in redox balance and gene expression in rat hypothalamus and anterior pituitary. *J Pineal Res* 41:238–246
- Princ FG, Juknat AA, Maxit AG, Cardalda C, Battle A (1997) Melatonin's antioxidant protection against delta-aminolevulinic acid-induced oxidative damage in rat cerebellum. *J Pineal Res* 23:40–46
- Quinn J, Kulhanek D, Nowlin J, Jones R, Pratico D, Rokach J, Stackman R (2005) Chronic melatonin therapy fails to alter amyloid burden or oxidative damage in old Tg2576 mice: implications for clinical trials. *Brain Res* 1037:209–213
- Radogna F, Diederich M, Ghibelli L (2010) Melatonin: a pleiotropic molecule regulating inflammation. *Biochem Pharmacol* 80:1844–1852
- Rajaratnam SM, Polymeropoulos MH, Fisher DM, Roth T, Scott C, Birznieks G, Klerman EB (2009) Melatonin agonist tasimelteon (VEC-162) for transient insomnia after sleep-time shift: two randomised controlled multicentre trials. *Lancet* 373:482–491

- Reiter RJ (1980) The pineal and its hormones in the control of reproduction in mammals. *Endocr Rev* 1:109–131
- Reiter RJ (1998) Oxidative damage in the central nervous system: protection by melatonin. *Prog Neurobiol* 56:359–384
- Reiter RJ, Tan DX (2003) What constitutes a physiological concentration of melatonin? *J Pineal Res* 34:79–80
- Reiter RJ, Manchester LC, Tan DX (2010) Neurotoxins: free radical mechanisms and melatonin protection. *Curr Neuropharmacol* 8:194–210
- Reyes Toso C, Ricci C, de Mignone IR, Reyes P, Linares LM, Alborno LE, Cardinali DP, Zaninovich AA (2003) In vitro effect of melatonin on oxygen consumption in liver mitochondria of rats. *Neuroendocrinol Lett* 24:341–344
- Ribelayga C, Pévet P, Simonneaux V (2000) HIOMT drives the photoperiodic changes in the amplitude of the melatonin peak of the Siberian hamster. *Am J Physiol Regul Integr Comp Physiol* 278:R1339–R1345
- Riemersma-van der Lek RF, Swaab DF, Twisk J, Hol EM, Hoogendijk WJ, van Someren EJ (2008) Effect of bright light and melatonin on cognitive and noncognitive function in elderly residents of group care facilities: a randomized controlled trial. *JAMA* 299:2642–2655
- Rod NH, Hansen J, Schernhammer E, Ritz B (2010) Major life events and risk of Parkinson's disease. *Mov Disord* 25:1639–1645
- Rodríguez C, Mayo JC, Sainz RM, Antolin I, Herrera F, Martin V, Reiter RJ (2004) Regulation of antioxidant enzymes: a significant role for melatonin. *J Pineal Res* 36:1–9
- Rosales-Corral S, Tan DX, Reiter RJ, Valdivia-Velazquez M, Martinez-Barboza G, Acosta-Martinez JP, Ortiz GG (2003) Orally administered melatonin reduces oxidative stress and proinflammatory cytokines induced by amyloid-beta peptide in rat brain: a comparative, in vivo study versus vitamin C and E. *J Pineal Res* 35:80–84
- Rosen R, Hu DN, Perez V, Tai K, Yu GP, Chen M, Tone P, McCormick SA, Walsh J (2009) Urinary 6-sulfatoxymelatonin level in age-related macular degeneration patients. *Mol Vis* 15:1673–1679
- Rosenstein RE, Pandi-Perumal SR, Srinivasan V, Spence DW, Brown GM, Cardinali DP (2010) Melatonin as a therapeutic tool in ophthalmology: implication for glaucoma and uveitis. *J Pineal Res* 49:1–13
- Rosenthal NE, Sack DA, Gillin JC, Lewy AJ, Goodwin FK, Davenport Y, Mueller PS, Newsome DA, Wehr TA (1984) Seasonal affective disorder. A description of the syndrome and preliminary findings with light therapy. *Arch Gen Psychiatry* 41:72–80
- Sack RL, Lewy AJ, Erb DL, Vollmer WM, Singer CM (1986) Human melatonin production decreases with age. *J Pineal Res* 3:379–388
- Sainz RM, Mayo JC, Rodríguez C, Tan DX, Lopez-Burillo S, Reiter RJ (2003) Melatonin and cell death: differential actions on apoptosis in normal and cancer cells. *Cell Mol Life Sci* 60:1407–1426
- Saravanan KS, Sindhu KM, Mohanakumar KP (2007) Melatonin protects against rotenone-induced oxidative stress in a hemiparkinsonian rat model. *J Pineal Res* 42:247–253
- Savaskan E, Ayoub MA, Ravid R, Angeloni D, Fraschini F, Meier F, Eckert A, Muller-Spahn F, Jockers R (2005) Reduced hippocampal MT2 melatonin receptor expression in Alzheimer's disease. *J Pineal Res* 38:10–16
- Scheer FA, Van Montfrans GA, Van Someren EJ, Mairuhu G, Buijs RM (2004) Daily nighttime melatonin reduces blood pressure in male patients with essential hypertension. *Hypertension* 43:192–197
- Scher J, Wankiewicz E, Brown GM, Fujieda H (2003) All amacrine cells express the MT1 melatonin receptor in human and macaque retina. *Exp Eye Res* 77:375–382
- Schernhammer E, Chen H, Ritz B (2006) Circulating melatonin levels: possible link between Parkinson's disease and cancer risk? *Cancer Causes Control* 17:577–582
- Serfaty M, Kennell-Webb S, Warner J, Blizard R, Raven P (2002) Double blind randomised placebo controlled trial of low dose melatonin for sleep disorders in dementia. *Int J Geriatr Psychiatry* 17:1120–1127
- Shaikh AY, Xu J, Wu Y, He L, Hsu CY (1997) Melatonin protects bovine cerebral endothelial cells from hyperoxia-induced DNA damage and death. *Neurosci Lett* 229:193–197
- Shirazi A, Mihandoost E, Mohseni M, Ghazi-Khansari M, Rabie MS (2011) Radio-protective effects of melatonin against irradiation-induced oxidative damage in rat peripheral blood. *Phys Med*. <http://dx.doi.org/10.1016/j.ejmp.2011.11.007>
- Siegrist C, Benedetti C, Orlando A, Beltran JM, Tuchscher L, Nosedà CM, Brusco LI, Cardinali DP (2001) Lack of changes in serum prolactin, FSH, TSH, and estradiol after melatonin treatment in doses that improve sleep and reduce benzodiazepine consumption in sleep-disturbed, middle-aged, and elderly patients. *J Pineal Res* 30:34–42
- Silva SO, Rodrigues MR, Carvalho SR, Catalani LH, Campa A, Ximenes VF (2004) Oxidation of melatonin and its catabolites, N1-acetyl-N2-formyl-5-methoxykynuramine and N1-acetyl-5-methoxykynuramine, by activated leukocytes. *J Pineal Res* 37:171–175
- Simonneaux V, Ribelayga C (2003) Generation of the melatonin endocrine message in mammals: a review of the complex regulation of melatonin synthesis by norepinephrine, peptides, and other pineal transmitters. *Pharmacol Rev* 55:325–395
- Singer C, Tractenberg RE, Kaye J, Schafer K, Gamst A, Grundman M, Thomas R, Thal LJ (2003) A multicenter, placebo-controlled trial of melatonin for sleep disturbance in Alzheimer's disease. *Sleep* 26:893–901
- Singhal NK, Srivastava G, Patel DK, Jain SK, Singh MP (2011) Melatonin or silymarin reduces maneb- and paraquat-induced Parkinson's disease phenotype in the mouse. *J Pineal Res* 50:97–109
- Skene DJ, Swaab DF (2003) Melatonin rhythmicity: effect of age and Alzheimer's disease. *Exp Gerontol* 38:199–206
- Skene DJ, Vivien-Roels B, Sparks DL, Hunsaker JC, Pévet P, Ravid D, Swaab DF (1990) Daily variation in the concentration of melatonin and 5-methoxytryptophol in the human pineal gland: effect of age and Alzheimer's disease. *Brain Res* 528:170–174
- Southgate G, Daya S (1999) Melatonin reduces quinolinic acid-induced lipid peroxidation in rat brain homogenate. *Metab Brain Dis* 14:165–171
- 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
- Spuch C, Antequera D, Isabel Fernandez-Bachiller M, Isabel Rodríguez-Franco M, Carro E (2010) A new tacrine-melatonin hybrid reduces amyloid burden and behavioral deficits in a mouse model of Alzheimer's disease. *Neurotox Res* 17:421–431
- Srinivasan V, Singh J, Pandi-Perumal SR, Brown GM, Spence DW, Cardinali DP (2010) Jet lag, circadian rhythm sleep disturbances, and depression: the role of melatonin and its analogs. *Adv Ther* 27:796–813
- Srinivasan V, Spence DW, Pandi-Perumal SR, Brown GM (2011) Cardinali DP (2011) Melatonin in mitochondrial dysfunction and related disorders. *Int J Alzheimer's Dis* vol. doi: [10.4061/2011/741974](https://doi.org/10.4061/2011/741974)
- Sterniczuk R, Dyck RH, Laferla FM, Antle MC (2010) Characterization of the 3xTg-AD mouse model of Alzheimer's disease: part 1. Circadian changes. *Brain Res* 1348:139–148
- Stevens RG, Hansen J, Costa G, Haus E, Kauppinen T, Aronson KJ, Castano-Vinyals G, Davis S, Frings-Dresen MH, Fritschl L,

- Kogevinas M, Kogi K, Lie JA, Lowden A, Peplonska B, Pesch B, Pukkala E, Schernhammer E, Travis RC, Vermeulen R, Zheng T, Coglianò V, Straif K (2011) Considerations of circadian impact for defining ‘shift work’ in cancer studies: IARC Working Group Report. *Occup Environ Med* 68:154–162
- Stopa EG, Volicer L, Kuo-Leblanc V, Harper D, Lathi D, Tate B, Satlin A (1999) Pathologic evaluation of the human suprachiasmatic nucleus in severe dementia. *J Neuropathol Exp Neurol* 58:29–39
- Subramanian P, Mirunalini S, Pandi-Perumal SR, Trakht I, Cardinali DP (2007) Melatonin treatment improves the antioxidant status and decreases lipid content in brain and liver of rats. *Eur J Pharmacol* 571:116–119
- Swabb DF, Fliers E, Partiman TS (1985) The suprachiasmatic nucleus of the human brain in relation to sex, age and senile dementia. *Brain Res* 342:37–44
- Swerdlow RH (2011) Brain aging, Alzheimer’s disease, and mitochondria. *Biochim Biophys Acta* 1812:1630–1639
- Synofzik M, Ronchi D, Keskin I, Basak AN, Wilhelm C, Gobbi C, Birve A, Biskup S, Zecca C, Fernandez-Santiago R, Kaugesaar T, Schols L, Marklund SL, Andersen PM (2012) Mutant superoxide dismutase-1 indistinguishable from wild type causes ALS. *Hum Mol Genet*. <http://dx.doi.org/10.1093/hmg/dds188>
- Tai SH, Hung YC, Lee EJ, Lee AC, Chen TY, Shen CC, Chen HY, Lee MY, Huang SY, Wu TS (2011) Melatonin protects against transient focal cerebral ischemia in both reproductively active and estrogen-deficient female rats: the impact of circulating estrogen on its hormetic dose-response. *J Pineal Res* 50:292–303
- Takeuchi N, Uchimura N, Hashizume Y, Mukai M, Etoh Y, Yamamoto K, Kotorii T, Ohshima H, Ohshima M, Maeda H (2001) Melatonin therapy for REM sleep behavior disorder. *Psychiatry Clin Neurosci* 55:267–269
- Tan DX, Manchester LC, Reiter RJ, Plummer BF, Hardies LJ, Weintraub ST, Vijayalaxmi Shepherd AM (1998) A novel melatonin metabolite, cyclic 3-hydroxymelatonin: a biomarker of in vivo hydroxyl radical generation. *Biochem Biophys Res Commun* 253:614–620
- Tan D, Manchester LC, Reiter RJ, Qi W, Hanes MA, Farley NJ (1999) High physiological levels of melatonin in the bile of mammals. *Life Sci* 65:2523–2529
- Tan DX, Manchester LC, Hardeland R, Lopez-Burillo S, Mayo JC, Sainz RM, Reiter RJ (2003) Melatonin: a hormone, a tissue factor, an autocoid, a paracoid, and an antioxidant vitamin. *J Pineal Res* 34:75–78
- Tan DX, Manchester LC, Sanchez-Barcelo E, Mediavilla MD, Reiter RJ (2010) Significance of high levels of endogenous melatonin in mammalian cerebrospinal fluid and in the central nervous system. *Curr Neuropharmacol* 8:162–167
- Tansey MG, McCoy MK, Frank-Cannon TC (2007) Neuroinflammatory mechanisms in Parkinson’s disease: potential environmental triggers, pathways, and targets for early therapeutic intervention. *Exp Neurol* 208:1–25
- Tapias V, Cannon JR, Greenamyre JT (2010) Melatonin treatment potentiates neurodegeneration in a rat rotenone Parkinson’s disease model. *J Neurosci Res* 88:420–427
- Taysi S, Memisogullari R, Koc M, Yazici AT, Aslankurt M, Gumustekin K, Al B, Ozabacigil F, Yilmaz A, Tahsin OH (2008) Melatonin reduces oxidative stress in the rat lens due to radiation-induced oxidative injury. *Int J Radiat Biol* 84:803–808
- Thomas B, Mohanakumar KP (2004) Melatonin protects against oxidative stress caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the mouse nigrostriatum. *J Pineal Res* 36:25–32
- Travis RC, Allen NE, Peeters PH, van Noord PA, Key TJ (2003) Reproducibility over 5 years of measurements of 6-sulphatoxymelatonin in urine samples from postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 12:806–808
- Tricoire H, Locatelli A, Chemineau P, Malpoux B (2002) Melatonin enters the cerebrospinal fluid through the pineal recess. *Endocrinology* 143:84–90
- Tsai MC, Chen WJ, Tsai MS, Ching CH, Chuang JI (2011) Melatonin attenuates brain contusion-induced oxidative insult, inactivation of signal transducers and activators of transcription 1, and upregulation of suppressor of cytokine signaling-3 in rats. *J Pineal Res* 51:233–245
- Tuney I, Montilla P, Del Carmen MM, Feijoo M, Salcedo M (2004) Protective effect of melatonin on 3-nitropropionic acid-induced oxidative stress in synaptosomes in an animal model of Huntington’s disease. *J Pineal Res* 37:252–256
- Turek FW, Gillette MU (2004) Melatonin, sleep, and circadian rhythms: rationale for development of specific melatonin agonists. *Sleep Med* 5:523–532
- Uchida K, Okamoto N, Ohara K, Morita Y (1996) Daily rhythm of serum melatonin in patients with dementia of the degenerate type. *Brain Res* 717:154–159
- Urata Y, Honma S, Goto S, Todoroki S, Iida T, Cho S, Honma K, Kondo T (1999) Melatonin induces gamma-glutamylcysteine synthetase mediated by activator protein-1 in human vascular endothelial cells. *Free Radic Biol Med* 27:838–847
- Urbanski HF, Sorwell KG (2011) Age-related changes in neuroendocrine rhythmic function in the rhesus macaque. *Age (Dordr)*. <http://dx.doi.org/10.1007/s11357-011-9352-z>
- van Someren EJ (2000) Circadian and sleep disturbances in the elderly. *Exp Gerontol* 35:1229–1237
- Vekrellis K, Xilouri M, Emmanouilidou E, Rideout HJ, Stefanis L (2011) Pathological roles of alpha-synuclein in neurological disorders. *Lancet Neurol* 10:1015–1025
- Venegas C, García JA, Escames G, Ortiz F, Lopez A, Doerrier C, García-Corzo L, Lopez LC, Reiter RJ, Acuña-Castroviejo D (2012) Extrapineal melatonin: analysis of its subcellular distribution and daily fluctuations. *J Pineal Res* 52:217–227
- Voordouw BC, Euser R, Verdonk RE, Alberda BT, de Jong FH, Drogendijk AC, Fauser BC, Cohen M (1992) Melatonin and melatonin–progesterin combinations alter pituitary-ovarian function in women and can inhibit ovulation. *J Clin Endocrinol Metab* 74:108–117
- Wada K, Chatzipanteli K, Busto R, Dietrich WD (1998) Role of nitric oxide in traumatic brain injury in the rat. *J Neurosurg* 89:807–818
- Wade AG, Ford I, Crawford G, McMahon AD, Nir T, Laudon M, Zisapel N (2007) Efficacy of prolonged release melatonin in insomnia patients aged 55–80 years: quality of sleep and next-day alertness outcomes. *Curr Med Res Opin* 23:2597–2605
- Waldhauser F, Steger H (1986) Changes in melatonin secretion with age and pubescence. *J Neural Transm Suppl* 21:183–197
- Waldhauser F, Waldhauser M, Lieberman HR, Deng MH, Lynch HJ, Wurtman RJ (1984) Bioavailability of oral melatonin in humans. *Neuroendocrinology* 39:307–313
- Wang X (2009) The antiapoptotic activity of melatonin in neurodegenerative diseases. *CNS Neurosci Ther* 15:345–357
- Wang X, Sirianni A, Pei Z, Cormier K, Smith K, Jiang J, Zhou S, Wang H, Zhao R, Yano H, Kim JE, Li W, Kristal BS, Ferrante RJ, Friedlander RM (2011) The melatonin MT1 receptor axis modulates mutant Huntingtin-mediated toxicity. *J Neurosci* 31:14496–14507
- Weishaupt JH, Bartels C, Polking E, Dietrich J, Rohde G, Poeggeler B, Mertens N, Sperling S, Bohn M, Huther G, Schneider A, Bach A, Siren AL, Hardeland R, Bahr M, Nave KA, Ehrenreich H (2006) Reduced oxidative damage in ALS by high-dose enteral melatonin treatment. *J Pineal Res* 41:313–323
- Wiesenberg I, Missbach M, Kahlen JP, Schrader M, Carlberg C (1995) Transcriptional activation of the nuclear receptor RZR alpha by the pineal gland hormone melatonin and identification



- of CGP 52608 as a synthetic ligand. *Nucleic Acids Res* 23:327–333
- Willis GL (2008) Parkinson's disease as a neuroendocrine disorder of circadian function: dopamine-melatonin imbalance and the visual system in the genesis and progression of the degenerative process. *Rev Neurosci* 19:245–316
- Willis GL, Armstrong SM (1999) A therapeutic role for melatonin antagonism in experimental models of Parkinson's disease. *Physiol Behav* 66:785–795
- Willis GL, Robertson AD (2004) Recovery of experimental Parkinson's disease with the melatonin analogues ML-23 and S-20928 in a chronic, bilateral 6-OHDA model: a new mechanism involving antagonism of the melatonin receptor. *Pharmacol Biochem Behav* 79:413–429
- Willis GL, Turner EJ (2007) Primary and secondary features of Parkinson's disease improve with strategic exposure to bright light: a case series study. *Chronobiol Int* 24:521–537
- Wilson SJ, Nutt DJ, Alford C, Argyropoulos SV, Baldwin DS, Bateson AN, Britton TC, Crowe C, Dijk DJ, Espie CA, Gringras P, Hajak G, Idzikowski C, Krystal AD, Nash JR, Selsick H, Sharpley AL, Wade AG (2010) British Association for Psychopharmacology consensus statement on evidence-based treatment of insomnia, parasomnias and circadian rhythm disorders. *J Psychopharmacol* 24:1577–1601
- Wu YH, Swaab DF (2007) Disturbance and strategies for reactivation of the circadian rhythm system in aging and Alzheimer's disease. *Sleep Med* 8:623–636
- Wu YH, Feenstra MG, Zhou JN, Liu RY, Torano JS, Van Kan HJ, Fischer DF, Ravid R, Swaab DF (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 J, Seregard S, Alverer PV (2006a) Photochemical damage of the retina. *Surv Ophthalmol* 51:461–481
- Wu YH, Zhou JN, Balesar R, Unmehopa U, Bao A, Jockers R, Van Heerikhuizen J, Swaab DF (2006b) Distribution of MT1 melatonin receptor immunoreactivity in the human hypothalamus and pituitary gland: colocalization of MT1 with vasopressin, oxytocin, and corticotropin-releasing hormone. *J Comp Neurol* 499:897–910
- Wu YH, Zhou JN, Van Heerikhuizen 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
- Wurtman RJ, Zhdanova I (1995) Improvement of sleep quality by melatonin. *Lancet* 346:1491
- Yang X, Yang Y, Fu Z, Li Y, Feng J, Luo J, Zhang Q, Wang Q, Tian Q (2011) Melatonin ameliorates Alzheimer-like pathological changes and spatial memory retention impairment induced by calyculin A. *J Psychopharmacol* 25:1118–1125
- Yuan H, Pang SF (1991) [<sup>125</sup>I]Iodomelatonin-binding sites in the pigeon brain: binding characteristics, regional distribution and diurnal variation. *J Endocrinol* 128:475–482
- Zeitzer JM, Daniels JE, Duffy JF, Klerman EB, Shanahan TL, Dijk DJ, Czeisler CA (1999) Do plasma melatonin concentrations decline with age? *Am J Med* 107:432–436
- Zhang Y, Dawson VL, Dawson TM (2000) Oxidative stress and genetics in the pathogenesis of Parkinson's disease. *Neurobiol Dis* 7:240–250
- Zhang Q, Ding H, Li W, Fan Z, Sun A, Luo J, Ke ZJ (2009) Senescence accelerated mouse strain is sensitive to neurodegeneration induced by mild impairment of oxidative metabolism. *Brain Res* 1264:111–118
- Zhong G, Naismith SL, Rogers NL, Lewis SJ (2011) Sleep-wake disturbances in common neurodegenerative diseases: a closer look at selected aspects of the neural circuitry. *J Neurol Sci* 307:9–14
- 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