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**THE USE OF CHRONOBOTICS IN THE RESYNCHRONIZATION OF THE
SLEEP/WAKE CYCLE. THERAPEUTICAL APPLICATION IN THE EARLY PHASES
OF ALZHEIMER'S DISEASE.**

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Abstract

Treatment of circadian rhythm disorders, whether precipitated by intrinsic factors (e.g., sleep disorders, blindness, mental disorders, aging) or by extrinsic factors (e.g., shift work, jet-lag) has led to the development of a new type of agents called "chronobiotics". The term "chronobiotic" defines a substance displaying the therapeutic activity of shifting the phase or increasing the amplitude of the circadian rhythms. The prototype of this therapeutic group is melatonin, whose administration synchronizes the sleep-wake cycle in blind people and in individuals suffering from circadian rhythm sleep disorders, like delayed sleep phase syndrome, jet lag or shift-work. Daily melatonin production decreases with age, and in several pathologies, attaining its lowest values in Alzheimer's disease (AD) patients. About half of dementia patients have severe disruptions in their sleep-wakefulness cycle. Melatonin replacement is effective to treat sundowning and other sleep wake disorders in fully developed AD, although controversial data on this point exist. Indeed, large interindividual differences between patients suffering

from AD exist and can explain these erratic results. Theoretically the effect of melatonin could be more consistent at an earlier stage of the disease, i.e., mild cognitive impairment (MCI), an etiologically heterogeneous syndrome that precedes dementia. PubMed was searched using Entrez for articles including clinical trials. Search terms were “Alzheimer” “mild cognitive impairment” and “melatonin”. Full publications were obtained and references were checked for additional material where appropriate. Only clinical studies with empirical treatment data were reviewed. Five double blind, randomized placebo-controlled trials and 1 open-label retrospective study (N= 651) all agree in indicating that treatment with daily evening melatonin improves sleep quality and cognitive performance in MCI. The analysis of published evidence and patents indicates that melatonin can be a useful ad-on therapeutic tool in the early phases of AD.

Keywords: Alzheimer’s disease, circadian rhythms, melatonin, mild cognitive impairment

INTRODUCTION

Many biological functions wax and wane in cycles that repeat each day, month or year. Such patterns do not reflect simply organism's passive response to environmental changes. Rather, they reflect the organism's biological rhythms, that is, its ability to keep track of time and to direct changes in function accordingly. Because the earth rotates on its axis, it presents two environments, i.e. light and darkness (L/D); because the earth's axis of rotation is tilted, durations of daily periods of darkness and light vary during the course of the year.

Through evolution, animals responded to these environmental changes by preferentially adapting to them. This is the origin of biological rhythms that repeat approximately every 24 hours, called circadian rhythms (from the Latin *circa*, for around, and *diēs*, for day), and of rhythms that oscillate annually, following the recursive appearance of the seasons. Thus, when animals switch between diurnal, nocturnal or seasonal modes of their behavior, they are not simply responding passively to changes in external lighting conditions. They are responding to signals generated by a circadian pacemaker which is written in their genes that is synchronized with the cycles of the earth's rotation, anticipates the transitions between day and night, and triggers appropriate changes in behavioral state and physiological substrates [1]. In this way, the circadian pacemaker creates a day and night within the organism that mirrors approximately the world outside.

THE CIRCADIAN TIMING SYSTEM IS COMPOSED OF MANY INDIVIDUAL, TISSUE-SPECIFIC CELLULAR CLOCKS

At a molecular level, these circadian clocks are based on clock genes, some of which encode proteins able to feedback and inhibit their own transcription [1]. The cellular oscillators consist of interlocked transcriptional and post-translational feedback loops that involve a small number of core clock genes (about 12 genes

identified currently) [1]. The positive drive to the daily clock is constituted by helix-loop-helix, PAS-domain containing transcription factor genes, called Bmal1 and Clock (or its paralog Npas2). The protein products of these genes form heterodimeric complexes that control the transcription of other clock genes, notably three Period (Per1/Per2/Per3) genes and two Cryptochrome (Cry1/Cry2) genes, which in turn provide the negative feedback signal that shuts down the Clock/Bmal1 drive to complete the circadian cycle. Other clock genes like Rev-erba, Rora, NR1D1 and timeless provide additional transcriptional / translational feedback loops to form the rest of the core clockwork, which has been characterized in rodents by a transgenic gene deletion methodology. Clock gene expression oscillates because of the delay in the feedback loops, regulated in part by phosphorylation of the clock proteins that control their stability, nuclear re-entry and transcription complex formation [1].

To generate coherent physiological and behavioral responses, the phases of the multitude of cellular clocks are orchestrated by a master circadian pacemaker residing in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus [2] Fig. (1). The central clock is a key regulator of many bodily functions that follow a circadian rhythm, such as sleep and wakefulness, thermoregulation, glucose homeostasis and fat metabolism. This circadian apparatus includes: (a) the SCN, (b) an array of SCN-generated circadian physiology outputs, and (c) molecular clocks in the cells of all peripheral tissues.

Without the action of external time cues ("Zeitgebers") the period of these oscillators is close but not exactly 24 h. The rhythm is adjusted to 24 h by the action of light, the main (but not the unique) Zeitgeber in humans. The SCN receives information about environmental lighting through photic input via a direct retinal innervation, the retinohypothalamic tract arising from intrinsically photoreceptive retinal ganglion cells that express the photopigment melanopsin Fig. (1). Other retinal projections originating in cones and rods project through the visual pathway to the intergeniculate leaflet of the lateral geniculate complex and, via the geniculohypothalamic tract, to the SCN [3].

Brief exposures to light are sufficient to entrain the SCN clockwork to solar time, adjusting the oscillator to a precise 24-h cycle. Individual SCN neurons are competent biological clocks, but the sustainability and synchronization of the molecular oscillator depend on orchestrating the spontaneous electrical activity within the SCN via neuropeptidergic signaling among SCN neurons. These diffusible signals include transforming growth factor α , epidermal growth factor, prokineticin-2 and cardiotrophin-like cytokine [4]. The intercellular communication of SCN neurons not only synchronizes rhythmicity of the SCN but is also required for the maintenance of the amplitude and precision of individual cellular oscillations.

A second synchronizer of the SCN clockwork is melatonin Fig. (1). In mammals, melatonin is synthesized in the pineal gland in a rhythmic manner with high levels during nighttime and low levels during daytime [5]. Projections of the SCN driving the daily melatonin rhythm inhibit the firing of neurons in the subparaventricular zone of the anterior hypothalamus [6]. From this zone a multisynaptic pathway starts which includes the medial forebrain bundle, reticular formation and intermediolateral cell column of the cervical spinal cord, the

superior cervical ganglion and postganglionic sympathetic fibers that end in the vicinity of pineal cells and that stimulate melatonin synthesis [3].

Melatonin modulates circadian rhythms in the SCN by acting on MT₁ and MT₂ melatonin receptors expressed by SCN neurons, thus creating a reciprocal interaction between the SCN and the pineal gland [7]. Melatonin's phase-altering effect is caused by its direct influence on the electrical and metabolic activity of the SCN, presumably involving γ -aminobutyric acid-related mechanisms [8,9]. The circadian rhythm in the secretion of melatonin has been shown to be able to synchronize the sleep/wake cycle in both normal and blind subjects (i.e., in the absence of the synchronizing effect of light) [10].

The SCN communicates day-night cycle phase information to the rest of the body through neuronal and humoral signals, including the autonomic nervous system and the neuroendocrine system [11]. Through them the peripheral circadian cellular clocks synchronize to the same phase. At the same time, the clocks of the periphery are able to respond to other environmental cues such as food intake and alter their phase according to these cues [1] Fig. (1).

The daily sleep/wake cycle is influenced by two separate processes: (1) the endogenous biological clock that drives the circadian rhythm of sleep/wake cycle; (2) a homeostatic component, or "sleep load", that influences sleep propensity, a state which is determined by the immediate history of sleep and wakefulness [12,13]. These two processes, which interact continuously, determine the consolidated bout of sleep at night and the consolidated bout of wakefulness during daytime. Observations of subjects whose circadian rhythms had been experimentally desynchronized have supported the inference that homeostatic processes drive non-rapid eye movement (REM), or slow, sleep while REM sleep is driven by the circadian component [14].

The role of SCN in the regulation of sleep/wake cycle is relevant, mainly promoting wakefulness [15], as first studied in the squirrel monkey [16] (Fig. (2)). The sinusoidal output signal produced by the SCN is described by its period (cycle length), phase (position in the cycle), and amplitude (range between highest and lowest signal). The output amplitude reflects the "strength" or robustness of the circadian timing system, which can also be described as the drive to restore homeostasis in response to stimuli or the extent to which circadian behavior is separated into distinct periods of activity and rest within one cycle [15].

The fact that the nocturnal increase of melatonin secretion occurs approximately 2 h in advance of the individual's habitual bedtime has been the basis to postulate that melatonin is involved in the physiological regulation of sleep [17] Fig. (2). Studies in humans under constant routine conditions have defined the so-called "biological night" that corresponds to the period during which melatonin is produced and secreted into the bloodstream. The beginning of the biological night is characterized by onset of the melatonin surge, an accompanying increase in sleep propensity as well as a decrease in core body temperature; the opposite occurs as the biological night and sleep end [18] Fig. (2). Due to its neuronal suppressive actions on MT₁ receptors [19] and as a result of its phase shifting activity, mediated via its actions on MT₁ and MT₂ receptors [20], melatonin modulates the electrical activity of the SCN. The firing rate of the SCN decreases during the transition from non-REM to REM sleep [21].

CIRCADIAN RHYTHM DISORDERS ARE PRECIPITATED BY INTRINSIC AND EXTRINSIC FACTORS

Among the innumerable periodic changes that underlie and support the overt circadian physiologic rhythms, the peak values occur in a characteristic sequence over the day ("phase map") in human healthy subjects [22]. Such a sequence and spacing reflects the order and temporal relationships of cause-effect in the normal interactions of the various bodily processes and is the very indicative of organism's health [23].

Disruption of amplitude or phase of circadian rhythms can be produced endogenously, like that seen in many psychiatric disorders, blindness, circadian sleep disorders or chronic diseases. On the other hand, phase maps may undergo transitory disruptions when humans are compelled to make a rapid phase adjustment as, for example, after a rapid move to a new geographic longitude or as a consequence of shift work. When sleep is displaced, as is the case with transmeridian travelers and shift workers, the normal phase relationship between the sleep/wake cycle and the endogenous circadian oscillator is perturbed, a situation which can lead to substantial deterioration in sleep quality. In contrast, normal individuals living on a day-oriented schedule show a balanced relationship between homeostatic and circadian processes that serves to promote uninterrupted bouts of approximately 8 hours of sleep and 16 hours of wakefulness per day. Therefore, the temporal alignment between the sleep/wake cycle and the endogenous circadian system is a determining factor in the quality of the subsequent sleep and waking episodes [24,25]. Sleep recording during e.g. ultrashort sleep/wake paradigms or free-running conditions indicated that the peak propensity for REM sleep is observed near the nadir of the core body temperature cycle, at the middle of a normal night. In comparison, the occurrence of slow wave sleep is much more influenced by the duration of prior waking than by circadian phase. However, a cross-influence occurs since REM sleep can also be influenced by sleep deprivation just as slow wave activity shows circadian modulation [24,25].

Treatment of circadian rhythm disorders needs of therapeutic agents with the capacity to shift the phase or increase the amplitude of rhythms. The name "chronobiotics" has been given to these drugs, whose prototype is melatonin [26,27]. Melatonin secretion is an "arm" of the biologic clock in the sense that it responds to signals from the SCN and in that the timing of the melatonin rhythm indicates the status of the clock, both in terms of phase (i.e., internal clock time relative to external clock time) and amplitude. From another point of view, melatonin is also a chemical code of night: the longer the night, the longer the duration of its secretion. In many species, this pattern of secretion serves as a time cue for seasonal rhythms [28]. The combined application of light in the morning and melatonin in the evening augments the amplitude of circadian rhythmicity, thus constituting the basis for an adjuvant chronobiological treatment useful in many acute and chronic diseases Fig. (3).

SLEEP-WAKE AND CIRCADIAN RHYTHM ABNORMALITIES IN ALZHEIMER'S DISEASE (AD) PATIENTS

The sleep-wake disturbances in AD patients become more marked with progression of the disease. Sleep-wake disturbances of elderly AD patients result from changes at different levels, such as reductions in the strength of environmental synchronizers or their perception, a lack of mental and physical activity, age- or disease-induced losses of functionality of the circadian clock. Cross-sectional studies have shown that sleep disturbances are associated with increased memory and cognitive impairment in AD patients [29].

AD patients with disturbed sleep-wake rhythms did not only exhibit reduced amounts of melatonin secreted, but also a higher degree of irregularities in the pattern of the melatonin secretory rhythm, such as variations in phasing of the peak [30]. Therefore, the melatonin rhythm has not only lost signal strength in clock resetting, but also reliability as an internal synchronizing time cue. Loss or damage of neurons in the hypothalamic SCN and other parts of the circadian timing system presumably account for the circadian rhythm abnormalities seen in demented patients [31-33], especially as the number of neurons in the SCN of AD patients is reduced [34]. Clinical findings strongly argue in favor of disruption of the circadian timing system in AD, since numerous overt rhythms are disturbed, including body temperature and concentrations of other hormones such as glucocorticoids [35,36]. Circadian alterations, which are detectable at an advanced stage of AD, also concern phase relationships, such as the phase difference between the rest-activity and core body temperature cycles, the last one being significantly delayed [31].

In facing a weakened circadian system the possibility of improving rhythmicity in AD patients by well-timed light treatment has been entertained [37]. In practical terms, this may be important as AD patients were found to be less exposed to environmental light than their age-matched controls, so that dysfunction of the SCN was aggravated by low strength of the synchronizing signal light. There is evidence that the combined treatment of bright light plus melatonin showed the best effects to attenuate cognitive deterioration and to improve sleep in old patients [38,39]. In other words, the AD patient is gradually deprived of the photic input and even more of the non-photoc, darkness-related internal signal melatonin.

Sundowning, the typical chronobiological phenomenon seen in AD, is observed in conjunction with disturbances of the sleep-wake cycle. Symptoms appeared in the late afternoon or early evening and include reduced ability to maintain attention to external stimuli, disorganized thinking and speech, a variety of motor disturbances including agitation, wandering and repetitious physical behaviors, and perceptual and emotional disturbances [37,40]. Bright light exposure in selected circadian phases markedly alleviated sundowning symptoms, such as wandering, agitation and delirium and improved sleep wave patterns in AD patients [41,42]. Therefore, it was logical to test whether melatonin could be an effective chronobiotic to improve this AD patient's condition.

MELATONIN AS A THERAPEUTIC AGENT IN AD

Melatonin (3 mg p.o. for 21 days) as a sleep-promoting agent was first tried in a small non-homogenous group of elderly patients with primary insomnia associated with dementia [43]. Seven out of 10 dementia patients having sleep disorders treated with melatonin (3 mg p.o. at bed time) showed a significant decrease in sundowning and reduced variability of sleep onset time. In another study, 14 AD patients who exhibited irregular sleep-wake cycles, treated with 6 mg for 4 weeks, showed a significantly reduced percentage of nighttime activity as compared to a placebo group [44]. The efficacy of 3 mg melatonin/day at bedtime in improving the sleep and alleviating sundowning was shown in 11 elderly AD patients [45] and in 24 patients in other studies [46,47].

Long-term administration of melatonin in the dose of 6-9 mg to 14 AD patients with sleep disorders and sundowning agitation for a period of 2-3 years improved sleep quality [48]. Sundowning, diagnosed clinically in all patients examined was no longer detectable in 12 patients. Another study on 45 AD patients with sleep disturbances, in which 6 mg of melatonin was given daily for 4 months, confirmed sleep improvement and suppression of sundowning [49]. Along with this amelioration, which can already be seen as an important improvement regarding both the patient and the caregiver, the evolution of cognitive alterations in AD patients receiving melatonin seemed to be halted as compared to AD patients not receiving melatonin [48,50]. The major findings of those open-label studies were confirmed in a double-blind, placebo-controlled study, with regard to sleep-wake rhythmicity, cognitive and non-cognitive functions [51].

A large multicenter, randomized, placebo-controlled clinical trial was undertaken to test melatonin efficacy in AD patients [52]. Two dose formulations of oral melatonin were applied and 157 subjects with AD and nighttime sleep disturbance were randomly assigned to one of the following treatment groups: (i) placebo, (ii) 2.5 mg slow-release melatonin, (iii) 10 mg melatonin, given daily for 2 months. In this study melatonin facilitated sleep in a certain number of individuals, but collectively the increase in nocturnal total sleep time and decreased wake after sleep onset, as determined by actigraphy were only apparent as trends in the melatonin-treated groups. On subjective measures, however, caregiver ratings of sleep quality showed significant improvement in the 2.5 mg sustained-release melatonin group relative to placebo [52].

Large interindividual differences between patients suffering from a neurodegenerative disease are not uncommon and can explain the erratic results seen with melatonin in fully developed AD. It should be also taken into account that melatonin, though having some sedating and sleep latency-reducing properties, does not primarily act as a sleeping pill, but mainly as a chronobiotic. Since the circadian oscillator system is obviously affected in AD patients showing severe sleep disturbances, the efficacy of melatonin should be expected to depend on disease progression. Indeed, melatonin failed to improve sleep or agitation in two double-blind randomized placebo-controlled trials in institutionalized patients with AD [53,54]. Thus a major question remains concerning melatonin's efficacy in advanced AD patients.

Overall, published data concerning melatonin treatment of AD patients were analyzed [55]. Eight reports (5 open-label studies, 2 case reports) (N= 89 patients) supported a possible efficacy of melatonin: sleep quality improved and in patients with AD sundowning was reduced and cognitive decay slowed progression. In 6 double blind, randomized placebo-controlled trials, a total number of 210 AD patients were examined. Sleep was objectively measured by wrist actigraphy (N= 5) and additionally neuropsychological assessment and sleep quality were subjectively evaluated (N= 6). Sleep quality increased and sundowning decreased significantly and cognitive performance improved in 4 studies (N= 143) whereas there was absence of effects in 2 studies (N= 67) [55].

Therefore, the question whether melatonin has a causal value in preventing or treating AD, affecting disease progression of the neuropathology and the driving mechanisms, remains unanswered. Double-blind multicenter studies are needed to further explore and investigate the potential and usefulness of melatonin as an antidementia drug. Its apparent usefulness in symptomatic treatment, concerning sleep, sundowning, etc., even in a progressed state, further underlines the need for such decisive studies.

MELATONIN AS A THERAPEUTIC AGENT IN MILD COGNITIVE IMPAIRMENT

As outlined, melatonin acts at different levels relevant to the development and manifestation of AD. The antioxidant, mitochondrial and antiamyloidogenic effects may be seen as a possibility of interfering with the onset of the disease. Therefore, the beginning of treatment is decisive [56]. Indeed, one cannot expect a profound inhibition of disease progression once a patient is already in an advanced demented state.

Mild cognitive impairment (MCI) is an etiologically heterogeneous syndrome characterized by cognitive impairment shown by objective measures adjusted for age and education in advance of dementia. Approximately 12% of MCI convert to AD or other dementia disorders every year. Since MCI may represent prodromal AD it should be adequately diagnosed and treated [57]. Indeed, the degenerative process in AD brain starts 20–30 years before the clinical onset of the disease. During this phase, plaques and tangles loads increase and at a certain threshold the first symptom appears. As already mentioned, CSF melatonin levels decrease even in preclinical stages when the patients do not manifest any cognitive impairment (at Braak stages I-II), suggesting that the reduction in CSF melatonin may be an early trigger and marker for AD. Therefore, MCI is the right moment for initiating any melatonin treatment aiming to affect progression of the disease.

The first report on melatonin treatment of 10 MCI patients (6 mg/day for 10 days) indicated that besides enhancing the rest-activity rhythm and improved sleep quality the ability to remember previously learned items improved along with a significant reduction in depressed mood [58]. In another double-blind, placebo-controlled pilot study performed in 26 individuals with age-related MCI, the administration of 1 mg melatonin or placebo at bed time for 4 weeks resulted in improvement of sleep and of scores on the California Verbal Learning Test-interference subtest [59].

In Argentina melatonin was introduced in 1995 as a registered medicament for the treatment of sleep disorders in the elderly, particularly in those whose endogenous melatonin levels are low. Hence, melatonin is often added to the regular treatment of old patients who complain of sleep disorders and memory disturbances in our environment. This gave us the opportunity to carry out a retrospective study of a group of 25 MCI patients who received melatonin (3 – 9 mg per day) for 9 to 18 months in comparison to a similar group of 25 MCI patients who did not receive it [60]. Patients treated with melatonin showed significantly better performance in Mini-Mental State Examination (MMSE) and the cognitive subscale of the Alzheimer's Disease Assessment Scale (ADAS-Cog). After application of a neuropsychological battery comprising a Mattis' test, Digit-symbol test, Trail A and B tasks and the Rey's verbal test, better performance was found in melatonin-treated patients, except for the Digit-symbol test which remained unchanged. Abnormally high Beck Depression Inventory scores decreased in melatonin treated patients, concomitantly with an improvement in wakefulness and sleep quality. The results suggested that melatonin could be a useful add-on drug for treating MCI in a clinic environment [60]. A follow up of that study comprising the retrospective analysis a group of 35 MCI patients who received melatonin for 9 to 24 months in comparison to 25 MCI patients who did not receive is in press [55]. Overall, the results confirm the observations supporting a role of melatonin as a useful add-on drug for treating MCI in a clinic environment.

A randomized controlled trial on the effect of bright light and melatonin on cognitive and noncognitive function in elderly residents of group care facilities was recently published [38]. The authors concluded that light has a benefit in improving some cognitive and noncognitive symptoms of MCI which was amplified by the conjoint administration of melatonin. Melatonin alone had an adverse effect on mood. In other two similar studies, one of them using the prolonged release preparation of melatonin (Circadin™) recently approved by the European Medicines Agency, melatonin resulted in significant and clinically meaningful improvements in sleep quality, morning alertness, sleep onset latency and quality of life in old patients with mild cognitive impairment [61,62]. In these studies melatonin treatment improved mood.

Table 1 shows a summary of published data concerning melatonin treatment of MCI patients. Five double blind, randomized placebo-controlled trials and 1 open-label retrospective study (N= 651) all agree in indicating that treatment with daily evening melatonin improves sleep quality and cognitive performance in MCI [55].

CURRENT & FUTURE DEVELOPMENTS

Normal circadian rhythms are synchronized to a regular 24 h environmental LD cycle and both the SCN and melatonin play a role for this adaptation. Desynchronization of circadian rhythms as occurs in dementia result in severe disturbances of sleep and in the phase position of plasma melatonin levels. Although the question whether melatonin disruption is a cause or a consequence of the disorder remains open, melatonin has being tried as a treatment for sleep-wake cycle disruption in AD. The effects of melatonin are more consistent in the

early stages of AD (MCI) rather than in fully develop AD-like dementia. Until recently, melatonin had not been licensed in Europe or USA for any indication. In 2007 a prolonged-release preparation of melatonin (Circadin™) was licensed as a treatment for sleep disorders in old people.

AD is an age-associated neurodegenerative disease reportedly exhibiting an increase in oxidative damage. Many mechanisms have been proposed as predisposing for excessive oxidative damage in AD, including the genetic background (e.g., expression levels and subforms of presenilins and apolipoprotein E), inflammatory processes associated with cytokine release, or neurotoxicity of metal ions [63,64]. The deposition of β -amyloid ($A\beta$) plaques is thought to destabilize neurons by mechanisms which require further clarification. Tangles are associated with hyperphosphorylation of tau, a microtubule-associated protein, and of neurofilament H/M subunits, processes that lead to misfolding and accumulation of altered proteins, along with a disruption of microtubules. In an animal model of Alzheimer-like hyperphosphorylation of tau that caused spatial memory retention impairment, the intraperitoneal administration of melatonin for 9 days prevented synaptophysin loss, memory retention deficits and hyperphosphorylation of tau and neurofilaments [65].

It must be noted, however, that the simplistic concept that reduces AD lesions to oxidative damage has been shown insufficient to explain the disease. Classical radical scavengers like vitamins E and C have been used for the treatment of AD patients with only limited success. Although some studies demonstrated a reduction in lipid peroxidation [66], epidemiological data showed only minor or no clear-cut clinical effects of classical antioxidants [67-72]. Moreover, these compounds remained relatively inefficient in preventing $A\beta$ toxicity and fibrillogenesis.

In this regard, melatonin and other structurally related indolic compounds, such as indole-3-propionic acid, proved to be more potent than classical antioxidants [73-76]. The antifibrillogenic effects of melatonin and its metabolites were observed not only *in vitro* but also *in vivo* in transgenic mouse models [77-79]. Protection from $A\beta$ toxicity was observed, especially at the mitochondrial level. In addition to its chronobiological properties, melatonin is a potent direct and indirect antioxidant, given rise to metabolites more potent than the native molecule, such as *N*¹-acetyl-*N*²-formyl-5-methoxykynuramine and *N*¹-acetyl-5-methoxykynuramine [80]. The antioxidant properties of melatonin are not shared by the melatonin agonists already in the market, like ramelteon [81].

Melatonin treatment promotes mainly non-REM sleep in the elderly [82] and may be beneficial in MCI and AD by supporting restorative phases of sleep. Regardless of the mechanistic details, all pertinent data unanimously direct to a sleep-promoting effect of melatonin in AD and MCI patients, as generally in elderly insomniacs.

It is clear that in order to postulate melatonin as a useful ad-on therapeutic tool in MCI, larger double-blind multicenter studies are needed. Its apparent usefulness in symptomatic treatment, concerning sleep, sundowning or cognitive impairment, even in a progressed state, further underlines the need for such decisive studies. To what extent the new melatonergic agents approved by the U.S. Food and Drug Administration or the European Medicines Agency (ramelteon,

agomelatine) [83] share the protective activity of melatonin in mild cognitive impairment remains to be defined.

PATENT SELECTION

The identification of the effect of melatonin on circadian rhythmicity was followed by a number of initial patents claiming inventions on the use of melatonin as a chronobiotic that are not longer legally active e.g. [84-87]. It must be noted that melatonin, as a natural product, cannot be patented itself but through its different uses.

The following recent patents are considered by the authors to be relevant on the topic of melatonin as a chronobiotic agent, used alone or in combination with other therapies.

[88]: This invention discloses a pharmaceutical composition containing oxytocin and melatonin in amounts therapeutically effective for inducing labor in a pregnant human patient.

[89]: A method for achieving a chronobiologic effect on a circadian rhythm in a human infant or fetus in utero is described. The method involves the administration of melatonin or dose-equivalent melatonin analogue or melatonin receptor agonist.

[90]: This invention refers to the use of melatonin and soy isoflavones for treating the sleep/wake cycle disorders in perimenopausal women.

[91]: A method for improving sleep in an individual comprising the administration of a composition comprising melatonin, lavender flower extract and Ferula extract is provided.

[92]: The present invention relates to low-dose formulations of melatonin, and methods of use thereof, which provide a sustained release of melatonin so as to rapidly increase plasma levels of melatonin and maintain a relatively high level.

[93]: A multi-layered solid dosage form for oral administration for a multi-phasic controlled release of melatonin is described.

[94]: The present invention provides a pharmaceutical composition for sublingual or oral administration of actives with low to poor aqueous solubility like melatonin.

[95]: The invention relates to a device for melatonin treatment involving an adapting means which adapts the daily dose upon measurement of a body parameter of the patient or the user of the device.

[96]: The present invention deals with a sustained release preparation of melatonin (now approved by EMEA for its use in humans).

[97]: A method for treating circadian rhythm disorders is described. The method involves the administration of melatonin, melatonin agonists or compounds that stimulate endogenous melatonin production.

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CONFLICT OF INTEREST STATEMENT AND DISCLOSURE STATEMENT

The authors declare that they have no proprietary, financial, professional, nor any other personal interest of any kind in any product or services and/or company that could be construed or considered to be a potential conflict of interest that might have influenced the views expressed in this manuscript.

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Table 1. Clinical studies on melatonin efficacy in MCI

Design	Subjects (M, F)	Treatment	Study's duration	Measured	Results	Reference
Double-blind, placebo-controlled, crossover study	10 (4, 6) patients with mild cognitive impairment (MCI)	6 mg melatonin p.o. daily at bed time	10 days	Actigraphy. Neuropsychological assessment.	Enhanced the rest-activity rhythm and improved sleep quality (reduced sleep onset latency and in the number of transitions from sleep to wakefulness. Total sleep time unaffected. The ability to remember previously learned items improved along with a significant reduction in depressed mood.	[58]
Double-blind, placebo-controlled pilot study	26 individuals with age-related MCI	1 mg melatonin p.o. or placebo at bed time	4 weeks	Sleep questionnaire and a battery of cognitive tests at baseline and at 4 weeks	Melatonin administration improved reported morning "restedness" and sleep latency after nocturnal awakening, and also improved scores on the California Verbal Learning Test-interference subtest.	[59]
Open-label, retrospective study	50 (13, 37) MCI outpatients	25 had received daily 3-9 mg of a fast-release melatonin preparation p.o. at bedtime. Melatonin was given in addition to the standard medication	9-18 months	Daily logs of sleep and wake quality. Initial and final neuropsychological assessment.	Patients treated with melatonin showed significantly better performance in neuropsychological assessment. Abnormally high Beck Depression Inventory scores decreased in melatonin-treated patients, concomitantly with an improvement in wakefulness and sleep quality.	[60]
Randomized,	354	prolonged	3 weeks	Leeds Sleep	Melatonin resulted in significant and	[61]

Design	Subjects (M, F)	Treatment	Study's duration	Measured	Results	Reference
double blind, placebo-controlled study	individuals with age-related cognitive decay	release melatonin (Circadin, 2 mg) or placebo, 2 h before bedtime		Evaluation and Pittsburgh Sleep Questionnaires, Clinical Global Improvement scale score and quality of life.	clinically meaningful improvements in sleep quality, morning alertness, sleep onset latency and quality of life	
Long-term, double-blind, placebo-controlled, 2 x 2 factorial randomized study	189 (19, 170) individuals with age-related cognitive decay	Long-term daily treatment with whole-day bright (1000 lux) or dim (300 lux) light. Evening melatonin (2.5 mg) or placebo administration	1 to 3.5 years	Standardized scales for cognitive and noncognitive symptoms, limitations of activities of daily living, and adverse effects assessed every 6 months.	Light attenuated cognitive deterioration and also ameliorated depressive symptoms. Melatonin shortened sleep onset latency and increased sleep duration but adversely affected scores for depression. The combined treatment of bright light plus melatonin showed the best effects.	[38]
Prospective, randomized, double-blind, placebo-controlled, study	22 (15, 7) individuals with age-related cognitive decay	Participants received 2 months of melatonin (5 mg p.o./ day) and 2 months of placebo	2 months	Sleep disorders were evaluated with the Northside Hospital Sleep Medicine Institute (NHSMI) test. Behavioral disorders were evaluated with the Yesavage Geriatric Depression Scale	Melatonin treatment significantly improved sleep quality scores. Depression also improved significantly after melatonin administration.	[62]

Design	Subjects (M, F)	Treatment	Study's duration	Measured	Results	Reference
				and Goldberg Anxiety Scale.		

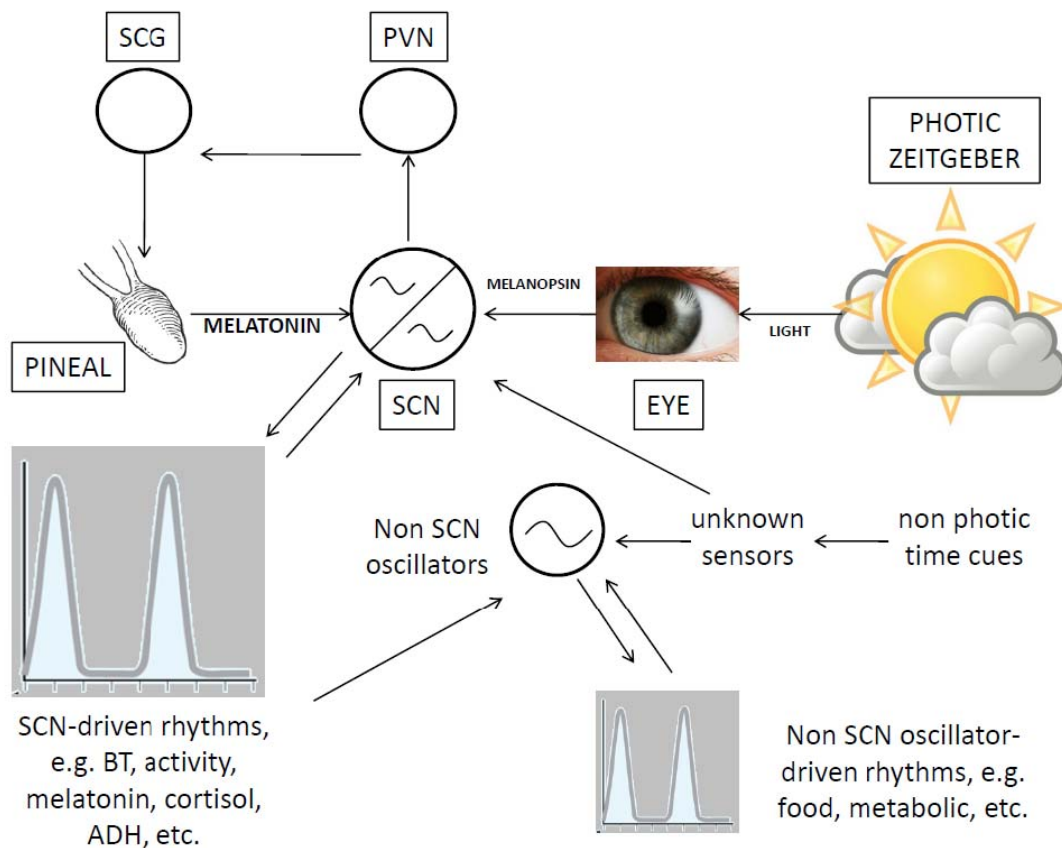


Figure 1. Circadian regulation. Light impinging on the eye send neural signals to a population of receptive neurons in the SCN. The SCN in turn acting via a complex indirect pathway sends a circadian signal to the pineal gland regulating synthesis of melatonin as well as to other brain sites to regulate brain oscillators. Melatonin feeds back on the SCN as well as on numerous other brain sites that contain melatonin receptors. Besides the SCN, other circadian oscillators entrained by nonphotic environmental time cues also drive a number of circadian rhythms. The rhythms, in turn, can feedback on the oscillators. PVN: paraventricular nucleus; BT: body temperature.

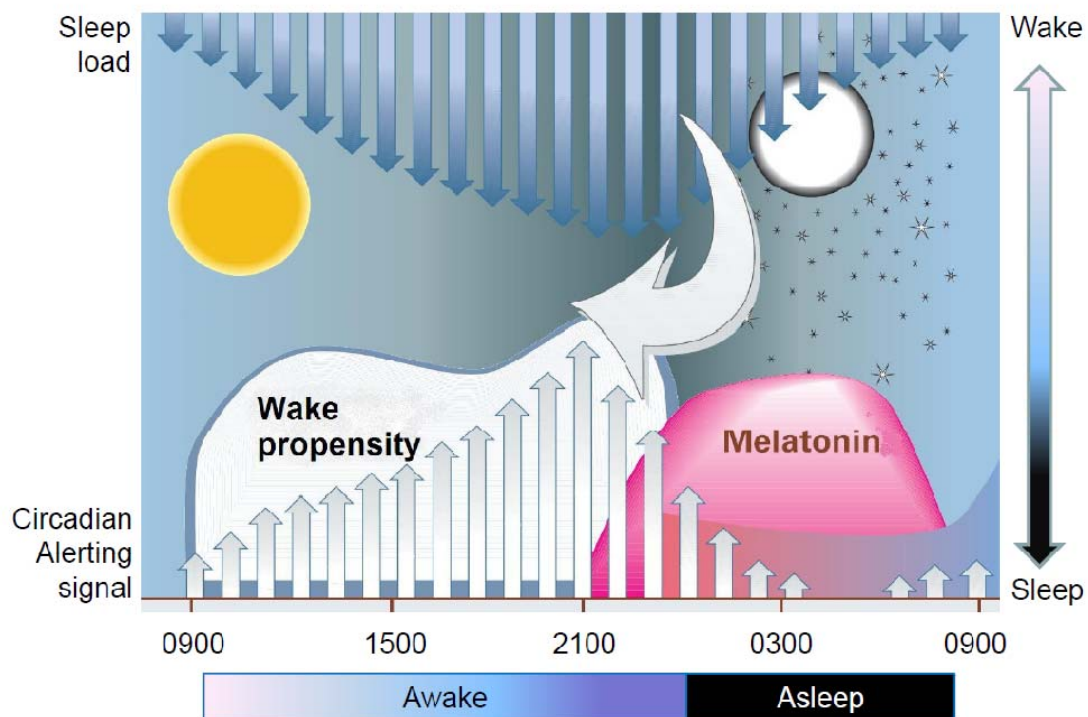


Figure 2. The primary physiological function of melatonin is to convey information concerning the daily cycle of light and darkness to body physiology. Melatonin is thought to affect the physiology of the circadian pacemaker of the SCN in two ways, facilitating the synchronization of circadian rhythms and attenuating the SCN-generated alerting signals. Thus melatonin production allows the homeostatic sleep load to exert its influence unopposed by the circadian alerting signal (arrow). As a result, wake propensity diminishes, and sleep ensues. As the night progresses, melatonin levels drop. As morning approaches, the alerting signal increases, promoting wakefulness.

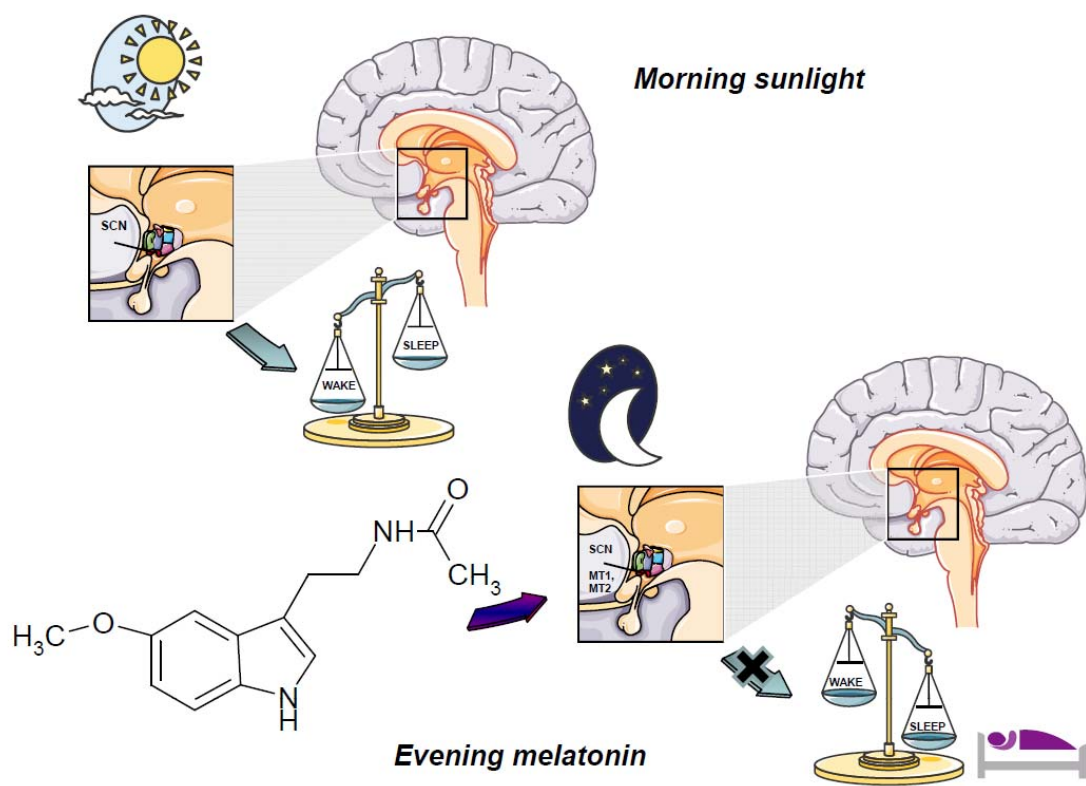


Figure 3. The basis of a chronobiological treatment. The simple measure of increasing the illumination level in the morning and administering melatonin at night ameliorates symptoms of disturbed cognition, mood, behavior, functional abilities, and sleep in old people [38].