

Review

Melatonin and the aging brain

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Abstract

The events associated with brain aging are enumerated with emphasis on increased oxidative and inflammatory processes and on mitochondrial dysfunction. Several of these factors are further increased in a wide range of overt age-related neurological diseases. This generality has given impetus to concepts concerning similar therapeutic approaches common to a series of neurodegenerative disorders. Animal and cell culture models of several such disorders have benefited from the application of melatonin. The mechanisms underlying the neuroprotective properties of melatonin are likely to involve activation of specific melatonin receptors. This can lead to modulation of transcription factors and consequent altered gene expression, resulting in enhancement of antioxidant enzymes and downregulation of basal levels of inflammation. Melatonin has potential utility both in slowing normal brain aging and in treatment of neurodegenerative conditions. This is reinforced by the low cost of melatonin and its very low toxic hazard.

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1. The aging brain is associated with oxidative stress, excess inflammation and mitochondrial dysfunction

Aging involves a very complex series of changes both at the genetic and the phenotypic level. Among the more universal mechanisms, are selective deletions of portions of mitochondrial DNA (Melov et al., 1999; Wei and Lee, 2002), changes in immune function, and the presence of an imbalance between pro-oxidant and antioxidant factors. Alterations in these parameters have been repeatedly shown to occur with cerebral aging (LeBel and Bondy, 1992; Ames et al., 1993; Lass et al., 1998; Calabrese et al., 2000). Age related changes in brain morphology include the appearance of lipofuscin and other insoluble materials such as amyloid plaques that are resistant to normal intracellular catabolic processes. Amyloid deposits are found in half of brains from the apparently normal elderly population as well as being characteristic of Alzheimer's disease (AD) (Price et al., 1991). These inclusions are composed of proteinaceous and carbohydrate components. Their accretion is likely to adversely affect cell function, in a

parallel manner to the more spectacular distortion of neuronal geometry seen in gangliosidoses. The source of these gradually accumulating materials is by formation of protein–carbohydrate complexes. Peptides within these complexes are cross-linked and this is the basis of their resistance to ubiquitinylation and degradation by proteases.

1.1. Reactive oxidizing species and brain aging

There is considerable evidence for a relation between age-related cellular changes in the CNS being in part consequent to an imbalance between pro-oxidant and anti-oxidant cerebral factors (LeBel and Bondy, 1992; Calabrese et al., 2000). All classes of macromolecules are increasingly subject to oxidative or nitrosylative damage with age (reviewed in Poon et al., 2004). These include proteins (Kim et al., 2006a,b; Davies, 2005; Sohal et al., 1993), nucleic acids (Fraga et al., 1990) and lipids (Roberts and Reckelhoff, 2001). While the establishment of an association between oxidative events and the deleterious consequences of aging has been repeatedly reported, the evidence for a causal relation whereby oxidant events lead to impaired neurological or behavioral status is less well documented. Treatment of aged gerbils with a free radical spin trapping agent, α -phenyl-*N*-tert-butyl nitron (PBN), has

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been reported to reverse age-related loss of memory skills as evidenced by use of a radial arm maze (Sack et al., 1996). In a rapidly aging mouse line, such a spin trap can also simultaneously reduce the extent of free radical-induced protein oxidation and improve cognition (Butterfield et al., 1996). Successful treatment of ischemia in stroke models using rats and marmosets has been followed by a phase 3 clinical trial for treatment of acute ischemic stroke with a derivative of PBN, disodium 4-[(*tert*-butylimino)methyl] benzene-1,3-disulfonate *N*-oxide (NXY-059) (Floyd, 2006).

1.2. Inflammation and brain aging

There is increasing evidence from this and other laboratories, that the level of activity of the cerebral immune response system is dysregulated in the aged animal. Several factors reflecting intrinsic immune status within the nervous system are chronically elevated with age and in the absence of exogenous stimuli (Terrazzino et al., 1997; Xie et al., 2003; Sharman et al., 2002a, 2004). In many ways, microglia act as the brain's macrophages. The number of activated microglia is substantially increased in the CNS white matter of aged rats (Perry et al., 1993) and primates (Sloane et al., 1999). The importance of microglia in adverse inflammatory processes is illustrated by the finding that blockade of microglial activation is protective against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxicity to dopaminergic neurons (Wu et al., 2002). Heightened basal surveillance is however not accompanied by a corresponding ability to respond to an inflammatory stimulus of exogenous origin. In fact, the reactivity of the intrinsic immune system within the CNS to such a material as lipopolysaccharide is considerably decreased in the elderly mouse (Sharman et al., 2002a). It is likely that attenuation of the signal/noise ratio of immune defenses, reflects an impaired ability to mount an effective response to pathogens. Chronically sustained low-level inflammatory activity is likely to produce adverse effects. The harmfulness of an extended and ineffective immune response is exemplified in the lung where the continuing presence of mineral particles leads to a futile phagocytic attack by alveolar macrophages on such irresolvable foci, which ultimately leads to severe pathological changes involving inflammatory cytokines (Rimal et al., 2005). Parallel futile inflammatory responses in the brain may be raised toward aggregated amyloid peptide and other insoluble proteinaceous inclusions (Bondy and Campbell, 2002). Microglial activation is heightened in aged animals (Sloane et al., 1999) and this may reflect a non-productive aberrant response to endogenous factors which cannot be resolved by mounting an immune response.

1.3. Mitochondrial dysfunction and brain aging

The mitochondrion, by virtue of an intensely active respiratory chain and monoamine oxidase activity, is the major source of reactive oxidizing species and also constitutes a major target of cumulative oxidative events. Most of the reactive oxygen species produced intracellularly, originate from leakage

out of the respiratory chain. While electron transport is a very efficient process, it has been estimated that around 2% of oxygen utilized escapes complete reduction to water and can form transient reactive intermediates (Boveris and Chance, 1973). There is evidence that the efficiency of mitochondrial respiration may diminish with age with a consequent increase in the production of superoxide and other oxidant species (Kokoszka et al., 2001; Sharman and Bondy, 2001). Following chemical stress, mitochondria from aged mice are more prone to produce reactive oxygen species than are mitochondria from young mice (Liu and Ames, 2005). The impairment of mitochondrial function thus carries the hazard of oxidant-related damage and the most proximal targets that are maximally at risk are likely to be molecules present within the mitochondrion.

Increased oxidative stress in the aged brain has been attributed to reduced efficiency of mitochondrial energy production (Ames et al., 2005; Floyd and Hensley, 2002; Cocco et al., 2005). Such compromised respiratory function can lead to oxidative stress and impaired antioxidant enzyme systems (Tian et al., 1998). Mitochondrial dysfunction has also been implicated in several common age-related neurodegenerative diseases including Alzheimer's, Parkinson's and Huntington's diseases and amyotrophic lateral sclerosis (Bowling and Beal, 1995). Primary targets of oxidative damage include mitochondrial membranes (which have an important inner, and outer layered structure), mitochondrial proteins, and the mitochondrial DNA (mtDNA). mtDNA is relatively unprotected in comparison to nuclear DNA since it is located near the mitochondrial inner membrane, not enclosed within basic histones, and since the mitochondrial DNA repair mechanism lacks the effectiveness and sophistication of its nuclear counterpart. Thus the level of DNA damage in aging humans is 10-fold greater in the mitochondrion than in the nucleus (Mecocci et al., 1993). In addition, the level of mitochondrial damage is 15-fold greater in people over 70 years of age than in the younger population (Mecocci et al., 1993). This is evidenced by greater levels of gene deletions and by a much higher level of 8-hydroxy-2-deoxyguanosine in mtDNA (Mecocci et al., 1997). As judged by deletion frequencies, the mutation rate of mtDNA is increased many fold in old relative to young mice (Wang et al., 1997). A high level of mutated mtDNA has been found to lead to an impairment of mitochondrial respiration (Sobreira et al., 1996). There is evidence that those mitochondria containing typical deletions, while less efficient in performing oxidative phosphorylation than normal mitochondria, are able to divide faster (Corral-Debrinski et al., 1994). This raises the issue of whether such "malignant mitochondria" increasingly replace healthy mitochondria with age.

Mitochondria and mitochondrial dysfunction also play key parts in the regulated processes of cellular self-destruction, apoptosis, autophagy and necrosis (Skulachev, 2006; Kim et al., 2006a,b). When functioning properly, regulatory control of these processes of cell death is exquisitely sensitive to imbalances in cellular homeostasis as gauged by such measures as Ca^{2+} levels (Halestrap, 2006) and cellular redox status (Haddad, 2004).

2. The treatment of age-related neurological disease

Recent therapeutic approaches to the most common disease associated with human brain aging, Alzheimer's disease (AD), have included both anti-oxidant and anti-inflammatory drugs. These directions are validated by epidemiological studies suggesting that the extended use of anti-inflammatory agents (for example by patients with arthritis) reduces the risk of AD (McGeer and McGeer, *in press*). Other epidemiological data have implicated the utility of antioxidants in lowering the incidence of AD (Pitchumoni and Doraiswamy, 1998). It may well be that these reductions in incidence of AD result from deceleration of changes occurring with normal brain aging, rather than from a direct mitigation of AD-specific changes. The search for agents, with properties that target the nervous system, and restore a biochemical and behavioral profile more closely reflecting that found in younger animals, may thus be of value in treatment of diseases such as AD.

The selection of appropriate chemicals whose extended usage may lead to beneficial changes within the central nervous system, poses some distinctive problems. For example, broad, non-specific reduction of oxidative effects, may impact negatively upon physiological events that rely on reactive oxygen species, such as the involvement of superoxide anion in the inflammatory response. Nitric oxide (NO) is a free radical which can combine with superoxide to form a very potent oxidant, peroxynitrite, but NO also plays an important role in the modulation of both intracellular and intercellular signals. Although protein nitrosylation is elevated with aging (Sloane et al., 1999; Sharman et al., 2002b), NO also has a series of key functions relating to blood supply and neurotransmission. Thus global inhibition of nitric oxide synthase in order protect to against neurotoxic damage (Itzhak and Ali, 1996) would be an inappropriate broad-based preventive strategy.

2.1. Melatonin may reduce some aspects of brain aging

The rationale for emphasizing melatonin as having potential for retardation of age-related oxidant events, is based both on

our prior data and the reports of others (Reiter et al., 1998; Cuzzocrea et al., 2000; Bondy et al., 2002). Following oral ingestion, melatonin is readily available to the brain (Lahiri et al., 2004). This is in contrast to the often-limited access to the brain, of other potentially beneficial lipophilic or water-soluble compounds. For example, α -tocopherol treatment of humans in the DATATOP study for mitigation of Parkinson's disease (PD) revealed that even after many months of ingestion, levels of this vitamin were not equilibrated within the cerebrospinal fluid, and still rising (Vatassery et al., 1998). In addition, melatonin is potentially of *specific* value to the CNS where it is able to induce antioxidant genes (Kotler et al., 1998) and to attenuate the LPS (lipopolysaccharide)-induced inflammatory responses (Sewerynek et al., 1995; Lezoualch et al., 1998; Sharman et al., 2002a,b). Melatonin has also been reported to ameliorate indices of anxiety induced by LPS (Nava and Carta, 2001). β -Amyloid-induced interleukin secretion is also suppressed by melatonin (Clapp-Lilly et al., 2001). Supercoiled mtDNA in brain can be converted into circular and linearized forms by ethanol and this can be attenuated by concurrent treatment with melatonin (Mansouri et al., 2001). Our laboratory and others have found good evidence for the potential utility of melatonin in partially restoring both the biochemical and behavioral profile of older animals (Reiter et al., 1998). Pinealectomy, which removes the major source of melatonin synthesis within the brain, appears to accelerate the aging process (Reiter et al., 1999; Payao et al., 2001). Furthermore, melatonin has been reported to be neuroprotective in a wide range of conditions (Table 1). Melatonin appears to target the nervous system in a rather selective manner.

Selection of the use of melatonin to slow down the onset of undesirable aspects of aging is further supported by the findings that the thymic involution associated with aging can be arrested by pineal grafting or by administration of melatonin (Provinciali et al., 1996; Tian et al., 2003) and it has been suggested that aging is essentially a pineal-programmed event (Pierpaoli, 1998).

The evidence for the potential benefits of melatonin for brain aging can be roughly divided into three major areas, namely: (i)

Table 1
Neurological disorders that melatonin has been reported to ameliorate

Disorder	Report of melatonin improvement	Disease model	Proposed beneficial mechanism
Parkinsonism	Antolin et al. (2002), Thomas and Mohanakumar (2004)	Mouse MPTP administration	Mitochondrial protection, antioxidant
Alzheimer's disease	Matsubara et al. (2003), Lahiri et al. (2005), Wu and Swaab (2005), Srinivasan et al. (2006), Feng et al. (2006)	Transgenic mouse	Anti-inflammatory antioxidant
Brain edema	Gorgulu et al. (2001)	Rat cortical cooling injury	Antioxidant
Alcoholism	Baydas et al. (2005a)	Rat ethanol administration	Neural adhesion molecules preserved, antioxidant
Depression	Weil et al. (2006)	MT1 receptor knockout mouse	MT1 receptor activation
Cerebral ischemia	Lee et al. (2005)	Mouse midcerebral artery 1 h occlusion	Antioxidant in penumbral zone
Hyperhomocysteinuria	Baydas et al. (2005b)	Methionine-induced hyperhomocysteinemia in rats	Antioxidant
Glioma	Martin et al. (2006)	C6 glioma cell culture	NF- κ B inhibition
Phenylketonuria	Martinez-Cruz et al. (2006)	Rat maternal hyperphenylalaninemia	Mitochondrial protection

Many reports exist relating to Alzheimer's disease (AD) and Parkinson's disease (PD), and only a few recent, representative works are cited.

Potential benefits of melatonin on overall systemic aging. This includes effects not confined to the CNS such as altered immune function and longevity. (ii) Utility of melatonin in the mitigation of undesirable changes associated with generic brain aging rather than with a specific neurological disease. (iii) Reported benefits of use of melatonin in specific neurological diseases or in their corresponding animal models.

2.1.1. Melatonin and the aging organism

There are several reports of an extension of lifespan of both vertebrate and invertebrate animals treated with melatonin (Oaknin-Bendahan et al., 1995; Bonilla et al., 2002; Anisimov et al., 2005) suggesting that this agent has effects on the general systemic metabolism rather than acting on a single organ. Extension of lifespan by melatonin has also been reported for several single-celled organisms (Hardeland and Poeggeler, 2003). Conversely, pinealectomy may reduce longevity (Bulian and Pierpaoli, 2000). This implied beneficial effect on aging is substantiated by the ability of melatonin to restore both the reproductive cycle of aged mice (Diaz et al., 2000) and the responsiveness of the systemic immune system of aged animals (Akbulut et al., 2001). The increasing incidence of cancer with age may be partially relieved by melatonin (Bulian and Pierpaoli, 2000). An inverse relation exists in several tissues, between melatonin levels and age-related oxidative damage to DNA, suggesting widespread antioxidant effects (Morioka et al., 1999).

2.1.2. Melatonin and deficits associated with brain aging

Some of the general changes associated with brain aging in the absence of clinical disease, include memory deficits, cerebral arterial thinning and deposition of lipofuscin and amyloid plaques. All of these changes may be partially prevented by extended melatonin treatment (Matsubara et al., 2003; Bondy et al., 2004; Dupuis et al., 2004; Lahiri et al., 2005; Abd El Mohsen et al., 2005). Melatonin can restore the ability of the aging brain to respond to an inflammatory stimulus (Sharman et al., 2002a). This parallels the effects of melatonin on the circulating immune system (described above).

2.1.3. Use of melatonin in specific neurological diseases or in their corresponding animal models

Many studies are reported on the effect of melatonin on various neurological and psychiatric disorders and their relevant animal models. Most of these disorders become more prevalent with age. Morphological, physiological, or behavioral indices of aging have been reported reduced by melatonin.

A review must judge the preponderance of evidence and summarize the most established findings to serve as a platform for design of new studies. Using such a rubric, an emerging generalization is that, considering age-related diseases with a slow rate of progression such as AD and PD as well as aging itself, any beneficial effects of melatonin are likely to require extended dosing, perhaps preceding the appearance of overt deficits. For example, in the case of AD a disease characterized by depressed levels of melatonin in the csf (Zhou et al., 2003), attenuation of the rate of plaque deposition may only be

Table 2

Amelioration of changes commonly associated with neurodegenerative disease, by melatonin

Adverse change	Selected reports of beneficial effect of melatonin
Oxidative damage	Reiter et al. (1998), Sharman et al. (2002b), Abd El Mohsen et al. (2005), Kim et al. (2000)
Neuroinflammation	Sharman et al. (2002a, 2004)
Excitotoxicity	Skaper et al. (1999), Cheung (2003)

possible if exposure to exogenous melatonin is initiated in the young animal, well before appearance of amyloid deposits (Quinn et al., 2005).

A wide variety of studies points to the potential usefulness of melatonin for treating Parkinson's disease, including inhibition of lipid peroxidation, hydroxyl formation and protection of nigral dopaminergic neurons in MPTP-treated rodents (reviewed by Mayo et al., 2005). Exogenous melatonin has been shown to protect against L-DOPA autooxidation in the rat and increase its availability in the striatum (Rocchitta et al., 2006). Conversely, the utility of melatonin in treatment of relatively acute animal models involving MPTP or 6-hydroxydopamine is contradicted by reports that the symptomatic treatment of PD may actually be worsened by acute melatonin application (reviewed by Willis, 2005) perhaps by inhibition of dopamine release (Zisapel, 2001).

Other pre-clinical animal studies have focused on the effect of melatonin on three major events associated with a wide range of neurological diseases as well as overall brain aging. This classical triad consists of oxidative damage to macromolecules, chronic inflammation and low level hyperexcitation (excitotoxicity). All of these are reported to be moderated by melatonin (Table 2). These three processes while different, are clearly closely inter-related and often occur together. It can be difficult to determine which is the primary event which leads to a cascade of reactive responses. However, the anti-excitatory properties of melatonin are likely to be secondary to the antioxidant (Skaper et al., 1999; Cheung, 2003).

The issue of the initial site of melatonin's actions and the possible chain of events ensuing will be discussed below.

3. Mechanisms that may underlie properties of melatonin impacting on the aging process

3.1. Melatonin as an anti-oxidant

Melatonin is present in bacteria, plants, eukaryotes, and fungi as well as all phyla of multicellular animals and it may be that its original evolutionary role was as an anti-oxidant (Hardeland and Poeggeler, 2003). Melatonin has been shown to possess antioxidant properties in both tissue culture and in intact animals. A much discussed issue is whether it acts in this manner directly or by way of activating critical pathways involved in the disposition of free radicals (Srinivasan et al., 2006). The evidence for a direct effect rests on the fact that melatonin can act as a powerful free radical scavenger in isolated cell-free systems (Beyer et al., 1998; Tan et al., 2002).

There are however reports that melatonin can act in a pro-oxidant in such systems (Bondy et al., 2002; Buyukavci et al., 2006). Melatonin is present within brain at a concentration (around 4 pM) that is only 5% of that found in serum (Lahiri et al., 2004), and – unless it were to be highly concentrated in a localized area – can make little scavenging contribution in comparison to predominant antioxidant species such as glutathione (present in millimolar amounts) and α -tocopherol (5 μ M, Sanchez-Moreno et al., 2004).

However, melatonin is present in cerebrospinal fluid (csf) at concentrations actually higher than serum, suggesting that the pineal may be a source of blood-borne melatonin (Rousseau et al., 1999). This would account for the diurnal flux of melatonin content in both blood and csf (Debus et al., 2002). The evidence for the involvement of activation of melatonin receptors in accounting for its antioxidant potential includes several reports on induction of antioxidant enzymes by melatonin described in Section 3.3.

3.2. Modulation of immune function by melatonin

Melatonin can reduce levels of GFAP, a marker of astroglial and intrinsic immune activation, within the brain (Baydas et al., 2002). Basal levels of expression of inflammatory cytokines are depressed by melatonin in aged mice. All of these evidences of immune activation are elevated with age (Sharman et al., 2002a, 2004; Yu et al., 2002; McGeer and McGeer, 2004; Griffin, 2006) and are even more pronounced in AD (Griffin, 2006). The causal relation between this and oxidative events is unclear since inflammation involves oxidative activity, and free radicals can recruit an immune response.

3.3. Melatonin receptors and enzyme induction

Within the brain, there are three major plasma membrane receptors for melatonin (Table 3). Two of these, MT1 and MT2 are coupled to G-proteins whose activation leads to depression of levels of cyclic AMP. The third, MT3 or NQO2, is a quinone reductase with poorly understood in vivo function. However, the existence of additional melatonin binding sites in the nucleus of many cell types suggests mechanisms of action other than through plasma membrane receptors (Filadelfi and Castrucci, 1996). The specificity of melatonin may reside in its properties as a neurohormone, which affects transcriptional events in the CNS (Kotler et al., 1998) rather than as a non-specific antioxidant.

A wide range of antioxidant enzymes is induced by melatonin including glutathione peroxidase, catalase and

superoxide dismutases (Baydas, 2006; Manda and Bhatia, 2003). These protein changes are paralleled by altered levels of gene expression of oxidative enzymes (Anisimov and Popovic, 2004). In addition levels of some prooxidant enzymes such as lipoxygenase and nitric oxide synthetase are depressed following melatonin treatment (Reiter et al., 2001; Sharman et al., 2002b). Several kinds of cytoplasmic melatonin receptor involve G-protein transduction and modulate transcription. MT1 activation depresses CREB, and stimulates ERK (Chan et al., 2002). MT2 levels are depressed in AD (Savaskan et al., 2005). Changes in these signaling pathways may form the basis of the alteration in gene expression effected by melatonin.

The interaction of melatonin with its MT3 receptor may contribute to understanding melatonin's action at pharmacological concentrations, for, despite tight binding to MT3 ($K_i = 280$ nM), melatonin inhibits MT3 only modestly ($IC_{50} = 43$ μ M). MT3 has been characterized as a “toxicity enzyme” (Mailliet et al., 2004), based on the reduced sensitivity of MT3-knockout mice to the toxic effects of the MT3 substrate menadione (Long et al., 2002). Thus melatonin inhibits the (possibly toxic) activity of MT3 activity at just the concentrations where it is found to be protective pharmacologically (Mailliet et al., 2004).

Knockout strains of mouse lacking either MT1 or MT2 have been developed (Jin et al., 2003). These mutants indicate that there is a limited functional redundancy between the receptor subtypes in the suprachiasmatic nucleus.

3.4. The link between aging and circadian events

While the endogenous circadian clock seems to decline more slowly with age, the body's ability to synchronize properly with external light cycles is diminished more rapidly (Weinert, 2000). There is a connection between the length of the photoperiod and aging. The mean lifespan of the prosimian primate *Microcebus murinus* is decreased 28% by an accelerated photoperiodic regimen (Perret, 1997). Moreover, a shortened diurnal cycle is associated with diminished nocturnal melatonin secretion (Aujard et al., 2001), and can hasten the appearance of several behavioral deficits associated with aging (Cayetanot et al., 2005). Light suppresses melatonin production in humans (Lewy et al., 1980). In consideration of other evidence presented, this linkage between reduced melatonin levels and accelerated aging may be more than merely correlative. Levels of both MT1 and MT2 receptors are very high within the suprachiasmatic nucleus (SCN), the site of circadian rhythms regulation. However, levels of expression of both MT1 and MT2 mRNAs in the SCN are identical in

Table 3
Melatonin receptor nomenclature and properties

Name	GenBank designation	UniProt designation	Other designations	Properties
Melatonin receptor type 1a	Mtnr1a	MTR1A (Q61184)	Mell1a, ML1a, ML1, MT1	353aa plasma membrane GPCR
Melatonin receptor type 1b	Mtnr1b	MTR1B (Q8CIQ6)	Mell1b, ML1b, MT2	364aa plasma membrane GPCR
NAD(P)H dehydrogenase, quinone 2; NRH:quinone oxidoreductase 2	NQO2	NQO2 (Q9J175)	ML2, MT3	230aa zinc-binding cytoplasmic enzyme

senescence-accelerated and senescence-resistant mouse strains (Asai et al., 2000).

3.5. Melatonin and mitochondria

There are many references documenting the ability of melatonin to maintain indices of mitochondrial health. These include down-regulation of Bax, caspases, and inhibition of mitochondrial DNA fragmentation, apoptosis, cytochrome c release and closure of the permeability transition pore. Depression of these adverse events is accompanied by induction of Bcl-2, and improved function of the respiratory chain and ATP synthesis (Yoo et al., 2002; Feng et al., 2006; Yon et al., 2006). Maintenance of mitochondrial glutathione levels has been also attributed to melatonin (Leon et al., 2005; Srinivasan et al., 2006).

Concepts concerning the possible mechanisms of melatonin's protection of mitochondrial function fall broadly into two classes, namely direct effects of melatonin upon mitochondria, and indirect effects following induction of repression of key proteins. There is little evidence of a direct effect of melatonin upon mitochondrial constituents. Direct inhibition of the mitochondrial permeability transition pore has been shown in isolated systems but this requires micromolar concentrations of melatonin (Andrabi et al., 2004). The weight of evidence suggests that mitochondrial protection is largely consequent to the induction of antioxidant enzymes and repression of apoptotic pathways. The low levels of melatonin present in tissues cannot afford much direct antioxidant power but much amplification is provided by any action by way of gene regulation.

3.6. Summary of mechanisms of melatonin action and suggestions for future work

The simplest way to account for the multiplicity of effects of melatonin is to posit an early alteration of gene expression,

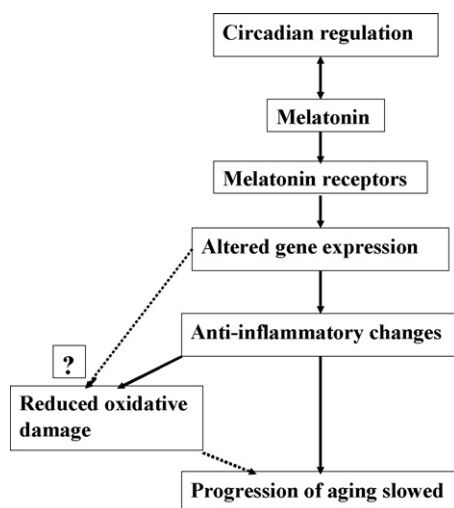


Fig. 1. Proposed key relationships between the regulation of melatonin levels and their potential effect on brain aging.

consisting of depression of mRNAs for immune-related cytokines and elevation of those for anti-oxidant proteins. This would lead to many of the reported enzymic changes resulting from melatonin treatment. Upstream of this may be activation of transcription factors after binding to cytoplasmic melatonin receptors, or changes due to melatonin acting directly on nuclear receptors. Melatonin receptors are not well characterized and no specific antagonists are available. Thus, circulating melatonin levels which fluctuate under circadian influence but which are depressed with age, could dynamically influence levels of oxidative and inflammatory activity (Fig. 1). Further research in characterizing melatonin receptors could lead to better delineation of the sequence of events by which melatonin exerts its effects.

4. Conclusion

The median age of the United States population is rapidly increasing and this will lead to a corresponding increase in the incidence of many age-related syndromes. Aging is often associated with both memory and motor deficits including impaired locomotor, postural and balancing skills. The potential for major increases in incidence of neurodegenerative disorders will be especially pronounced in view of the declining cardiovascular death rate. Retardation of the appearance of changes found with non-pathological aging, could postpone the clinical onset of diseases such as Parkinsonism and Alzheimer's disease. It may be that one of the most rewarding approaches to mitigation of the societal effects of these diseases lies in the deceleration of changes associated with normal cerebral senescence that are not specifically associated with any neurological disease. While the onset of neurological disease generally does not represent merely an acceleration of normal aging, it is obviously based on a platform of aging. Both normal aging and pathological processes in part involve changes in the same loci. The identification and protection of such common targets can be valuable in the development of strategies designed to delay the manifestation of common neurodegenerative disorders. The slow progression over a substantial portion of an individual's adult lifespan with these conditions suggests that, to be effective, treatments such as dietary supplementation need to be followed over many years. This lengthy treatment of essentially "well" patients requires that both the effectiveness and safety of such regimens be rigorously investigated – initially in animal models – before their widespread use can be confidently advocated.

Since only 9% of Americans eat the recommended five servings of fruits and vegetables daily (Ames et al., 1993), the opportunity for retarding neural aging by modifying the intake of exogenous nutrients, is high. Dietary supplementation as a means of delaying age-related neural disorders is more likely to be adhered to than any regimen based on caloric restriction which is known to retard aging processes. However, no dietary supplement has yet been found as effective as caloric restriction in extending lifespan (Lee et al., 2004). The consumption of melatonin on a regular basis may help to mitigate some aspects of brain aging and appears to pose very little downside risk. Any

potential benefits will occur over a long time, and will thus be hard to definitively document in humans. Data derived from animal studies, suggest the value of melatonin. Aging is a multifactorial process and the advocacy of a single remedial agent, is clearly incomplete. Unlike the focused pharmacological remediation for a specific disorder, amelioration of the wider range of changes associated with normal aging is obviously best addressed with a multiplicity of nutritional and physiological modifications. However, melatonin has utility as a means of retarding some aspects of brain aging. In addition to the experimental evidence described above, melatonin has several positive features that enhance its candidacy as a therapeutic agent. Melatonin is an evolutionarily ancient neurohormone that has very low toxicity, and no carcinogenic properties and this makes it a very safe compound. It is also readily available and has a low cost. Melatonin is likely to constitute an inexpensive and non-hazardous means of maintaining the functionality of the aging brain.

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References

- Abd El Mohsen, M.M., Irvani, M.M., Spencer, J.P., Rose, S., Fahim, A.T., Motawi, T.M., Ismail, N.A., Jenner, P., 2005. Age-associated changes in protein oxidation and proteasome activities in rat brain: modulation by antioxidants. *Biochem. Biophys. Res. Commun.* 336, 386–391.
- Akbulut, K.G., Gonul, B., Akbulut, H., 2001. The effects of melatonin on humoral immune responses of young and aged rats. *Immunol. Invest.* 30, 17–20.
- Ames, B.N., Atamna, H., Killilea, D.W., 2005. Mineral and vitamin deficiencies can accelerate the mitochondrial decay of aging. *Mol. Aspects Med.* 26, 363–378.
- Ames, B.N., Shigenaga, M.K., Hagen, T.M., 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc. Natl. Acad. Sci. U.S.A.* 90, 7915–7922.
- 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.
- Anisimov, S.V., Popovic, N., 2004. Genetic aspects of melatonin biology. *Rev. Neurosci.* 15, 209–230.
- Anisimov, V.N., Popovich, I.G., Zabezhinskii, M.A., Rozenfel'd, S.V., Khavinson, V.Kh., Semenchenko, A.V., Iashin, A.I., 2005. Effect of epitalon and melatonin on life span and spontaneous carcinogenesis in senescence accelerated mice (SAM). *Vopr. Onkol.* 51, 93–98.
- Antolin, I., Mayo, J.C., Sainz, R.M., del Brio Mde, L., Herrera, F., Martin, V., Rodriguez, C., 2002. Protective effect of melatonin in a chronic experimental model of Parkinson's disease. *Brain Res.* 943, 163–173.
- Asai, M., Ikeda, M., Akiyama, M., Oshima, I., Shibata, S., 2000. Administration of melatonin in drinking water promotes the phase advance of light–dark cycle in senescence-accelerated mice, SAMR1 but not SAMP8. *Brain Res.* 876, 220–224.
- Aujard, F., Dkhissi-Benyahya, O., Fournier, I., Claustrat, B., Schilling, A., Cooper, H.M., Perret, M., 2001. Artificially accelerated aging by shortened photoperiod alters early gene expression (Fos) in the suprachiasmatic nucleus and sulfatoxymelatonin excretion in a small primate, *Microcebus murinus*. *Neuroscience* 105, 403–412.
- Baydas, G., Reiter, R.J., Nedzvetskii, V.S., Nerush, P.A., Kirichenko, S.V., 2002. Altered glial fibrillary acidic protein content and its degradation in the hippocampus, cortex and cerebellum of rats exposed to constant light: reversal by melatonin. *J. Pineal Res.* 33, 134–139.
- Baydas, G., Yasar, A., Tuzcu, M., 2005a. Comparison of the impact of melatonin on chronic ethanol-induced learning and memory impairment between young and aged rats. *J. Pineal Res.* 39, 346–352.
- Baydas, G., Ozer, M., Yasar, A., Tuzcu, M., Koz, S.T., 2005b. Melatonin improves learning and memory performances impaired by hyperhomocysteinemia in rats. *Brain Res.* 1046, 187–194.
- Beyer, C.E., Steketee, J.D., Saphier, D., 1998. Antioxidant properties of melatonin—an emerging mystery. *Biochem. Pharmacol.* 56, 1265–1272.
- Bondy, S.C., Campbell, A., 2002. Initiation of futile pathways common to many neurological diseases. In: Marwah, J., Lo, E. (Eds.), *Neuroprotection*. Prominent Press, pp. 299–309.
- Bondy, S.C., Lahiri, D.K., Perreau, V.M., Sharman, K.Z., Campbell, A., Zhou, J., Sharman, E.H., 2004. Retardation of brain aging by chronic treatment with melatonin. *Ann. N.Y. Acad. Sci.* 1035, 197–215.
- Bondy, S.C., Yang, Y.E., Walsh, T.J., Gie, Y.W., Lahiri, D.K., 2002. Dietary modulation of age-related changes in cerebral pro-oxidant status. *Neurochem. Int.* 40, 123–130.
- Bonilla, E., Medina-Leendertz, S., Diaz, S., 2002. Extension of life span and stress resistance of *Drosophila melanogaster* by long-term supplementation with melatonin. *Exp. Gerontol.* 37, 629–638.
- Boveris, A., Chance, B., 1973. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *Biochem. J.* 134, 707–716.
- Bowling, A.C., Beal, M.F., 1995. Bioenergetic and oxidative stress in neurodegenerative diseases. *Life Sci.* 56, 1151–1171.
- Bulian, D., Pierpaoli, W., 2000. The pineal gland and cancer. I: Pinelectomy corrects congenital hormonal dysfunctions and prolongs life of cancer-prone C3H/He mice. *J. Neuroimmunol.* 108, 131–135.
- Butterfield, D.A., Martin, L., Carney, J.M., Hensley, K., 1996. A beta (25–35) peptide displays H₂O₂-like reactivity towards aqueous Fe²⁺, nitroxide spin probes, and synaptosomal membrane proteins. *Life Sci.* 58, 217–228.
- Buyukavci, M., Ozdemir, O., Buck, S., Stout, M., Ravindranath, Y., Savasan, S., 2006. Melatonin cytotoxicity in human leukemia cells: relation with its pro-oxidant effect. *Fundam. Clin. Pharmacol.* 20, 73–79.
- Calabrese, V., Bates, T.E., Stella, A.M., 2000. NO synthase and NO-dependent signal pathways in brain aging and neurodegenerative disorders: the role of oxidant/antioxidant balance. *Neurochem. Res.* 25, 1315–1341.
- Cayetanot, F., Van Someren, E.J., Perret, M., Aujard, F., 2005. Shortened seasonal photoperiodic cycles accelerate aging of the diurnal and circadian locomotor activity rhythms in a primate. *J. Biol. Rhythms* 20, 461–469.
- Chan, A.S., Lai, F.P., Lo, R.K., Voyno-Yasenetskaya, T.A., Stanbridge, E.J., Wong, Y.H., 2002. Melatonin mt1 and MT2 receptors stimulate c-Jun N-terminal kinase via pertussis toxin-sensitive and -insensitive G proteins. *Cell Signal.* 14, 249–257.
- Cheung, R.T., 2003. The utility of melatonin in reducing cerebral damage resulting from ischemia and reperfusion. *J. Pineal Res.* 34, 153–160.
- Clapp-Lilly, K.L., Smith, M.A., Perry, G., Duffy, L.K., 2001. Melatonin reduces interleukin secretion in amyloid-beta stressed mouse brain slices. *Chem. Biol. Interact.* 134, 101–107.
- Cocco, T., Sgobbo, P., Clemente, M., Lopriore, B., Grattagliano, I., Di Paola, M., Villani, G., 2005. Tissue-specific changes of mitochondrial functions in aged rats: effect of a long-term dietary treatment with *N*-acetylcysteine. *Free Radic. Biol. Med.* 38, 796–805.
- Corral-Debrinski, M., Horton, T., Lott, M.T., Shoffner, J.M., McKee, A.C., Beal, M.F., Graham, B.H., Wallace, D.C., 1994. Marked changes in mitochondrial DNA deletion levels in Alzheimer brains. *Genomics* 23, 471–476.
- Cuzzocrea, S., Costantino, G., Gitto, E., Mazzon, E., Fulia, F., Serraino, I., Cordaro, S., Barberi, I., De Sarro, A., Caputi, A.P., 2000. Protective effects of melatonin in ischemic brain injury. *J. Pineal Res.* 29, 217–227.
- Davies, M.J., 2005. The oxidative environment and protein damage. *Biochim. Biophys. Acta* 1703, 93–109.
- Diaz, E., Pazo, D., Esquifino, A.I., Diaz, B., 2000. Effects of ageing and exogenous melatonin on pituitary responsiveness to GnRH in rats. *J. Reprod. Fertil.* 119, 151–156.

- Debus, O.M., Lerchl, A., Bothe, H.W., Bremer, J., Fiedler, B., Franssen, M., Koehring, J., Steils, M., Kurlmann, G., 2002. Spontaneous central melatonin secretion and resorption kinetics of exogenous melatonin: a ventricular CSF study. *J. Pineal Res.* 33, 213–217.
- Dupuis, F., Regrigny, O., Atkinson, J., Liminana, P., Delagrangé, P., Scalbert, E., Chillon, J.M., 2004. Impact of treatment with melatonin on cerebral circulation in old rats. *Br. J. Pharmacol.* 141, 399–406.
- 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.
- Filadelfi, A.M., Castrucci, A.M., 1996. Comparative aspects of the pineal/melatonin system of poikilothermic vertebrates. *J. Pineal Res.* 20, 175–186.
- Floyd, R.A., 2006. Nitrones as therapeutics in age-related diseases. *Aging Cell* 1, 51–57.
- Floyd, R.A., Hensley, K., 2002. Oxidative stress in brain aging. Implications for therapeutics of neurodegenerative diseases. *Neurobiol. Aging* 23, 795–807.
- Fraga, C.G., Shigenaga, M.K., Park, J.W., Degan, P., Ames, B.N., 1990. Oxidative damage to DNA during aging: 8-hydroxy-2'-deoxyguanosine in rat organ DNA and urine. *Proc. Natl. Acad. Sci. U.S.A.* 87, 4533–4537.
- Gorgulu, A., Palaoglu, S., Ismailoglu, O., Tuncel, M., Surucu, M.T., Erbil, M., Kilinc, K., 2001. Effect of melatonin on cerebral edema in rats. *Neurosurgery* 49, 1434–1441.
- Griffin, W.S., 2006. Inflammation and neurodegenerative diseases. *Am. J. Clin. Nutr.* 83, 470S–474S.
- Haddad, J.J., 2004. Redox and oxidant-mediated regulation of apoptosis signaling pathways: immuno-pharmaco-redox conception of oxidative siege versus cell death commitment. *Int. Immunopharmacol.* 4, 475–493.
- Halestrap, A.P., 2006. Calcium, mitochondria and reperfusion injury: a pore way to die. *Biochem. Soc. Trans.* 34, 232–237.
- Hardeland, R., Poeggeler, B., 2003. Non-vertebrate melatonin. *J. Pineal Res.* 34, 233–234.
- Itzhak, Y., Ali, S.F., 1996. The neuronal nitric oxide synthase inhibitor, 7-nitroindazole, protects against methamphetamine-induced neurotoxicity in vivo. *J. Neurochem.* 67, 1770–1773.
- Jin, X., von Gall, C., Pieschl, R.L., Gribkoff, V.K., Stehle, J.H., Reppert, S.M., Weaver, D.R., 2003. Targeted disruption of the mouse Mel(1b) melatonin receptor. *Mol. Cell Biol.* 23, 1054–1060.
- Kim, C.H., Zou, Y., Kim, D.H., Kim, N.D., Yu, B.P., Chung, H.Y., 2006a. Proteomic analysis of nitrated and 4-hydroxy-2-nonenal-modified serum proteins during aging. *J. Gerontol. A Biol. Sci. Med. Sci.* 61, 332–338.
- Kim, R., Emi, M., Tanabe, K., 2006b. Role of mitochondria as the gardens of cell death. *Cancer Chemother. Pharmacol.* 57, 545–553.
- Kim, S.J., Reiter, R.J., Qi, W., Tan Dx, D., Cabrera, J., 2000. Melatonin prevents oxidative damage to protein and lipid induced by ascorbate-Fe(3+)-EDTA: Comparison with glutathione and alpha-tocopherol. *Neuro. Endocrinol. Lett.* 21, 269–276.
- Kokoszka, J.E., Coskun, P., Esposito, L.A., Wallace, D.C., 2001. Increased mitochondrial oxidative stress in the Sod2(+/-) mouse results in the age-related decline of mitochondrial function culminating in increased apoptosis. *Proc. Natl. Acad. Sci. U.S.A.* 98, 2278–2283.
- Kotler, M., Rodriguez, C., Sainz, R.M., Antolin, I., Menendez-Pelaez, A., 1998. Melatonin increases gene expression for antioxidant enzymes in rat brain cortex. *J. Pineal Res.* 24, 83–89.
- Lahiri, D.K., Ge, Y.W., Sharman, E.H., Bondy, S.C., 2004. Age-related changes in serum melatonin in mice: higher levels of combined melatonin and 6-hydroxymelatonin sulfate in the cerebral cortex than serum, heart, liver and kidney tissues. *J. Pineal Res.* 36, 217–223.
- Lahiri, D.K., Chen, D.M., Lahiri, P., Bondy, S., Greig, N.H., 2005. Amyloid, cholinesterase, melatonin, and metals and their roles in aging and neurodegenerative diseases. *Ann. N.Y. Acad. Sci.* 1056, 430–449.
- Lass, A., Sohal, B.H., Weindruch, R., Forster, M.J., Sohal, R.S., 1998. Caloric restriction prevents age-associated accrual of oxidative damage to mouse skeletal muscle mitochondria. *Free Radic. Biol. Med.* 25, 1089–1097.
- LeBel, C.P., Bondy, S.C., 1992. Oxidative damage and cerebral aging. *Prog. Neurobiol.* 38, 601–609.
- Lee, C.K., Pugh, T.D., Klopp, R.G., Edwards, J., Allison, D.B., Weindruch, R., Prolla, T.A., 2004. The impact of alpha-lipoic acid, coenzyme Q10 and caloric restriction on life span and gene expression patterns in mice. *Free Radic. Biol. Med.* 36, 1043–1057.
- Lee, E.J., Lee, M.Y., Chen, H.Y., Hsu, Y.S., Wu, T.S., Chen, S.T., Chang, G.L., 2005. Melatonin attenuates gray and white matter damage in a mouse model of transient focal cerebral ischemia. *J. Pineal Res.* 38, 42–52.
- Leon, J., Acuna-Castroviejo, D., Escames, G., Tan, D.X., Reiter, R.J., 2005. Melatonin mitigates mitochondrial malfunction. *J. Pineal Res.* 38, 1–9.
- Lewy, A.J., Wehr, T.A., Goodwin, F.K., Newsome, D.A., Markey, S.P., 1980. Light suppresses melatonin secretion in humans. *Science* 210, 1267–1269.
- Lezoualch, F., Sparapani, M., Behl, C., 1998. *N*-Acetyl-serotonin (normelatonin) and melatonin protect neurons against oxidative challenges and suppress the activity of the transcription factor NF-kappaB. *J. Pineal Res.* 24, 168–178.
- Liu, J., Ames, B.N., 2005. Reducing mitochondrial decay with mitochondrial nutrients to delay and treat cognitive dysfunction, Alzheimer's disease, and Parkinson's disease. *Nutr. Neurosci.* 8, 67–89.
- Long, D.J., Iskander, K., Gaikwad, A., Arin, M., Roop, D.R., Knox, R., Barrios, R., Jaiswal, A.K., 2002. Disruption of dihydronicotinamide riboside:quinone oxidoreductase 2 (NQO2) leads to myeloid hyperplasia of bone marrow and decreased sensitivity to menadione toxicity. *J. Biol. Chem.* 277, 46131–46139.
- Mailliet, F., Ferry, G., Vella, F., Thiam, K., Delagrangé, P., Boutin, J.A., 2004. Organs from mice deleted for NRH:quinone oxidoreductase 2 are deprived of the melatonin binding site MT3. *FEBS Lett.* 578, 116–120.
- Manda, K., Bhatia, A.L., 2003. Melatonin-induced reduction in age-related accumulation of oxidative damage in mice. *Biogerontology* 4, 133–139.
- Mansouri, A., Demeilliers, C., Amsellem, S., Pessayre, D., Fromenty, B., 2001. Acute ethanol administration oxidatively damages and depletes mitochondrial DNA in mouse liver, brain, heart, and skeletal muscles: protective effects of antioxidants. *J. Pharmacol. Exp. Ther.* 298, 737–743.
- Martin, V., Herrera, F., Carrera-Gonzalez, P., Garcia-Santos, G., Antolin, I., Rodriguez-Blanco, J., Rodriguez, C., 2006. Intracellular signaling pathways involved in the cell growth inhibition of glioma cells by melatonin. *Cancer Res.* 66, 1081–1088.
- Martinez-Cruz, F., Osuna, C., Guerrero, J.M., 2006. Mitochondrial damage induced by fetal hyperphenylalaninemia in the rat brain and liver: its prevention by melatonin, Vitamin E, and Vitamin C. *Neurosci. Lett.* 392, 1–4.
- 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., Antolin, I., Rodriguez, C., Reiter, R.J., 2005. Melatonin and Parkinson's disease. *Endocrine* 27 (2), 169–178.
- McGeer, P.L., McGeer, E.G., 2004. Inflammation and the degenerative diseases of aging. *Ann. N.Y. Acad. Sci.* 1035, 104–116.
- McGeer, P.L., McGeer, E.G. NSAIDs and Alzheimer disease: Epidemiological, animal model and clinical studies. *Neurobiol. Aging* (epub ahead of print), in press.
- Mecocci, P., MacGarvey, U., Kaufman, A.E., Koontz, D., Shoffner, J.M., Wallace, D.C., Beal, M.F., 1993. Oxidative damage to mitochondrial DNA shows marked age-dependent increases in human brain. *Ann. Neurol.* 34, 609–616.
- Mecocci, P., Beal, M.F., Cecchetti, R., Polidori, M.C., Cherubini, A., Chionne, F., Avellini, L., Romano, G., Senin, U., 1997. Mitochondrial membrane fluidity and oxidative damage to mitochondrial DNA in aged and AD human brain. *Mol. Chem. Neuropathol.* 31, 53–64.
- Melov, S., Schneider, J.A., Coskun, P.E., Bennett, D.A., Wallace, D.C., 1999. Mitochondrial DNA rearrangements in aging human brain and in situ PCR of mtDNA. *Neurobiol. Aging* 20, 565–571.
- Morioka, N., Okatani, Y., Wakatsuki, A., 1999. Melatonin protects against age-related DNA damage in the brains of female senescence-accelerated mice. *J. Pineal Res.* 27, 202–209.
- Nava, F., Carta, G., 2001. Melatonin reduces anxiety induced by lipopolysaccharide in the rat. *Neurosci. Lett.* 307, 57–60.

- Oaknin-Bendahan, S., Anis, Y., Nir, I., Zisapel, N., 1995. Effects of long-term administration of melatonin and a putative antagonist on the ageing rat. *Neuroreport* 6, 785–788.
- Payao, S.L., de Carvalho, C.V., da Silva, E.R., Lopes, C., Markus, R.P., Winter, L.M., Smith, M.A., 2001. Pinealectomy-associated decrease in ribosomal gene activity in rats. *Biogerontology* 2, 105–108.
- Perret, M., 1997. Change in photoperiodic cycle affects life span in a prosimian primate (*Microcebus murinus*). *J. Biol. Rhythms* 12, 136–145.
- Perry, V.H., Matyszak, M.K., Fearn, S., 1993. Altered antigen expression of microglia in the aged rodent CNS. *Glia* 7, 60–67.
- Pierpaoli, W., 1998. Neuroimmunomodulation of aging. A program in the pineal gland. *Ann. N.Y. Acad. Sci.* 840, 491–497.
- Pitchumoni, S.S., Doraiswamy, P.M., 1998. Current status of antioxidant therapy for Alzheimer's Disease. *J. Am. Geriatr. Soc.* 46, 1566–1572.
- Poon, H.F., Calabrese, V., Scapagnini, G., Butterfield, D.A., 2004. Free radicals and brain aging. *Clin. Geriatr. Med.* 20, 329–359.
- Price, J.L., Davis, P.B., Morris, J.C., White, D.L., 1991. The distribution of tangles, plaques and related immunohistochemical markers in healthy aging and Alzheimer's disease. *Neurobiol. Aging* 12, 295–312.
- Provinciali, M., Di Stefano, G., Bulian, D., Tibaldi, A., Fabris, N., 1996. Effect of melatonin and pineal grafting on thymocyte apoptosis in aging mice. *Mech. Ageing Dev.* 90, 1–19.
- Quinn, J., Kulhanek, D., Nowlin, J., Jones, R., Pratico, D., Rokach, J., Stackman, R., 2005. Chronic melatonin therapy fails to alter amyloid burden or oxidative damage in old Tg2576 mice: implications for clinical trials. *Brain Res.* 1037, 209–213.
- Reiter, R.J., Acuna-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., Guerrero, J.M., Garcia, J.J., Acuna-Castroviejo, D., 1998. Reactive oxygen intermediates, molecular damage, and aging. Relation to melatonin. *Ann. N.Y. Acad. Sci.* 854, 410–424.
- Reiter, R.J., Tan, D., Kim, S.J., Manchester, L.C., Qi, W., Garcia, J.J., Cabrera, J.C., El-Sokkary, G., Rouvier-Garay, V., 1999. Augmentation of indices of oxidative damage in life-long melatonin-deficient rats. *Mech. Ageing Dev.* 110, 157–173.
- Rimal, B., Greenberg, A.K., Rom, W.N., 2005. Basic pathogenetic mechanisms in silicosis: current understanding. *Curr. Opin. Pulm. Med.* 11, 169–173.
- Roberts 2nd, L.J., Reckelhoff, J.F., 2001. Measurement of F2-isoprostanes unveils profound oxidative stress in aged rats. *Biochem. Biophys. Res. Commun.* 287, 254–256.
- Rocchitta, G., Migheli, R., Esposito, G., Marchetti, B., Desole, M.S., Miele, E., Serra, P.A., 2006. Endogenous melatonin protects L-DOPA from autoxidation in the striatal extracellular compartment of the freely moving rat: potential implication for long-term L-DOPA therapy in Parkinson's disease. *J. Pineal Res.* 40, 204–213.
- Rousseau, A., Petren, S., Planthoin, J., Eklundh, T., Nordin, C., 1999. Serum and cerebrospinal fluid concentrations of melatonin: a pilot study in healthy male volunteers. *J. Neural Transm.* 106, 883–888.
- Sack, C.A., Soccia, D.J., Crandall, B.M., Arendash, G.W., 1996. Antioxidant treatment with phenyl-alpha-tert-butyl nitron (PBN) improves the cognitive performance and survival of aging rats. *Neurosci. Lett.* 205, 181–184.
- Sanchez-Moreno, C., Dorfman, S.E., Lichtenstein, A.H., Martin, A., 2004. Dietary fat type affects vitamins C and E and biomarkers of oxidative status in peripheral and brain tissues of golden Syrian hamsters. *J. Nutr.* 134, 655–660.
- Savaskan, E., Ayoub, M.A., Ravid, R., Angeloni, D., Fraschini, F., Meier, F., Eckert, A., Muller-Spahn, F., Jockers, R., 2005. Reduced hippocampal MT2 melatonin receptor expression in Alzheimer's disease. *J. Pineal Res.* 38, 10–16.
- Sewerynek, E., Abe, M., Reiter, R.J., Barlow-Walden, L.R., Chen, L., McCabe, T.J., Roman, L.J., Diaz-Lopez, B., 1995. Melatonin administration prevents lipopolysaccharide-induced oxidative damage in phenobarbital-treated animals. *J. Cell Biochem.* 58, 436–444.
- Sharman, E., Bondy, S.C., 2001. Effects of dietary antioxidants and age on cortical electron transport chain activity. *Neurobiol. Aging* 22, 629–634.
- Sharman, K.G., Sharman, E., Bondy, S.C., 2002a. Dietary melatonin selectively reverses age-related changes in cortical basal cytokine mRNA levels, and their responses to an inflammatory stimulus. *Neurobiol. Aging* 23, 633–638.
- Sharman, E.H., Vaziri, N.D., Ni, Z., Sharman, K.G., Bondy, S.C., 2002b. Reversal of biochemical and behavioral parameters of brain aging by melatonin and acetyl L-carnitine. *Brain Res.* 95, 223–230.
- Sharman, E., Sharman, K.G., Lahiri, D.K., Bondy, S.C., 2004. Age-related changes in murine CNS mRNA gene expression are modulated by dietary melatonin. *J. Pineal Res.* 36, 165–170.
- Skaper, S.D., Floreani, M., Ceccon, M., Facci, L., Giusti, P., 1999. Excitotoxicity, oxidative stress, and the neuroprotective potential of melatonin. *Ann. N.Y. Acad. Sci.* 890, 107–118.
- Skulachev, V.P., 2006. Bioenergetic aspects of apoptosis, necrosis and mitopoptosis. *Apoptosis* 11, 473–485.
- Sloane, J.A., Hollander, W., Moss, M.B., Rosene, D.L., Abraham, C.R., 1999. Increased microglial activation and protein nitration in white matter of the aging monkey. *Neurobiol. Aging* 20, 395–405.
- Sobreira, C., Davidson, M., King, M.P., Miranda, A.F., 1996. Dihydrorhodamine 123 identifies impaired mitochondrial respiratory chain function in cultured cells harboring mitochondrial DNA mutations. *J. Histochem. Cytochem.* 44, 571–579.
- Sohal, R.S., Agarwal, S., Dubey, A., Orr, W.C., 1993. Protein oxidative damage is associated with life expectancy of houseflies. *Proc. Natl. Acad. Sci. U.S.A.* 90, 7255–7259.
- 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.
- Tan, D.X., Reiter, R.J., Manchester, L.C., Yan, M.T., El-Sawi, M., Sainz, R.M., Mayo, J.C., Kohen, R., Allegra, M., Hardeland, R., 2002. Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. *Curr. Top. Med. Chem.* 2, 181–197.
- Terrazzino, S., Perego, C., De Luigi, A., De Simoni, M.G., 1997. Interleukin-6, tumor necrosis factor and corticosterone induction by central lipopolysaccharide in aged rats. *Life Sci.* 61, 695–701.
- Thomas, B., Mohanakumar, K.P., 2004. Melatonin protects against oxidative stress caused by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in the mouse nigrostriatum. *J. Pineal Res.* 36, 25–32.
- Tian, L., Cai, Q., Wei, H., 1998. Alterations of antioxidant enzymes and oxidative damage to macromolecules in different organs of rats during aging. *Free Radic. Biol. Med.* 24, 1477–2184.
- Tian, Y.M., Zhang, G.Y., Dai, Y.R., 2003. Melatonin rejuvenates degenerated thymus and redresses peripheral immune functions in aged mice. *Immunol. Lett.* 88, 101–104.
- Vatassery, G.T., Fahn, S., Kuskowski, M.A., 1998. Alpha tocopherol in CSF of subjects taking high-dose vitamin E in the DATATOP study. *Parkinson Study Group. Neurology* 50, 1900–1902.
- Wang, E., Wong, A., Cortopassi, G., 1997. The rate of mitochondrial mutagenesis is faster in mice than humans. *Mutat. Res.* 377, 157–166.
- Wei, Y.H., Lee, H.C., 2002. Oxidative stress, mitochondrial DNA mutation, and impairment of antioxidant enzymes in aging. *Exp. Biol. Med.* 227, 671–682.
- Weil, Z.M., Hotchkiss, A.K., Gatien, M.L., Picke-Dahl, S., Nelson, R.J., 2006. Melatonin receptor (MT1) knockout mice display depression-like behaviors and deficits in sensorimotor gating. *Brain Res. Bull.* 68, 425–429.
- Weinert, D., 2000. Age-dependent changes of the circadian system. *Chronobiol. Int.* 17, 261–283.
- Willis, G.L., 2005. The role of ML-23 and other melatonin analogues in the treatment and management of Parkinson's disease. *Drug News Perspect.* 18, 437–444.
- Wu, D.C., Jackson-Lewis, V., Vila, M., Tieu, K., Teismann, P., Vadseth, C., Choi, D.K., Ischiropoulos, H., Przedborski, S., 2002. Blockade of microglial activation is neuroprotective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson disease. *J. Neurosci.* 22, 1763–1771.
- Wu, Y.H., Swaab, D.F., 2005. The human pineal gland and melatonin in aging and Alzheimer's disease. *J. Pineal Res.* 38, 145–152.
- Xie, Z., Morgan, T.E., Rozovsky, I., Finch, C.E., 2003. Aging and glial responses to lipopolysaccharide in vitro: greater induction of IL-1 and IL-6, but smaller induction of neurotoxicity. *Exp. Neurol.* 182, 135–141.

- Yon, J.H., Carter, L.B., Reiter, R.J., Jevtovic-Todorovic, V., 2006. Melatonin reduces the severity of anesthesia-induced apoptotic neurodegeneration in the developing rat brain. *Neurobiol. Dis.* 21, 522–530.
- Yoo, Y.M., Yim, S.V., Kim, S.S., Jang, H.Y., Lea, H.Z., Hwang, G.C., Kim, J.W., Kim, S.A., Lee, H.J., Kim, C.J., Chung, J.H., Leem, K.H., 2002. Melatonin suppresses NO-induced apoptosis via induction of Bcl-2 expression in PGT-beta immortalized pineal cells. *J. Pineal Res.* 33, 146–150.
- Yu, W.H., Go, L., Guinn, B.A., Fraser, P.E., Westaway, D., McLaurin, J., 2002. Phenotypic and functional changes in glial cells as a function of age. *Neurobiol. Aging* 23, 105–115.
- Zhou, J.N., Liu, R.Y., Kamphorst, W., Hofman, M.A., Swaab, D.F., 2003. Early neuropathological Alzheimer's changes in aged individuals are accompanied by decreased cerebrospinal fluid melatonin levels. *J. Pineal Res.* 35, 125–130.
- Zisapel, N., 2001. Melatonin-dopamine interactions: from basic neurochemistry to a clinical setting. *Cell. Mol. Neurobiol.* 21, 605–616.