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Tel.-Fax: (54) (0)11 43781151
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MELATONIN AS A PROTOTYPE OF CHRONOBIOTIC-CYTOPROTECTIVE DRUGS.

Daniel P. Cardinali
Department of Physiology, Faculty of Medicine, University of Buenos Aires, Buenos Aires, Argentina.
(dcardinali@fmed.uba.ar)
(www.daniel-cardinali.medem.com)

The circadian clock is one of the most indispensable biological functions

During the past decade, enormous progress has been made in elucidating the molecular components of the biological clock. The molecular mechanisms that underlie the function of the clock are universally present in all cells and consist of gene-protein-gene feedback loops in which proteins can down regulate their own transcription and stimulate the transcription of other clock proteins. Although anchored genetically, circadian rhythms are synchronized by (entrained) and maintain certain phase relationships to exogenous factors, especially the sleep portion of the light-dark (L/D) schedule. These rhythms will persist with a period different from 24 h when external time cues are suppressed or removed, such as during complete social isolation or in constant light or darkness [1].

Only a few environmental cues, like L/D cycles, are effective entraining agents ("Zeitgebers") for the circadian oscillator. An entraining agent can actually reset, or phase shift, the internal clock. Depending on when an organism is exposed to such an entraining agent, circadian rhythms can be advanced, delayed, or not shifted at all. Therefore, involved in adjusting the daily activity pattern to the appropriate time of day is a rhythmic variation in the influence of the Zeitgeber as a resetting factor.

In mammals, a hierarchically major circadian oscillator is located in the suprachiasmatic nuclei (SCN) of the hypothalamus **(Fig. 1)**. Lesions of the SCN eliminate circadian-driven rhythms. Inversely, SCN transplants to animals whose own SCN had been ablated, can restore circadian rhythms. Every single SCN cell exerts a waxing and waning of the firing rate with a predictable circadian rhythm. Synchronized by paracrine signals the SCN produces an output signal that "drives" endogenously generated daily oscillations in hormones, sleep – wakefulness, alertness, performance, and many other physiological functions. The sinusoidal output signal produced by the SCN can be 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 [1].

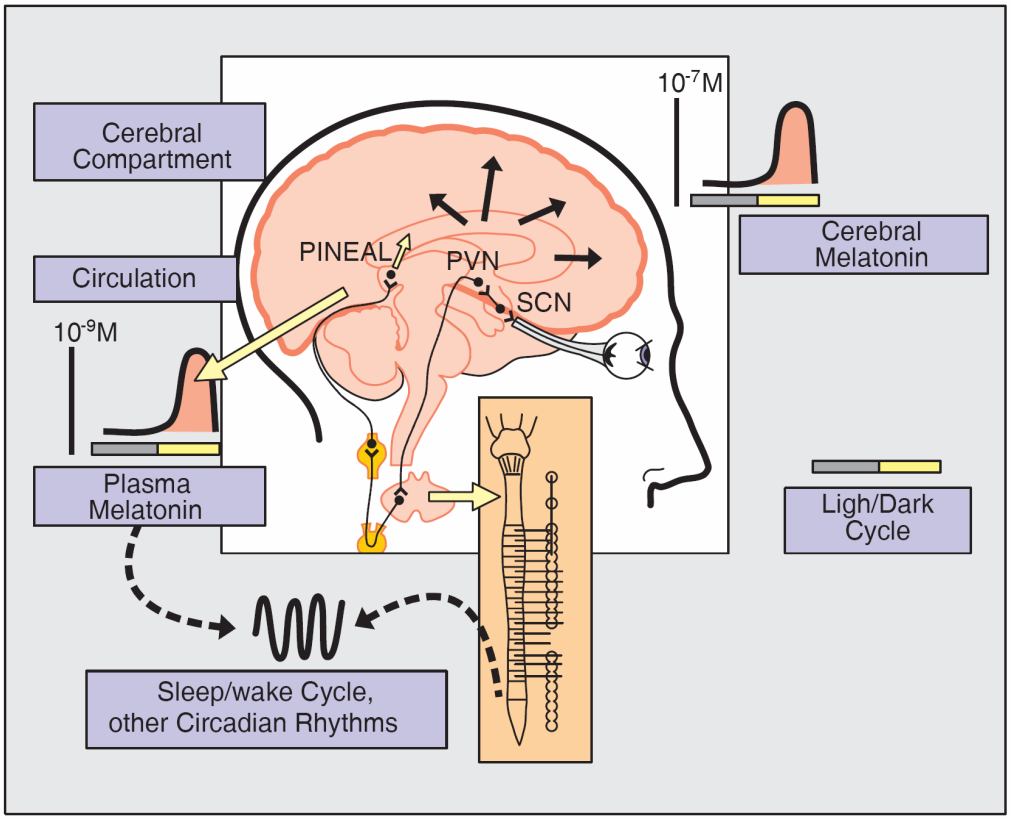

Figure 1: *Possible mechanism of action of melatonin in the transmission of photoperiodic information. Photic information is conveyed to the suprachiasmatic nuclei (SCN), principally through the retino-hypothalamic tract, where it synchronizes the activity of the circadian oscillator to exactly 24 h. Neuronal efferent pathways from the SCN directly distribute circadian information to different brain areas, including the paraventricular nucleus (PVN) abd the pineal gland, that generates the melatonin rhythm. The main neural route for environmental lighting control of melatonin secretion includes the intermediolateral column of the thoracic chord gray and the superior cervical ganglion (SCG). The generated melatonin rhythm is used by the SCN to distribute its rhythmic information.*

Photic entrainment of the SCN pacemaker is mediated by a specialized subset of intrinsically photosensitive ganglion cells that are spread throughout the retina rather than having a foveal concentration. Those specialized ganglion cells also receive input from rods and cones, acting as a redundant input pathway for synchronizing the circadian system, but can still function even if the rods and cones are so severely damaged that the individual is blind. Based on denervation or nerve stimulation studies, a simple model of pineal regulation was envisioned, comprising two premises: (i) the neural route for environmental lighting control of melatonin secretion is the neuronal circuit “retina - retinohypothalamic tract - suprachiasmatic nuclei (SCN) - periventricular hypothalamus - intermediolateral column of the thoracic chord gray - superior cervical ganglion - internal carotid nerves - pineal gland”; (ii) norepinephrine released from sympathetic terminals at night activates postsynaptic β-adrenoceptors coupled to the adenylate cyclase-cAMP system, therefore increasing melatonin synthesis and release. However, the presence of functional α-adrenoceptors as well the characterization of central peptidergic pinealopetal pathways point out to a complexity of mechanisms regulating melatonin biosynthesis **(Fig. 2)**.

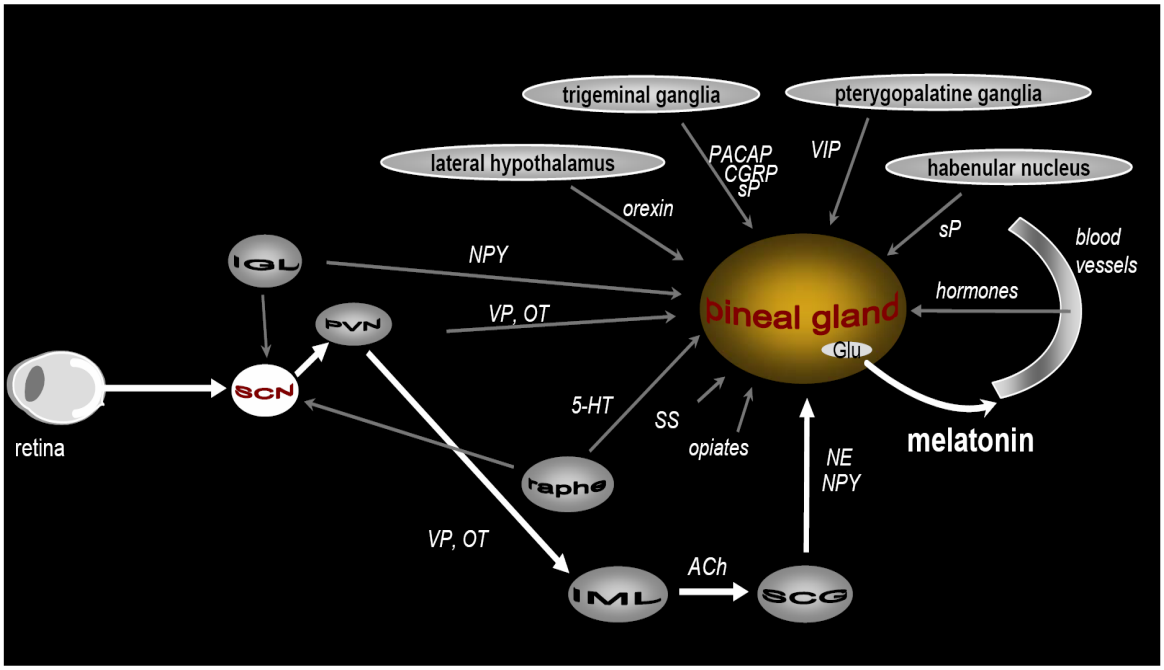

Figure 2: *Schematic representation of the various neural and endocrine inputs of the mammalian pineal gland. The main neural pathway, which transmits light information to the pineal gland **(see Fig. 1)**, is shown with thick arrows. In addition, numerous other neural or endocrine inputs are known to reach the pineal gland. 5-HT: serotonin; ACh: acetylcholine; CGRP: calcitonin gene-related peptide; Glu: glutamate; IGL: intergeniculate leaflet of the geniculate body; IML: intermediolateral column of the thoracic chord gray; NE: norepinephrine; NPY: neuropeptide Y; PACAP: pituitary adenylate cyclase activating peptide; PVN: paraventricular nuclei of the hypothalamus; SCG: superior cervical ganglia; SCN: suprachiasmatic nuclei; SS: somatostatin; sP: substance P; VIP: vasoactive intestinal peptide; VP: vasopressin.*

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[1]. 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 quintessence of organism's health. 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. Under such circumstances the various individual 24-h components comprising the circadian phase map do not reset their phases to the new environmental times at the same rate, and become somewhat displaced in their relations to one another. To reset them to the new local time requires several days of exposure to the local phase setters.

Two interacting processes regulate the timing, duration and depth, or intensity, of sleep: a homeostatic process that maintains the duration and intensity of sleep within certain boundaries and a circadian rhythm that determines the timing of sleep [2] **(Fig. 3)**. The homeostatic process depends on immediate history: the interval since the previous

sleep episode and the intensity of sleep in that episode. This drive to enter sleep increases, possibly exponentially, with the time elapsed since previous sleep episode. It declines exponentially once sleep has been initiated. The homeostatic sleep drive controls slow wave sleep rather than rapid eye movement (REM) sleep. In contrast, the phase and amplitude of the circadian rhythm are independent of the history of previous sleep but are generated by the major pacemaker, the SCN. The circadian variation of human sleep propensity is roughly the inverse of the core body temperature rhythm: maximum propensity for sleep and the highest continuity of sleep occur in proximity to the minimum temperature [2].

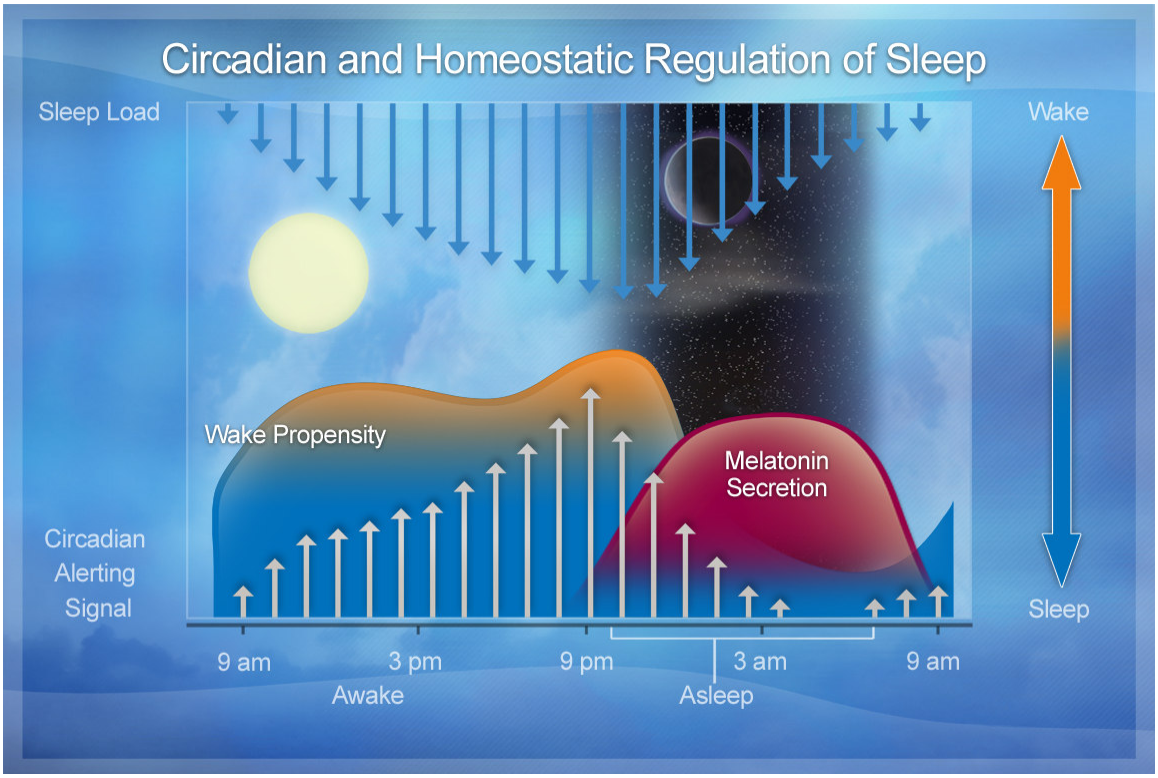

Figure 3: *Melatonin is thought to affect the physiology of the SCN in two ways, facilitating the synchronization of circadian rhythms and attenuating the SCN-generated alerting signals. In this capacity, night-time melatonin production allows the homeostatic sleep load to exert its influence unopposed by the circadian alerting signal. As a result, wake propensity diminishes, and sleep ensues. Nocturnal onset of melatonin secretion correlates well with the opening of the "sleep gate." As the night progresses, melatonin levels drop. As morning approaches, the alerting signal increases, promoting wakefulness.*

Drugs that influence the circadian apparatus are named "chronobiotics"

The prototype of this type of drugs is melatonin. Melatonin is produced in most organisms from algae to mammals, and its role varies considerably across the phylogenetic spectra [3]. In humans it plays a major function in the coordination of circadian rhythmicity, remarkable the sleep/wake cycle [4]. 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 [4].

Like the effects induced by the external Zeitgeber light, effects by the internal Zeitgeber melatonin are also time-dependent. The greatest density of high-affinity melatonin receptors in humans is located in the SCN. Entraining free-running circadian rhythms by administering melatonin is only possible if the SCN is intact. Daily timed administration of melatonin to rats shifts the phase of the circadian clock, and this phase shifting may partly explain melatonin effect on sleep in humans, or "chronobiotic effect" [5,6]. Indirect support for such a physiological role derived from clinical studies on blind subjects showing free running of their circadian rhythms while a more direct support for this hypothesis was provided by the demonstration that the phase response curve for melatonin was opposite (i.e. 180 degrees out of phase) to that of light

Within the SCN, melatonin reduces neuronal activity in a time-dependent manner. In rodents, the effects of melatonin on SCN activity are mediated by at least two different receptors. They are insensitive during the day, but sensitive at dusk and dawn (MT2; causes phase shifts) and during early night period (MT1; decreases neuronal firing rate) [7]. Melatonin secreted during nighttime provides enough inertia to resist minor perturbations of the circadian timing system.

The evening increase in melatonin secretion is associated with an increase in the propensity for sleep [8] **(Fig. 3)**. Secretion of melatonin during the day, as occurs in diverse pathologic or occupational health situations, is strongly associated with daytime sleepiness or napping, and the administration of melatonin during the day induces sleepiness. Melatonin (in a dose of 3-5 mg daily, timed to advance the phase of the internal clock) can maintain synchronization of the circadian rhythm to a 24-hour cycle in sighted persons who are living in conditions likely to induce a free-running rhythm, and it synchronizes the rhythm in some persons after a short period of free-running. In blind subjects with free-running rhythms, it has been possible to stabilize, or entrain, the sleep/wake cycle to a 24-hour period by giving melatonin, with resulting improvements in sleep and mood [5]. In normal aged subjects [9] and in demented patients with desynchronization of sleep/wake cycle [10] melatonin administration is helpful to reduce the variation of onset time of sleep. The phase shifting effects of melatonin were also sufficient to explain its effectiveness as a treatment for circadian-related sleep disorders such as jet lag or the delayed phase sleep syndrome [6,11].

Melatonin vs. melatonin receptor agonists in the treatment of sleep disorders

In a recent review paper on the rationale for development of specific melatonin agonists, Turek and Gillette proposed that an agent with a longer half-life may have a better opportunity to activate brain melatonin receptors for influencing sleep properties than melatonin itself [12]. Slow release melatonin has been reported to improve whole night sleep and was more effective than placebo in improving the sleep of patients. On subjective measures, caregiver ratings of sleep quality showed significant improvement in the 2.5-mg sustained-release melatonin group relative to placebo in Alzheimer´s disease (AD) patients [13].

Agomelatine and ramelteon are two of the melatonin agonists that have been found useful in the treatment of sleep problems mostly by shortening sleep onset latency. Agomelatine is a high affinity MT_1 agonist and has a similar action as that of melatonin in influencing electrical activity of SCN neurons. Agomelatine has the capacity to re-entrain circadian rhythms in response to phase-shift in L/D cycle. This melatonin agonist is in clinical development for use in anxiety disorders associated with depression due to its additional activity on 5-HT1c receptors. Ramelteon (Rozerem™) has a longer half life than melatonin, has high affinity for MT1 and MT2 receptors [14]. In July 2005 the U.S. Food and Drug Administration (FDA) has approved Rozerem™ as 8-mg tablets for the treatment of insomnia characterized by difficulty with sleep onset. Ramelteon is thus the first and only prescription sleep medication that has shown no evidence of abuse and dependence and, as a result, has not been designated as a controlled substance by the U.S. Drug Enforcement Administration (DEA). The FDA approval allows physicians to prescribe the melatonin agonist for long-term use in adults based on the lack of abuse and dependence potential.

Melatonin has chronobiotic and neuroprotective properties in AD patients

AD is an age-associated neurodegenerative disease with severe disruption of the sleep/wake rhythm and an irregular and decreased melatonin secretion. The circadian melatonin rhythm disappeared in both pre-symptomatic and diagnosed AD patients [15]. The melatonin decrease was specifically profound in AD patients carrying the apolipoprotein E-epsilon 4/4 genotype, which is a predictor of early onset AD. Furthermore, a reduced hippocampal MT2 melatonin receptor expression has been reported in AD patients. The decrease in melatonin production seen during preclinical AD stages (Braak stages I-II) has been linked to dysfunctional regulation of pineal gland by SCN. These changes in melatonin secretion contribute to the frequent initial symptoms in “AD-like” patients, including sleep disruption and nightly restlessness [16].

In view of the altered melatonin secretion and disrupted circadian rhythmicity found in AD it was logical to treat AD patients with melatonin. The first report in the international literature was made by us in 1997 derived from studies started at the time of melatonin’s approval as a medicament in Argentina [17]. Since then the efficacy of melatonin for treatment of AD patients has been demonstrated by several studies [13,16,18-22]. A significant observation in same of these studies was the halted evolution of the cognitive and amnesic alterations expected in comparable populations of patients not receiving melatonin [20,21,23]. Indeed melatonin has very strong cytoprotective properties in a number of neurodegenerative disorders via its antioxidant and antiapoptotic effects [24,25]. In addition, melatonin effect was not only based on antioxidant properties, but also on interference with the phosphorylation system, especially stress kinases.

Animal studies have been very indicative of the ability of melatonin to prevent β-amyloid toxicity. For example, melatonin had the ability to protect against the changes in circadian rhythmicity induced by microinjection of β amyloid peptide 25 – 35 into the SCN of golden hamsters [26]. Melatonin prevents the death of neuroblastoma cells exposed to β amyloid polypeptide [25]. Animal models of AD have also been used to study the possible antioxidant and antiapoptotic actions of melatonin in arresting the neuronal lesions. Melatonin inhibited Aβ deposition in APP 695 transgenic mice [27]. In the APP695 transgenic mouse, senile plaques appear in the cortex as early as at 8 months of age.

These mice display behavioral impairments and memory deficits. Evaluation of long term administration of melatonin revealed that melatonin (10 mg/kg) alleviated learning and memory deficits. It also reduced the number of apoptotic neurons [27]. In another model of transgenic mice, the senile plaques consisting of deposited Aβ are capable to induce the secretion of interleukins IL-6 and IL-1β [28]. In this study, melatonin attenuated the Aβ-induced secretion of IL-1β and IL-6, again a beneficial antiinflammatory effect related to antioxidative protection.

Concluding remarks

Melatonin provides an innovative neuroprotective strategy by combining its effects on sleep with its cytoprotective properties **(Fig. 4)**. Melatonin protects against several mechanisms of neuronal death, including oxyradical-mediated damage and apoptosis implicated in the pathogenesis of a number of neurodegenerative diseases like AD, Parkinsonism, Huntington's chorea and neurological conditions like stroke, epilepsy and brain trauma.

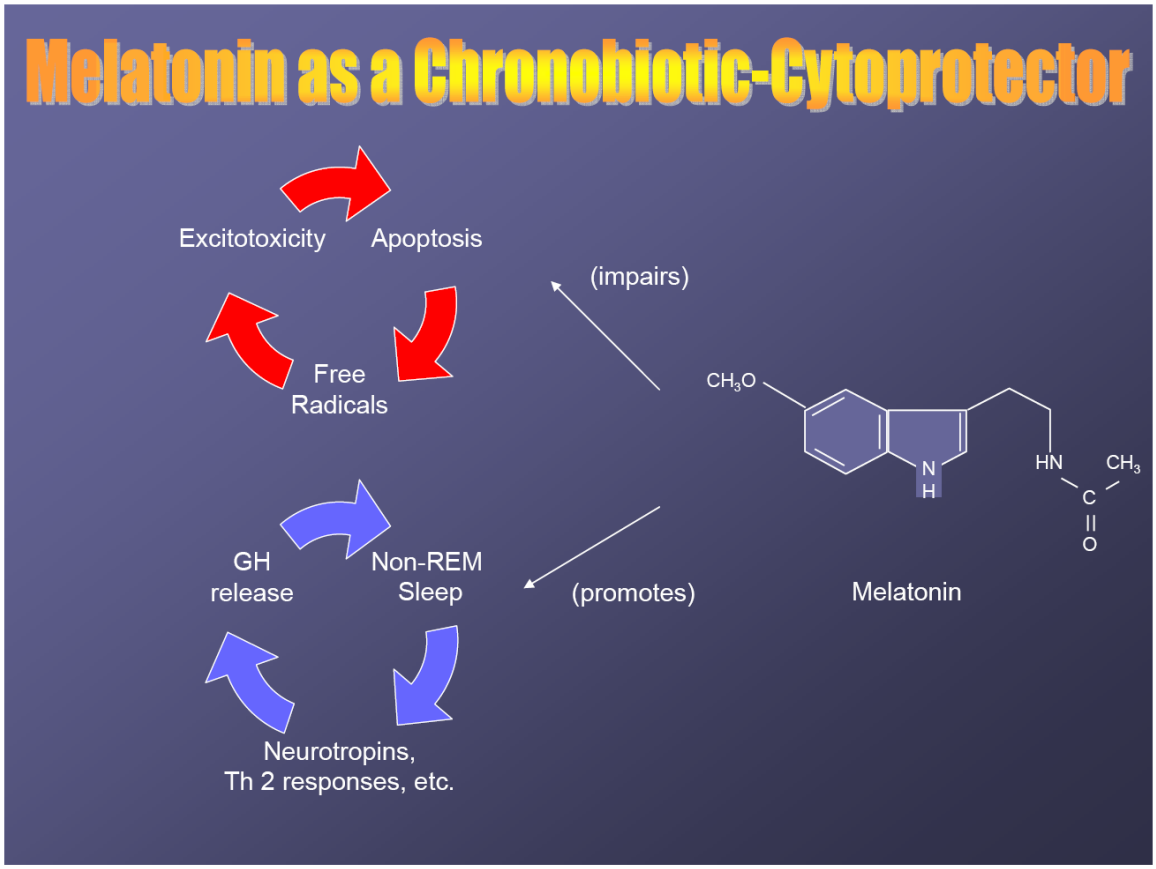

Figure 4: *Melatonin gives neuroprotection against three mechanisms of neuronal death: free radical-mediated damage, apoptosis and glutamate excitotoxicity. In addition, and through restoration of non REM sleep, melatonin presumably results in neurotropin synthesis and GH release.*

Melatonin has also a strong cytoprotective activity in a number of situations including osteoporosis [29], ischemia-reperfusion and diabetic microangiopathy [30]. Through restoration of slow wave sleep [8] melatonin treatment can result in better regulation of neuronal metabolism. Indeed a better sleep must be considered as a neuroprotective strategy that can potentially improve the course and outcome of several brain disorders, and thus the quality of life of the affected individuals and their family members.

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