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# Melatonin and brain inflammaging

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ABSTRACT

Melatonin is known to possess several properties of value for healthy aging, as a direct and indirect antioxidant, protectant and modulator of mitochondrial function, antiexcitotoxic agent, enhancer of circadian amplitudes, immune modulator and neuroprotectant. It is levels tend to decrease in the course of senescence and are more strongly reduced in several neurodegenerative disorders, especially Alzheimer's disease, and in diseases related to insulin resistance such as diabetes type 2. Although the role of melatonin in aging and age-related diseases has been repeatedly discussed, the newly emerged concept of inflammaging, that is, the contribution of low-grade inflammation to senescence progression has not yet been the focus of melatonin research. This review addresses the multiple protective actions of melatonin and its kynuramine metabolites that are relevant to the attenuation of inflammatory responses and progression of inflammaging in the brain, i.e. avoidance of excitotoxicity, reduction of free radical formation by support of mitochondrial electron flux, prevention of NADPH oxidase activation and suppression of inducible nitric oxide synthase, as well as downregulation of proinflammatory cytokines. The experimental evidence is primarily discussed on the basis of aging and senescence-accelerated animals, actions in the immune system, and the relationship between melatonin and sirtuins, having properties of aging suppressors. Sirtuins act either as accessory components or downstream factors of circadian oscillators, which are also under control by melatonin. Inflammaging is assumed to strongly contribute to neurodegeneration of the circadian master clock observed in advanced senescence and, even more, in Alzheimer's disease, a change that affects countless physiological functions.



*Abbreviations:* AANAT, aralkylamine *N*-acetyltransferase; Aβ, amyloid-β; AD, Alzheimer's disease; AFMK, $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine; AIM2, absent in melanoma 2; ALS, amyotrophic lateral sclerosis; AMK, $N^1$-acetyl-5-methoxykynuramine; AMMC, 3-acetamidomethyl-6-methoxycinnolinone; APP, amyloid precursor protein; BMAL1, brain and muscle aryl hydrocarbon receptor nuclear translocator-like 1 (alias: ARNTL, ARNTL1); CCL, chemokine (C–C motif) ligand; CD, cluster of differentiation; CLOCK, circadian locomotor output cycles kaput; COX, cyclooxygenase; DDR, DNA damage response; ETC, electron transport chain; FTLD, frontotemporal lobar degeneration; GABA, γ-aminobutyric acid; GPx, glutathione peroxidase; GSH, reduced glutathione; GSK-3β, glycogen synthase kinase 3β; GSSG, oxidized glutathione; HD, Huntington's disease; HMG, high mobility group chromatin protein; IFN, interferon; Ig, immunoglobulin; IL, interleukin; iNOS, inducible NO synthase; IRP, immune risk profile; JNK, c-Jun N-terminal kinase; LPS, (bacterial) lipopolysaccharide; IκB-α, inhibitor of NF-κB, alpha; $MT_1$, melatonin receptor 1; $MT_2$, melatonin receptor 2; mtDNA, mitochondrial DNA; mtTFA, mitochondrial transcription factor A; mtPTP, mitochondrial permeability transition pore; NAMPT, nicotinamide phosphoribosyltransferase; NF-κB, nuclear factor κB; NK cells, natural killer cells; NKT cells, natural killer T-cells; NLRP, nucleotide-binding domain and leucine-rich repeat containing protein; nNOS, neuronal NO synthase; Nox, NADPH oxidase; Nrf2, nuclear factor (erythroid-derived 2)-like 2 (alias: NFE2L2); PD, Parkinson's disease; Per2, period-2; $PGE_2$, prostaglandin E2; PI3K, phosphatidylinositol 3-kinase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SASP, senescence-associated secretory phenotype; SCN, suprachiasmatic nucleus; SIRT1, sirtuin 1; SIRT3, sirtuin 3; TNF, tumor necrosis factor; $\Delta\Psi_{mt}$, mitochondrial membrane potential..

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**Contents**



*List of symbols*

| | |
|---|---|
| α | alpha |
| β | beta |
| γ | gamma |
| κ | kappa |
| Δ | delta |
| Ψ | psi |

## 1. Introduction

The term 'inflammaging' has been coined to denominate the contribution of inflammatory processes to the progression of aging (Franceschi et al., 2000; Boren and Gershwin, 2004; Capri et al., 2006; Salvioli et al., 2006; Cevenini et al., 2013). The importance of inflammation in senescence and its role in the development of age-associated diseases is being increasingly perceived. Genetic predispositions such as an immune risk profile (IRP), which comprises an increased tendency toward inflammatory responses, may set limits to health and lifespan, whereas an "inverted IRP" found in centenarians may be the basis of successful aging (Strindhall et al., 2007; Candore et al., 2010). In fact, a well-functioning immune system is believed to be the strongest predictor of human longevity and healthy aging (Franceschi and Bonafè, 2003; Candore et al., 2006; DelaRosa et al., 2006; Ponnappan and Ponnappan, 2011). However, immunosenescence is not simply a process of deterioration, which proceeds more or less rapidly in the various individuals. Instead, the immune system is remodeled with aging in all subjects, a necessity that results from unavoidable changes in immune cell populations, which will be discussed in the following section. Apart from gradual functional losses, which are unavoidable despite the immune remodeling, immunosenescence has two additional undesired consequences, a higher incidence of autoimmune responses (Goronzy et al., 2013) and increased levels of inflammatory mediators (Candore et al., 2010). This latter observation is even made in centenarians. However, in these successfully aged subjects, the higher quantities of proinflammatory factors are associated with augmentations of anti-inflammatory cytokines and a protective genotype (Candore et al., 2010).

Inflammation is not only a matter of normal senescence, but is more strongly observed in a number of diseases, in particular, of the neurodegenerative type, as will be discussed in detail. With regard to the pathological aspects, brain inflammaging is a topic that is attracting increasing interest. As will be outlined in the respective section, the phenomenology of brain inflammation not caused by infections, but by degenerative processes may differ from acute or chronic inflammation elsewhere in the body and appear, in a sense, atypical. It may be of a slowly progressing, lingering type with moderate microglia activation that is sustained by the degenerative processes and oxidative stress resulting from release of reactive oxygen species (ROS) and reactive nitrogen species (RNS) by immune cells, astrocytes and neurons that is further enhanced by damage to mitochondria. Inflammaging, especially in neurodegenerative diseases, is multiply intertwined with other potentially deteriorating processes, among which mitochondrial dysfunction is of prime importance (De la Fuente and Miquel, 2009; Hardeland, 2013a). The connection between the immune system and oxidative stress has been extended by a recently discovered mechanism based on the senescence-associated secretory phenotype (SASP), which leads to a low-level but persistent inflammation, as will be outlined in the next section.

Melatonin is multiply interrelated to brain function, to the immune system and to the defense against damage by ROS and RNS. In its role as the hormone of the pineal gland, it is not only released to the circulation, from where it can enter the CNS, but also directly, via the pineal recess, to the third ventricle of the brain (Tricoire et al., 2002, 2003a,b). It is additionally synthesized in various immune cells (Carrillo-Vico et al., 2013; Hardeland, 2013a). Melatonin receptors are found in many places within the CNS and also in the majority of the immune cells (summarized in: Hardeland et al., 2011). It does not only participate in the regulation of sleep and circadian rhythms, but exerts numerous additional actions in the CNS, notably as an antiexcitatory agent, a regulator of hormone secretion, of metabolic pathways and intracerebral blood flow. In the immune system including microglia, it modulates the release of various pro- and anti-inflammatory cytokines as well as other immune stimulatory factors and can directly activate monocytes (Guerrero and Reiter, 2002; Carrillo-Vico et al., 2013; Hardeland, 2013a). It attenuates oxidative stress by various mechanisms that comprise avoidance of free radical-generating conditions, downregulation of strongly elevated inducible NO synthase (iNOS) and neuronal NO synthase

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(nNOS), upregulation of antioxidant enzymes and free radical scavenging (Reiter et al., 2001, 2002, 2003; Hardeland, 2005; Hardeland et al., 2011).

With regard to the eminent role of mitochondria in cell death, autophagy and free radical generation by electron dissipation from the electron transport chain (ETC), mitochondrial effects of melatonin are also of particular importance in the context of inflammation, which can lead to blockade of the ETC, enhanced electron overflow, breakdown of the mitochondrial membrane potential, and apoptosis. Protective actions of melatonin concerning maintenance of electron flux, decrease of electron leakage and structural integrity of the organelle have been extensively described and summarized (Hardeland et al., 2009a; Hardeland, 2013a). These effects have been observed under various conditions, from acute inflammation to normal and accelerated aging.

Without discussing at this point the gerontological significance of melatonin in detail, it should be mentioned that strong indications exist for its support of healthy aging (Poeggeler, 2005; Hardeland, 2013a). In laboratory animals, however, an extension of lifespan is, when demonstrable, only marginal. Several beneficial effects of melatonin have been observed in numerous models of neurodegenerative disorders, including Alzheimer's disease (AD), although a halting of disease progression is not or only poorly demonstrable in humans (Srinivasan et al., 2006; Cardinali et al., 2010).

A potentially important aspect of the relationship between melatonin and aging, especially inflammaging, concerns the changes of melatonin secretion that are frequently observed in the course of senescence. In many subjects, the nocturnal secretion of melatonin decreases with age, though with considerable interindividual variation. Reductions in melatonin levels are even more expressed in various diseases and disorders, most strongly in neurodegenerative diseases such as AD and other forms of dementia (Skene et al., 1990; Skene and Swaab, 2003; Hardeland, 2012, 2013a). In other words, melatonin, a regulatory molecule that can attenuate stimuli initiating inflammatory responses, that reduces damage to cells, mitochondria and DNA, and presumably prevents excessive conversion of cells to the DNA-damaged, senescent phenotype with SASP, undergoes a substantial decline in many aging individuals and, even more, in patients affected by neurodegeneration. The relationship between the decrease in melatonin and brain inflammaging merits future attention.

## 2. Inflammaging: new concepts and mechanisms

Earlier concepts of aging focused on various different mechanisms and hypotheses, such as assumed life-determining limits of energy expenditure, telomere attrition leading to reduced cell growth and progressive losses in number and proliferative potential of stem cells that cause deficits in tissue repair. All these processes do contribute to aging, but they should not be regarded as isolated, monocausal alterations that explain aging on an exclusive basis, but are rather interconnected and may become relevant in different stages of senescence, as recently discussed elsewhere (Hardeland, 2013a). The multiple nexus to other processes also apply to the frequently cited free radical theory of aging (Harman, 1956, 2006; Afanas'ev, 2010), including its variants focusing on mitochondrial dysfunction (Harman, 1972; Miquel et al., 1992; Gruber et al., 2008; Gomez-Cabrera et al., 2012). With regard to the immune system, attention was first focused on its functional losses. However, the perception that the immune system is not just decaying, but undergoing remodeling processes that include tendencies toward enhanced proinflammatory signaling have led to a new understanding of aging in conjunction with enhanced inflammation, as denominated by inflammaging. The importance of this view is supported by several findings, such as enhanced inflammation in neurodegenerative diseases, the effects of inflammatory signals and free radicals released by immune cells on neurons and astrocytes, and, more recently, the discovery of SASP with its consequences for sustained low-level inflammation.

### 2.1. Causes resulting from immunosenescence

Immunosenescence is one of the causes of chronic low-grade inflammation. The remodeling of the immune system is a necessity to which several processes contribute. One of these is progressive thymic involution, which leads to losses in the number of both $CD4^+$ and $CD8^+$ T lymphocytes as well as a disturbed balance between naïve, memory and effector T cells (Linton and Thoman, 2001; Franceschi and Bonafè, 2003; Pfister and Savino, 2008; Pinti et al., 2010; Goronzy et al., 2013). Typically an exhaustion of $CD95^-$, virgin T lymphocytes is observed. This concerns especially the subset of $CD8^+$ cells, which are largely replaced by clonal expansion of $CD28^-$ cells of lower proliferative activity (Sansoni et al., 2008). With regard to $CD8^+$ T lymphocytes, type 1 cytokines are mainly elevated, such as interleukin-2 (IL-2), interferon-γ (IFNγ) and tumor necrosis factor-α (TNFα), and, to a smaller extent, type 2 cytokines, such as IL-4, IL-6, and IL-10. In balance, this contributes to a higher inflammatory state, however, with large interindividual differences. This divergence may be related to the degree of thymic involution and the maintenance of a population of "recent thymic emigrants" (Pinti et al., 2010). Age-dependent changes have been also reported for $CD4^+$ T lymphocytes, mostly in the $CD95^+$ $CD28^+$ subset, i.e., activated/memory cells, among which TNFα-positive cells are decreased, but IL-4-positive cells increased, reflecting, in these cells, a shift from type 1 to type 2 cytokines (Alberti et al., 2006).

Alterations in T cell subpopulations also affect the functionality and composition of B lymphocytes. A second cause is life-long exposure to numerous foreign antigens, which leads to changes in B cell subpopulations (Listi et al., 2006; Colonna-Romano et al., 2010). B cell immunosenescence results in decreases of immunoglobulin M (IgM) and IgD production, whereas increased levels of IgG1 have been reported (Buffa et al., 2013). A presumably relevant regulatory dysfunctionality is apparent in strong losses of naïve $IgD^+$ B cells, which are replaced by exhausted memory $IgD^-$B cells (Listi et al., 2006). Interestingly, in centenarian offspring, naïve $IgD^+$ $CD27^-$B cells were more abundant, whereas exhausted $IgD^-CD27^-$B were mostly unchanged, findings that may indicate a favorable genetic predisposition (Colonna-Romano et al., 2010). A recently described, TNF-producing $CD19^+$ $CD38^-CD24^-$ memory B cell subset was found to be lower in centenarian offspring, which would also indicate a lower inflammatory status (Buffa et al., 2013).

The alterations observed during immunosenescence do not concern only the adaptive, but also the innate immune system (Mocchegiani et al., 2007, 2009; Shaw et al., 2010; Gayoso et al., 2011), although the latter is usually less affected, which is especially apparent in very old individuals (Mocchegiani et al., 2009; Dewan et al., 2012). Centenarians were reported to have a relatively higher number of natural killer (NK) and natural killer T (NKT) cells, especially NKT cells bearing the γδ-T cell receptor, in contrast to less successfully aging subjects. Moreover, these cells responded more strongly to activation in centenarians by releasing IFNγ (Mocchegiani et al., 2007, 2009), which would underline the importance of a robust innate immune system. However, this potential for a more efficient defense is partially compensated, at the inflammatory side, by a lower expression of the IL-6 receptor. This finding is therefore of particular importance as sustained elevated IL-6 release, which occurs under chronic inflammatory conditions, leads to high metallothionein expression and, thereby, to poor zinc availability because of its sequestration by this protein

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(Mocchegiani et al., 2007). Indeed, zinc is important to several aspects of the immune system, including function of the thymus.

### 2.2. Senescence-associated secretory phenotype (SASP)

SASP has turned out to be a continually active source of low-grade inflammation and oxidative stress. Because it depends on DNA damage, which becomes more likely with increasing age and accumulates with time, its significance to aging tissues including the CNS steadily rises. Senescent cells carrying damaged DNA are usually mitotically arrested, a mechanism that keeps them alive and metabolically active, but prevents a neoplastic fate. However, these cells display a chronic DNA damage response (DDR) (Fumagalli and d'Adda di Fagagna, 2009). Apart from other effects concerning the cytoskeleton and formation of protein aggregates, the most important change in the behavior of these senescent, arrested cells concerns the release of proinflammatory cytokines, the hallmark of SASP (Coppé et al., 2008, 2010; Young and Narita, 2009). These factors, which are notably secreted by nonimmune cells, attract and/or activate immune cells. On the one hand, they may lead to the elimination of a number of senescent cells, but, on the other hand, they cause low-grade inflammation. Since inflammation is a source of oxidative stress, the number of cells damaged by ROS and RNS is, on balance, steadily rising. In the extreme, ROS and RNS derived by the SASP mechanism can even induce cancer in previously nondamaged cells, in the worst case, in stem cells, which turn into telomerase-expressing cancer stem cells, which are usually not arrested (Davalos et al., 2010; Acosta et al., 2013). Thus, a mechanism that primarily prevents tumor formation, can secondarily become tumorigenic.

Interestingly, aging astrocytes express SASP characteristics (Salminen et al., 2011). Thus, low-level inflammation and, eventually, induction of cancer stem cells by SASP are also relevant to the aging brain.

### 2.3. Neuronal overexcitation and microglia activation

Neuronal overexcitation and inflammatory responses are intimately associated. These processes can be stimulated from either side. While glutamate excitotoxicity can lead to microglia activation (Campuzano et al., 2008; Arlicot et al., 2014; Lin et al., 2014; Nomaru et al., 2014), primary immune responses involving the microglia may, in turn, lead to excitotoxicity (Takeuchi et al., 2005; Brown, 2007; Brown and Neher, 2010). Astrocytes are frequently coactivated with microglial cells. They also contribute to both excitotoxicity and microglia activation, by mechanisms that involve impaired glutamate uptake, inflammatory signals, and/or oxidative/nitrosative stress (Tilleux and Hermans, 2007; Brown, 2007; Salminen et al., 2011; Morales et al., 2014). As will be discussed in Section 5, melatonin is capable of attenuating the detrimental changes at all these levels. A particular problem results from the fact that inflammatory responses can be initiated from different sides. Inflammasomes that may cause both release of proinflammatory cytokines such as IL-1β and IL-18 and apoptotic or pyroptotic cell death are found in neurons (NLRP1 and AIM2), astrocytes (NLRP2) and microglia (NLRP3) (de Rivero Vaccari et al., 2014). Histone H1 released from dying cells of whatever type acts as an additional pro-inflammatory signal and chemoattractant (Gilthorpe et al., 2013). Moreover, stimulation of proinflammatory processes in different cell types implies the possibility of positive feedback loops between neurons, astrocytes and microglia that expand the grade and area of inflammation and may be further aggravated by recruitment of other immune cells.

However, it seems important to remain aware of the conditionality of these immune-stimulatory processes and their activating interconnections. In particular, microglia is neither a basically inactive population of cells, as believed earlier, nor a uniformly responding entity. Even the ramified microglia has been shown to be continuously active in terms of movement and safeguarding the central nervous microenvironment (Blaylock, 2013). Activation of microglia can lead to different phenotypes, from neurodestructive and phagocytic cells to others that are primarily neuroprotective or growth promoting (Blaylock, 2013). To perceive these differences is important insofar as most of the information concerning the connections between overexcitation and microglial activity has been obtained in animal models of excitotoxicity, brain injury or immunological challenge, e.g., by endotoxemia. A crucial question concerns the applicability of these findings to the slow, lingering alterations that occur in the course of aging, with regard to both asymptomatic changes and age-associated pathologies. According to current knowledge, the factors that initiate age-related deviations in neuronal activity and low-grade inflammation may frequently differ from the inducers applied in the experimental models, but the intercellular communication routes and the signaling pathways seem to be largely the same. At least, one hallmark of inflammaging, namely, SASP, has been demonstrated in astrocytes (Salminen et al., 2011) and, therefore, exists in an aging brain that is not compromised by more severe insults. When additional pro-inflammatory signals come into play in the course of neuropathological changes, the neuroinflammatory traits may be different from those observed after chemically induced excitotoxicity, brain injury or endotoxemia, but may still contribute to a considerable extent to the progressing anatomical and functional deterioration in the CNS.

### 2.4. Inflammaging progression in neurodegenerative pathologies

While inflammaging should be basically understood as a normal, but for quite some time limited process in the nondiseased brain, in parallel to similar changes occurring in other tissues, it is brain-specifically accelerated and aggravated under neuropathological conditions. A sustained, progressive, primarily low-grade inflammation is observed in presumably all neurodegenerative disorders, with differences in details, pathways and affected cells (Lyman et al., 2014; Lynch, 2014; Nuzzo et al., 2014; Urrutia et al., 2014). Neuroinflammation has been demonstrated in Huntington's disease (HD) (Chandra et al., 2014; Crotti et al., 2014), amyotrophic lateral sclerosis (ALS) (Bowerman et al., 2013; Zhao et al., 2013; Rizzo et al., 2014), Friedreich's ataxia (Lu et al., 2009), Parkinson's disease (PD) (More et al., 2013; Nolan et al., 2013; Taylor et al., 2013), frontotemporal lobar degeneration (FTG or FTLD) (Miller et al., 2013; Ridolfi et al., 2013), and Alzheimer's disease (AD) (Fuster-Matanzo et al., 2013; Ridolfi et al., 2013; Liu and Chan, 2014; Lopategui Cabezas et al., 2014).

The inflammatory component in neurodegenerative disorders was not obvious from the beginning. Meanwhile, inflammaging has been interpreted as a prodromal process to AD (Giunta et al., 2008). However, as discussed elsewhere (Srinivasan et al., 2006), differences exist between tissue acutely inflamed because of infection and the AD brain, in which some classical hallmarks are usually absent, such as neutrophil infiltration and edema, whereas other characteristics including microglia activation, release of pro-inflammatory cytokines (IL-1β, IL-6, IL-15, IL-18, TNFα), appearance of acute-phase proteins (e.g., C-reactive protein) and lymphocyte recruitment are clearly demonstrable. In addition, enhanced NO formation, which is largely caused by microglial upregulation of iNOS, elevated ROS generation by microglial NADPH oxidase (Brown and Neher, 2010) and impairments of mitochondrial electron flux, lead to critical levels of peroxynitrite formed by combination of NO and superoxide, and secondary radicals from the decay of peroxynitrite adducts, such as $NO_2$, hydroxyl and carbonate radicals (Hardeland and Coto-Montes,

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2010). The resulting nitroxidative stress and damage is reflected by widespread protein modifications induced by peroxynitrite or peroxynitrite-derived free radicals (Smith et al., 1997; Hensley et al., 1998). For further details concerning mitochondrial dysfunction, oxidative and nitrosative stress see Srinivasan et al. (2006) and Hardeland and Coto-Montes (2010). This includes the contribution of toxic mono- and oligomers of amyloid-β (Aβ) peptides, Aβ metal complexes that undergo redox cycling, and the depletion of copper and zinc by amyloid plaques, which impairs the formation of functionally active Cu,Zn-superoxide dismutase (summarized in: Hardeland, 2011a).

Inasmuch as early phases of neurodegenerative disorders are poorly distinguishable from normal inflammaging or other forms of low grade inflammation, these pathologies display important mechanisms of aggravation, in terms of vicious cycles. These comprise accumulation of toxic products, such as Aβ peptides, oligomers and plaques in AD, microglial proliferation and phagocytosis after their activation, and progressive formation of pro-inflammatory signal molecules. Elevated levels of IL-6 and TNFα are demonstrable in the CNS as well as in the circulation, reflecting dysregulation of inflammatory pathways, and are meanwhile regarded as markers of frailty (Michaud et al., 2013). Increases in IL-15, a cytokine that stimulates activities and proliferation of cytotoxic T-cells, NK cells and B-cells, are associated in the CNS with microglial activation and are especially observed in AD and PD, but also under other neuroinflammatory conditions (Rentzos and Rombis, 2012). In AD, a specific deteriorating role may be attributed to IL-18, a cytokine that is not only a key mediator of inflammation involved in neurodegeneration (Bossù et al., 2010), but has been also shown to promote Aβ formation (Sutinen et al., 2012). Interrupting the pro-inflammatory vicious cycles represents a main challenge for the combat against neurodegenerative diseases.

## 3. Melatonin's age-dependent decline

The pleiotropically acting hormone melatonin which influences countless functions either directly or via circadian oscillators, has additional relevance to aging because of its roles as an immunomodulator (Carrillo-Vico et al., 2013), downregulator of nNOS and iNOS, antiexcitatory and partially anti-inflammatory agent (Hardeland et al., 2011), protector of mitochondrial electron flux (Acuña-Castroviejo et al., 2003, 2007; Hardeland et al., 2009a; Hardeland, 2013a), and multiply acting antioxidant (Reiter et al., 2001, 2002, 2003; Hardeland, 2005; Tan et al., 2007). Deviations in the levels and/or circadian patterns of this molecule have to be expected to cause a plethora of physiological effects. On a statistical basis, the nocturnal maximum of melatonin decreases in the course of aging, although the interindividual variability is relatively large (Sack et al., 1986; Karasek and Reiter, 2002; Skene and Swaab, 2003). While several individuals maintain a fairly well pronounced rhythm with only moderate reductions of nocturnal values, others display nocturnal values close to those during daytime. For causes of reduced melatonin formation see Hardeland (2012). As soon as age-related reductions of melatonin become more profound, numerous changes are observed (Hardeland, 2012), among these considerable alterations in the immune system, as summarized earlier (Cardinali et al., 2008a). Decreased levels of melatonin are not only observed in pineal gland, blood and some other body fluids, but also in the cerebrospinal fluid (Brown et al., 1979; Liu et al., 1999). As long as a melatonin rhythm is still detectable in elderly individuals, the nocturnal peak of plasma melatonin is frequently phase-advanced relative to young subjects, indicating changes in the circadian oscillator system (Skene and Swaab, 2003). The interindividual variability of melatonin levels at advanced age raises the questions of whether maintenance of substantial melatonin rhythmicity can be taken as a marker of successful aging, and whether a more pronounced decrease is partially or more importantly causal to other physiological or even cognitive deteriorations.

In several neurodegenerative disorders, especially AD and other types of senile dementia, levels of melatonin are frequently more strongly decreased than in age-matched controls (Skene et al., 1990; Liu et al., 1999; Skene and Swaab, 2003; Srinivasan et al., 2005; Wu and Swaab, 2007; Hardeland, 2012). In quite a number of these patients, the melatonin rhythm is practically abolished. Decreased melatonin levels are also observed in various other disorders and diseases, but notably not PD (summarized in: Hardeland, 2012). These include neurological pathologies, painful conditions, and a number of metabolic disorders or diseases that seem, at first glance, unrelated to brain functions. This concerns especially diabetes type 2 (O'Brien et al., 1986; Peschke et al., 2007). Interestingly, the connection of this disease to melatonin is supported by other findings on altered melatonin signaling and concomitant age-associated impairments of cognitive functions, and the development of diabetes as a consequence of defective clock functions (Marcheva et al., 2010; Hardeland et al., 2012). These aspects have recently gained considerable actuality, as nexuses between insulin resistance, neuroinflammation and AD have become apparent (Clark and Vissel, 2013; Jiang et al., 2013; De Felice et al., 2014; de la Monte and Tong, 2014; Ferreira et al., 2014).

## 4. Two-sided role of melatonin in inflammation: proinflammatory vs. anti-inflammatory actions

In its role as an immune modulator, melatonin has been reviewed several times (Guerrero and Reiter, 2002; Carrillo-Vico et al., 2005, 2006, 2013; Szczepanik, 2007; Cardinali et al., 2008a; Hardeland et al., 2011; Hardeland, 2013a). Its functions are diverse. As summarized in the reviews mentioned, it is produced by and acts on various types of leukocytes. Among these, T lymphocytes and NK cells may be of particular interest in the context of brain inflammaging. Although melatonin formation was demonstrated in monocytes, corresponding findings have not been published to date for microglia. In monocytes, it enhances ROS formation and cytotoxicity, representing one of the strongest direct prooxidant effects of melatonin reported (Morrey et al., 1994). Similar findings have been also observed in the promonocytic cell line U937 (Cristofanon et al., 2009; Radogna et al., 2009). Again, a direct demonstration of this effect is missing in microglia, although clarification of this possibility would be of considerable importance for the understanding of inflammatory dynamics in the brain. It may be that the threshold for this type of activation is higher in microglia than in other cells of the monocytic line and, therefore, not relevant to low-grade inflammation.

Numerous effects of melatonin known from melatonin in the peripheral parts of the immune system may well be relevant to immune functions in general and to inflammaging as well, but not necessarily to brain inflammation. This may especially concern the expansion of lymphocyte subtypes and changes in leukocyte abundance or activity in the various peripheral immune tissues including lymph nodes, gut, spleen and thymus (Carrillo-Vico et al., 2013; Cardinali et al., 2008a; Hardeland et al., 2011; Hardeland, 2013a). Nevertheless, the changes observed in the peripheral immune system during senescence may well influence health and indirectly affect the CNS.

If judged under the rubric of cytokine release, one arrives at the conclusion that melatonin can alternately exert pro- and anti-inflammatory effects (Carrillo-Vico et al., 2013; Hardeland, 2013a). Among the immune-stimulatory and potentially or demonstrably pro-inflammatory cytokines, elevations of IL-1β, IL-2, IL-6, IL-12,

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TNFα, and IFNγ can be mentioned (García-Mauriño et al., 1997, 1998, 1999; Barjavel et al., 1998; Carrillo-Vico et al., 2005; Lardone et al., 2006). An additional classic finding describes the counteraction by melatonin of the inhibitory effect of prostaglandin $E_2$ ($PGE_2$) on IL-2 production (Carrillo-Vico et al., 2003). Moreover, a decrease in the anti-inflammatory IL-10 was reported (Kühlwein and Irwin, 2001) as well as a reduction of IL-2-induced IL-10 secretion (Lissoni et al., 1996). However, these results seem to largely depend on cells, systems studied and especially conditions relevant to the grade of inflammation. In particular, downregulations of IL-1β, IL-6, IL-8, IL-12, and TNFα have been repeatedly documented under conditions of high oxidative stress, ischemia/reperfusion, brain trauma, hemorrhagic shock, and various forms of high-grade inflammation including sepsis (summarized in: Hardeland, 2013a). Two additional anti-inflammatory effects have to be taken into consideration. First cyclooxygenase-2 (COX-2) expression has been shown to be downregulated by melatonin in macrophages via nuclear factor-κB (NF-κB) (Mayo et al., 2005b; Deng et al., 2006; Korkmaz et al., 2012). The same signaling pathway seems to be involved in the suppression of iNOS in macrophages (Deng et al., 2006; Korkmaz et al., 2012), an effect observed in various organs and cells (López et al., 2006; Escames et al., 2006a,b, 2007), and notably also in microglia and astrocytes (Jiménez-Ortega et al., 2009; Tapias et al., 2009).

The contrasting results on either pro- or anti-inflammatory actions seem to indicate that the former effects are observed under basal conditions, whereas the latters are typical for experimental high-grade inflammation. Under these aspects, melatonin has been regarded as an immune modulator with buffering properties, allowing immune stimulation in response to, e.g., infectious challenges, but mitigating severe damage in high-grade inflammation (Carrillo-Vico et al., 2013). Another interpretation has assumed that melatonin may only promote early phases of inflammation, but attenuates its continuation to avoid chronification of a disease (Radogna et al., 2010).

Therefore, the decisive question is how melatonin acts under conditions of low-grade inflammation, especially during inflammaging and specifically in the CNS, and under the consideration of SASP. This problem extends toward its utility in advanced neuroinflammation, e.g., in the different stages of AD. To date, a definitive answer based on evidence cannot be given. In particular, the effects of melatonin on SASP-dependent inflammation are practically unknown, because the proinflammatory cytokines are not mainly derived from immune cells, but from previously normal nonimmune cells that have been mitotically arrested after mutation and display a chronic DDR. However, there are several indications for a preferably anti-inflammatory role of melatonin in the course of aging. In old female rats, melatonin decreased the proinflammatory cytokines TNFα, IL-1β and, to a considerable extent, IL-6, whereas it strongly increased the anti-inflammatory IL-10 in the liver (Kireev et al., 2008). In the senescence-accelerated mouse strain SAMP8 (Chang et al., 2009) and in aged murine neurons (Tajes et al., 2009), melatonin upregulated sirtuin 1 (SIRT1). Recently, melatonin was shown to upregulate SIRT1 in the dentate gyrus of aged, ovariectomized rats, in conjunction with substantial decreases in proinflammatory cytokines (Kireev et al., 2014). It remains open to which extent these latter effects have been directly exerted by melatonin or indirectly by the sirtuin. SIRT1 is also known to have anti-inflammatory properties and its inactivation by posttranslational modification is believed to promote inflammaging (Hwang et al., 2013). Similar increases of SIRT1 were obtained in the pancreas of SAMP8 mice (Cuesta et al., 2013). Although SIRT1 and melatonin appear to be negatively coupled, when studied in tumor cells, this may not be a contradiction when analyzed on a chronobiological basis (Hardeland, 2013a). The observed upregulation of this sirtuin by melatonin in the context of aging might indicate an additional anti-inflammatory action of the methoxyindole in senescence. Notably, both melatonin and the SIRT1 ligand resveratrol were reported to protect cultured neurons from SAMP8 mice against frailty-inducing agents, including buthionine sulfoximine, which depletes reduced glutathione, and mitochondrial toxins (Cristòfol et al., 2012). The toxicological effects were more pronounced in SAMP8 than in the normally aging, otherwise isogenic SAMR1 mice. These authors also described upregulation of SIRT1 by both melatonin and resveratrol and counteractions by the SIRT1 inhibitor sirtinol. These findings are in line with directly studied anti-inflammatory effects of melatonin in SAMP8 mice obtained, however, in peripheral organs. In the liver, IL-1β and TNFα were downregulated and IL-10 upregulated by melatonin (Cuesta et al., 2010). Corresponding anti-inflammatory effects were observed in the pancreas (Cuesta et al., 2011) and heart (Forman et al., 2010, 2011) of this strain. Collectively, these studies are in favor of the idea that melatonin behaves in a primarily anti-inflammatory way in the context of normal aging. What has been observed in the peripheral organs, may also apply to the brain, although the experimental basis for this concept is still limited. Whether the same may be the case under advanced neurodegenerative conditions, remains to be studied.

## 5. Prevention of inflammation initiation by melatonin

With regard to neuroinflammation, it is important to distinguish between the causes of initiation and the self-sustaining, continually aggravating processes in disease progression. This distinction is also of relevance to the perspectives of medicinal intervention. The chances for a successful therapy are presumably much better in early than in advanced stages. Progressed inflammation has already led to numerous deteriorations and, in diseases with pathological accumulations of misfolded proteins and intracellular cytoskeletal tangles, to dysfunctions and dysregulations that perpetually re-stimulate inflammatory responses.

Beyond infectious causes, inflammation via primary microglia activation that is not initiated by an interplay with neurons or astrocytes seems to be the exception. However, this possibility cannot be generally ruled out with regard to SASP, since damage of DNA does not only occur upon oxidative stress, but also takes place as a stochastical process that hits here and there at one or other cell. Nevertheless, one of the most frequent causes of microglia activation and resulting inflammation is neuronal overexcitation, e.g., by overshooting glutamatergic stimulation, which may also be caused by insufficient glutamate reuptake by astrocytes. Another important cause of neuroinflammation is mitochondrial dysfunction, which may be associated with overexcitation, enhanced NO formation and $Ca^{2+}$ overload. It leads to enhanced electron dissipation and, thus, formation of ROS, peroxynitrite and additional peroxynitrite-derived free radicals (Hardeland et al., 2009a; Hardeland, 2009a,). Although apoptotic cell death does not lead to inflammation, and although mitochondrial dysfunction can be attenuated by mitophagy, the spreading of ROS and RNS may suffice to initiate microglia activation. Moreover, the mitochondrial changes are associated with imbalanced fusion/fission homeostasis and peripheral loss of these organelles causing functional impairments of neurotransmission (Hardeland, 2009b). Melatonin is known to antagonize both excitotoxicity and mitochondrial dysfunction (Hardeland et al., 2009a; Hardeland and Coto-Montes, 2010). Therefore, it may halt early stages of neuroinflammation.

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### 5.1. Antiexcitatory actions

The antiexcitatory actions of melatonin comprise different mechanisms. They have not only been studied in the context of sedation, but also of anticonvulsive effects (Fariello et al., 1977; Golombek et al., 1992a,b, 1996; Muñoz-Hoyos et al., 1998; Molina-Carballo et al., 2007; Solmaz et al., 2009) and they may be also partially related to anxiolytic, antihyperalgesic and antinociceptive properties of the methoxyindole (Golombek et al., 1991a,b, 1993; Pang et al., 2001; Papp et al., 2006; Ulugol et al., 2006; Srinivasan et al., 2010). The antiexcitatory effects should not be confused with sleep-inducing and -promoting effects, because they are also observed in nocturnally active animals, in which high melatonin levels are associated with physical exercise and neural activity. Instead, the antiexcitatory function should rather be understood in terms of setting limits to overexcitation and, thus, to avoid all the negative consequences of excitotoxicity and possibly resulting in nitroxidative stress, mitochondrial impairments and microglia activation.

The anticonvulsant actions of melatonin seem to be, at least partially, mediated by the membrane receptors, $MT_1$ and/or $MT_2$, because this property was also reported for ramelteon, a synthetic, $MT_1/MT_2$-selective melatonergic agonist (Fenoglio-Simeone et al., 2009). The antiexcitatory/antiexcitotoxic functions steered by melatonergic signaling may likewise largely depend on these receptors, although this has not always been demonstrated directly. Among these, the inhibition of nNOS (León et al., 2000; Acuña-Castroviejo et al., 2005; Entrena et al., 2005; Jiménez-Ortega et al., 2009) may be of particular importance. However, this effect seems to be only effective as soon as nNOS is strongly upregulated, since the normal circadian maximum of NO in the rat CNS almost coincides with that of melatonin (Clément et al., 2003; Hardeland et al., 2003). The value of this mechanism should be mainly seen in the prevention of overexcitation. Other antiexcitatory actions concern the modulation of GABA and glutamate receptors (Rosenstein and Cardinali, 1990; Molina-Carballo et al., 2007), in particular, decreases in cytosolic $Ca^{2+}$ via $GABA_c$ (Prada et al., 2005) or metabotropic glutamate $mGlu_3$ receptors (Prada and Udin, 2005), and GABAergic facilitation (Cardinali et al., 2008b). Using patch clamp techniques and measurements of cytosolic $Ca^{2+}$, inhibitory effects on high voltage-activated calcium channels were described in dorsal root ganglion neurons (Ayar et al., 2001). Moreover, modulations of the opioid system (Golombek et al., 1991a; Ulugol et al., 2006; Srinivasan et al., 2010, 2012), changes in $K^+$ currents (Liu et al., 2007; Corthell et al., 2014), and, in retinal ganglion cells, an $MT_2$-dependent potentiation of glycine receptor-mediated inhibitory post-synaptic currents (Zhang et al., 2007; Zhao et al., 2010; Ji et al., 2011) have been reported. Although the glycine-related effects are site-specific and may not be applicable to other CNS regions, they underline the more general antiexcitatory role of melatonin.

### 5.2. Mitochondrial protection

Mitochondrial function, integrity and intracellular distribution are central to various aspects of healthy aging, such as attenuation of aging-associated oxidative damage, free radical-induced microglia activation, neuronal overexcitation, losses of connectivity because of peripheral mitochondrial depletion and cell death. Melatonin interferes in multiple ways with these processes of deterioration. Thus, its mitochondrial actions that are relevant to aging and age-related diseases will have to be discussed in this section.

Mitochondrial dysfunction is both cause and consequence of excessive free radical formation and inflammatory responses. Therefore, any reduction of free radicals by melatonin, via diminished production of ROS and RNS, upregulation of antioxidant enzymes, avoidance of $Ca^{2+}$ overload, improvements of mitochondrial electron flux, radical scavenging or anti-inflammatory effects, contributes to the preservation of mitochondrial integrity and function. Antiapoptotic actions of melatonin are also associated with its modulation of the balance between pro- and antiapoptotic mitochondrial proteins of the intrinsic pathway of apoptosis, an influence on cardiolipin peroxidation, and prevention of a long-lasting breakdown of the mitochondrial membrane potential ($\Delta\Psi_{mt}$). For details regarding especially the progression of aging see Hardeland (2013a).

The protection of mitochondria is also important because of the high vulnerability of these organelles by oxidative and nitrosative damage. However, this is frequently misinterpreted in terms of its causes. The main reason is not an insufficient protection of mitochondrial DNA (mtDNA), which is, in fact, not naked, but rather relatively densely covered by mtTFA, the mitochondrial transcription factor A, which is structurally related to high mobility group (HMG) chromatin proteins and also serves functions in nucleoid structure, damage sensing and mitochondrial replication (Kang and Hamasaki, 2005). Other proteins bound to mtDNA are antioxidant enzymes that constitute integral components of the mitochondrial nucleoid and convey on-site protection (Kienhöfer et al., 2009). Moreover, investigations in mtDNA mutator mice revealed that the production of free radicals was not demonstrably increased in these animals during aging, relative to controls, although mitochondrial mutations had accumulated (Thompson, 2006).

Protection of mitochondria by melatonin has been described under various conditions. Two findings of potential relevance to changes that occur during aging are mtDNA copy numbers and attenuation of mitophagy, although they were not obtained in aged animals. Kainic acid-induced neurotoxicity in the mouse hippocampus, associated with mitophagy and α-synuclein aggregation, was antagonized by melatonin, which normalized the cellular content of mtDNA and cytochrome c (Chang et al., 2012). In a mouse model of opiate addiction, the progressive reduction of hippocampal mtDNA was reversed by melatonin, along with improvements of neuronal structure and functions (Feng et al., 2013). An important question is that of whether numerous findings obtained in models of sepsis, endotoxemia and other types of high-grade inflammation are really relevant to basic processes of aging. Damage and ETC blockade by the different ROS and RNS as well as their intramitochondrial sites of action and consequences to electron overflow have been extensively discussed elsewhere (Hardeland, 2009a,b, 2013a; Hardeland et al., 2009b; Hardeland and Coto-Montes, 2010). Because of its crucial importance, the role of cardiolipin in the breakdown of mitochondrial function should be emphasized. This compound is peroxidized earlier and more strongly than other mitochondrial lipids, which is explained by its interaction with cytochrome c, because the resulting proteolipid complex gains the function of a peroxidase (Basova et al., 2007; Ott et al., 2007; Bayir et al., 2007; Kagan et al., 2009a,b). The peroxidation of cardiolipin was shown to precede that of other mitochondrial lipids, as demonstrated by a lipidomic approach (Samhan-Arias et al., 2011; Kagan, 2013). Moreover, damaged mitochondrial membranes externalize, by virtue of phospholipid scramblase-3, cardiolipin to the outer membrane, which represents an elimination signal for mitophagy (Chu et al., 2013). Cardiolipin peroxidation has been reported to be prevented by melatonin (Petrosillo et al., 2006, 2008; Luchetti et al., 2007), but it remains uncertain whether this effect is based on a direct inhibition of the cytochrome c/cardiolipin peroxidase rather than radical scavenging (cf. Hardeland, 2013a). As discussed there, an alternative explanation might be deduced from the known upregulation of mitochondrial glutathione peroxidase (GPx),

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because overexpression of the respective subform, GPx4, strongly antagonizes cardiolipin peroxidation and cytochrome c release, whereas peroxidation was increased in heterozygous knockouts (Liang et al., 2009).

Formation of the primary free radical, the superoxide anion ($O_2^{\bullet-}$), by mitochondria is a key step for processes leading to intra- and extramitochondrial damage, which includes dismutation to $O_2$ and $H_2O_2$, a source of hydroxyl radicals ($^{\bullet}OH$), or combination with $^{\bullet}NO$ to peroxynitrite ($ONOO^-$), which decomposes after protonation to $^{\bullet}NO_2$ and $^{\bullet}OH$ or, as a $CO_2$ adduct ($ONOOCO_2^-$) to $^{\bullet}NO_2$ and the carbonate radical ($CO_3^{\bullet-}$), as summarized elsewhere (Hardeland and Coto-Montes, 2010; Hardeland, 2011b). Electron leakage from the ETC can occur either by overflow at Complexes I and III, especially because of bottlenecks of electron flux (Hardeland, 2009a; Hardeland et al., 2009a), or by permeability transition. The opening of the mitochondrial permeability transition pore (mtPTP) was originally regarded as a fatal event that ends up in apoptosis or, at least, mitophagy. However, mtPTP opening has been found to already occur under relatively basal conditions and was described as "superoxide flashes" (Wang et al., 2008). This process can be stimulated by oxidative stress (Sheu et al., 2008). Meanwhile, the duration of the opening has turned out to be decisive. Usually a transient $\Delta\Psi_{mt}$ breakdown of short duration does not lead to apoptosis, but may be rather favorable, because it also represents a means for releasing unfavorable amounts of $Ca^{2+}$ from the mitochondrial matrix, whereas long-lasting $Ca^{2+}$ overload typically initiates cell death. Interestingly, melatonin inhibited prolonged permeability transition in astrocytes, whereas it still allowed transient mtPTP opening (Jou, 2011). This is in good agreement with melatonin's counteraction of apoptosis induction by the $Ca^{2+}$ ionophore, ionomycin (Jou et al., 2004). Moreover, melatonin was reported to directly inhibit mtPTP opening, at an $IC_{50}$ of 0.8 μM (Andrabi et al., 2004), a concentration that would require mitochondrial accumulation of melatonin. This has, in fact, already been observed (López et al., 2009; Paradies et al., 2010; Venegas et al., 2012), at physiological levels of the circulating hormone in vivo (Messner et al., 1998). The fate of superoxide anions released to the intermembrane space has been reported to be influenced by melatonin, which activates Cu,Zn-superoxide dismutase present in this intermembrane compartment (Iñarrea et al., 2011). However, this mechanism should not be relevant under conditions of superoxide flashes, because $O_2^{\bullet-}$also activates this enzyme and is present in sufficiently high quantities after a permeability transition (Hardeland, 2013a).

Mitochondria are protected by melatonin in manifold ways. Instead of actions observed in models of high-grade inflammation, such as downregulation of iNOS, enhanced expression of respirasomal components and antioxidant enzymes which are reviewed elsewhere (Acuña-Castroviejo et al., 2003, 2005, 2007; Hardeland, 2005, 2009a,b; Hardeland et al., 2009a, 2011), the following considerations should be the focus of aging-related findings. A senescence-associated decay of mitochondrial functions is generally observed, a process which is particularly aggravated in neurodegenerative disorders, in which inflammation contributes considerably to the deterioration of neuronal function. In brain mitochondria of aged rats, melatonin prevented cardiolipin peroxidation and dysfunction resulting there from (Petrosillo et al., 2008). Moreover, it upregulated, in a comparable setting, superoxide dismutase (Öztürk et al., 2012). In the same study, a decrease of GPx levels was reported that may be interpreted as a normalization, because a senescence-associated increase of oxidative stress leads to a rise in GPx. However, this finding contrasts with results obtained in brain mitochondria from the senescence-accelerated mouse strain SAMP8, in which GPx decreased by age and was upregulated by melatonin (Carretero et al., 2009). Similar results in other organs of SAMP8 mice are summarized elsewhere (Hardeland, 2013a). Most of the protection experiments concerning SAMP8 mitochondria have been carried out in peripheral organs, but data from brain regions do exist. Melatonin was shown to prevent the age-related rigidization of mitochondrial membranes (García et al., 2011), and elevated $\Delta\Psi_{mt}$ (Cristòfol et al., 2012). In the hippocampus of SAMP8 mice, the numerical number and surface volume of mitochondria was increased by melatonin (Cheng et al., 2008). Collectively, data from brain (Carretero et al., 2009) and other organs of SAMP8 mice (summary: Hardeland et al., 2013) show numerous additional improvements by melatonin, such as increased activities of Complexes I, III, and IV, elevations of respiratory control index, efficiency in ATP synthesis, ATP content and/or ATP/ADP ratio, ATP/O ratio, higher state 3 respiration and lower state 4 respiration, reduced electron leakage, increased activities of GPx and glutathione reductase, augmentations of reduced glutathione (GSH) or the GSH/oxidized glutathione (GSSG) ratio, and attenuated lipid peroxidation. In conclusion, the numerous positive effects of melatonin at the mitochondrial level indicate that the initiation of inflammatory processes via dysfunction of these organelles and resulting overproduction of free radicals is strongly antagonized by melatonin.

## 6. Anti-inflammatory actions of melatonin in the CNS

The question of whether and, if so, to what extent melatonin may reduce inflammatory responses in the CNS is of crucial importance. The answer has to give credit to the fact that melatonin can exert both pro- and anti-inflammatory effects, as outlined in a previous section. Without considering the specific situation in aging, the general experience has been that melatonin rather promotes low-grade but strongly antagonizes high-grade inflammation, as described by the "buffering hypothesis" (Carrillo-Vico et al., 2013). During normal aging and in the earlier stages of neurodegenerative disorders, the immunologically relevant changes have to be classified as low-grade inflammation and may also be regarded as, to a certain extent, atypical. Nevertheless, the evidence from studies in aging animals mostly speak for a predominantly anti-inflammatory, antioxidative and mitochondrial-protective role of melatonin (Petrosillo et al., 2008; Cheng et al., 2008; Tajes et al., 2009; Carretero et al., 2009; Öztürk et al., 2012). Unfortunately, most of the direct evidence for anti-inflammatory actions that are based on reductions of pro-inflammatory cytokines has been obtained in peripheral organs (Cuesta et al., 2010, 2011; Forman et al., 2010, 2011). However, a more general effect of melatonin that concerns various aspects of senescence including inflammaging may be central importance, in peripheral organs as well as in the brain. Signaling via NF-κB has been shown to drive the majority of genes related to SASP and to be multiply involved in processes of aging, also in the CNS (Adler et al., 2007; Freund et al., 2011; Franceschi and Campisi, 2014). Melatonin has been shown to be a potent suppressor of NF-κB expression (Deng et al., 2006; Korkmaz et al., 2012).

Moreover, conclusions on a mostly anti-inflammatory role of melatonin in aging are supported by its antagonism to insulin resistance (Cuesta et al., 2013), which is in good agreement with counteractions against metabolic syndrome, prediabetic states and diabetes type 2 (Nishida et al., 2002; Nishida, 2005; Peschke et al., 2007; Peschke, 2008; Robeva et al., 2008; Korkmaz et al., 2009; Shieh et al., 2009; Peschke and Mühlbauer, 2010; Hardeland et al., 2012). These new insights concerning a relationship between insulin resistance and low-grade neuroinflammation, which consequences to the development of AD (Clark and Vissel, 2013; Jiang et al., 2013; De Felice et al., 2014; de la Monte and Tong, 2014; Ferreira et al., 2014) indicates a substantial role of melatonin as an anti-inflammatory agent in the context of aging. An overview of

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melatonin's actions that antagonize brain inflammaging is presented in Fig. 1.

### 6.1. Actions in experimental models

In the brain, anti-inflammatory actions of melatonin have been described in several animal and cell culture models. Some studies shall be mentioned in advance, in which melatonin's anti-inflammatory actions were investigated in high-grade inflammation induced by infection, transient focal ischemia, or endotoxemia, although such models may not be translated to normal aging and only with caution to advanced stages of neurodegenerative diseases. In acute *Klebsiella pneumoniae* meningitis, melatonin strongly inhibited, in the rat hippocampus, microglia activation (criterion: isolectin-B4 histochemistry), the microglial rise in cytosolic $Ca^{2+}$, and reduced, in both hippocampus and serum, the levels of TNFα, IL-1β and IL-6 (Wu et al., 2011). The reduction of inflammation was associated with lower numbers of apoptotic and higher counts of cytochrome oxidase-positive neurons, indicating a support of mitochondrial function. In a median nerve injury model, TNFα, IL-1β and IL-6 were decreased by melatonin in the rat cuneate nucleus (Chiang et al., 2013). In the dentate gyrus of ovariectomized rats, which exhibit higher levels of proinflammatory cytokines than age-matched control animals, melatonin also decreased TNFα, IL-1β and IL-6 and, additionally, upregulated SIRT1 mRNA expression (Kireev et al., 2014). Substantial attenuations of microglia activation were also observed after hypoxia-ischemia (Balduini et al., 2012) or ischemia-reperfusion, along with reduced neutrophil and macrophage/monocyte infiltration (Lee et al., 2007). Melatonin antagonized several lipopolysaccharide (LPS)-induced immunological changes, such as expression of COX-2, microsomal PGE synthase-1, and iNOS in the rat astrocytoma cell line C6 (Niranjan et al., 2012), rises in TNFα and IL-1β in several rat brain regions (Tyagi et al., 2010), and expression of three chemokines (CCL2, CCL5, CCL9) in the murine microglial cell line BV2 (Min et al., 2012). Another finding of possibly fundamental importance for the protective role of melatonin in neuroinflammation has been obtained in LPS-treated rats (Pinato et al., 2013). In the cerebellum, which has previously discussed as one of the extrapineal CNS sites of melatonin formation (Hardeland et al., 2011), these authors showed that LPS induced aralkylamine *N*-acetyltransferase (AANAT) and melatonin formation in this brain region, but not in cortex and hippocampus, although circulating melatonin in the blood plasma was decreased by this treatment. The induction of cerebellar melatonin formation was not prevented by pinealectomy. The physiological melatonin levels formed in response to LPS were sufficient to protect cerebellum but not cortex and hippocampus against neuronal loss, and the effect was abolished by the melatonergic antagonist luzindole (Pinato et al., 2013).

Several publications have studied anti-inflammatory actions of melatonin in microglial cell lines. In BV-2 cells, Yan et al. (2013) investigated the increase of oxidative stress by fluoride, showing rises in NADPH oxidase, iNOS activity, the release of TNFα and IL-1β as well as downstream effects on c-Jun N-terminal kinase (JNK) phosphorylation, which were antagonized by melatonin. In HAPI cells, melatonin inhibited the amphetamine-induced rise in iNOS (Tocharus et al., 2008) and the methamphetamine-induced overexpression of TNFα, IL-1β and IL-6 (Tocharus et al., 2010). Similar upregulations of iNOS and TNFα by methamphetamine and their suppression by melatonin were also observed in SH-SY5Y neuroblastoma cells (Permpoonputtana and Govitrapong, 2013).

With regard to the newly emerged nexus between insulin resistance and neuroinflammation, as mentioned in Section 3, a

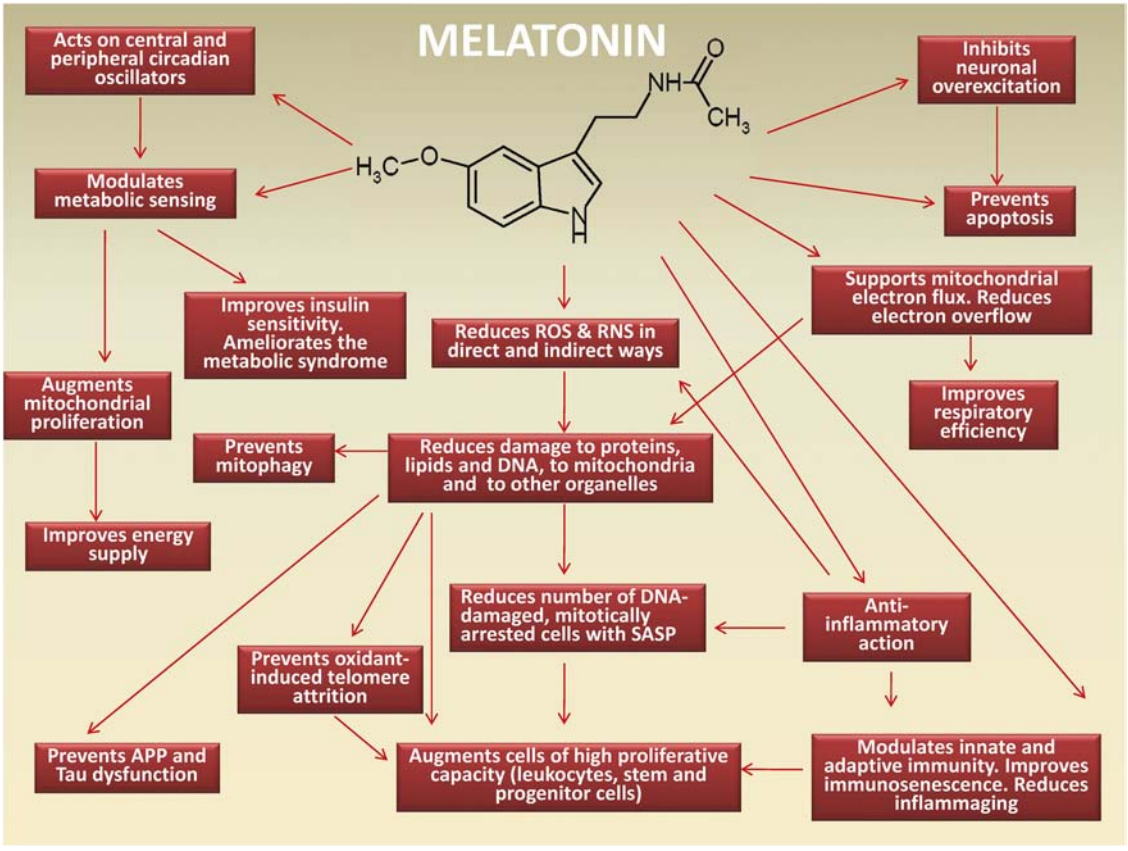


**Fig. 1.** Overview of the multiple actions of melatonin that antagonize brain inflammaging.

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study on experimental diabetic neuropathy is of particular interest. In the sciatic nerve, melatonin suppressed a number of inflammatory responses, including rises in NF-κB expression and inhibitor of NF-κB-α (IκB-α) phosphorylation, the decrease of nuclear factor (erythroid-derived 2)-like 2 (Nrf2), increased expression of iNOS and COX-2, as well as elevated levels of TNFα and IL-6 (Negi et al., 2011). Although some of melatonin's actions may have been related to its antioxidative effects, others are clearly associated with a counteraction of inflammation. This distinction may be only of interest from a mechanistic point of view, because antioxidant and anti-inflammatory actions are in any case intertwined in systems that can respond by inflammation.

### 6.2. Actions in neurodegenerative diseases

Effects of melatonin in neurodegenerative diseases have been repeatedly reviewed in their multiple aspects (Mayo et al., 2005a; Srinivasan et al., 2005, 2006, 2011; Cardinali et al., 2010; Pappolla et al., 2000; Esposito and Cuzzocrea, 2010; Rosales-Corral et al., 2012; Lin et al., 2013). Several of the beneficial effects described are poorly or only indirectly linked to inflammation. This may be by support of the circadian system, which is deteriorating by age and even more under conditions of neurodegeneration, and in which melatonin can improve sleep and behavioral symptoms such as sundowning. Other indirect effects concern the support of mitochondrial function and intraneuronal distribution, along with reductions in ROS formation and mitophagy, prevention of tau hyperphosphorylation, antioxidant and antiamyloidogenic actions. Although this functional spectrum is, at certain points, either connected to inflammation or even may, under conditions of improvement by melatonin, substantially reduce neuroinflammation, the whole body of evidence would exceed the scope of this article and shall not be repeated here in full. Instead, the focus shall be laid on described reductions of inflammatory signals. In this context, it should also be recalled that melatonin is more strongly reduced in most neurodegenerative disorders, with the exception of PD, relative to age-matched control subjects (Hardeland, 2012), as discussed is Section 3. Therefore, neurodegeneration is frequently associated with a relative melatonin deficiency that has to lead to specific functional losses which may be relieved by melatonin supplementation. It should be also noted that the relationship between melatonin and SIRT1, as it appears to exist in aging nontumor cells (Chang et al., 2009; Tajes et al., 2009; Cristòfol et al., 2012; Cuesta et al., 2013; Kireev et al., 2014), may extend to the also observed age-associated decreases in SIRT1 and its cosubstrate, $NAD^+$, changes that have been implicated in neurodegenerative disorders, too (Imai and Guarente, 2014; Herskovits and Guarente, 2014; Rehan et al., 2014). These reductions in SIRT1, $NAD^+$, and the rate-limiting enzyme of $NAD^+$ formation, nicotinamide phosphoribosyltransferase (NAMPT), are additionally associated with circadian dysfunction and mitochondrial impairments, changes that may be corrected by melatonin.

An important, both direct and indirect inflammatory signal with particular importance to AD are Aβ oligomers, with a contribution of amyloid plaques. Microglia activation is induced by Aβ peptides/oligomers (Tan et al., 2012) and can be easily achieved by $Aβ_{1–42}$ injection (McLarnon, 2014). In both astrocytes and neurons, $Aβ_{1–40}$ and $Aβ_{1–42}$ oligomers stimulate NADPH oxidase, thereby increasing ROS production and causing secondary effects of oxidative stress (Narayan et al., 2014). Moreover, astrocytes respond to $Aβ_{25–35}$ by upregulating $Aβ_{1–42}$ formation and secretion (Dal Prà et al., 2014). In addition to the peptides, amyloid plaques are also capable of activating microglia and cause release of proinflammatory cytokines (Rodriguez et al., 2014). Not only are micro- and astroglia activated by Aβ oligo- and polymers, but also neurons were shown to be stimulated to express inflammatory molecules, including TNFα, IL-1β, COX-2, and the T-cell and monocyte attractant chemokine CX3CL1 (Hanzel et al., 2014). For this reason, any reduction of Aβ formation should also attenuate neuroinflammation and oxidative stress. While several antifibrillogenic effects by melatonin are known (reviewed in: Srinivasan et al., 2006; Rosales-Corral et al., 2012), only indirect evidence is available concerning the formation of Aβ. In the pheochromocytoma cell line, melatonin caused a decrease in the expression of β-amyloid precursor protein (APP) mRNA (Song and Lahiri, 1997; Lahiri, 1999). To what extent these findings can be translated to and reproduced in the brain, is of considerable importance.

Several publications have described beneficial effects of melatonin in AD models of single, double or triple transgenic mice (Matsubara et al., 2003; Feng et al., 2006; Olcese et al., 2009; Dragicevic et al., 2011; García-Mesa et al., 2012; Peng et al., 2013). However, the relationship to brain inflammaging largely remains a matter of indirect interpretations. Perhaps, findings of reduced Aβ aggregation and oxidative stress markers in melatonin-treated transgenic animals (Olcese et al., 2009) may be indicative of an anti-inflammatory action. This conclusion may be in line with similar results obtained after injection of $Aβ_{1–42}$, $Aβ_{1–40}$ or $Aβ_{25–35}$ peptides (Masilamoni et al., 2008; Gunasingh et al., 2008; Jun et al., 2013), although some of these findings have to be critically seen with regard to dosage.

A specific aspect of microglia activation by $Aβ_{1–42}$ consists of NADPH oxidase (Nox) activation. Melatonin was shown to inhibit this process by a previously unknown antioxidant effect. It reduced the translocation of the Nox subunit $p47^{phox}$ to the plasma membrane by preventing its phosphorylation via the phosphatidylinositol 3-kinase (PI3K)/Akt cascade and, thereby its association with the subunits $gp91^{phox}$ and $p67^{phox}$ that are essential parts of the active Nox complex (Zhou et al., 2008). As a result, the formation of superoxide and secondary free radicals generated is considerably reduced and, thus, the oxidative stress component of inflammation diminished.

Effects of melatonin concerning the reduction of proinflammatory cytokines formed in response to $Aβ_{1–40}$ were investigated in an organotypic mouse brain slice culture (Clapp-Lilly et al., 2001). Melatonin inhibited the $Aβ_{1–40}$-induced rises of both IL-1β and IL-6. Interestingly, no melatonin effect was observed in the absence of the Aβ peptide, but instead unexpectedly caused a rise in IL-6 when given at the high concentration of 500 μM. The latter finding indicates another type of proinflammatory action by melatonin when administered in excess, a possibility that has not been sufficiently considered in a number of studies. Similar repressions of interleukin secretion were observed in a more recent study in organotypic slices from rat hippocampus, in which $Aβ_{25–35}$ was applied (Bender Hoppe et al., 2010). This peptide, which also stimulates $Aβ_{1–42}$ secretion (Dal Prà et al., 2014), caused microglial and astrocytic activation. The resulting enhanced release of TNFα and IL-6 was blocked by melatonin, along with other beneficial effects such as reduction of glycogen synthase kinase-3β (GSK-3β) activation and tau hyperphosphorylation. Attenuation of both GSK-3β activation and tau hyperphosphorylation by melatonin has been repeatedly observed, in different experimental settings, and seems to also comprise effects beyond the prevention of cytokine release (Deng et al., 2005; Li et al., 2005; Yin et al., 2006; Gutierrez-Cuesta et al., 2007; Peng et al., 2013). GSK-3β is known to act as an accessory component of cellular circadian oscillators (Hirota et al., 2008; Sahar et al., 2010; Kozikowski et al., 2011; Kinoshita et al., 2012). With regard to the effects of melatonin on central and peripheral circadian clocks (Hardeland et al., 2012; Hardeland, 2013b), the pineal hormone can be assumed to influence GSK-3β activity additionally via the oscillators. In

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SAMP8 mice, melatonin also inhibited another tau kinase, cyclin-dependent kinase 5 (cdk5), and reduced the cleavage of its activator p35 to its hyperactivator p25 (Gutierrez-Cuesta et al., 2007). The suppression of p25 formation has been observed in experimental models of both Alzheimer's (Srinivasan et al., 2006) and Parkinson's disease (Alvira et al., 2006).

The suppression of TNFα secretion by melatonin may be additionally important to the production of Aβ peptides. Combinations of TNFα and IFNγ were shown to inhibit the release of soluble APP and to enhance the formation of $A\beta_{1-40}$ and $A\beta_{1-42}$ in neurons and astrocytes (Blasko et al., 1999, 2000). With regard to the proinflammatory cytokines, the time course is of particular interest. Although some changes were already present at 6 or 12 h, the increases in TNFα and IL-6 were extremely higher after 48 rather than 24 h of $A\beta_{25-35}$ exposure (Bender Hoppe et al., 2010), a finding that may be interpreted in terms of a vicious cycle of the neuroinflammatory process. Nevertheless, the suppression of cytokine secretion by melatonin was still quite effective after 48 h, which may indicate melatonin's potential for breaking the positive feedback loop of the vicious cycle.

## 7. Formation of methoxylated kynuramines in the brain and the contribution of inflammation

One of the melatonin catabolic pathways is initiated by pyrrole ring cleavage, to yield $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine (AFMK), which is deformylated to $N^1$-acetyl-5-methoxykynuramine (AMK). AFMK formation is possible by a remarkably large number of mechanisms, including enzymatic reactions (Hardeland et al., 2004, 2009b), e.g., by indoleamine 2,3-dioxygenase and myeloperoxidase, as well as various pseudoenzymatic and nonenzymatic reactions, including oxidation by free radicals and other oxidants. Deformylation of AFMK to AMK is also possible by enzymatic catalysis, involving arylamine formamidases or hemoperoxidases, and nonenzymatic reactions (Hardeland, 2010). Both AFMK and AMK had been discovered after injection of melatonin into the cisterna magna of rats (Hirata et al., 1974). Since the other melatonin metabolite known at this time, 6-hydroxymelatonin, was not detected in that study, AFMK and AMK were concluded to represent major brain metabolites of melatonin. Meanwhile, it is known that 6-hydroxymelatonin is also formed in the brain, but, surprisingly, sulfated to 6-sulfatoxymelatonin (for details and discussion see: Hardeland, 2010). Meanwhile, some doubts had arisen as to whether these high amounts of AFMK and AMK detected in the original publication are generally present in the brain, although the finding is reliable. A possibility may consist in the activation of melatonin-catabolizing enzymes or generation of free radicals in the presence of very high concentrations of the methoxyindole (Hardeland, 2010). However, little is known about the effects of melatonin in unchallenged microglia, whereas the counteraction of proinflammatory treatments and signals has been repeatedly studied. Although macrophages, peripheral monocytes, cultured monocyte-derived cell lines and also postnatal microglia were reported to be activated by melatonin, resting microglia was believed to not respond (Shafer et al., 2001). Melatonin was found to be efficiently oxidized to AFMK by activated macrophages (Silva et al., 2004), but corresponding studies on microglia have not yet been conducted.

A relationship between AFMK and inflammation became evident when high AFMK concentrations were detected in the CSF of patients with viral meningitis (Silva et al., 2005). Interestingly, a negative correlation emerged between AFMK levels and the concentrations of the pro-inflammatory cytokines IL-1β and IL-8. Individuals with 10–50 nM AFMK had considerably higher levels of these interleukins than patients with more than 50 nM AFMK. It seems important to also take notice of the remarkably high amounts of AFMK detected in these subjects, since 50 nM is by orders of magnitude higher than nocturnal plasma concentrations of melatonin, which are maximally in the range of 1 nM in young individuals and frequently much less in middle-aged or elderly persons. This seems to indicate an accumulation of AFMK over time. If AFMK is formed as a consequence of inflammation, including oxidative stress resulting thereof, the negative correlation with pro-inflammatory cytokines might indicate an AFMK-related or -mediated dampening of the inflammatory responses. This assumption however, requires direct demonstration.

Nevertheless, it might be worth following this possibility in the future. AFMK is also capable of scavenging free radicals (Tan et al., 2001; Burkhardt et al., 2001), although the antioxidant properties of its product AMK exceed those of AFMK (Ressmeyer et al., 2003; Hardeland et al., 2004). Moreover, several reports have demonstrated protection by AFMK against oxidotoxicity (Tan et al., 2001; Onuki et al., 2005; Manda et al., 2007), excitotoxicity (Tan et al., 2001; Onuki et al., 2005), mitochondrial dysfunction (Dragicevic et al., 2011), and prevention of radiation-induced inhibition of neurogenesis and memory impairment (Manda et al., 2008). The mitochondrial effects were notably observed in APP-expressing neuroblastoma cells. Mitochondrial protection was also shown in other systems with AMK (Acuña-Castroviejo et al., 2003; Tapias et al., 2009). AMK is also of particular interest as an anti-inflammatory agent. It was reported to be a more potent COX inhibitor than acetylsalicylic acid (Kelly et al., 1984) and to downregulate COX-2 expression in macrophages (Mayo et al., 2005). Moreover, it downregulates iNOS (Tapias et al., 2009), in addition to antiexcitatory actions as a potent inhibitor of nNOS (Entrena et al., 2005; León et al., 2006). It also scavenges NO very efficiently, thereby forming a stable product, 3-acetamidomethyl-6-methoxycinnolinone (AMMC), that does not re-donate NO like other nitrosated compounds including 1-nitrosomelatonin (Guenther et al., 2005; Hardeland et al., 2007). To what extent the potentially anti-inflammatory, antiexcitatory and mitochondrial protective actions of AMK are of relevance to the brain under conditions of low-grade inflammation and in neurodegenerative diseases remains to be clarified.

## 8. Inflammaging and the decay of the circadian pacemaker

A particular aspect of brain inflammaging concerns the impaired function of the circadian pacemaker, the hypothalamic suprachiasmatic nucleus (SCN), which is observed during normal aging and, even more, in neurodegenerative disorders. This change is frequently underrated, but may be of high relevance, because it systemically affects a host of other functions in the body. Several alterations can be involved in SCN insufficiency, from reduction of blue light perception to losses in signal transmission to the SCN (review: Hardeland et al., 2012), but a major factor is that of SCN degeneration. Because of the crucial role of the SCN in the control of mammalian pineal gland, dysfunction of the hypothalamic master clock strongly contributes to aging- or disease-related reductions of nocturnal melatonin secretion and changes in melatonin's secretory patterns and phasing (review: Hardeland, 2012). Although SCN deterioration is more strongly pronounced in AD and other forms of dementia, it seems that the relevance of this phenomenon is already high in normal aging (Skene et al., 1990; Skene and Swaab, 2003; Wu and Swaab, 2007; Hardeland, 2012, 2013a). In part, this may be related to changes in melatonin, but additional impairments of other rhythmic functions likely arise when a master clock decays. It has also been shown that the replacement of the poorly functional SCN of a senescent hamster by transplantation of a juvenile SCN not only restored the previously decomposed circadian rhythmicity, but caused a

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rejuvenation in terms of physical appearance and also extended the lifespan of the recipient (Hurd and Ralph, 1998). These findings impressively show how important the SCN and a well-operating circadian system are for preventing aging-related impairments.

The specific mechanisms of inflammaging in the SCN are poorly understood, compared to other brain regions. One may, of course, assume that the known processes of microglia and astrocyte activation contribute to the deterioration of the SCN. Nevertheless, an additional, chronobiological mechanism has emerged during the last years. The aging suppressor SIRT1 has been shown to act as an accessory component of cellular cicadian oscillators. It interacts transiently with the core oscillator complex BMAL1:CLOCK, is part of an amplifying positive feedback loop that involves NAMPT and $NAD^+$ and further influences the expression of the core oscillator gene *Period2* (*Per2*) (Nakahata et al., 2008, 2009; Grimaldi et al., 2009; Ramsey et al., 2009; Wijnen, 2009; Imai, 2010). More recently, it was shown that another sirtuin, SIRT3, can also participate in the $NAD^+$ cycle, thereby driving the rhythmicity of mitochondrial activity (Peek et al., 2013). Moreover, SIRT1 was shown to decrease with aging in the SCN of wild-type mice, with consequences to BMAL1 and CLOCK levels and circadian output functions (Chang and Guarente, 2013). Overexpression of SIRT1 prevented the aging-dependent circadian changes, whereas SIRT1 deletion in young mice led to decreased *Bmal1* and *Per2* expression and temporal patterns reminiscent of those in senescent animals (Chang and Guarente, 2013). Therefore, sirtuins such as SIRT1 and SIRT3 are obviously required for the maintenance of an appropriately operating circadian system at different levels including the coordinative function of the SCN and the crucial role of mitochondria in cellular metabolic adaptation. A major question that arises from these recent findings is that of the cause of the aging-related decline in S1RT1. Another parameter that decreases similarly during senescence is the melatonin level. Therefore, it is of utmost importance to confirm or reject results on upregulation of S1RT1 by melatonin in the context of aging, as discussed in Section 4, whereas suppression was repeatedly observed in tumor cells. In addition to its chronobiological role, SIRT1 has anti-inflammatory properties and inflammaging is believed to be promoted by its absence (Hwang et al., 2013). If a positive relationship between melatonin and SIRT1 in aging nontumor cells is confirmed, either of these molecules may contribute to an attenuation of inflammation and, thus, inflammaging in the SCN. The next question of whether melatonin or SIRT1 tends to decline first, is to date entirely open. A SIRT1-depleted malfunctioning SCN would poorly stimulate the pineal gland, but also a reduction in melatonin secretion may impair SIRT1 expression in the SCN. It seems possible that both of these possibilities, i.e., primary decrease in either melatonin secretion or in SIRT1 expression, may lead to a vicious cycle that not only impairs the circadian pacemaker but ultimately other brain regions and organs, too, thus reducing the resistance to oxidative insults and aging-associated inflammatory responses.

## 9. Conclusion

Low-grade inflammation is a hallmark of senescence and contributes to brain inflammaging. Interindividual variability in the grade and progression of the underlying processes is high and seems to depend on a spectrum ranging from an IRP to an ‘‘inverted IRP‘‘, but may be overlaid by lifestyle and the occurrence of other diseases (Hardeland, 2013a). Brain inflammaging is considerably enhanced in neurodegenerative diseases, most impressively in AD. Interestingly, the degree of inflammation seems to be negatively correlated with the level of melatonin. This relationship also exists in diabetes type 2, which has now gained relevance with regard to the possible role of insulin resistance in the development and, perhaps, progression of AD (Clark and Vissel, 2013; Jiang et al., 2013; De Felice et al., 2014; de la Monte and Tong, 2014; Ferreira et al., 2014). However, with regard to the decline of melatonin, the distinction between cause and consequence is by far unclear to date. In several animal models including senescence-accelerated mice, melatonin has been reported to be protective at various levels, including mitochondrial protection and reduction of proinflammatory cytokines. The latter effect has also been obtained in models using Aβ peptides, as outlined in Section 6.2. The primarily anti-inflammatory actions of melatonin, as reported, are surprising insofar as melatonin can behave conditionally either in a pro- or anti-inflammatory way, with a mainly proinflammatory profile in low-grade inflammation (Carrillo-Vico et al., 2013). The reasons for why primarily anti-inflammatory actions have been described in the aging brain deserves clarification. Several reasons may be discussed, but require experimental support. One possibility may be related to dosage. In the aging or senescence-accelerated animals, melatonin was usually administered via the drinking water, frequently at doses of 1 mg/kg/day. Although the time course of external melatonin in the blood was usually not followed, the effective dose may have been in favor of anti-inflammatory actions, whereas lower endogenous levels may tend to enhance proinflammatory responses. However, marked acute inflammatory responses were observed, in experimental granulomatosis, also at an elevated dosis of 4 mg/kg (de la Rocha et al., 2004). Nevertheless, a treatment option may result from the anti-inflammatory actions observed in other experiments. Another possibility may be based on the assumption by Radogna et al. (2010), who discussed sequential actions of melatonin under low-grade inflammatory conditions. According to their concept, melatonin would behave in proinflammatory way under acute low-grade inflammation, but as an anti-inflammatory agent after chronification. Interestingly, melatonin also decreased proinflammatory cytokines in another type of low-grade inflammation induced by high-fat diet. In rats receiving 750 μg melatonin/day via the drinking water, levels of IL-1β, IL-6, TNFα, IFNγ and also C-reactive protein were decreased, whereas the previously reduced anti-inflammatory cytokines IL-4 and IL-10 were increased (Cano Barquilla et al., 2014). A limitation of inflammatory responses by physiological levels of melatonin may be also assumed because of elevated proinflammatory cytokine expression in aortic endothelial cells of pinealectomized rats (Wang et al., 2013), findings that would require confirmation in brain tissue and exclusion of other influences such as changes in the circadian system.

One of the problematic properties of chronic neuroinflammation has to be seen in its potential for aggravation by vicious cycles. Again, this seems to be particularly serious in AD, in which the released Aβ peptides further stimulate neuroinflammation (Tan et al., 2012; Hanzel et al., 2014; Rodriguez et al., 2014) and ROS production (Narayan et al., 2014), while inflammatory signals and cells may further enhance Aβ levels and deposits (Eikelenboom et al., 2006; von Bernhardi, 2007; Choo et al., 2013). Whether a system containing positive feedback loops that progressively aggravate the disease condition can be halted at all after a certain stage has been reached may be doubted. The generally poor therapeutic outcome in the treatment of moderate to severe AD is indicative of this problem. Nevertheless, the stage of disease and start of intervention may turn out to be decisive. This has also become evident in an AD model using transgenic mice. When the animals were treated with melatonin relatively early in their life, this was partially successful in retarding symptoms and death (Matsubara et al., 2003), whereas no relevant improvements where stated when the treatment started later in life (Quinn et al., 2005). In any case the presence of a pathogenic transgene may not allow a higher curative efficacy, and what applies to a transgenic mice may

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not be equally valid for a diseased human. Nevertheless, melatonin may be nothing more than an agent for palliative improvements if treatment starts too late. If there is any chance for delaying symptoms in humans, given an early onset of therapy, remains to be shown. However, what may be critically seen in the case of advanced neurodegenerative disorders, may turn out to be of value during normal aging. However, this would require extensive clinical studies, in which subjects would have to be treated over long periods, from a timepoint on when the first symptoms of age-dependent circadian dysfunctions become apparent, such as sleep disturbances or nocturia. It may also be difficult to control the treatment of persons who not yet perceive the changes as impairments of health over a long period of time.

With regard to the mechanistic aspects of melatonin effects in neuroinflammation and, more specifically, in brain inflammaging, a number of gaps have to be closed. If insulin resistance turns out to be as important in neuroinflammation as in diabetes type 2 and metabolic syndrome, improvements of insulin sensitivity by melatonin, as known from the latter area and discussed in Section 6, should be directly studied in the brain. Moreover, the spectrum of cytokines under control by melatonin should be expanded under conditions of aging and chronic neuroinflammation. First, it should be noted that microglia activation is not exclusively deleterious, but can also contribute to the clearance of senile plaques (Schlachetzki and Hüll, 2009; Choo et al., 2013). As done in other studies on AD, anti-inflammatory cytokines should be studied as well. Moreover, one of the key regulators of neuroinflammation, IL-18, should be included in further investigations on immune modulation by melatonin in the CNS. Finally, another field with crucial relevance to inflammaging, namely, the roles of DDR and SASP deserve specific attention in future research on the aging CNS. This concerns both the in-depth analysis of the contribution of these newly emerged pathways to brain inflammaging and the elucidation of melatonin's role in this specific area, in which this direct and indirect antioxidant, neuroprotective and often anti-inflammatory agent may be beneficial.

## References


Acosta, J.C., Banito, A., Wuestefeld, T., Georgilis, A., Janich, P., Morton, J.P., Athineos, D., Kang, T.W., Lasitschka, F., Andrulis, M., Pascual, G., Morris, K.J., Khan, S., Jin, H., Dharmalingam, G., Snijders, A.P., Carroll, A.P., Capper, D., Pritchard, C., Inman, G.J., Longerich, T., Sansom, O.J., Benitah, S.A., Zender, L., Gil, J., 2013. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nat. Cell Biol. 15, 978–990.

Acuña-Castroviejo, D., Escames, G., León, J., Carazo, A., Khaldy, H., 2003. Mitochondrial regulation by melatonin and its metabolites. Adv. Exp. Med. Biol. 527, 549–557.

Acuña-Castroviejo, D., Escames, G., López, L.C., Hitos, A.B., León, J., 2005. Melatonin and nitric oxide: two required antagonists for mitochondrial homeostasis. Endocrine 27, 159–168.

Acuña-Castroviejo, D., Escames, G., Rodríguez, M.I., López, L.C., 2007. Melatonin role in the mitochondrial function. Front. Biosci. 12, 947–963.

Adler, A.S., Sinha, S., Kawahara, T.L., Zhang, J.Y., Segal, E., Chang, H.Y., 2007. Motif module map reveals enforcement of aging by continual NF-kappaB activity. Genes Dev. 21, 3244–3257.

Afanas'ev, I., 2010. Signaling and damaging functions of free radicals in aging-free radical theory, hormesis, and TOR. Aging Dis. 1, 75–88.

Alberti, S., Cevenini, E., Ostan, R., Capri, M., Salvioli, S., Bucci, L., Ginaldi, L., De martinis, M., Franceschi, C., Monti, D., 2006. Age-dependent modifications of Type 1 and Type 2 cytokines within virgin and memory $CD4^+$ cells in humans. Mech. Ageing Dev. 127, 560–566.

Alvira, D., Tajes, M., Verdaguer, E., Acuña-Castroviejo, D., Folch, J., Camins, A., Pallas, M., 2006. Inhibition of the cdk5/p25 fragment formation may explain the antiapoptotic effects of melatonin in an experimental model of Parkinson's disease. J. Pineal Res. 40, 251–258.

Andrabi, S.A., Sayeed, I., Siemen, D., Wolf, G., Horn, T.F., 2004. Direct inhibition of the mitochondrial permeability transition pore: a possible mechanism responsible for anti-apoptotic effects of melatonin. FASEB J. 18, 869–871.

Arlicot, N., Tronel, C., Bodard, S., Garreau, L., de la Crompe, B., Vandevelde, I., Guilloteau, D., Antier, D., Chalon, S., 2014. Translocator protein (18 kDa) mapping with [$^{125}$I]-CLINDE in the quinolinic acid rat model of excitotoxicity: a longitudinal comparison with microglial activation, astrogliosis, and neuronal death. Mol. Imaging 13, 4–11.

Ayar, A., Martin, D.J., Ozcan, M., Kelestimur, H., 2001. Melatonin inhibits high voltage activated calcium currents in cultured rat dorsal root ganglion neurones. Neurosci. Lett. 313, 73–77.

Balduini, W., Carloni, S., Perrone, S., Bertrando, S., Tataranno, M.L., Negro, S., Proietti, F., Longini, M., Buonocore, G., 2012. The use of melatonin in hypoxic-ischemic brain damage: an experimental study. J. Matern. Fetal Neonatal Med. 25 (Suppl. 1), 119–124.

Barjavel, M.J., Mamdouh, Z., Raghbate, N., Bakouche, O., 1998. Differential expression of the melatonin receptor in human monocytes. J. Immunol. 160, 1191–1197.

Basova, L.V., Kurnikov, I.V., Wang, L., Ritov, V.B., Belikova, N.A., Vlasova, I.I., Pacheco, A.A., Winnica, D.E., Peterson, J., Bayir, H., Waldeck, D.H., Kagan, V.E., 2007. Cardiolipin switch in mitochondria: shutting off the reduction of cytochrome c and turning on the peroxidase activity. Biochemistry 46, 3423–3434.

Bayir, H., Tyurin, V.A., Tyurina, Y.Y., Viner, R., Ritov, V., Amoscato, A.A., Zhao, Q., Zhang, Y.J., Janaesko-Feldman, K.L., Alexander, H., Basova, L.V., Clark, R.S., Kochanek, P.M., Kagan, V.E., 2007. Selective early cardiolipin peroxidation after traumatic brain injury: an oxidative lipidomics analysis. Ann. Neurol. 62, 154–169.

Bender Hoppe, J., Frozza, R.L., Horn, A.P., Argenta Comiran, R., Bernardi, A., Campos, M.M., Oliveira Battastini, A.M., Salbego, C., 2010. Amyloid-β neurotoxicity in organotypic culture is attenuated by melatonin: involvement of GSK-3β, *tau* and neuroinflammation. J. Pineal Res. 48, 230–238.

Blasko, I., Marx, F., Steiner, E., Hartmann, T., GrubeckLoebenstein, B., 1999. TNFα plus IFNγ induce the production of Alzheimer β-amyloid peptides and decrease the secretion of APPs. FASEB J. 13, 63–68.

Blasko, I., Veerhuis, R., Stampfer-Kountchev, M., Saurwein-Teissl, M., Eikelenboom, P., Grubeck. Loebenstein, B., 2000. Costimulatory effects of interferon-γ and interkeukin-1β or tumor necrosis factor α on the synthesis of Aβ1-40 and Aβ1-42 by human astrocytes. Neurobiol. Dis. 7 , 692-689.

Blaylock, R.L., 2013. Immunology primer for neurosurgeons and neurologists. Part 2: Innate brain immunity. Surg. Neurol. Int. 4, 118.

Boren, E., Gershwin, M.E., 2004. Inflamm-aging: autoimmunity, and the immune-risk phenotype. Autoimmun. Rev. 3, 401–406.

Bossù, P., Ciaramella, A., Salani, F., Vanni, D., Palladino, I., Caltagirone, C., Scapigliati, G., 2010. Interleukin-18, from neuroinflammation to Alzheimer's disease. Curr. Pharm. Des. 16, 4213–4224.

Bowerman, M., Vincent, T., Scamps, F., Perrin, F.E., Camu, W., Raoul, C., 2013. Neuroimmunity dynamics and the development of therapeutic strategies for amyotrophic lateral sclerosis. Front. Cell. Neurosci. 7, 214.

Brown, G.C., 2007. Mechanisms of inflammatory neurodegeneration: iNOS and NADPH oxidase. Biochem. Soc. Trans. 35 (Pt. 5), 1119–1121.

Brown, G.C., Neher, J.J., 2010. Inflammatory neurodegeneration and mechanisms of microglial killing of neurons. Mol. Neurobiol. 41, 242–247.

Brown, G.M., Young, S.N., Gauthier, S., Tsui, H., Grota, L.J., 1979. Melatonin in human cerebrospinal fluid in daytime; its origin and variation with age. Life Sci. 25, 929–936.

Buffa, S., Pellicanò, M., Bulati, M., Martorana, A., Goldeck, G., Caruso, C., Pawelec, G., Colonna-Romano, G., 2013. A novel B cell population revealed by a CD38/CD24 gating strategy: $CD38^-CD24^-$B cells in centenarian offspring and elderly people. Age (Dordr.) 35, 2009–2024.

Burkhardt, S., Reiter, R.J., Tan, D.-X., Hardeland, R., Cabrera, J., Karbownik, M., 2001. DNA oxidatively damaged by chromium(III) and $H_2O_2$ is protected by the antioxidants melatonin, $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine, resveratrol and uric acid. Int. J. Biochem. Cell Biol. 33, 775–783.

Campuzano, O., Castillo-Ruiz, M.M., Acarin, L., Castellano, B., Gonzalez, B., 2008. Distinct pattern of microglial response, cyclooxygenase-2, and inducible nitric oxide synthase expression in the aged rat brain after excitotoxic damage. J. Neurosci. Res. 86, 3170–3183.

Candore, G., Colonna-Romano, G., Balistreri, C.R., Di Carlo, D., Grimaldi, M.P., Listi, F., Nuzzo, D., Vasto, S., Lio, D., Caruso, C., 2006. Biology of longevity; role of the innate immune system. Rejuvenation Res. 9, 143–148.

Candore, G., Caruso, C., Colonna-Romano, G., 2010. Inflammation, genetic background and longevity. Biogerontology 11, 565–573.

Cano Barquilla, P., Pagano, E.S., Jiménez-Ortega, V., Fernández-Mateos, P., Esqifino, A.I., Cardinali, D.P., 2014. Melatonin normalizes clinical and biochemical parameters of mild inflammation in diet-induced metabolic syndrome in rats. J. Pineal Res. 57, 280–290.

Capri, M., Monti, D., Salvioli, S., Lescai, F., Altilia, S., Sevini, F., Valensin, S., Ostan, R., Bucci, L., Franceschi, C., 2006. Complexity of anti-immunosenescence strategies in humans. Artif. Organs 30, 730–742.

Cardinali, D.P., Esquifino, A.I., Srinivasan, V., Pandi-Perumal, S.R., 2008a. Melatonin and the immune system in aging. Neuroimmunomodulation 15, 272–278.

Cardinali, D.P., Pandi-Perumal, S.R., Niles, L.P., 2008b. Melatonin and its receptors: biological function in circadian sleep–wake regulation. In: Monti, J.M., Pandi-Perumal, S.R., Sinton, C.M. (Eds.), Neurochemistry of Sleep and Wakefulness. Cambridge University Press, Cambridge, UK, pp. 283–314.

Cardinali, D.P., Furio, A.M., Brusco, L.I., 2010. Clinical aspects of melatonin intervention in Alzheimer's disease progression. Curr. Neuropharmacol. 8, 218–227.

Carretero, M., Escames, G., López, L.C., Venegas, C., Dayoub, J.C., García, L., Acuña-Castroviejo, D., 2009. Long-term melatonin administration protects brain mitochondria from aging. J. Pineal Res. 47, 192–200.

Carrillo-Vico, A., García-Mauriño, S., Calvo, J.R., Guerrero, J.M., 2003. Melatonin counteracts the inhibitory effect of PGE2 on IL-2 production in human lymphocytes via its mt1 membrane receptor. FASEB J. 17, 755–757.

Carrillo-Vico, A., Guerrero, J.M., Lardone, P.J., Reiter, R.J., 2005. A review of the multiple actions of melatonin on the immune system. Endocrine 27, 189–200.


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Carrillo-Vico, A., Reiter, R.J., Lardone, P.J., Herrera, J.L., Fernández-Montesinos, R., Guerrero, J.M., Pozo, D., 2006. The modulatory role of melatonin on immune responsiveness. Curr. Opin. Investig. Drugs 7, 423–431.

Carrillo-Vico, A., Lardone, P.J., Álvarez-Sánchez, N., Rodríguez-Rodríguez, A., Guerrero, J.M., 2013. Melatonin: buffering the immune system. Int. J. Mol. Sci. 14, 8638–8683.

Cevenini, E., Monti, D., Franceschi, C., 2013. Inflamm-aging. Curr. Opin. Clin. Nutr. Metab. Care 16, 14–20.

Chandra, A., Johri, A., Beal, M.F., 2014. Prospects for neuroprotective therapies in prodromal Huntington's disease. Mov. Disord. 29, 285–293.

Chang, H.C., Guarente, L., 2013. SIRT1 mediates central circadian control in the SCN by a mechanism that decays with aging. Cell 153, 1448–1460.

Chang, H.M., Wu, U.I., Lan, C.T., 2009. Melatonin preserves longevity protein (sirtuin 1) expression in the hippocampus of total sleep-deprived rats. J. Pineal Res. 47, 211–220.

Chang, C.F., Huang, H.J., Lee, H.C., Hung, K.C., Wu, R.T., Lin, A.M., 2012. Melatonin attenuates kainic acid-induced neurotoxicity in mouse hippocampus via inhibition of autophagy and α-synuclein aggregation. J. Pineal Res. 52, 312–321.

Cheng, S., Ma, C., Qu, H., Fan, W., Pang, J., He, H., 2008. Differential effects of melatonin on hippocampal neurodegeneration in different aged accelerated senescence prone mouse-8. Neuro Endocrinol. Lett. 29, 91–99.

Chiang, R.P.-Y., Huang, C.-T., Tsai, Y.-J., 2013. Melatonin reduces median nerve injury-induced mechanical hypersensitivity via inhibition of microglial p38 mitogen-activated protein kinase activation in rat cuneate nucleus. J. Pineal Res. 54, 232–244.

Choo, X.Y., Alukaidey, L., White, A.R., Grubman, A., 2013. Neuroinflammation and copper in Alzheimer's disease. Int. J. Alzheimers Dis. 2013, 145345.

Chu, C.T., Ji, J., Dagda, R.K., Jiang, J.F., Tyurina, Y.Y., Kapralov, A.A., Tyurin, V.A., Yanamala, N., Shrivastava, I.H., Mohammadyani, D., Qiang Wang, K.Z., Zhu, J., Klein-Seetharaman, J., Balasubramanian, K., Amoscato, A.A., Borisenko, G., Huang, Z., Gusdon, A.M., Cheikhi, A., Steer, E.K., Wang, R., Baty, C., Watkins, S., Bahar, I., Bayir, H., Kagan, V.E., 2013. Cardiolipin externalization to the outer mitochondrial membrane acts as an elimination signal for mitophagy in neuronal cells. Nat. Cell Biol. 15, 1197–1205.

Clapp-Lilly, K.L., Smith, M.A., Perry, G., Duffy, L.K., 2001. Melatonin reduces interleukin secretion in amyloid-β stressed mouse brain slices. Chem. Biol. Interact. 134, 101–107.

Clark, I.A., Vissel, B., 2013. Treatment implications of the altered cytokine-insulin axis in neurodegenerative disease. Biochem. Pharmacol. 86, 862–871.

Clément, P., Gharib, A., Cespuglio, R., Sarda, N., 2003. Changes in sleep–wake cycle architecture and cortical nitric oxide release during ageing in the rat. Neuroscience 116, 863–870.

Colonna-Romano, G., Buffa, S., Bulati, M., Candore, G., Lio, D., Pellicanò, M., Vasto, S., Caruso, C., 2010. B cells compartment in centenarian offspring and old people. Curr. Pharm. Des. 16, 604–608.

Coppé, J.P., Patil, C.K., Rodier, F., Sun, Y., Muñoz, D.P., Goldstein, J., Nelson, P.S., Desprez, P.Y., Campisi, J., 2008. Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and p53 tumor suppressor. PLoS Biol. 6, 2853–2868.

Coppé, J.P., Desprez, P.Y., Krtolica, A., Campisi, J., 2010. The senescence-associated secretory phenotype: the dark side of tumor suppression. Annu. Rev. Pathol. 5, 99–118.

Corthell, J.T., Olcese, J., Trombley, P.Q., 2014. Melatonin in the mammalian olfactory bulb. Neuroscience 261, 74–84.

Cristofanon, S., Uguccioni, F., Cerella, C., Radogna, F., Dicato, M., Ghibelli, L., Diederich, M., 2009. Intracellular prooxidant activity of melatonin induces a survival pathway involving NF-κB activation. Ann. N.Y. Acad. Sci. 1171, 472–478.

Cristòfol, R., Porquet, D., Corpas, R., Coto-Montes, A., Serret, J., Camins, A., Pallàs, M., Sanfeliu, C., 2012. Neurons from senescence-accelerated SAMP8 mice are protected against frailty by the sirtuin 1 promoting agents melatonin and resveratrol. J. Pineal Res. 52, 271–281.

Crotti, A., Benner, C., Kerman, B.E., Gosselin, D., Lagier-Tourenne, C., Zuccato, C., Cattaneo, E., Gage, F.H., Cleveland, D.W., Glass, C.K., 2014. Mutant Huntingtin promotes autonomous microglia activation via myeloid lineage-determining factors. Nat. Neurosci. 17, 513–521.

Cuesta, S., Kireev, R., Forman, K., Garcia, C., Escames, G., Ariznavarreta, C., Vara, E., Tresguerres, J.A.F., 2010. Melatonin improves inflammation processes in liver of senescence-accelerated prone male mice (SAMP8). Exp. Gerontol. 45, 950–956.

Cuesta, S., Kireev, R., García, C., Forman, K., Escames, G., Vara, E., Tresguerres, J.A.F., 2011. Beneficial effect of melatonin treatment on inflammation, apoptosis and oxidative stress on pancreas of a senescence accelerated mice model. Mech. Ageing Dev. 132, 573–582.

Cuesta, S., Kireev, R., García, C., Rancan, L., Tresguerres, J.A.F., 2013. Melatonin can improve insulin resistance and aging-induced alterations in senescence-accelerated prone male mice (SAMP8). Age (Dordr.) 35, 659–671.

Dal Prà, I., Chiarini, A., Gui, L., Chakravarthy, B., Pacchiana, R., Gardenal, E., Whitfield, J.F., Armato, U., 2014. Do astrocytes collaborate with neurons in spreading the infectious Aβ and tau drivers of Alzheimer's disease? Neuroscientist, http://dx.doi.org/10.1177/1073858414529828.

Davalos, A.R., Coppé, J.P., Campisi, J., Desprez, P.Y., 2010. Senescent cells as a source of inflammatory factors for tumor progression. Cancer Metastasis Rev. 29, 273–283.

De Felice, F.G., Lourenco, M.V., Ferreira, S.T., 2014. How does brain insulin resistance develop in Alzheimer's disease? Alzheimers Dement. 10 (Suppl. 1), S26–S32.

De la Fuente, M., Miquel, J., 2009. An update of the oxidation-inflammation theory of aging: the involvement of the immune system in oxi-inflamm-aging. Curr. Pharm. Des. 15, 3003–3026.

de la Monte, S.M., Tong, M., 2014. Brain metabolic dysfunction at the core of Alzheimer's disease. Biochem. Pharmacol. 88, 548–559.

de la Rocha, N.E., Rotelli, A.E., Guardia, T., Pelzer, L.E., 2004. Different effects of melatonin on experimental granulomatous inflammation. Inflammation 28, 189–193.

DelaRosa, O., Pawelec, G., Peralbo, R., Wikby, A., Mariani, E., Mocchegiani, E., Tarazona, R., Solana, R., 2006. Immunological biomarkers of ageing in man: changes in both innate and adaptive immunity are associated with health and longevity. Biogerontology 7, 471–481.

Deng, W.G., Tang, S.T., Tseng, H.P., Wu, K.K., 2006. Melatonin suppresses macrophage cyclooxygenase-2 and inducible nitric oxide synthase expression by inhibiting p52 acetylation and binding. Blood 108, 518–524.

Deng, Y.Q., Xu, G.G., Duan, P., Zhang, Q., Wang, J.Z., 2005. Effects of melatonin on wortmannin-induced tau hyperphosphorylation. Acta Pharmacol. Sin. 26, 519–526.

de Rivero Vaccari, J.P., Dietrich, W.D., Keane, R.W., 2014. Activation and regulation of cellular inflammasomes: gaps in our knowledge for central nervous system injury. J. Cereb. Blood Flow Metab. 34, 369–375.

Dewan, S.K., Zheng, S.B., Xia, S.J., Bill, K., 2012. Senescent remodeling of the immune system and its contribution to the predisposition of the elderly to infections. Chin. Med. J. (Engl.) 125, 3325–3331.

Dragicevic, N., Copes, N., O'Neill-Moffitt, G., Jin, J., Buzzeo, R., Mamcarz, M., Tan, J., Cao, C., Olcese, J.M., Arendash, G.W., Bradshaw, P.C., 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.

Eikelenboom, P., Veerhuis, R., Scheper, W., Rozemuller, A.J., van Gool, W.A., Hoozemans, J.J., 2006. The significance of neuroinflammation in understanding Alzheimer's disease. J. Neural Transm. 113, 1685–1695.

Entrena, A., Camacho, M.E., Carrión, M.D., López-Cara, L.C., Velasco, G., León, J., Escames, G., Acuña-Castroviejo, D., Tapias, V., Gallo, M.A., Vivo, A., Espinosa, A., 2005. Kynurenamines as neural nitric oxide synthase inhibitors. J. Med. Chem. 48, 8174–8181.

Escames, G., López, L.C., Ortíz, F., Ros, E., Acuña-Castroviejo, D., 2006a. Age-dependent lipopolysaccharide-induced iNOS expression and multiorgan failure in rats: effects of melatonin treatment. Exp. Gerontol. 41, 1165–1173.

Escames, G., López, L.C., Tapias, V., Utrilla, P., Reiter, R.J., Hitos, A.B., León, J., Rodríguez, M.I., Acuña-Castroviejo, D., 2006b. Melatonin counteracts inducible mitochondrial nitric oxide synthase-dependent mitochondrial dysfunction in skeletal muscle of septic mice. J. Pineal Res. 40, 71–78.

Escames, G., López, L.C., Ortíz, F., López, A., García, J.A., Ros, E., Acuña-Castroviejo, D., 2007. Attenuation of cardiac mitochondrial dysfunction by melatonin in septic mice. FEBS J. 274, 2135–2147.

Esposito, E., Cuzzocrea, S., 2010. Antiinflammatory activity of melatonin in central nervous system. Curr. Neuropharmacol. 8, 228–242.

Fariello, R.G., Bubenik, G.A., Brown, G.M., Grota, L.J., 1977. Epileptogenic action of intraventricularly injected antimelatonin antibody. Neurology 27, 567–570.

Feng, Y.-M., Jia, Y.-F., Su, L.-Y., Wang, D., Lv, L., Xu, L., Yao, Y.G., 2013. Decreased mitochondrial DNA copy number in the hippocampus and peripheral blood during opiate addiction is mediated by autophagy and can be salvaged by melatonin. Autophagy 9, 18–29.

Feng, Z., Qin, C., Chang, Y., Zhang, J.T., 2006. Early melatonin supplementation alleviates oxidative stress in a transgenic mouse model of Alzheimer's disease. Free Radic. Biol. Med. 40, 101–109.

Fenoglio-Simeone, K., Mazarati, A., Sefidvash-Hockley, S., Shin, D., Wilke, J., Milligan, H., Sankar, R., Rho, J.M., Maganti, R., 2009. Anticonvulsant effects of the selective melatonin receptor agonist ramelteon. Epilepsy Behav. 16, 52–57.

Ferreira, S.T., Clarke, J.R., Bomfim, T.R., De Felice, F.G., 2014. Inflammation, defective insulin signaling, and neurological dysfunction in Alzheimer's disease. Alzheimers Dement. 10 (Suppl. 1), S76–S83.

Forman, K., Vara, E., García, C., Kireev, R., Cuesta, S., Acuña-Castroviejo, D., Tresguerres, J.A.F., 2010. Beneficial effects of melatonin on cardiological alterations in a murine model of accelerated aging. J. Pineal Res. 49, 312–320.

Forman, K., Vara, E., García, C., Kireev, R., Cuesta, S., Escames, G., Tresguerres, J.A.F., 2011. Effects of combined treatment with growth hormone and melatonin in the cardiological aging on male SAMP8 mice. J. Gerontol. A. Biol. Sci. Med. Sci. 66, 823–834.

Franceschi, C., Bonafè, M., 2003. Centenarians as a model for healthy aging. Biochem. Soc. Trans. 31, 457–461.

Franceschi, C., Campisi, J., 2014. Chronic inflammation (inflammaging) and its potential contribution to age-associated diseases. J. Gerontol. A. Biol. Sci. Med. Sci. 69, S4–S9.

Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000. Inflamm-aging. An evolutionary perspective in immunosenescence. Ann. N.Y. Acad. Sci. 908, 244–254.

Freund, A., Patil, C.K., Campisi, J., 2011. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. EMBO J. 30, 1536–1548.

Fumagalli, M., d'Adda di Fagagna, F., 2009. SASPense and DDRama in cancer and ageing. Nat. Cell Biol. 11, 921–923.

Fuster-Matanzo, A., Llorens-Martín, M., Hernández, F., Avila, J., 2013. Role of neuroinflammation in adult neurogenesis and Alzheimer disease: therapeutic approaches. Mediators Inflamm. 2013, 260925.

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García, J.J., Piñol-Ripoll, G., Martínez-Ballarín, E., Fuentes-Broto, L., Miana-Mena, F.J., Venegas, C., Caballero Escames, G., Coto-Montes, A., Acuña-Castroviejo, D., 2011. Melatonin reduces membrane rigidity and oxidative damage in the brain of SAMP8 mice. Neurobiol. Aging 32, 2045–2054.

García-Mauriño, S., González-Haba, M.G., Calvo, J.R., Rafii-El-Idrissi, M., Sánchez-Margalet, V., Goberna, R., Guerrero, J.M., 1997. Melatonin enhances IL-2, IL-6, and IFN-γ production by human circulating $CD4^+$ cells: a possible nuclear receptor-mediated mechanism involving T helper type 1 lymphocytes and monocytes. J. Immunol. 159, 574–581.

García-Mauriño, S., González-Haba, M.G., Calvo, J.R., Goberna, R., Guerrero, J.M., 1998. Involvement of nuclear binding sites for melatonin in the regulation of IL-2 and IL-6 production by human blood mononuclear cells. J. Neuroimmunol. 92, 76–84.

García-Mauriño, S., Pozo, D., Carrillo-Vico, A., Calvo, J.R., Guerrero, J.M., 1999. Melatonin activates Th1 lymphocytes by increasing IL-12 production. Life Sci. 65, 2143–2150.

García-Mesa, Y., Giménez-Llort, L., López, L.C., Venegas, C., Cristòfol, R., Escames, G., Acuña-Castroviejo, D., Sanfeliu, C., 2012. Melatonin plus physical exercise are highly neuroprotective in the 3×Tg-AD mouse. Neurobiol. Aging 33 , 1124.e13–1124.e29.

Gayoso, I., Sanchez-Correa, B., Campos, C., Alonso, C., Pera, A., Casado, J.G., Morgado, S., Tarazona, R., Solana, R., 2011. Immunosenescence of human natural killer cells. J. Innate Immun. 3, 337–343.

Gilthorpe, J.D., Oozeer, F., Nash, J., Calvo, M., Bennett, D.L., Lumsden, A., Pini, A., 2013. Extracellular histone H1 is neurotoxic and drives a pro-inflammatory response in microglia. F1000Res 2, 148.

Giunta, B., Fernandez, F., Nikolic, W.V., Obregon, D., Rrapo, E., Town, T., Tan, J., 2008. Inflammaging as a prodrome to Alzheimer's disease. J. Neuroinflammation 5, 51.

Golombek, D.A., Escolar, E., Burin, L.J., De Brito Sánchez, M.G., Cardinali, D.P., 1991a. Time-dependent melatonin analgesia in mice: inhibition by opiate or benzodiazepine antagonism. Eur. J. Pharmacol. 194, 25–30.

Golombek, D.A., Escolar, E., Cardinali, D.P., 1991b. Melatonin-induced depression of locomotor activity in hamsters: time dependency and inhibition by the central type benzodiazepine antagonist Ro 15-1788. Physiol. Behav. 49, 1091–1098.

Golombek, D.A., Escolar, E., Burin, L.J., De Brito Sánchez, M.G., Fernández Duque, D., Cardinali, D.P., 1992a. Chronopharmacology of melatonin: inhibition by benzodiazepine antagonism. Chronobiol. Int. 9, 124–131.

Golombek, D.A., Fernández Duque, D., De Brito Sánchez, M.G., Burin, L., Cardinali, D.P., 1992b. Time-dependent anticonvulsant activity of melatonin in hamsters. Eur. J. Pharmacol. 210, 253–258.

Golombek, D.A., Martíni, M., Cardinali, D.P., 1993. Melatonin as an anxiolytic in rats: time dependence and interaction with the central GABAergic system. Eur. J. Pharmacol. 237, 231–236.

Golombek, D.A., Pévet, P., Cardinali, D.P., 1996. Melatonin effects on behavior: possible mediation by the central GABAergic system. Neurosci. Biobehav. Rev. 20, 403–412.

Gomez-Cabrera, M.C., Sanchis-Gomar, F., Garcia-Valles, R., Pareja-Galeano, H., Gambini, J., Borras, C., Viña, J., 2012. Mitochondria as sources and targets of damage in cellular aging. Clin. Chem. Lab. Med. 50, 1287–1295.

Goronzy, J.J., Li, G., Yang, Z., Weyand, C.M., 2013. The Janus head of T cell aging – autoimmunity and immunodeficiency. Front. Immunol. 4, 131.

Grimaldi, B., Nakahata, Y., Kaluzova, M., Masubuchi, S., Sassone-Corsi, P., 2009. Chromatin remodeling, metabolism and circadian clocks: the interplay of CLOCK and SIRT1. Int. J. Biochem. Cell Biol. 41, 81–86.

Gruber, J., Schaffer, S., Halliwell, B., 2008. The mitochondrial free radical theory of aging—where do we stand? Front. Biosci. 13, 6554–6579.

Guenther, A.L., Schmidt, S.I., Laatsch, H., Fotso, S., Ness, H., Ressmeyer, A.-R., Poeggeler, B., Hardeland, R., 2005. Reactions of the melatonin metabolite AMK ($N^1$-acetyl-5-methoxykynuramine) with reactive nitrogen species: formation of novel compounds, 3-acetamidomethyl-6-methoxycinnolinone and 3-nitro-AMK. J. Pineal Res. 39, 251–260.

Guerrero, J.M., Reiter, R.J., 2002. Melatonin-immune system relationships. Curr. Top. Med. Chem. 2, 167–179.

Gunasingh, M.J., Philip, J.E., Ashok, B.S., Kirubagaran, R., Jebaraj, W.C., Davis, G.D., Vignesh, S., Dhandayuthapani, S., Jayakumar, R., 2008. Melatonin prevents amyloid protofibrillar induced oxidative imbalance and biogeneic amine catabolism. Life Sci. 83, 96–102.

Gutierrez-Cuesta, J., Sureda, F.X., Romeu, M., Canudas, A.M., Caballero, B., Coto-Montes, A., Camins, A., Pallàs, M., 2007. Chronic administration of melatonin reduced cerebral injury biomarkers in SAMP8. J. Pineal Res. 42, 394–402.

Hanzel, C.E., Pichet-Binette, A., Pomentel, L.S., Iulita, M.F., Allard, S., Ducatenzeiler, A., Do Carmo, S., Cuello, A.C., 2014. Neuronal driven pre-plaque inflammation in a transgenic rat model of Alzheimer's disease. Neurobiol. Aging 35, 2249–2262.

Hardeland, R., 2005. Antioxidative protection by melatonin: multiplicity of mechanisms from radical detoxification to radical avoidance. Endocrine 27, 119–130.

Hardeland, R., 2009a. Melatonin, mitochondrial electron flux and leakage: recent findings and resolution of contradictory results. Adv. Stud. Biol. 1, 207–230.

Hardeland, R., 2009b. Neuroprotection by radical avoidance: search for suitable agents. Molecules 14, 5054–5102.

Hardeland, R., 2010. Melatonin metabolism in the central nervous system. Curr. Neuropharmacol. 8, 168–181.

Hardeland, R., 2011a. Cognitive enhancers in moderate to severe Alzheimer's disease. Clin. Med. Insights Ther. 3, 459–476.

Hardeland, R., 2011b. Melatonin and its metabolites as anti-nitrosating and anti-nitrating agents. J. Exp. Integr. Med. 1, 67–81.

Hardeland, R., 2012. Melatonin in aging and disease—multiple consequences of reduced secretion, options and limits of treatment. Aging Dis. 3, 194–225.

Hardeland, R., 2013a. Melatonin and the theories of aging: a critical appraisal of melatonin's role in antiaging mechanisms. J. Pineal Res. 55, 325–356.

Hardeland, R., 2013b. Chronobiology of melatonin beyond the feedback to the suprachiasmatic nucleus – consequences to melatonin dysfunction. Int. J. Mol. Sci. 14, 5817–5841.

Hardeland, R., Coto-Montes, A., 2010. New vistas on oxidative damage and aging. Open Biol. J. 3, 39–52.

Hardeland, R., Coto-Montes, A., Poeggeler, B., 2003. Circadian rhythms, oxidative stress, and antioxidative defense mechanisms. Chronobiol. Int. 20, 921–962.

Hardeland, R., Ressmeyer, A.-R., Zelosko, V., Burkhardt, S., Poeggeler, B., 2004. Metabolites of melatonin: formation and properties of the methoxylated kynuramines AFMK and AMK. In: Haldar, C., Singh, S.S. (Eds.), Recent Advances in Endocrinology and Reproduction: Evolutionary, Biotechnological and Clinical Applications. Banaras Hindu University, Varanasi, pp. 21–38.

Hardeland, R., Backhaus, C., Fadavi, A., 2007. Reactions of the NO redox forms $NO^+$, $^{\bullet}NO$ and HNO (protonated $NO^-$) with the melatonin metabolite $N^1$-acetyl-5-methoxykynuramine. J. Pineal Res. 43, 382–388.

Hardeland, R., Poeggeler, B., Pappolla, M.A., 2009a. Mitochondrial actions of melatonin—an endeavor to identify their adaptive and cytoprotective mechanisms. Abh. Sächs. Akad. Wiss. Math.-Nat. Kl. 65 (Pt.3), 14–31.

Hardeland, R., Tan, D.-X., Reiter, R.J., 2009b. Kynuramines, metabolites of melatonin and other indoles: the resurrection of an almost forgotten class of biogenic amines. J. Pineal Res. 47, 109–126.

Hardeland, R., Cardinali, D.P., Srinivasan, V., Spence, D.W., Brown, G.M., Pandi-Perumal, S.R., 2011. Melatonin – a pleiotropic, orchestrating regulator molecule. Prog. Neurobiol. 93, 350–384.

Hardeland, R., Madrid, J.A., Tan, D.-X., Reiter, R.J., 2012. Melatonin, the circadian multioscillator system and health: the need for detailed analyses of peripheral melatonin signaling. J. Pineal Res. 52, 139–166.

Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11, 298–300.

Harman, D., 1972. The biologic clock: the mitochondria? J. Am. Geriatr. Soc. 20, 145–147.

Harman, D., 2006. Free radical theory of aging: an update: increasing the functional life span. Ann. N.Y. Acad. Sci. 1067, 10–21.

Hensley, K., Maidt, M.L., Yu, Z., Sang, H., Markesbery, W.R., Floyd, R.A., 1998. Electrochemical analysis of protein nitrotyrosine and dityrosine in the Alzheimer brain indicates region-specific accumulation. J. Neurosci. 18, 8126–8132.

Herskovits, A.Z., Guarente, L., 2014. SIRT1 in neurodevelopment and brain senescence. Neuron 81, 471–483.

Hirata, F., Hayaishi, O., Tokuyama, T., Senoh, S., 1974. *In vitro* and *in vivo* formation of two new metabolites of melatonin. J. Biol. Chem. 249, 1311–1313.

Hirota, T., Lewis, W.G., Liu, A.C., Lee, J.W., Schultz, P.G., Kay, S.A., 2008. A chemical biology approach reveals period shortening of the mammalian circadian clock by specific inhibition of GSK-3β. Proc. Natl. Acad. Sci. U. S. A. 105, 20746–204751.

Hurd, M.W., Ralph, M.R., 1998. The significance of circadian organization for longevity in the golden hamster. J. Biol. Rhythms 13, 430–436.

Hwang, J.W., Yao, H., Caito, S., Sundar, I.K., Rahman, I., 2013. Redox regulation of SIRT1 in inflammation and cellular senescence. Free Radic. Biol. Med. 61C, 95–110.

Imai, S., 2010. Clocks in the NAD world: NAD as a metabolic oscillator for the regulation of metabolism and aging. Biochim. Biophys. Acta 1804, 1584–1590.

Imai, S., Guarente, L., 2014. $NAD^+$ and sirtuins in aging and disease. Trends Cell Biol. 24, 464–471.

Iñarrea, P., Casanova, A., Alava, M.A., Iturralde, M., Cadenas, E., 2011. Melatonin and steroid hormones activate Cu,Zn-superoxide dismutase by means of mitochondrial cytochrome P450. Free Radic. Biol. Med. 50, 1575–1581.

Ji, M., Zhao, W.J., Dong, L.D., Miao, Y., Yang, X.L., Sun, Y.H., Wang, Z., 2011. RGS2 and RGS4 modulate melatonin-induced potentiation of glycine currents in rat retinal ganglion cells. Brain Res. 1411, 1–8.

Jiménez-Ortega, V., Cano, P., Cardinali, D.P., Esquifino, A.I., 2009. 24-Hour variation in gene expression of redox pathway enzymes in rat hypothalamus: effect of melatonin treatment. Redox Rep. 14, 132–138.

Jiang, T., Yu, J.T., Zhu, X.C., Tan, L., 2013. TREM2 in Alzheimer's disease. Mol. Neurobiol. 48, 180–185.

Jou, M.J., 2011. Melatonin preserves the transient mitochondrial permeability transition for protection during mitochondrial $Ca^{2+}$ stress in astrocyte. J. Pineal Res. 50, 427–435.

Jou, M.-J., Peng, T.-I., Reiter, R.J., Jou, S.-B., Wu, H.-Y., Wen, S.-T., 2004. Visualization of the antioxidative effects of melatonin at the mitochondrial level during oxidative stress-induced apoptosis of rat brain astrocytes. J. Pineal Res. 37, 55–70.

Jun, Z., Li, Z., Fang, W., Fengzhen, Y., Puyuan, W., Wenwen, L., Zhi, S., Bondy, S.C., 2013. Mleatonin decreases levels of S100β and NFKB, increases levels of synaptophysin in a rat model of Alzheimer's disease. Curr. Aging Sci. 6, 142–149.

Kagan, V.E., 2013. Emerging free radical optimism: translational oxidative lipidomics. Eur. J. Clin. Invest. 43 (Suppl. 1), 57.

Kagan, V.E., Bayir, A., Bayir, H., Stoyanovsky, D., Borisenko, G.G., Tyurina, Y.Y., Wipf, P., Atkinson, J., Greenberger, J.S., Chapkin, R.S., Belikova, N.A., 2009a. Mitochondria-targeted disruptors and inhibitors of cytochrome c/cardiolipin peroxidase complexes: a new strategy in anti-apoptotic drug discovery. Mol. Nutr. Food Res. 53, 104–114.

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Kagan, V.E., Bayir, H.A., Belikova, N.A., Kapralov, O., Tyurina, Y.Y., Tyurin, V.A., Jiang, J., Stoyanovski, D.A., Wipf, P., Kochanek, P.M., Greenberger, J.S., Pitt, B., Shvedova, A.A., Borisenko, G., 2009b. Cytochrome c/cardiolipin relations in mitochondria: a kiss of death. Free Radic. Biol. Med. 46, 1439–1453.

Kang, D., Hamasaki, N., 2005. Mitochondrial transcription factor A in the maintenance of mitochondrial DNA. Overview of its multiple roles. Ann. N.Y. Acad. Sci. 1042, 101–108.

Karasek, M., Reiter, R.J., 2002. Melatonin and aging. Neuroendocrinol. Lett. 23 (Suppl. 1), 14–16.

Kelly, R.W., Amato, F., Seamark, R.F., 1984. N-acetyl-5-methoxy kynurenamine, a brain metabolite of melatonin, is a potent inhibitor of prostaglandin biosynthesis. Biochem. Biophys. Res. Commun. 121, 372–379.

Kienhöfer, J., Häussler, D.J., Ruckelshausen, F., Muessig, E., Weber, K., Pomentel, D., Ulrich, V., Bürkle, A., Bachschmid, M.M., 2009. Association of mitochondrial antioxidant enzymes with mitochondrial DNA as integral nucleoid constituents. FASEB J. 23, 2034–2044.

Kinoshita, C., Miyazaki, K., Ishida, N., 2012. Chronic stress affects PERIOD2 expression through glycogen synthase kinase-3β phosphorylation in the central clock. Neuroreport 23, 98–102.

Kireev, R.A., Tresguerres, A.C., Garcia, C., Arriznavarreta, C., Vara, E., Tresguerres, J.A.F., 2008. Melatonin is able to prevent the liver of old castrated female rats from oxidative and pro-inflammatory damage. J. Pineal Res. 45, 394–402.

Kireev, R.A., Vara, E., Viña, J., Tresguerres, J.A.F., 2014. Melatonin and oestrogen treatments were able to improve neuroinflammation and apoptotic processes in dentate gyrus of old ovariectomized female rats. Age (Dordr.) 36, 9707.

Korkmaz, A., Topal, T., Tan, D.-X., Reiter, R.J., 2009. Role of melatonin in metabolic regulation. Rev. Endocr. Metab. Disord. 10, 261–270.

Korkmaz, A., Rosales-Corral, S., Reiter, R.J., 2012. Gene regulation by melatonin linked to epigenetic phenomena. Gene 503, 1–11.

Kozikowski, A.P., Gunosewoyo, H., Guo, S., Gaisina, I.N., Walter, R.L., Ketcherside, A., McClung, C.A., Mesecar, A.D., Caldarone, B., 2011. Identification of a glycogen synthase kinase-3β inhibitor that attenuates hyperactivtiy in CLOCK mutant mice. ChemMedChem 6, 1593–1602.

Kühlwein, E., Irwin, M., 2001. Melatonin modulation of lymphocyte proliferation and Th1/Th2 cytokine expression. J. Neuroimmunol. 117, 51–57.

Lahiri, D.K., 1999. Melatonin affects the metabolism of the β-amyloid precursor protein in different cell types. J. Pineal Res. 26, 137–146.

Lardone, P.J., Carrillo-Vico, A., Naranjo, M.C., De Felipe, B., Vallejo, A., Karasek, M., Guerrero, J.M., 2006. Melatonin synthesized by Jurkat human leukemic T cell line is implicated in IL-2 production. J. Cell. Physiol. 206, 273–279.

Lee, M.-Y., Kuan, Y-H., Chen, H.-Y., Chen, T.-Y., Chen, S.-T., Huang, C.-C., Yang, I.-P., Hsu, Y.-S., Wu, T.-S., Lee, E.-J., 2007. Intravenous administration of melatonin reduced the intracerebral inflammatory response following transient focal cerebral ischemia in rats. J. Pineal Res. 42, 297–309.

León, J., Macías, M., Escames, G., Camacho, E., Khaldy, H., Martín, M., Espinosa, A., Gallo, M.A., Acuña-Castroviejo, D., 2000. Structure-related inhibition of calmodulin-dependent neuronal nitric-oxide synthase activity by melatonin and synthetic kynurenines. Mol. Pharmacol. 58, 967–975.

León, J., Escames, G., Rodríguez, M.I., López, L.C., Tapias, V., Entrena, A., Camacho, E., Carrión, M.D., Gallo, M.A., Espinosa, A., Tan, D.-X., Reiter, R.J., Acuña-Castroviejo, D., 2006. Inhibition of neuronal nitric oxide synthase activity by N1-acetyl-5-methoxykynuramine, a brain metabolite of melatonin. J. Neurochem. 98, 2023–2033.

Li, X.C., Wang, Z.F., Zhang, J.X., Wang, Q., Wang, J.Z., 2005. Effect of melatonin on calyculin A-induced tau hyperphosphorylation. Eur. J. Pharmacol. 510, 25–30.

Liang, H., Ran, Q., Jang, Y.C., Holstein, D., Lechleiter, J., McDonald-Marsh, T., Musatov, A., Song, W., Van Remmen, H., Richardson, A., 2009. Glutathione peroxidase 4 differentially regulates the release of apoptogenic proteins from mitochondria. Free Radic. Biol. Med. 47, 312–320.

Lin, L., Huang, Q.X., Yang, S.S., Chu, J., Wang, J.Z., Tian, Q., 2013. Melatonin in Alzheimer's disease. Int. J. Mol. Sci. 14, 14575–45931.

Lin, T.Y., Huang, W.J., Wu, C.C., Lu, C.W., Wang, S.J., 2014. Acacetin inhibits glutamate release and prevents kainic-acid-induced neurotoxicity in rats. PLOS ONE 9, e88644.

Linton, P., Thoman, M.L., 2001. T cell senescence. Front. Biosci. 6, D248–D261.

Lissoni, P., Pittalis, S., Rovelli, F., Roselli, M., Ardizzoia, A., 1996. Modulation of cytokine production from TH2-lymphocytes and monocytes by the pineal neurohormone melatonin. Oncol. Rep. 3, 541–543.

Listi, F., Candore, G., Modica, M.A., Russo, M., Di Lorenzo, G., Esposito-Pelliteri, M., Colonna-Romano, G., Aquino, A., Bulati, M., Lio, D., Franceschi, C., Caruso, C., 2006. A study of serum immunoglobulin levels in elderly persons that provides new insights into B cell immunosenescence. Ann. N.Y. Acad. Sci. 1089, 487–495.

Liu, L., Chan, C., 2014. The role of the inflammasome in Alzheimer's disease. Ageing Res. Rev. 15C, 6–15.

Liu, R.Y., Zhou, J.N., van Heerikhuize, J., Hofman, M.A., Swaab, D.F., 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.

Liu, L.Y., Hoffman, G.E., Fei, X.W., Li, Z., Zhang, Z.H., Mei, Y.A., 2007. Delayed rectifier outward $K^+$ current mediates the migration of rat cerebellar granule cells stimulated by melatonin. J. Neurochem. 102, 333–344.

Lopategui Cabezas, I., Herrera Batista, A., Pentón Rol, G., 2014. The role of glial cells in Alzheimer disease: potential therapeutic implications. Neurologia 29, 305–309.

López, L.C., Escames, G., Tapias, V., Utrilla, P., León, J., Acuña-Castroviejo, D., 2006. Identification of an inducible nitric oxide synthase in diaphragm mitochondria from septic mice: its relation with mitochondrial dysfunction and prevention by melatonin. Int. J. Biochem. Cell Biol. 38, 267–278.

López, A., García, J.A., Escames, G., Venegas, C., Ortíz, F., López, L.C., Acuña-Castroviejo, D., 2009. Melatonin protects the mitochondria from oxidative damage reducing oxygen consumption, membrane potential, and superoxide anion production. J. Pineal Res. 46, 188–198.

Lu, C., Schoenfeld, R., Shan, Y., Tsai, H.J., Hammock, B., Cortopassi, G., 2009. Frataxin deficiency induces Schwann cell inflammation and death. Biochim. Biophys. Acta 1792, 1052–1061.

Luchetti, F., Canonico, B., Mannello, F., Masoni, C., D'Emilio, A., Battistelli, M., Papa, S., Falcieri, E., 2007. Melatonin reduces early changes in intramitochondrial cardiolipin during apoptosis in U937 cell line. Toxicol. In Vitro 21, 293–301.

Lyman, M., Lloyd, D.G., Ji, X., Vizcaychipi, M.P., Ma, D., 2014. Neuroinflammation: the role and consequences. Neurosci. Res. 79, 1–12.

Lynch, M.A., 2014. The impact of neuroimmune changes on development of amyloid pathology: relevance to Alzheimer's disease. Immunology 141, 292–301.

Manda, K., Ueno, M., Anzai, K., 2007. AFMK, a melatonin metabolite, attenuates X-ray-induced oxidative damage to DNA, proteins and lipids in mice. J. Pineal Res. 42, 386–393.

Manda, K., Ueno, M., Anzai, K., 2008. Space radiation-induced inhibition of neurogenesis in the hippocampal dentate gyrus and memory impairment in mice: ameliorative potential of the melatonin metabolite, AFMK. J. Pineal Res. 45, 430–438.

Marcheva, B., Ramsey, K.M., Buhr, E.D., Kobayashi, Y., Su, H., Ko, C.H., Ivanova, G., Omura, C., Mo, S., Vitaterna, M.H., Lopez, J.P., Philipson, L.H., Bradfield, C.A., Crosby, S.D., JeBailey, L., Wang, X., Takahashi, J.S., Bass, J., 2010. Disruption of the clock components CLOCK and BMAL1 leads to hypoinsulinaemia and diabetes. Nature 466, 627–631.

Masilamoni, J.G., Jesudason, E.P., Dhandayuthapani, S., Ashok, B.S., Vignesh, S., Jebaraj, W.C., Paul, S.F., Jayakumar, S., 2008. The neuroprotective role of melatonin against amyloid beta peptide injected mice. Free Radic. Res. 42, 661–673.

Matsubara, E., Bryant-Thomas, T., Pacheco Quinto, J., Henry, T.L., Poeggeler, B., Herbert, D., Cruz-Sanchez, F., Chyan, Y.-J., Smith, M.A., Perry, G., Shoji, M., Abe, K., Leone, A., Grundke-Ikbal, I., Wilson, G.L., Ghiso, J., Williams, C., Refolo, L.M., Pappolla, M.A., Chain, D.G., 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, J.C., Sainz, R.M., Tan, D.-X., Antolín, I., Rodríguez, C., Reiter, R.J., 2005a. Melatonin and Parkinson's disease. Endocrine 27, 169–178.

Mayo, J.C., Sainz, R.M., Tan, D.-X., Hardeland, R., León, J., Rodriguez, C., Reiter, R.J., 2005b. Anti-inflammatory actions of melatonin and its metabolites, N1-acetyl-N2-formyl-5-methoxykynuramine (AFMK) and N1-acetyl-5-methoxykynuramine (AMK), in macrophages. J. Neuroimmunol. 165, 139–149.

McLarnon, J.G., 2014. Correlated inflammatory responses and neurodegeneration in peptide-injected animal models of Alzheimer's disease. Biomed. Res. Int. 2014, 923670.

Messner, M., Hardeland, R., Rodenbeck, A., Huether, G., 1998. Tissue retention and subcellular distribution of continuously infused melatonin in rats under near physiological conditions. J. Pineal Res. 25, 251–259.

Michaud, M., Balardy, L., Moulis, G., Gaudin, C., Peyrot, C., Vellas, B., Cesari, M., Nourhashemi, F., 2013. Proinflammatory cytokines, aging, and age-related diseases. J. Am. Med. Dir. Assoc. 14, 877–882.

Miller, Z.A., Rankin, K.P., Graff-Redford, N.R., Takada, L.T., Sturm, V.E., Cleveland, C.M., Criswell, L.A., Jaeger, P.A., Stan, T., Heggeli, K.A., Hsu, S.C., Karydas, A., Khan, B.K., Grinberg, L.T., Gomo-Tempini, M.L., Boxer, A.L., Rosen, H.J., Kramer, J.H., Coppola, G., Geschwind, D.H., Rademakers, R., Seeley, W.W., Wyss-Coray, T., Miller, B.L., 2013. TDP-43 frontotemporal lobar degeneration and autoimmune disease. J. Neurol. Neurosurg. Psychiatry 84, 956–962.

Min, K.J., Jang, J.H., Kwon, T.K., 2012. Inhibitory effects of melatonin on the lipopolysaccharide-induced CC chemokine expression in BV2 murine microglial cells are mediated by suppression of Akt-induced NF-κB and STAT/GAS activity. J. Pineal Res. 52, 296–304.

Miquel, J., de Juan, E., Sevila, I., 1992. Oxygen-induced mitochondrial damage and aging. EXS 62, 47–57.

Mocchegiani, E., Giacconi, R., Muti, R., Cipriano, C., Costarelli, L., Tesei, S., Gasparini, N., Malavolta, M., 2007. Zinc-bound metallothioneins and immune plasticity: lessons from very old mice and humans. Immun. Ageing 4, 7.

Mocchegiani, E., Giacconi, R., Cipriano, C., Malavolta, M., 2009. NK and NKT cells in aging and longevity: role of zinc and metallothioneins. J. Clin. Immunol. 29, 416–425.

Molina-Carballo, A., Muñoz-Hoyos, A., Sánchez-Forte, M., Uberos-Fernández, J., Moreno-Madrid, F., Acuña-Castroviejo, D., 2007. Melatonin increases following convulsive seizures may be related to its anticonvulsant properties at physiological concentrations. Neuropediatrics 38, 122–125.

Morales, I., Guzmán-Martínet, L., Cerda-Troncoso, C., Farías, G.A., Maccioni, R.B., 2014. Neuroinflammation in the pathogenesis of Alzheimer's disease. A rational framework for the search of novel therapeutic approaches. Front. Cell. Neurosci. 8, 112.

More, S.V., Kumar, H., Kim, I.S., Song, S.Y., Choi, D.K., 2013. Cellular and molecular mediators of neuroinflammation in the pathogenesis of Parkinson's disease. Mediators Inflamm. 2013, 952375.

Morrey, K.M., McLachlan, J.A., Serkin, C.D., Bakouche, O., 1994. Activation of human monocytes by the pineal hormone melatonin. J. Immunol. 153, 2671–2680.

Muñoz-Hoyos, A., Sánchez-Forte, M., Molina-Carballo, A., Escames, G., Martín-Medina, E., Reiter, R.J., Molina-Font, J.A., Acuña-Castroviejo, D., 1998. Melatonin's role

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as an anticonvulsant and neuronal protector: experimental and clinical evidence. J. Child Neurol. 13, 501–509.
Nakahata, Y., Kaluzova, M., Grimaldi, B., Sahar, S., Hirayama, J., Chen, D., Guarente, L.P., Sassone-Corsi, P., 2008. The NAD+-dependent deacetylase SIRT1 modulates CLOCK-mediated chromatin remodeling and circadian control. Cell 134, 329–340.
Nakahata, Y., Sahar, S., Astarita, G., Kaluzova, M., Sassone-Corsi, P., 2009. Circadian control of the NAD+ salvage pathway by CLOCK-SIRT1. Science 324, 654–657.
Narayan, P., Holmström, K.M., Kim, D.H., Whitcomb, D.J., Wilson, M.R., St George-Hyslop, P., Wood, N.W., Dobson, C.M., Cho, K., Abramov, A.Y., Kleneman, D., 2014. Rare individual amyloid-β oligomers act on astrocytes to initiate neuronal damage. Biochemistry 53, 2442–2453.
Negi, G., Kumar, A., Sharma, S.S., 2011. Melatonin modulates neuroinflammation and oxidative stress in experimental diabetic neuropathy. Effects on NF-κB and Nrf2. J. Pineal Res. 50, 124–131.
Niranjan, R., Nath, C., Shukla, R., 2012. Melatonin attenuated mediators of neuroinflammation and α-7 nicotinic acetylcholine receptor mRNA expression in lipopolysaccharide (LPS) stimulated astrocytoma cells, C6. Free Radic. Res. 46, 1167–1177.
Nishida, S., 2005. Metabolic effects of melatonin on oxidative stress and diabetes mellitus. Endocrine 27, 131–136.
Nishida, S., Segawa, T., Murai, I., Nakagawa, S., 2002. Long-term melatonin administration reduces hyperinsulinemia and improves the altered fatty-acid compositions in type 2 diabetic rats via the restoration of Delta-5 desaturase activity. J. Pineal Res. 32, 26–33.
Nolan, Y.M., Sullivan, A.M., Toulouse, A., 2013. Parkinson's disease in the nuclear age of neuroinflammation. Trends Mol. Med. 19, 187–196.
Nomaru, H., Sakumi, K., Katogi, A., Ohnishi, Y.N., Kajitani, K., Tsuchimoto, D., Nestler, E.J., Nakabeppu, Y., 2014. Fosb gene products contribute to excitotoxic microglial activation by regulating the expression of complement C5a receptors in microglia. Glia 62, 1284–1298.
Nuzzo, D., Picone, P., Caruana, L., Vasto, S., Barera, A., Caruso, C., Di Carlo, M., 2014. Inflammatory mediators as biomarkers in brain disorders. Inflammation 37, 639–648.
O'Brien, I.A., Lewin, I.G., O'Hare, J.P., Arendt, J., Corrall, R.J., 1986. Abnormal circadian rhythm of melatonin in diabetic autonomic neuropathy. Clin. Endocrinol. (Oxf.) 24, 359–364.
Olcese, J.M., Cao, C., Mori, T., Mamcarz, M.B., Maxwell, A., Runfeldt, M.J., Wang, L., Zhang, C., Lin, X., Zhang, G., Arendash, G.W., 2009. Protection against cognitive deficits and markers of neurodegeneration by long-term oral administrations of melatonin in a transgenic model of Alzheimer disease. J. Pineal Res. 47, 82–96.
Onuki, J., Almeida, E.A., Medeiros, M.H.G., Di Mascio, P., 2005. Inhibition of 5-aminolevulinic acid-induced DNA damage by melatonin, $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine, quercetin or resveratrol. J. Pineal Res. 38, 107–115.
Ott, M., Gogvadze, V., Orrenius, S., Zhivotovsky, B., 2007. Mitochondria, oxidative stress and cell death. Apoptosis 12, 913–922.
Öztürk, G., Akbulut, K.G., Güney, S., Acuña-Castroviejo, D., 2012. Age-related changes in the rat brain mitochondrial antioxidative enzyme ratios: modulation by melatonin. Exp. Gerontol. 47, 706–711.
Pang, C.S., Tsang, S.F., Yang, J.C., 2001. Effects of melatonin, morphine and diazepam on formalin-induced nociception in mice. Life Sci. 68, 943–951.
Papp, M., Litwa, E., Gruca, P., Mocaër, E., 2006. Anxiolytic-like activity of agomelatine and melatonin in three animal models of anxiety. Behav. Pharmacol. 17, 9–18.
Pappolla, M.A., Chyan, Y., Poeggeler, B., Frangione, B., Wilson, G., Ghiso, J., Reiter, R.J., 2000. An assessment of the antioxidant and the antiamyloidogenic properties of melatonin: implications for Alzheimer's disease. J. Neural Transm. 107, 203–231.
Paradies, G., Petrosillo, G., Paradies, V., Reiter, R.J., Ruggiero, F.M., 2010. Melatonin, cardiolipin and mitochondrial bioenergetics in health and disease. J. Pineal Res. 48, 297–310.
Peek, C.B., Affinati, A.H., Ramsey, K.M., Kuo, H.Y., Yu, W., Sena, L.A., Ilkaveya, O., Marcheva, B., Kobayashi, Y., Omura, C., Levine, D.C., Bacsik, D.J., Gius, D., Newgard, C.B., Goetzman, E., Chandel, N.S., Denu, J.M., Mrksich, M., Bass, J., 2013. Circadian clock NAD+ cycle drives mitochondrial oxidative metabolism in mice. Science 342, 1243417.
Peng, C.X., Hu, J., Hong, X.P., Wu, Y.Y., Zhu, L.Q., Wang, J.Z., 2013. Disease-modified glycogen synthase kinase-3β intervention by melatonin arrests the pathology and memory deficits in an Alzheimer's animal model. Neurobiol. Aging 34, 1555–1563.
Permpoonputtana, K., Govitrapong, P., 2013. The anti-inflammatory effect of melatonin on methamphetamine-induced proinflammatory mediators in human neuroblastoma dopamine SH-SY5Y cell lines. Neurotox. Res. 23, 189–199.
Peschke, E., 2008. Melatonin, endocrine pancreas and diabetes. J. Pineal Res. 44, 26–40.
Peschke, E., Mühlbauer, E., 2010. New evidence for a role of melatonin in glucose regulation. Best Pract. Res. Clin. Endocrinol. Metab. 24, 829–841.
Peschke, E., Stumpf, I., Bazwinsky, I., Litvak, L., Dralle, H., Mühlbauer, E., 2007. Melatonin and type 2 diabetes – a possible link? J. Pineal Res. 42, 350–358.
Petrosillo, G., Di Venosa, N., Pistolese, M., Casanova, G., Tiravanti, E., Colantuono, G., Federici, A., Paradies, G., Ruggiero, F.M., 2006. Protective effect of melatonin against mitochondrial dysfunction associated with cardiac ischemia-reperfusion: role of cardiolipin. FASEB J. 20, 269–276.
Petrosillo, G., Fattoretti, P., Matera, M., Ruggiero, F.M., Bertoni-Freddari, C., Paradies, G., 2008. Melatonin prevents age-related mitochondrial dysfunction in rat brain via cardiolipin protection. Rejuvenation Res. 11, 935–943.
Pfister, G., Savino, W., 2008. Can the immune system still be efficient in the elderly? An immunological and immunoendocrine therapeutic perspective. Neuroimmunomodulation 15, 351–364.
Pinato, L., da Silveira Cruz-Machado, S., Franco, D.G., Campos, L.M.G., Cecon, E., Fernandes, P.A.C.M., Bittencourt, J.C., Markus, R.P., 2013. Selective protection of the cerebellum against intracerebroventricular LPS is mediated by local melatonin synthesis. Brain Struct. Funct., http://dx.doi.org/10.1007/s00429-013-0686-4.
Pinti, M., Nasi, M., Lugli, E., Gibellini, L., Bertoncelli, L., Roat, E., De Biasi, S., Mussini, C., Cossarizza, A., 2010. T cell homeostasis in centenarians: from the thymus to the periphery. Curr. Pharm. Des. 16, 597–603.
Poeggeler, B., 2005. Melatonin, aging, and age-related diseases: perspectives for prevention, intervention, and therapy. Endocrine 27, 201–212.
Ponnappan, S., Ponnappan, U., 2011. Aging and immune function: molecular mechanisms to interventions. Antioxid. Redox Signal. 14, 1551–1585.
Prada, C., Udin, S.B., 2005. Melatonin decreases calcium levels in retinotectal axons of *Xenopus laevis* by indirect activation of group III metabotropic glutamate receptors. Brain Res. 1053, 67–76.
Prada, C., Udin, S.B., Wiechmann, A.F., Zhdanova, I.V., 2005. Stimulation of melatonin receptors decreases calcium levels in Xenopus tectal cells by activating $GABA_C$ receptors. J. Neurophysiol. 94, 968–978.
Quinn, J., Kulhanek, D., Nowlin, J., Jones, R., Praticò, 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., Paternoster, L., De Nicola, M., Cerella, C., Ammendola, S., Bedini, A., Tarzia, G., Aquilano, K., Ciriolo, M., Ghibelli, L., 2009. Rapid and transient stimulation of intracellular reactive oxygen species by melatonin in normal and tumor leukocytes. Toxicol. Appl. Pharmacol. 239, 37–45.
Radogna, F., Diederich, M., Ghibelli, L., 2010. Melatonin: a pleiotropic molecule regulating inflammation. Biochem. Pharmacol. 80, 1844–1852.
Ramsey, K.M., Yoshino, J., Brace, C.S., Abrassart, D., Kobayashi, Y., Marcheva, B., Hong, H.K., Chong, J.L., Buhr, E.D., Lee, C., Takahashi, J.S., Imai, S., Bass, J., 2009. Circadian clock feedback cycle through NAMPT-mediated NAD+ biosynthesis. Science 324, 651–654.
Rehan, L., Laszki-Szczachor, K., Sobieszańska, M., Polak-Jonkisz, D., 2014. SIRT1 and NAD as regulators of aging. Life Sci. 105, 1–6.
Reiter, R.J., Acuña-Castroviejo, D., Tan, D.-X., Burkhardt, S., 2001. Free radical-mediated molecular damage. Mechanisms for the protective actions of melatonin in the central nervous system. Ann. N.Y. Acad. Sci. 939, 200–215.
Reiter, R.J., Tan, D.-X., Burkhardt, S., 2002. Reactive oxygen and nitrogen species and cellular and organismal decline: amelioration with melatonin. Mech. Ageing Dev. 123, 1007–1019.
Reiter, R.J., Tan, D.-X., Mayo, J.C., Sainz, R.M., Leon, J., Czarnocki, Z., 2003. Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. Acta Biochim. Pol. 50, 1129–1146.
Rentzos, M., Rombis, A., 2012. The role of IL-15 in central nervous system disorders. Acta Neurol. Scand. 125, 77–82.
Ressmeyer, A.-R., Mayo, J.C., Zelosko, V., Sáinz, R.M., Tan, D.-X., Poeggeler, B., Antolín, I., Zsizsik, B.K., Reiter, R.J., Hardeland, R., 2003. Antioxidant properties of the melatonin metabolite $N^1$-acetyl-5-methoxykynuramine (AMK): scavenging of free radicals and prevention of protein destruction. Redox Rep. 8, 205–213.
Ridolfi, E., Barone, C., Scarpini, E., Galimberti, D., 2013. The role of the innate immune system in Alzheimer's disease and frontotemporal lobar degeneration: an eye on microglia. Clin. Dev. Immunol. 2013, 939786.
Rizzo, F., Riboldi, G., Salani, S., Nizzardo, M., Simone, C., Corti, S., Hedlund, E., 2014. Cellular therapy to target neuroinflammation in amyotrophic lateral sclerosis. Cell. Mol. Life Sci. 71, 999–1015.
Robeva, R., Kirilov, G., Tomova, A., Kumanov, P., 2008. Melatonin–insulin interactions in patients with metabolic syndrome. J. Pineal Res. 44, 52–56.
Rodriguez, G.A., Tai, L.M., LaDu, M.J., Rebeck, G.W., 2014. Human APOE4 increases microglia reactivity at Aβ plaques in a mouse model of Aβ deposition. J. Neuroinflammation 11, 111.
Rosales-Corral, S.A., Acuña-Castroviejo, D., Coto-Montes, A., Boga, J.A., Manchester, L.C., Fuentes-Broto, L., Korkmaz, A., Ma, S., Tan, D.-X., Reiter, R.J., 2012. Alzheimer's disease: pathological mechanisms and the beneficial role of melatonin. J. Pineal Res. 52, 167–202.
Rosenstein, R.E., Cardinali, D.P., 1990. Central gabaergic mechanisms as target for melatonin activity. Neurochem. Int. 17, 373–379.
Sack, R.L., Lewy, A.J., Erb, D.L., Vollmer, W.M., Singer, C.M., 1986. Human melatonin production decreases with age. J. Pineal Res. 3, 379–388.
Sahar, S., Zocchi, K., Kinoshita, C., Borrelli, E., Sassone-Corsi, P., 2010. Regulation of BMAL1 protein stability and circadian function by GSK3β-mediated phosphorylation. PLoS ONE 5, e8561.
Salminen, A., Ojala, J., Kaamiranta, K., Haapasalo, A., Hiltunen, M., Soininen, H., 2011. Astrocytes in the aging brain express characteristics of senescence-associated secretory phenotype. Eur. J. Neurosci. 34, 3–11.
Salvioli, S., Capri, M., Valensin, S., Tieri, P., Monti, D., Ottavani, E., Franceschi, C., 2006. Inflamm-aging, cytokines and aging: state of the art, new hypotheses on the role of mitochondria and new perspectives from systems biology. Curr. Pharm. Des. 12, 3161–3171.
Samhan-Arias, A.K., Tyurina, Y.Y., Kagan, V.E., 2011. Lipid antioxidants: free radical scavenging versus regulation of enzymatic lipid peroxidation. J. Clin. Biochem. Nutr. 48, 91–95.
Sansoni, P., Vescovini, R., Fagnoni, F., Biasini, C., Zanni, L., Telera, A., Lucchini, G., Passeri, G., Monti, D., Franceschi, C., Passeri, M., 2008. The immune system in extreme longevity. Exp. Gerontol. 43, 61–65.

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Schlachetzki, J.C., Hüll, M., 2009. Microglial activation in Alzheimer's disease. Curr. Alzheimer Res. 6, 554–563.
Shafer, L.L., McNulty., J.A., Young, M.R., 2001. Assessment of melatonin's ability to regulate cytokine production by macrophage and microglia cell types. J. Neuroimmunol. 120, 84–93.
Shaw, A.C., Joshi, S., Greenwood, H., Panda, A., Lord, J.M., 2010. Aging of the innate immune system. Curr. Opin. Immunol. 22, 507–513.
Sheu, S.S., Wang, W., Cheng, H., Dirksen, R.T., 2008. Superoxide flashes: illuminating new insights into cardiac ischemia/reperfusion injury. Future Cardiol. 4, 551–554.
Shieh, J.-M., Wu, H.-T., Cheng, K.-C., Cheng, J.-T., 2009. Melatonin ameliorates high fat diet-induced diabetes and stimulates glycogen synthesis via a PKCζ-Akt-GSK3β pathway in hepatic cells. J. Pineal Res. 47, 339–344.
Silva, S.O., Rodrigues, M.R., Ximenes, V.F., Bueno-da-Silva, A.E., Amarante-Mendes, G.P., Campa, A., 2004. Neutrophils as a specific target for melatonin and kynuramines: effects on cytokine release. J. Neuroimmunol. 156, 146–152.
Silva, S.O., Ximenes, V.F., Livramento, J.A., Catalani, L.H., Campa, A., 2005. High concentrations of the melatonin metabolite, $N^1$-acetyl-$N^2$-formyl-5-methoxykynuramine, in cerebrospinal fluid of patients with meningitis: a possible immunomodulatory mechanism. J. Pineal Res. 39, 302–306.
Skene, D.J., Swaab, D.F., 2003. Melatonin rhythmicity: effect of age and Alzheimer's disease. Exp. Gerontol. 38, 199–206.
Skene, D.J., Vivien-Roels, B., Sparks, D.L., Hunsaker, J.C., Pévet, P., Ravid, D., Swaab, D.F., 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.
Smith, M.A., Richey Harris, P.L., Sayre, L.M., Beckman, J.S., Perry, G., 1997. Widespread peroxynitrite-mediated damage in Alzheimer's disease. J. Neurosci. 17, 2653–2657.
Solmaz, I., Gürkanlar, D., Gökçil, Z., Göksoy, C., Ozkan, M., Erdoğan, E., 2009. Antiepileptic activity of melatonin in guinea pigs with pentylenetetrazol-induced seizures. Neurol. Res. 31, 989–995.
Song, W., Lahiri, D.K., 1997. Melatonin alters the metabolism of the β-amyloid precursor protein in the neuroendocrine cell line PC12. J. Mol. Neurosci. 9, 75–92.
Srinivasan, V., Pandi-Perumal, S.R., Maestroni, G.J.M., Esquifino, A.I., Hardeland, R., Cardinali, D.P., 2005. Role of melatonin in neurodegenerative diseases. Neurotox. Res. 7, 293–318.
Srinivasan, V., Pandi-Perumal, S.R., Cardinali, D.P., Poeggeler, B., Hardeland, R., 2006. Melatonin in Alzheimer's disease and other neurodegenerative disorders. Behav. Brain Funct. 2, 15.
Srinivasan, V., Pandi-Perumal, S.R., Spence, D.W., Moscovitch, A., Trakht, I., Brown, G.M., Cardinali, D.P., 2010. Potential use of melatonergic drugs in analgesia: mechanisms of action. Brain Res. Bull. 81, 362–371.
Srinivasan, V., Cardinali, D.P., Srinivasan, U.S., Kaur, C., Brown, G.M., Spence, D.W., Hardeland, R., Pandi-Perumal, S.R., 2011. Therapeutic potential of melatonin and its analogs in Parkinson's disease: focus on sleep and neuroprotection. Ther. Adv. Neurol. Disord. 4, 297–317.
Srinivasan, V., Zakaria, R., Jeet Singh, H., Acuña-Castroviejo, D., 2012. Melatonin and its agonists in pain modulation and its clinical application. Arch. Ital. Biol. 150, 274–289.
Strindhall, J., Nilsson, B.O., Löfgren, S., Ernerudh, J., Pawelec, G., Johansson, B., Wikby, A., 2007. No immune risk profile among individuals who reach 100 years of age: findings from the Swedish NONA immune longitudinal study. Exp. Gerontol. 42, 753–761.
Sutinen, E.M., Pirtillä, T., Anderson, G., Salminen, A., Ojala, J.O., 2012. Pro-inflammatory interleukin-18 increases Alzheimer's disease-associated amyloid-β production in human neuron-like cells. J. Neuroinflammation 9, 199.
Szczepanik, M., 2007. Melatonin and its influence on immune system. J. Physiol. Pharmacol. 58 (Suppl. 6), 115–124.
Tajes, M., Gutierrez-Cuesta, J., Ortuño-Sahagun, D., Camins, A., Pallàs, M., 2009. Anti-aging properties of melatonin in an in vitro murine senescence model: involvement of the sirtuin 1 pathway. J. Pineal Res. 47, 228–237.
Takeuchi, H., Mizuno, T., Zhang, G., Wang, J., Kawanokuchi, J., Kuno, R., Suzumura, A., 2005. Neuritic beading induced by activated microglia is an early feature of neuronal dysfunction toward neuronal death by inhibition of mitochondrial respiration and axonal transport. J. Biol. Chem. 280, 10444–10454.
Tan, D.-X., Manchester, L.C., Burkhardt, S., Sainz, R.M., Mayo, J.C., Kohen, R., Shohami, E., Huo, Y.-S., Hardeland, R., Reiter, R.J., 2001. *N*1-Acetyl-*N*2-formyl-5-methoxykynuramine, a biogenic amine and melatonin metabolite, functions as a potent antioxidant. FASEB J. 15, 2294–2296.
Tan, D.-X., Manchester, L.C., Terron, M.P., Flores, L.J., Reiter, R.J., 2007. One molecule, many derivatives: a never-ending interaction of melatonin with reactive oxygen and nitrogen species? J. Pineal Res. 42, 28–42.
Tan, B., Choi, R.H., Chin, T.J., Kaur, C., Ling, E.A., 2012. Manipulation of microglial activity as a therapy for Alzheimer's disease. Front. Biosci. 4, 1402–1412.
Tapias, V., Escames, G., López, L.C., López, A., Camacho, E., Carrión, M.D., Entrena, A., Gallo, M.A., Espinosa, A., Acuña-Castroviejo, D., 2009. Melatonin and its brain metabolite $N^1$-acetyl-5-methoxykynuramine prevent mitochondrial nitric oxide synthase induction in Parkinsonian mice. J. Neurosci. Res. 87, 3002–3010.
Taylor, J.M., Main, B.S., Crack, P.J., 2013. Neuroinflammation and oxidative stress: co-conspirators in the pathology of Parkinson's disease. Neurochem. Int. 62, 803–819.
Thompson, L.V., 2006. Oxidative stress, mitochondria and mtDNA-mutator mice. Exp. Gerontol. 41, 1220–1222.
Tilleux, S., Hermans, E., 2007. Neuroinflammation and regulation of glial glutamate uptake in neurological disorders. J. Neurosci. Res. 85, 2059–2070.
Tocharus, J., Chongthammakun, S., Govitrapong, P., 2008. Melatonin inhibits amphetamine-induced nitric oxide synthase mRNA overexpression in microglial cell lines. Neurosci. Lett. 439, 134–137.
Tocharus, J., Khonthun, C., Chongthammakun, S., Govitrapong, P., 2010. Melatonin attenuates methamphetamine-induced overexpression of pro-inflammatory cytokines in microglial cell lines. J. Pineal Res. 48, 347–352.
Tricoire, H., Locatelli, A., Chemineau, P., Malpaux, B., 2002. Melatonin enters the cerebrospinal fluid through the pineal recess. Endocrinology 143, 84–90.
Tricoire, H., Malpaux, B., Møller, M., 2003a. Cellular lining of the sheep pineal recess studied by light-, transmission-, and scanning electron microscopy: morphologic indications for a direct secretion of melatonin from the pineal gland to the cerebrospinal fluid. J. Comp. Neurol. 456, 39–47.
Tricoire, H., Møller, M., Chemineau, P., Malpaux, B., 2003b. Origin of cerebrospinal fluid melatonin and possible function in the integration of photoperiod. Reprod. Suppl. 61, 311–321.
Tyagi, E., Agrawal, R., Nath, C., Shukla, R., 2010. Effect of melatonin on neuroinflammation and acetylcholinesterase activity induced by LPS in rat brain. Eur. J. Pharmacol. 640, 206–210.
Ulugol, A., Dokmeci, D., Guray, G., Sapolyo, N., Ozyigit, F., Tamer, M., 2006. Antihyperalgesic, but not antiallodynic, effect of melatonin in nerve-injured neuropathic mice: possible involvements of the L-arginine-NO pathway and opioid system. Life Sci. 78, 1592–1597.
Urrutia, P.J., Mena, N.P., Núñez, M.T., 2014. The interplay between iron accumulation, mitochondrial dysfunction, and inflammation during the execution step of neurodegenerative disorders. Front. Pharmacol. 5, 38.
Venegas, C., García, J.A., Escames, G., Ortiz, F., López, A., Doerrier, C., García-Corzo, L., López, L.C., Reiter, R.J., Acuña-Castroviejo, D., 2012. Extrapineal melatonin: analysis of its subcellular distribution and daily fluctuations. J. Pineal Res. 52, 217–227.
von Bernhardi, R., 2007. Glial cell dysregulation: a new perspective on Alzheimer disease. Neurotox. Res. 12, 215–232.
Wang, W., Fang, H., Groom, L., Cheng, A., Zhang, W., Liu, J., Wang, X., Li, K., Han, P., Zheng, M., Yin, J., Wang, W., Mattson, M.P., Kao, J.P., Lakatta, E.G., Sheu, S.S., Ouyang, K., Chen, J., Dirksen, R.T., Cheng, H., 2008. Superoxide flashes in single mitochondria. Cell 134, 279–290.
Wang, Y., Liu, X., Wang, W., Song, W., Chen, L., Fang, Q., Yan, X., 2013. The expression of inflammatory cytokines on the aorta endothelia are up-regulated in pinealectomized rats. Inflammation 36, 1363–1373.
Wijnen, H., 2009. Circadian rhythms. A circadian loop asSIRTs itself. Science 324, 598–599.
Wu, Y.H., Swaab, D.F., 2007. Disturbance and strategies for reactivation of the circadian rhythm system in aging and Alzheimer's disease. Sleep Med. 8, 623–636.
Wu, U.-I., Mai, F.-D., Sheu, J.-N., Chen, L.-Y., Liu, Y.-T., Huang, H.-C., Chang, H.-M., 2011. Melatonin inhibits microglial activation, reduces pro-inflammatory cytokine levels, and rescues hippocampal neurons of adults rats with acute *Klebsiella pneumoniae* meningitis. J. Pineal Res. 50, 159–170.
Yan, L., Liu, S., Wang, C., Wang, F., Song, Y., Yan, N., Xi, S., Liu, Z., Sun, G., 2013. JNK and NADPH oxidase involved in fluoride-induced oxidative stress in BV-2 microglia cells. Mediators Inflamm. 2013, 895975.
Yin, J., Liu, Y.H., Xu, Y.F., Zhang, Y.J., Chen, J.G., Shu, B.H., Wang, J.Z., 2006. Melatonin arrests peroxynitrite-induced tau hyperphosphorylation and the overactivation of protein kinases in rat brain. J. Pineal Res. 41, 124–129.
Young, A.R.J., Narita, M., 2009. SASP reflects senescence. EMBO Rep. 10, 228–230.
Zhang, M., Cao, L.H., Yang, X.L., 2007. Melatonin modulates glycine currents of retinal ganglion cells in rat. Neuroreport 18, 1675–1678.
Zhao, W.J., Zhang, M., Miao, Y., Yang, X.L., Wang, Z., 2010. Melatonin potentiates glycine currents through a PLC/PKC signalling pathway in rat retinal ganglion cells. J. Physiol. 588, 2605–2619.
Zhao, W., Beers, D.R., Appel, S.H., 2013. Immune-mediated mechanisms in the pathoprogression of amyotrophic lateral sclerosis. J. Neuroimmune Pharmacol. 8, 888–899.
Zhou, J., Zhang, S., Zhao, X., Wei, T., 2008. Melatonin impairs NADPH oxidase assembly and decreases superoxide anion production in microglia exposed to amyloid-$\beta_{1-42}$. J. Pineal Res. 45, 157–165.

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