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Melatonin: a universal time messenger

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Abstract

Temporal organization plays a key role in humans, and presumably all species on Earth. A core building block of the chronobiological architecture is the master clock, located in the suprachi asmatic nuclei [SCN], which organizes "when" things happen in sub-cellular biochemistry, cells, organs and organisms, including humans. Conceptually, time messenging should follow a 5 step-cascade. While abundant evidence suggests how steps 1 through 4 work, step 5 of "how is central time information transmitted througout the body?" awaits elucidation. Step 1: Light provides information on environmental (external) time; Step 2: Ocular interfaces between light and biological (internal) time are intrinsically photosensitive retinal ganglion cells [ipRGS] and rods and cones; Step 3: Via the retinohypothalamic tract external time information reaches the light-dependent master clock in the brain, viz the SCN; Step 4: The SCN translate environmental time information into biological time and distribute this information to numerous brain structures via a melanopsin-based network. Step 5: Melatonin, we propose, transmits, or is a messenger of, internal time information to all parts of the body to allow temporal organization which is orchestrated by the SCN. Key reasons why we expect melatonin to have such role include: First, melatonin, as the chemical expression of darkness, is centrally involved in time- and timing-related processes such as encoding clock and calendar information in the brain; Second, melatonin travels throughout the body without limits and is thus a ubiquitous molecule. The chemial conservation of melatonin in all tested species could make this molecule a candidate for a universal time messenger, possibly constituting a legacy of an all-embracing evolutionary history.

COMMENTARY

INTRODUCTION

In 2008 (Erren & Reiter 2008), we suggested that melatonin could serve as a messenger of time, thus explaining "its critical role for the timing and sequencing of biological rhythms" (Erren *et al.* 2003). In this paper, we propose that melatonin is not only a mediator between environmental and biological times at daily and seasonal scales but also a candidate for a universal time messen-

ger *throughout* the body. If our conceptual extension is valid, our proposal could contribute to answering the fundamental question "how do the suprachiasmatic nuclei [SCN] notify the immense variety of bodily systems of its rhythmic instructions?"

Pittendrigh (1960), as one of the nestors of modern chronobiology, emphasized with reference to a conversation with the computer revolutionary von Neumann that "temporal organization" is information-dependent (Pittendrigh 1993). While the question where and how environmental light information is coded within the master clock appears elucidated, how temporal information is distributed from the SCN throughout the body remains unresolved. We propose that the final link in an assumed 5-step-cascade of temporal organization, i.e., the messenging of internal time information throughout the body of humans (and of other vertebrate species), could involve melatonin as a key candidate.

FIVE STEPS OF TIME MESSENGING

Step 1: Light conveys information about environmental (external) time;

Step 2: Ocular interfaces between light and biological (internal) time are intrinsically photosensitive [ip] melanopsin-expressing retinal ganglion cells [RGCs] and rods and cones;

Step 3: Light information travels via the retinohypothalamic tracts to the master clock in the brain, viz the SCN;

Step 4: Within the SCN, external time information is translated into internal time and then distributed to numerous brain structures, thereby contributing to the photic synchronization of circadian rhythmicity (Hattar, Kumar *et al.* 2006).

Step 5: Melatonin, we propose, transmits – or is a messenger of – central time information to all parts of the body.

Insofar we develop our proposal as an extension of what was synthesized two decades ago: "The melatonin rhythm: both a clock and a calendar" (Reiter 1993). Beyond being a time provider at daily and seasonal scales, melatonin could serve as a universal time messenger which transmits central biological time information throughout the body of vertebrate species. To support our proposal we shall

- describe melatonin as a biological messenger
- develop why melatonin rhythms can be both a universal time provider and time messenger
- synthesize abundant evidence for time messenging Steps 1–4
- offer tentative evidence for time messenging Step 5

MELATONIN AS A BIOLOGICAL MESSENGER

Signaling within an organism is necessary for its existence. Indeed, cell signalling is a fundamental biological process which allows organization over time rather than permitting chaos. Cell signaling cascades include first and second messengers. First messengers are signals which arrive at cell surfaces where they are received by receptors. Second messengers are needed if the first messenger is not membrane soluble. Second messenger signals such as cyclic adenosine monophosphate (cAMP), cyclic guanosine monophosphate (cGMP), calcium ions, nitric oxide, or phospholipids are produced to relay the first messenger signal into the interior of the cell for responses.

That melatonin is a first messenger in vertebrates is undisputable. Clearly, in species that have a discrete morphologically-circumscribed pineal gland, and even those that do not have a well-defined pineal structure, a blood melatonin rhythm exists with highest levels of melatonin always existing at night (Roth, Gern *et al.* 1980, Reiter 1986). Since blood is shunted to every cell in the organism, the circadian melatonin signal is likewise available to every cell and it is capable of influencing its physiology, i.e., providing clock information. Moreover, other bodily fluids, e.g., ovarian follicular fluid, cerebrospinal fluid (CSF), aqueous humor, also exhibit light:dark-dependent circadian rhythms and they are therefore capable of conveying time-of-day information to any tissue they encounter (Brzezinski *et al.* 1987, Yu *et al.* 1990, Skinner & Malpaux 1999, Tricoire *et al.* 2003). The CSF melatonin cycle is particularly robust and it has the primary responsibility of providing feedback information to the biological clock itself, i.e., the SCN (Reiter *et al.* 2014). These feedback actions allow the master clock to more precisely time circadian rhythms throughout the body since the CSF melatonin impact on the SCN strengthens the circadian message the clock sends out.

The ability of cells to read the circadian melatonin signal is attested to by the widespread distribution of membrane receptors for melatonin which likely allows the day:night melatonin rhythm to mediate cyclic metabolic changes in these cells (Slominski *et al.* 2012). Additionally, nuclear and cytosolic binding sites for melatonin have been identified; whether the interaction of melatonin with these sites has any impact in changing cellular physiology has not been adequately tested (Hardeland 2008). Likewise, how melatonin's non-receptor mediated actions (when it functions as a free radical scavenger) elicit circadian alterations in cellular function has gone uninvestigated (Galano *et al.* 2013; Zhang & Zhang 2014). Considering the numerous means by which the melatonin rhythm contacts cells, even in the absence of membrane melatonin receptors in some tissues (Weaver & Reppert 1990; Shalabi *et al.* 2013), these cells could still "read" the melatonin signal.

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Another proposed means by which the SCN may convey time information to peripheral clocks is via the autonomic nervous system. This could indeed be an alternate route, but this would be less practical since the sympathetic and parasympathetic neurons are limited in terms of their peripheral distribution, i.e., they do not contact every cell. Also, there are some strains of inbred mice that reportedly lack a nocturnal melatonin rise (Goto *et al.* 1989). A detailed analysis of melatonin levels throughout a 24 hour cycle indicates, however, that they may have a short duration night time peak that could provide timing information (Conti & Maestroni 1996).

Melatonin's potential actions as a second messenger within cells are much less clear. It would not seem to be a second messenger in the classical sense, i.e., as a downstream effector associated with a cell membrane receptor. However, since melatonin may be synthesized in the mitochondria of every cell (Venegas *et al.* 2012; Tan *et al.* 2013), where it is used exclusively by the cells that produced it, it may function as a second messenger. Since it is likely that some stimuli probably induce melatonin production in peripheral cells of vertebrates, as already shown in plant cells (Reiter *et al.* 2015), after which the indoleamine impacts local cellular physiology, it could function as a type of second messenger. This, however, requires verification.

MELATONIN AS A UNIVERSAL TIME PROVIDER AND MESSENGER

That melatonin rhythms constitute the equivalent to being both a clock and a calendar was proposed two decades ago (Reiter 1993). At that time it was already established that variations of how melatonin is produced and secreted over 24 hours and over seasons provides both daily and seasonal, i.e., clock and calendar, information to organisms. Today we know that melatonin receptors reside in the master cellular clocks of the SCN where they receive clock messages in form of, or coded as, melatonin cycle signals. Melatonin receptors which may mediate differential circadian and seasonal time information have been identified in all organs investigated so far. Thus, it appeared straightforward in 2008, to extend the "1993 clock and calendar concept" to include "how" the temporal information is relayed and passed down from the brain to all bodily parts. Today, it seems obvious to elaborate the suggested causal cascades of transferring time and timing information throughout the body.

Even before the melatonin rhythm was shown to provide circadian information to cells, i.e., to function as a clock, the seasonally-changing melatonin cycle was documented as being a central and necessary intermediate between natural environmental photoperiodic changes and seasonal reproductive events in photoperiod-sensitive mammals (Reiter 1973; Brainard *et al.* 1982). Loss of the blood melatonin rhythm as a consequence of surgical removal of the pineal gland results in cessation of the circannual reproductive cycle in these species (Reiter 1974). These findings clearly showed that the melatonin rhythm has embedded in it information about the time of year, i.e., it functions as a calendar (Reiter 1993). This action of melatonin is mediated at the level of the hypothalamo-pituitary axis and is dependent on specific membrane receptors for this indoleamine which induce the expression of genes, the products of which regulate seasonal alterations in reproductive capability (Saenz de Miera *et al.* 2014; Wood & Loudon 2014).

Evidence for time messenging: Steps 1–4

For centuries it was assumed that ocular photoreception was confined to spatio-temporal imaging of the environment. Beyond light's crucial role for image-forming processes, it is now established that the retinas of rodents and primates are an integral part of the mechanisms whereby circadian rhythmicity is modulated by light. Light's ability to entrain 24-hour biological cycles relies *inter alia* on a photoreceptive network in the inner retina which was functionally discovered only recently. Retinal ganglion cells [RGCs] were already described in 1840 by Hannover but they were presumed to be non-responsive to light. However, highly specialized subsets of RGCs, constituting only 1–2% of the neurons which form the retinal ganglion cell layer, contain melanopsin (Dacey *et al.* 2005; Melyan *et al.* 2005; Panda *et al.* 2005; Qiu *et al.* 2005) and are intriniscally photosensitive (ip). These ipRGCs play a critical role in understanding what was known for decades, namely that the eyes are the sensory organs responsible for entrainment by light (Moore 1978). The specialized RGCs depolarize with a peak spectral sensitivity in the 460–480 nanometer [nm] range, corresponding to blue wavelengths, and they do this even when synaptic input from rod and cone photoreceptors is blocked. In these RGCs, pituitary adenylate cyclase-activating polypeptide (PACAP) is contained and glutamate, coding chemically for "darkness" and "light" information, is co-stored (Hannibal *et al.* 1997).

Starting three decades ago, experiments in rodents with degenerate retinas, i.e., without rods and cones, were compatible with the notion that classic photoreceptors contribute to but are not necessary for circadian responses to light, including the suppression of melatonin and the synchronization of circadian rhythms (Pevet *et al.* 1984; Webb *et al.* 1985; Foster *et al.* 1991). That there are two sets of photoreceptors for primarily visual (image-forming) and non-visual (non-image-forming) purposes in the human retina was suggested for instance by experiments showing that 460 nm monochromatic light phase shifted the human circadian pacemaker much more effectively than 555 nm light (Lockley *et al.* 2003).

The central processes of the melanopsin-containing cells, as with axons of all retinal ganglion cells, are part

of the retinohypathalamic tract (RHT) of the optic nerve (Moore *et al.* 1995). RHT axons are directly linked with the SCN as the central biological clock and with the intergeniculate leaflet. The neurons of the latter project back to the SCN (Hattar *et al.* 2002). These pathways evince the SCN of the current ambient light condition. Within the SCN, light induces the excitation of neuronal activity. In addition, light phase shifts the circadian rhythm inherent in the SCN. When light stimulates the ipRGCs, the excitatory neurotransmitter glutamate is released from the RHT onto the SCN neurons, initiating the immediate excitation of most neurons in the central biological clock (Meijer *et al.* 1986; Ding *et al.* 1994).

Molecular components of the circadian oscillators in the SCN are – at least in part – elucidated. Two interlocking transcriptional feedback loops (Emery & Reppert 2004; Antle & Silver 2005) are at work in the central clock. The first intracellular transcriptional–translational feedback loop includes several clock genes [Clock, Bmal1, Cryptochrome genes (Cry1,2) and Period genes (Per1–3)]. The circadian regulation of Bmal1 is involved in the second feedback loop. Clearly, the SCN constitute the master circadian oscillator in mammals, including man. Equally clearly, numerous cells in many, if not all, peripheral tissues are presumed to possess similar oscillators as the circadian rhythm-related genes are not confined to the SCN but exist exist as so-called slave oscillators (Emery & Reppert 2004; Antle & Silver 2005).

There are widespread central projections of melanopsin-expressing RGCs within the brain, thereby contributing to the photic synchronization of circadian rhythms (Hattar *et al.* 2006). With particular regard to the central master clock, there are extensive neuronal out- and inputs from and to the SCN within and outside the hypothalamus. Neuronal links between the SCN and the pineal gland, where the melatonin rhythm is generated, include synaptic connections in the hypothalamic paraventricular nuclei, the preganglionic sympathetic neurons of the upper thoracic cord and postganglionic sympathetic parikarya located in the superior cervical ganglia; the axons of these cells eventually terminate on the melatonin-producing cells of the pineal gland, the pinealocytes (Watts & Swanson 1987; Reiter 1993). Via this neuronal network, the ambient light/dark environment as conveyed centrally by the melanopsin-expressing retinal ganglion cells controls the production of melatonin in the pineal gland in all mammals.

Evidence for time messenging: Step 5

The pinealocytes discharge the melatonin they produce via two routes as confirmed by the corresponding circadian rhythms of melatonin in the blood and in the cerebrospinal fluid (CSF) (Reiter 1986; Skinner & Malpaux 1999; Legros *et al.* 2014). The melatonin rhythms in these two fluids, however, differ in terms of their preciseness and amplitude. In the peripheral circulation, the pineal-derived melatonin rhythm is of much lower amplitude than that in the CSF with melatonin values rarely exceeding 150 pg/ml in the former. This rhythm, which comes into contact with every cell in the organism aids in regulating the slave oscillators (Hardeland *et al.* 2012). In contrast, the nocturnal levels of melatonin in the third ventricular CSF are orders of magnitude higher than those in the blood. Moreover, the nocturnal rise in CSF melatonin exhibits a sharp and large increase associated with lights off and rapidly dissipates with lights on (Skinner & Malpaux 1999; Legros *et al.* 2014). This renders the CSF melatonin cycle much more precise than the peripheral melatonin rhythm which exhibits a more sluggish rise at darkness onset and begins to drop in advance of lights on (Reiter *et al.* 2014). This pronounced melatonin cycle in the CSF is believed to impart a strong circadian signal on the SCN, nuclei that are embedded in the walls of the third ventricle and easily accessed by CSF melatonin. What this means is that whereas the blood melatonin rhythm serves the function of synchronizing slave oscillators throughout the organism, the signal is relatively weak. On the other hand, modulation of the central rhythm generator, the SCN, is believed to be the responsibility of a highly precise and robust melatonin rhythm in the CSF (Pevet 2014; Reiter *et al.* 2014; Coomans *et al.* 2015; Vriend & Reiter 2015).

CONCLUSIONS

Over the past two decades, molecular mechanisms which determine circadian clockworks in mammals have been increasingly elucidated. It is understood that the master biological clock in the SCN is similar to circadian clocks in peripheral clocks. However, while the former appears to be self-sustained, the latter must be synchronyzed by the SCN. While neural and humoral cues appear to be involved (Balsalobre 2002; Lowrey & Takahashi 2004), how this "centrifugal" time messenging from one central to billions of peripheral clocks is factually achieved is still unclear. When trying to understand how time and timing information is provided throughout the bodies of vertebrate species, two reasons could make melatonin a promising time messenger candidate: first, melatonin, as the chemical expression of darkness (Reiter 1991), is critically involved in time- and timing-related processes such as encoding clock and calendar information; second, melatonin travels throughout the body without limits and could readily exert the ubiquitous actions described herein.

From an evolutionary perspective, manageable simplicity often persists in nature. If melatonin is the time messenger we propose it could be, no major alterations of time messenging systems may have been required across vertebrate species. Remarkably, this could be a legacy and reminder of an all-embracing evolutionary history.

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