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The melatonin rhythm: both a clock and a calendar

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Abstract. The paper briefly reviews the data which shows that the circadian production and secretion of melatonin by the pineal gland can impart both daily, i.e., clock, and seasonal, i.e., calendar, information to the organism. The paper summarizes the 3 patterns of nocturnal melatonin production that have been described. Clearly, regardless of the pattern of nocturnal melatonin production a particular species normally displays, the duration of nightime elevated melatonin is proportional to the duration of the night length. Since daylength under natural conditions changes daily the melatonin rhythm, which adjusts to the photoperiod sends time of year information to the organism. The melatonin receptors which subserve the clock message sent by the pineal gland in the form of a melatonin cycle may reside in the biological clock itself, namely, the suprachiasmatic nuclei (SCN). The melatonin receptors that mediate seasonal changes in reproductive physiology are presumably those that are located on the pars tuberalis cells of the anterior pituitary gland. Besides these receptors which likely mediate clock and calendar information, melatonin receptors have been described in other organs. Interestingly, the distribution of melatonin receptors is highly species-specific. Whereas the clock and calendar information that the melatonin cycle imparts to the organism relies on cell membrane receptors, a fact that is of some interest considering the high lipophilicity of melatonin, recent studies indicate that other functions of melatonin may require no receptor whatsoever.
Key words. Pineal gland; melatonin rhythm; circadian rhythms; seasonal reproduction; melatonin receptors; biological clock.

Introduction

Soon after its discovery in the bovine pineal gland[24], the control of melatonin production was shown to be photoperiod-dependent[38]. Interestingly, this prediction was made initially on the basis of a presumed 24 h rhythm in the activity of the melatonin forming enzyme, i.e., hydroxyindole-*O*-methyltransferase (HIOMT), in the rat pineal gland[84]. In these studies the claim was advanced that HIOMT activity and therefore, presumably, melatonin synthesis were higher at night than during the day. Whereas later studies have shown that production of pineal melatonin is clearly higher at night than during the day[49,68], the idea that HIOMT activity exhibits a dependable circadian rhythm has not withstood the test of time.

In all vertebrates[1,5,11–13,39,53,78], and possibly in many invertebrates[36] as well, a circadian rhythm of melatonin production occurs with highest levels always being associated with the night. In mammals the nocturnal rise in blood melatonin is derived primarily from the pineal gland; however, in non-mammalian vertebrates other organs, e.g., the eyes, may contribute significantly to the 24 h serum melatonin rhythm. In invertebrates a pineal gland is lacking and therefore the melatonin cycle is clearly derived from another source[36]. Even in mammals where melatonin is produced in organs other than the pineal[21] and sometimes in a rhythmic manner, the 24 h cycle of melatonin in the blood is clearly a function of the release of the hormone from the pineal gland. Besides being absolutely linked to the light:dark cycle, melatonin production is always highest at night regardless of the activity pattern the species displays, i.e., whether it is diurnally active, nocturnally active or whether it exhibits a crepuscular activity pattern. This means, of course, that in some species melatonin levels are highest when the animal is sleeping while in other species highest levels are achieved when the animal is most active. In humans, melatonin is generally considered to be a sleep promoting agent.

Although it is well documented that nighttime leads to a rise in melatonin synthesis in the pineal of virtually all mammals[49], unless there is a genetic absence of the enzymes mediating its production, the pattern of the nocturnal rise varies among species. Three different patterns of nocturnal melatonin production have been described (fig. 1)[48,49]. A type A pattern of melatonin production is typified by a delay of several hours in the onset of melatonin synthesis after darkness onset; thereafter, the melatonin level rises to its peak quickly and soon thereafter returns to daytime values, at about the time of lights on. Animals with this pattern of nocturnal melatonin production have a relatively short interval when melatonin values are above basal levels. An example of a species with this type of melatonin rhythm is the Syrian hamster (*Mesocricetus auratus*); of the 3 patterns that have been described, the type A pattern seems the least common.

Animals with a type B melatonin pattern experience a gradual rise in pineal production of the indole begin-

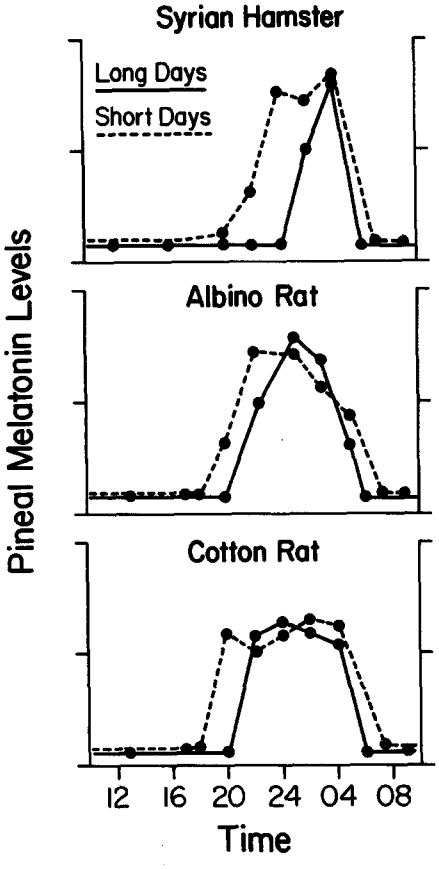

Figure 1. Three different patterns (A, B and C) of melatonin production have been described in the mammalian pineal gland. See text for details. Also listed here are representative animals which possess the various type rhythms: Syrian hamster (type A); albino rat (type B); cotton rat (type C). These rhythms were initially described in animals maintained under long day conditions, i.e., 14 h of light per day. If the daylength is shortened, i.e., night length increased, (short days in the figure) the duration of elevated melatonin is prolonged regardless of the melatonin pattern a species normally displays.

ning at about the time of lights off; peak melatonin levels are reached during the middle of the dark phase followed by a gradual reduction in indole production during the second half of the night. The two best known species that exhibit this common pattern of nocturnal melatonin synthesis are the domestic rat (*Rattus norvegicus*) and the human.

Mammals with a type C pattern of nocturnal melatonin synthesis are also common and they experience high melatonin levels for virtually the entire night. Thus, within 30 min after darkness onset peak melatonin production is reached; these high plateau levels are maintained for essentially the entire period of darkness and late in the dark phase melatonin values decline precipitiously. This pattern of melatonin production is present in the sheep and the cotton rat among other species.

Regardless of whether a given species exhibits a type A, B or C nocturnal melatonin pattern, an extension of the dark phase (such as a winter night) also prolongs the melatonin peak[47,72,82]. Thus, in all mammals at least the duration of elevated melatonin is positively correlated with the duration of the night.

The functional significance of the different nocturnal patterns of melatonin remains unknown. However, whereas the relevance of the different melatonin patterns is an enigma, the patterns may provide information concerning the cell biology of the induction of melatonin synthesis in various species. It is generally conceded that at or shortly after the time of lights off, norepinephrine (NE) is released from the post ganglionic sympathetic nerve endings that terminate in the pineal gland (see below) resulting in an increased intrapinealocyte concentration of the second messenger cyclic adenosine monophosphate (cAMP) which induces the rise in melatonin production[48,68]. The rapidity with which cAMP stimulation induces melatonin synthesis varies greatly among species. Thus, in some animals the nighttime rise in melatonin production essentially accompanies the increase in cAMP levels (e.g., in the sheep with a type C melatonin pattern) whereas in other species (e.g., the Syrian hamster which has a type A pattern of nocturnal melatonin synthesis) the rise in the intracellular second messenger cAMP precedes the marked synthesis of melatonin by several hours[63,64]. An explanation for the different species-specific time requirements for cAMP to induce a rise in melatonin synthesis is not available although it obviously relates to post cAMP induction mechanisms which are much longer in the pineal gland of the Syrian hamster than in the sheep[48].

Generation of the melatonin rhythm

The circadian production of melatonin in the mammalian pineal gland is a result of a neural message arriving at the gland from the suprachiasmatic nuclei (SCN) in the hypothalamus (fig. 2); in turn, due to the rapid release of newly synthesized melatonin, the blood melatonin cycle is a function of its synthesis within the pineal[49]. Because of this close association between melatonin synthesis and secretion, blood melatonin concentrations are generally considered to accurately reflect the quantity being produced in the gland at virtually the same time[47]. This close relationship between blood levels of a hormone and its concentration in its gland of origin is rather unusual. More typically, it is difficult to estimate the amount of hormone in a gland based exclusively on its concentration in the blood because the parent gland usually retains substantial reserves of the hormone in storage. However, since the pineal gland, rather than storing melatonin, releases it very shortly after it synthesizes the indole, blood concentrations are indicative of its current synthesis rate. Thus, any factor that in mammals limits pineal melatonin synthesis likewise leads to lower than normal melatonin levels in the blood. Two such conditions have been described in

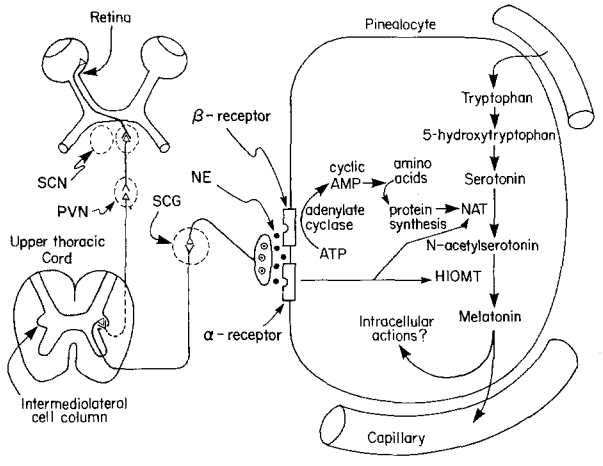

Figure 2. Neural connections between the eyes (retina) and the pineal gland, represented in this figure as a single pinealocyte. Synapses occur in this pathway at the level of the suprachiasmatic nuclei (SCN) (the biological clock), the paraventricular nuclei (PVN), the intermediolateral cell columns, and the superior cervical ganglia (SCN). Norepinephrine (NE) is released at night from the intrapineal sympathetic nerve endings; NE interacts with β- and α-adrenergic receptors eventually inducing the conversion of adenosine triphosphate (ATP) to cyclic AMP which in turn eventually causes the activation of *N*-acetyltransferase; the final enzyme in the pathway of melatonin synthesis is hydroxyindole-*O*-methyltransferase (HIOMT).

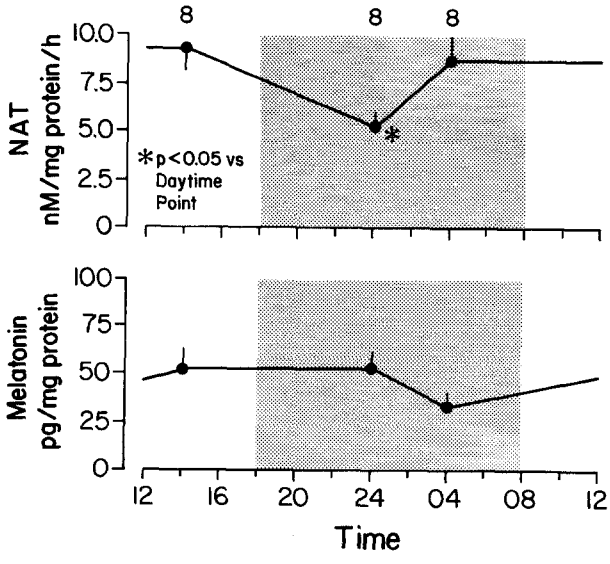

Figure 3. Twenty-four hour levels of NAT (*N*-acetyltransferase) activity and melatonin content of the pineal gland of the domestic pig (*Sus scrofa*). Despite the presence of ample activities of both NAT and HIOMT (data not shown), pineal melatonin levels do not rise at night. In fact, NAT activity which normally also increases during darkness, in the present case exhibited a small reduction. From Reiter et al.[52].

mammals, one being the genetic lack of the enzymes that convert serotonin to melatonin[17] and the second being a generalized deficiency in the Ca^{2+} stimulated, Mg^{2+} dependent ATPase (the calcium pump)[58]. Highly inbred mouse strains are the species that are described as lacking the genetic machinery to form melatonin[17] while the calcium pump problem occurs in the so-called cardiomyopathetic hamster. One other mammalian species[28], the domestic pig, lacks a nocturnal rise in melatonin (fig. 3) despite the presence of the enzymes required for its synthesis; the activities of these enzymes are stable throughout a given light:dark cycle[52].

The SCN, also sometimes referred to as the biological clock, certainly have a great deal to do with the generation of the cyclic production of melatonin. These nuclei, situated in the anterior hypothalamus on the base of the brain, send efferent fibers to the pineal gland by way of neuronal cell bodies located in the paraventricular nuclei, the intermediolateral cell column in the upper thoracic cord and in the superior cervical ganglia (fig. 2)[47]. The pineal gland in mammals, albeit not in birds, requires the sympathetic innervation to maintain its function[54]. Normally, in the absence of light, i.e., during the night, the SCN neurally signals the pineal gland via the neurons just described to produce melatonin[68]. During the day, light via the retinohypothalamic tract (RHT) inhibits the SCN from stimulating the pineal gland. The RHT arises from ganglion cells of the retina which pass to the SCN through the optic nerves. The neurotransmitter at the interface of the RHT with neurons in the SCN is most likely the excitatory neurotransmitter glutamate[30]. Considering the interaction of the mammalian retinas with the production of pineal melatonin, the pineal gland is truly an end organ of the visual system not unlike the visual cortex.[47].

The absolute reliance of the functional integrity of the mammalian pineal gland on its sympathetic innervation, eventually coming from the retina, is emphasized by transplantation studies. When the pineal gland is surgically removed from its normal site and transplanted elsewhere, e.g., under the kidney capsule, it grows well but it does not produce much, if any, melatonin and certainly not in a cyclic manner[40,83]. The only early study suggesting a restoration of normal pineal function when multiple glands were transplanted into a recipient rat has never been confirmed and the observations were probably erroneous[15]. Even when the pineal gland is left at its normal site in the skull but it is sympathetic denervated by superior cervical ganglionectomy the gland, by all physiological measurements currently available, is non-functional[54]. Thus, the arrival of transduced light:dark information at the level of the pineal gland through the SCN and peripheral sympathetic nervous system is an essential feature of the circadian production and secretion of melatonin.

The point has already been made that melatonin production is precisely nocturnal and, indeed, the hormone has been referred to as the chemical expression of darkness[49]. With the onset of darkness at night, NE is released from intrapineal sympathetic nerve terminals. The NE then interacts with both α- and β-adrenergic

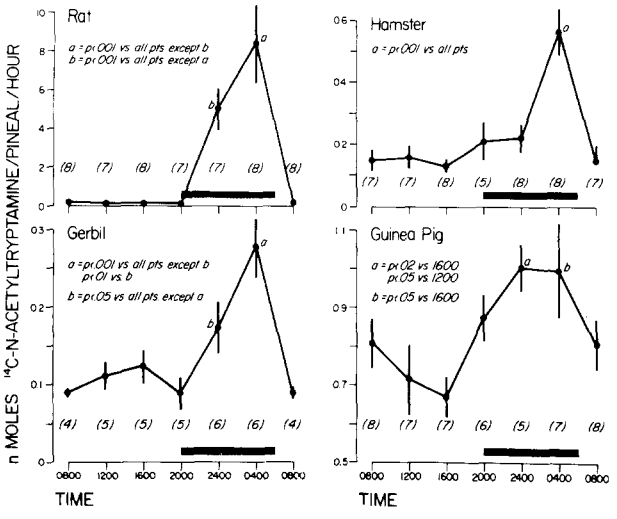

Figure 4. This figure illustrates the species-specific nocturnal increases in pineal *N*-acetyltransferase (NAT) activity in 4 species of rodents. Note the different scales on the vertical axes. In the rat the nighttime rise in NAT activity may be 100× greater than daytime levels while in the pineal gland of the guinea pig the nocturnal increase is roughly 2-fold compared to daytime levels. From Rudeen et al.[62].

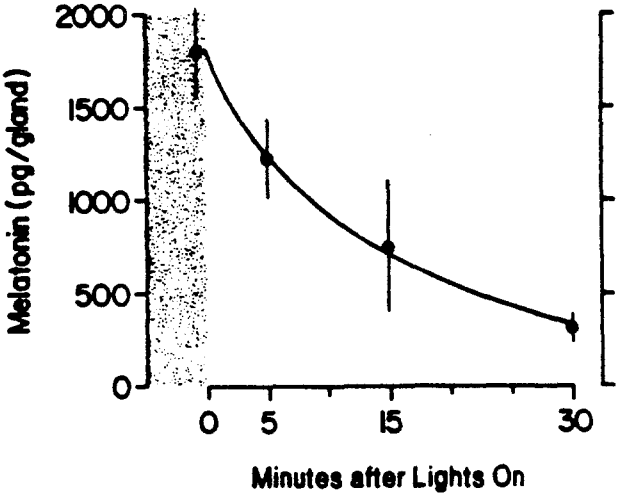

Figure 5. Suppression of rat pineal melatonin levels by acute exposure to light at night, here represented by the shaded area. Within 10 min (or less) after light onset pineal melatonin levels are reduced to 50%; by 20–30 min daytime melatonin levels are achieved. Light induced suppression of nocturnal melatonin levels is characteristic of all mammals including man.

receptors in the pinealocyte membrane leading to a large intracellular increase in cAMP (fig. 2)[48]. The subsequent induction of *N*-acetyltransferase (NAT) activity, the rate of which limits the amount of melatonin produced, requires, as noted above, a few minutes to several hours. The net result of NE release is thus a rise in NAT activity, the amplitude of which is also species-specific (fig. 4)[62]. This enzyme converts serotonin to *N*-acetylserotonin which is subsequently *O*-methylated to *N*-acetyl-5-methoxytryptamine, commonly known as melatonin. As mentioned above, once melatonin is produced it is quickly released into the numerous capillaries that pervade the gland.

The melatonin rhythm is remarkably stable and seems to be conserved under many physiological conditions, presumably attesting to its extreme importance to the organism. One example of how well the melatonin rhythm is preserved is provided. Serotonin, the precursor of melatonin, exists in concentrations in the pineal gland 100-fold greater than levels in the brain, where serotonin is an important neurotransmitter with numerous functions. Furthermore, if animals are treated with a drug that stimulates the metabolism of tryptophan (the amino acid from which serotonin is synthetized) to the point where the animals become deficient in the amino acid, brain serotonin values drop in advance of any reduction in pineal serotonin concentrations[10]. This is particularly remarkable considering, as already mentioned, pineal levels of the monoamine exceed those in the brain by 100 times[48]; yet serotonin seems to be preferentially preserved, pointing to the essential nature of the circadian melatonin cycle.

There is one environmental factor that greatly attenuates the nighttime rise in melatonin production; this is visible light. The acute exposure of non-human mammals or humans to light at night precipitously reduces pineal melatonin production and blood melatonin levels (fig. 5). Acute light exposure during darkness virtually immediately shuts down the enzymatic machinery required for the production of the indoleamine; under these conditions melatonin concentrations in the pineal plummet to half maximal values in less than 10 min; 30 min after light onset daytime melatonin levels are achieved. This occurs, at least in rats, even though the duration of light exposure is very short, e.g., 1 sec[55].

The ability of light at night to suppress the nocturnal production of melatonin is common to all species investigated but the intensity of light required to do so varies widely among species[45]. The albino rat, of the species investigated, is most sensitive in terms of the response of the pineal to nighttime light exposure. In this species, white light intensity of 0.0005 μW/cm² inhibits the pineal from producing melatonin[81]. At the other end of the spectrum, rather high intensity light (>1,850 μW/cm²) is required to initiate inhibition for pineal melatonin production in the ground squirrel[45,48]. The human falls between these two extremes and, indeed, in the human the degree of nocturnal suppression of circulating melatonin levels is directly related to the brightness of the light to which individuals are exposed[29]. In general, the retina-SCN-pineal system of nocturnally active animals is more sensitive to light inhibition than is this system in diurnally active species. The discrimination of light intensity is presumably done at the level of the so-called visual cells in the SCN[30]. Visual cells are more numerous in SCN of nocturnal animals and their elec-

trical activity is more readily adjusted by light stimuli arriving in the nuclei via the RHT.

The likely mechanism by which acute light exposure quickly turns off pineal melatonin synthesis is as follows. The detection of light by the retinal photoreceptors immediately signals the SCN that it is 'day'; the SCN signals the pineal gland, NE is no longer released from the intrapineal sympathetic neurons, intrapinealocyte cAMP levels fall, NAT is no longer expressed and as a consequence melatonin production drops. All these events obviously occur in a very short time frame.

Among the wavelengths classically referred to as visible light, it seems that light in the blue/green wavelength range is maximally inhibitory to the pineal[51,69]. However, these are by no means the only wavelengths that influence the circadian production of melatonin. Thus, wavelengths in the ultraviolet range (<400 nm)[35] and red light (>600 nm)[20] also are unexpectedly capable of reducing nocturnal melatonin production. Finally, extremely low frequency electromagnetic field exposure (in the range of 60 Hz) as well as pulsed static magnetic fields have been reported to attenuate the nocturnal production of melatonin[50,57]. The consequences of the limited suppression of pineal melatonin levels by these very low energy, extremely low frequency wavelengths remains to be definitively determined.

Finally, because of its marked effect on the circadian production of melatonin, another manipulation which perturbs the rhythm will be mentioned here. If during darkness when pineal melatonin synthesis is elevated, rats are forced to swim, pineal melatonin levels plummet as if the animals had been exposed to light[73]. Paradoxically, pineal NAT, the rate limiting enzyme in melatonin production, remains elevated as do blood levels of the hormone (fig. 6). This discrepancy is explained on the basis of sustained high production of melatonin with a very rapid discharge of the hormone from the pineal. Initially this was thought to be a stress response but subsequent studies revealed this not to be the case. Thus, adrenalectomy (which greatly lowers circulating glucocorticoids and catecholamines, the major stress hormones) has no effect on the pineal response to nighttime swimming. Likewise, hypophysectomy which eliminates adrenocorticotropic hormone (ACTH) from the blood does not alter the ability of swimming to diminish pineal melatonin levels. The release of artrial natriuretic peptide (ANP) from the heart (a normal consequence of exercise) is apparently not causative in the response[85] and, finally, the activity of the peripheral sympathetic nervous system is clearly unrelated to the drop in pineal melatonin associated with nighttime swimming[73]. Despite the dramatic and unusual nature of the response of pineal melatonin synthesis to swimming, all attempts to define the mechanisms that induce the changes observed have failed.

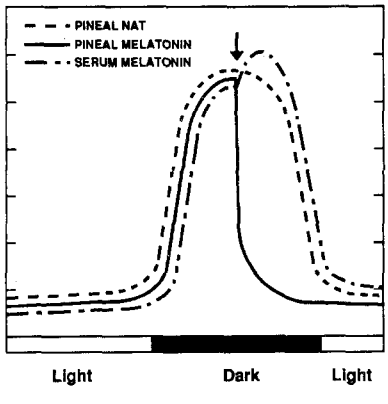

Figure 6. Pineal *N*-acetyltransferase (NAT) activity and pineal and blood melatonin levels in rats forced to swim at night. Pineal melatonin levels drop precipitously (as in rats exposed to light at night; see figure 5) but pineal NAT activity and blood melatonin levels remain elevated. The data suggest that newly synthesized melatonin is quickly released from the pineal gland during swimming.

The mammalian pineal melatonin cycle clearly is dependent upon a neural message arriving at the gland from the SCN while light modulates the signal that the SCN sends (fig. 2). Over eons of time mammals have been exposed, for all practical purposes, only to light and darkness that were a function of the rising and setting of the sun. Under these conditions the melatonin cycle was precisely controlled by the natural light:dark environment. With the advent of artificial light sources, manipulation of the regular recurring melatonin rhythm is a routine phenomenon. Thus, the exposure of animals including man to artificial light or transmeridian travel which changes day and/or night length severely compromises the circadian production of melatonin. Such changes undoubtedly have substantial down stream effects as will be discussed below.

In non-mammalian vertebrates, many of which display a light synchronized melatonin cycle, the SCN are not involved in influencing the rhythm[74]. In these species the 24 h melatonin cycle is presumably driven by light either directly via clock mechanisms in the pineal gland itself (such as in non-mammalian vertebrates) or in the extreme case of unicellular organisms[36], directly at the subcellular level, perhaps also via some physiological clock.

The melatonin rhythm as a clock

The precisely regulated 24 h melatonin rhythm imparts important time-of-day information to any organ system that can 'read' the message (fig. 7). Thus, the rhythm provides information concerning both day (low melatonin) and night (high melatonin) and, at least theoretically, it may also indicate evening (rising melatonin) and morning (falling melatonin); in this context the information derived from the melatonin rhythm may have anticipatory value.

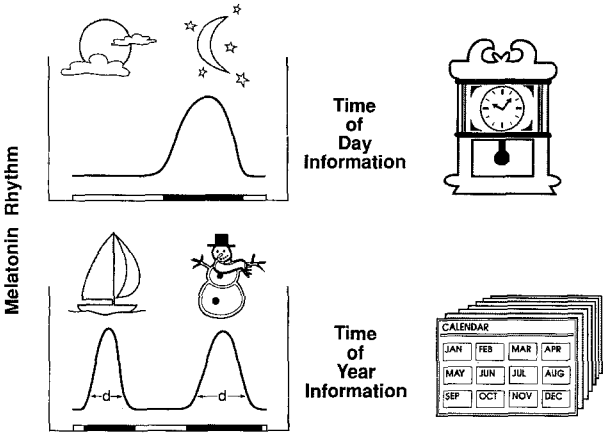

Figure 7. Diagrammatic representation of the two types of messages the melatonin rhythm imparts to the organism, i.e., clock and calendar information. The day:night difference in blood melatonin levels provide time-of-day information to those organs that 'read' the message while the seasonal variations in daylength, with the resulting changes in the duration of elevated nocturnal melatonin, provide time-of-year information.

Interestingly the pineal melatonin cycle, as dictated by neural messages from the SCN, is not inherently 24 h in duration. Studies on blind humans and in subjects living in Antarctica during the winter months (absence of solar light) suggest that the clock governing the melatonin rhythm actually runs with a period closer to 25 h than to 24 h[22,25]. Hence, under constant dark conditions the melatonin peak occurs approximately 1 h later on consecutive days. Even in sighted individuals living in temperate regions, during the winter months when night length is longest, the nocturnal melatonin peak becomes delayed. This relates to a condition referred to as seasonal affective disorder (SAD) of the winter subtype[61]. If these individuals are given phototherapy (usually bright light exposure soon after awakening in the morning), the melatonin cycle (and other circadian rhythms) are readjusted back to 24 h and the psychological depression disappears. For phototherapy to be effective in SAD patients the daytime light exposure must be continued on a daily basis throughout the winter months[26]. As spring approaches, the longer days regulate the endogenous circadian melatonin cycle more precisely and phototherapy is discontinued until the subsequent winter.

Since the eyes are involved in photoreception which synchronizes the activity of the SCN/pineal complex, in profoundly blind subjects phototherapy is of no value. Interestingly, however, the melatonin cycle in these individuals can be synchronized by the exogenous administration of melatonin at a precise time of day on a daily basis[34]. Clearly, melatonin has the capability of influencing the clock which regulates the endogenous cycle of melatonin production[74,75].

It is usually assumed that melatonin's action on the circadian clock is manifested at the level of the SCN, which are believed to be the or a major portion of the 24 h biological clock in mammals. In many, but not all, mammals membrane-bound melatonin receptors are abundantly present on cells in the SCN[65]. Whether these receptors are actually on the clock cells themselves or on interneurons that subsequently influence timing mechanisms in other cells remains unknown. Also, the intracellular mechanisms whereby melatonin alters the function of the biological clock has not been clarified. In in vitro preparations of the SCN, the ability of melatonin to synchronize the output of these cells has been elegantly demonstrated[14].

The point has already been made that the SCN of many species, including the fetal human brain (adult human brains have not been investigated)[59], are endowed with numerous melatonin receptors which presumably react to circulating melatonin levels and thereby provide clock information for other circadian rhythms. If in fact melatonin synchronizes circadian rhythms by this means, is would be expected that the SCN of all mammals would prosses melatonin receptors. This, however, seems not to be the case. In the mustelids, the SCN do not bind radioactive melatonin suggesting they are devoid of melatonin receptors. Rather little is known concerning the synchronization of circadian rhythms in mustelids by melatonin; if the indoleamine functions in such a capacity in these species it is possible it does so independently of SCN receptors for melatonin.

One recent demonstration that individual neurons independently generate rhythms with a 24 h periodicity leaves open the possibility that melatonin could act at the level of individual neurons anywhere in the brain to determine circadian fluctuations in cellular physiology[31]. This option, however, has not been tested in cells which exhibit spontaneous circadian activity.

That melatonin synchronizes other 24 h rhythms is well documented. In animals it synchronizes locomotor activity rhythms when they are maintained under constant conditions. Likewise, melatonin has been used to synchronize the disrupted rhythms, namely jet lag, that result from transmeridian travel[2]. Melatonin's ability to overcome signs of jet lag lends support to the idea that it can adjust the function of the biological clock[3]. Just as the indole is used in the treatment of jet lag, it is anticipated that it will also have utility in ameliorating the disturbed rhythms of shift workers.

The melatonin rhythm as a calendar

Under natural photoperiodic conditions the duration of elevated melatonin levels at night varies daily coincident with the variations in the light:dark cycle[16,82]. At the extremes of latitude these variations are more exaggerated than at points nearer to the equator but, no matter the latitude at which an animal resides, annual changes in daylength are unavoidable. Only at the time of the

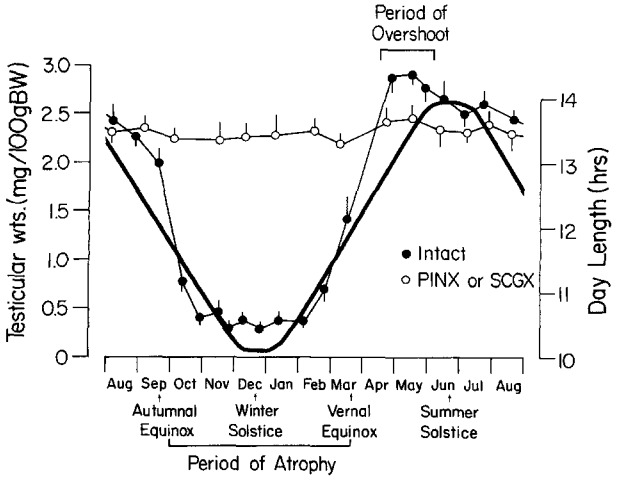

Figure 8. Testicular size (●) and daylength (heavy black line) vary in a parallel manner throughout the year, shown here in long day breeding rodents such as the Syrian hamster, the species on which this figure is based. That the pineal gland, via its secretion of melatonin, provides the necessary seasonal signal (calendar information) is demonstrated by the fact that either pinealectomy (PINX) or superior cervical ganglionectomy (SCGX), which destroy the cyclic production of melatonin, prevent the seasonal variations in reproduction competence. This figure is based on Syrian hamsters living outdoors in the northern hemisphere; in the southern hemisphere the rhythms would be 6 months out of phase with the rhythms shown here. From Reiter[43].

equinoxes are day and night length of equal duration at all points on the earth.

These annual changes in daylength, especially because of their ability to alter the circadian production of melatonin, have important biological implications. Photoperiodic species use the light:dark changes to mediate fluctuations in seasonal reproductive potential (fig. 8)[41-43]. For the most part, the center piece of seasonal reproduction is the time of the birth of the young. This is most frequently the spring. Mammals have evolved a variety of ploys, e.g., long or short gestation periods, delayed implantation, etc., to ensure parturition at the time optimal for survival of the offspring. In many cases this seasonality is dictated by the prevailing photoperiodic environment. When this is the case, the pineal gland usually serves as the intermediary between the external environment and the reproductive system.

These relationships were initially described in the Syrian hamster[18,41,46,66]. Under naturally long photoperiods and warm temperatures of the laboratory hamsters are continual breeders and have litters throughout the year. However, when they are placed in short photoperiods (< 12.5 h light per day) under laboratory conditions or under short winter photoperiods under field conditions the neuroendocrine-reproductive axis of both males and females of the species involute over a period of several weeks (fig. 8)[41,43]. That these changes are mediated by the pineal gland is easily documented since surgical removal of the pineal gland prevents totally the suppressive effects of short days on reproductive physiology[18]. Also, that melatonin is the hormonal messenger of the pineal gland in these seasonal responses is proven by the fact that appropriately timed melatonin injections reproduce the effects of short day exposure[43,71]. Thus, melatonin clearly passes calendar, i.e., seasonal, information about the photoperiod (fig. 7) to the neuroendocrine-reproductive system. This is manifested not only as a change in the size and function of the sex organs but likewise in appropriate alterations in all the hormones associated with reproductive physiology[43].

Following the demonstration that melatonin is a key factor in adjusting seasonal reproductive capability in the Syrian hamster, many other mammals were used to examine these relationships[27,60,77]. Virtually without exception the effects of photoperiod on annual reproductive physiology was shown to depend on the function of the pineal gland and its chief hormone melatonin.

The earliest studies in this field were typically conducted on what are referred to as long day breeders that have short gestation periods. For example, the Syrian hamster regenerates its gonads in the spring, mates and the female delivers her pups 16 days later. However, many animals actually breed during the short days of the fall and winter, e.g., sheep, deer, etc[23,60]. These photoperiodic species, like the long day breeders, also utilized the photoperiodic message which is transduced into the chemical messenger melatonin at the level of the pineal gland[27].

The initial studies with long day breeders such as the Syrian hamster left some investigators with the impression that melatonin was primarily inhibitory to reproductive physiology, i.e., it functioned as an antigonadotropin. However, as soon as it was discovered that many species breed during the short days of the year (when melatonin levels are elevated for a prolonged period) it readily became apparent that melatonin was clearly not exclusively antigonadotropic. Currently, it is generally conceded that melatonin is an essential mediator of seasonal reproduction in photoperiodic species and should not be considered either an exclusive anti- or pro-gonadotropic factor, although under given circumstances it can have either effect[70].

Even in long day breeders where in many cases prolonged elevated melatonin is generally considered inhibitory to reproductive physiology, there are species variations. The Turkish hamster (*Mesocricetus brandti*), which is phenotypically very similar to the Syrian hamster and which has an overlapping habitat with the Syrian hamster in the wild, use the melatonin message very differently although the end result is the same. Thus, whereas melatonin is generally considered to be inhibitory to reproduction in the Syrian hamster, in the Turkish hamster a certain amount of melatonin is required to maintain the gonads in a highly functional condition[9]. Thus, pinealectomy (loss of the circadian melatonin rhythm) in this species leads to gonadal atrophy.

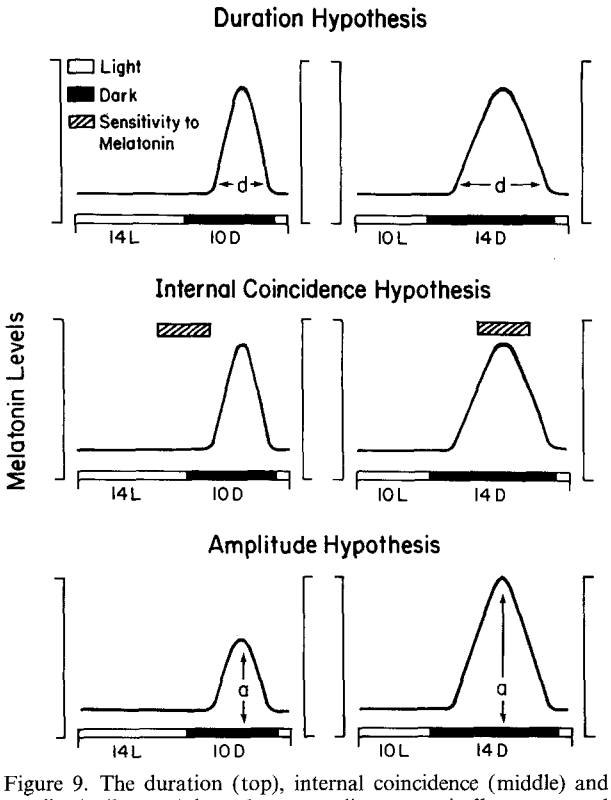

Figure 9. The duration (top), internal coincidence (middle) and amplitude (bottom) hypotheses are diagrammatically represented in this figure. The duration hypothesis claims the important message that the melatonin rhythm conveys is determined by the duration (d) of elevated melatonin, which varies with changing daylengths. The internal coincidence hypothesis states that reproductive responses occur only when high melatonin levels are coincident with a sensitivity period to melatonin (cross hatched bar, middle panel). Finally, the amplitude hypothesis assumes it is the amplitude (a) of the nocturnal melatonin peak that determines the actions of the hormone. From Reiter[48].

A great deal of effort has been expended to define the nature of the message that is encoded in the melatonin rhythm, i.e., is it the duration of elevated melatonin (referred to as the duration hypothesis), a coincidence between high melatonin levels and an increased sensitivity to melatonin (the internal coincidence hypothesis), or the amplitude of the nocturnal rise in melatonin (amplitude hypothesis) that is important (fig. 9)?

In general the duration hypothesis has the greatest experimental support[6,7,16]. Hence, the daily infusion of melatonin into pinealectomized animals duplicates the effects of photoperiod on the status of the reproductive system when the length of the infusion is adjusted to match the appropriate photoperiod[8]. These studies on the whole are technically clever and the data are convincing; as a result they have enjoyed widespread acceptance. There is little doubt that the duration hypothesis has validity and relates to the control of seasonal reproduction.

On the other hand, at least in the Djungarian hamster (*Phodopus surgorus*) a given duration of elevated melatonin can signal either functional or non-functional reproductive organs[19]. These findings suggested to the author that whereas duration of elevated melatonin is an important aspect of the message, the direction of change, i.e., whether the elevated melatonin signal is becoming longer of shorter, also contributed to how the message is interpreted. These findings in the Djungarian hamster seem to contrast somewhat with data obtained using the Syrian hamster. When Syrian hamsters are placed in short, but lengthening, photoperiods immediately after the winter solstice, the reproductive organs undergo regression[44]. Thus, under the conditions of this study it was insignificant that the daily period of elevated melatonin was becoming shorter; rather the only important part of the message was that the animal interpreted the nightly melatonin elevations to be long. Clearly, there are nuances of the duration hypothesis that have not been clarified.

In reference to the internal coincidence and amplitude hypotheses (fig. 9), the experimental data are not as strong as for the durational theory. Yet, there is suggestive evidence in each case. Accordingly, very short intervals of elevated melatonin associated with a critical period during the light:dark cycle reportedly induces reproductive regression in the Syrian hamster[67]. In terms of the amplitude of the nocturnal melatonin peak, human pubertal onset is speculatively related to the drop in the absolute height of nighttime circulating melatonin levels[80]. Likewise, in adult humans higher than normal nocturnal melatonin values may be associated with irregular menstruation and anovulation[6]. Because of findings such as this, melatonin is being proposed as a contraceptive agent in humans[79].

In the final analysis the seasonal message that the pineal gland sends to the reproductive system may not be a simple one. There is little doubt that the message is encoded in the melatonin rhythm but at different times and under different physiological conditions the melatonin cycle may be interpreted differently.

The reproductive system is not the only one that exhibits seasonal alterations which are dependent on the photoperiod and the pineal gland, it just happens to be the one that has been most extensively investigated. Such widely diverse functions as lipid metabolism, thyroid activity, temperature regulation, food consumption, locomotor activity, etc., may utilize the melatonin message to entrain them. In fact, any physiological event that exhibits an obvious circannual periodicity should be considered somehow related to the function of the pineal gland.

The melatonin receptors involved in mediating the effects of melatonin on the reproductive and endocrine systems are presumed to be those located in the pars tuberalis of the anterior pituitary gland[65,76]. These entodermally derived cells are in close proximity to the primary portal plexus and the terminals of the hypothalamic releasing hormone neurosecretory cells in the median eminence.

Melatonin theoretically controls the release of substances, e.g., gonadotropins or other factors, that act in a paracrine manner in the nearby median eminence thereby regulating the release of the hypothalamic releasing hormones, e.g., gonadotropin releasing hormone (GnRH)[32]. In this manner melatonin could obviously regulate the functional status of the gonads and control the reproductive capability of an animal on a seasonal basis.

Final comment

The pineal gland in vertebrates clearly has the capability of providing clock (24 h) and calendar (yearly) information for species living under natural photoperiodic conditions. Even in humans, who greatly exploit artificial light sources, there is evidence that daily and seasonal physiological events may be influenced by the natural photoperiods. The effects of the pineal gland and melatonin that have been described in this paper are believed to be mediated by membrane receptors located in discrete areas, e.g., the SCN and the pars tuberalis[76]. There are, however, melatonin receptors in other sites and the distribution of binding sites for melatonin varies widely among vertebrates[65]. This suggests that a variety of yet undefined functions which depend on membrane receptors may exist in these species. Nothing is known about melatonin binding sites in invertebrates although several species are known to produce melatonin in a circadian pattern.

For years the author has suspected that the actions of melatonin transcend the functions described in this brief review. Furthermore, it seemed unusual that a hormone which is so highly lipophilic, and therefore enters all cell compartments with ease, would depend on membrane receptors for its action. This issue has now come to a head with the demonstration that melatonin is a potent hydroxyl radical scavenger[37,56]. This function of melatonin clearly is accomplished without any membrane receptor and the only requirement is that melatonin be in the vicinity when hydroxyl radicals are produced[56]. Thus, whereas there are important functions carried out by melatonin which rely on the receptors that have been identified, the most basic action of melatonin, that of a free radical scavenger is not restrained by the need for a binding site on the cell membrane[37,56]. Furthermore, this discovery proves that melatonin is an important hormone in every aerobic organism that produces it, not only in photoperiodic species.

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