actions. Among the topics that have been investigated are the role of charged carriers in desorbing molecules from semiconductor surfaces (*29*), the influence of surface microstructure on photochemical reactions (*11*), the role of collective surface electromagnetic waves on the deposition process (*30*), and the effect of surfaces on ultraviolet molecular spectra (*31*), to name only a few. These studies have not only uncovered unexpected physical and chemical phenomena, but some of them have, in turn, led to novel techniques for microelectronics production. Applications to other disciplines, such as catalysis, can be expected in the future.

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Melatonin: A Coordinating Signal for Mammalian Reproduction?

Lawrence Tamarkin, Curtis J. Baird, O. F. X. Almeida

The pineal gland and its anatomical relation (Fig. 1) were known as early as the third century B.C., when Herophilus suggested that it might serve as a sphincter regulating the flow of thought in the ventricular system of the brain. Galen, about 450 years later, pointed out that the pineal was unlike brain tissue in structure, lay outside the cerebral ventricles, and probably had a function similar to that of the lymph nodes. In the 17th century René Descartes revived the ancient concept of the pineal as the "seat of the soul," and this view dominated scientific thought for the next 250 years (*1*).

Systematic investigations of the mammalian pineal began in the 1880's. Ahlborn, de Graaf, and others described its gross anatomy, vascularization, innervation, histology, and embryology and noted its similarity to the "third eye" or photosensory epiphyseal organ of lower vertebrates (*1, 2*). Around 1900 the biological role of the pineal began to be studied by means of glandular extirpation and administration of glandular extracts (*3*). Perhaps the most significant developments at that time were Heubner's report of precocious puberty in a boy with a pinealoma and Marburg's theory that the pineal regulates onset of puberty (*1*). Their papers triggered investigations into interactions between the pineal and reproduction.

In 1943 Bargmann (*4*) proposed that the endocrine function of the pineal was regulated by light via the central nervous system. This concept, dubbed neuroendocrine transduction by Wurtman and Axelrod (*5*), has proven to be accurate. The pineal receives environmental information through the brain and relays it to the body by means of its humoral secretions. While the pineal contains a host of indoleamines and peptides, this article will focus on the indoleamine *N*-acetyl-5-methoxytryptamine or melatonin, the only one of these compounds for which a function has been ascertained.

The Melatonin Signal

Melatonin is synthesized from a second pineal indoleamine, serotonin, through the action of two enzymes: *N*-acetyltransferase (NAT), which is responsible for the *N*-acetylation of serotonin, and hydroxyindole-*O*-methyltransferase (HIOMT), which is responsible for the *O*-methylation of the indole ring (*6*). Quay (*7*) observed that levels of serotonin in the pineal are high during the day and low at night and that alterations in the lighting cycle caused corresponding changes in pineal serotonin

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content. He also found a daily rhythm in pineal melatonin that was the inverse of the serotonin rhythm. These observations suggested that photoperiod regulates pineal indole rhythms and that the nighttime decline in pineal serotonin is due to increased melatonin production.

More recent studies in the rat (*8*) indicate that the oxidative metabolism of serotonin, probably by monoamine oxidase, is shifted to *N*-acetylation, probably by NAT, at night. Current thinking holds that the large nocturnal increase in NAT activity may be responsible for driving the melatonin rhythm, whereas HIOMT activity may determine, at least in part, the amplitude of the nocturnal increase in melatonin. Concentrations of melatonin have been found to increase at night in pineal, blood, cerebrospinal fluid, and urine in all mammalian species studied to date (*6, 9*).

The neural pathways by which photoperiodic information reaches the pineal (Fig. 1) were elucidated by a series of lesion experiments in which the daily rhythms of pineal indoles and enzymes served as markers. Although these studies were conducted in the rat, there is evidence that similar pathways occur in other mammals (*2, 10*). Nerve impulses stimulated by light impinging on the eyes are transmitted via a retinohypothalamic tract to the hypothalamic suprachiasmatic nuclei (SCN), which function as autonomous, central, circadian oscillators (*11*), and then to the paraventricular nuclei (*12*). From these hypothalamic nuclei the impulses traverse fibers in the medial forebrain bundle and reticular formation to the intermediolateral nucleus of the spinal cord. From there they pass to preganglionic adrenergic fibers of the sympathetic nervous system, which conduct them to the superior cervical ganglia (SCG). The final sympathetic input to the pineal arises from the SCG.

Conversion of the neural input into an endocrine output by the pineal may be summarized as follows (*6*): sympathetic nerves release norepinephrine at their terminals on the pineal cells in a rhythmic fashion that reflects the daily change in light and darkness. This neurotransmitter is bound by membrane β-adrenergic receptors (*13*), the cyclic nucleotide system is activated (*14*), NAT is synthesized (or activated), and synthesis of melatonin follows. It is not known whether melatonin secretion from the pinealocytes is under neural control or whether it simply diffuses into the surrounding vasculature and ventricular system to provide a daily signal to the body.

Modulation of the Signal by Light

Light serves a dual role in pineal biochemistry. The first role is a suppressive one, as demonstrated by experiments in several species in which NAT activity or melatonin synthesis was inhibited when light was presented at a sufficient intensity during the daily period of darkness (*15, 16*). In continuous darkness, however, the melatonin rhythm persists with an approximately 24-hour periodicity (*17, 18*). This observation is related to the circadian nature of pineal rhythms. The second role of light is to entrain or synchronize the pineal rhythm with the environment, perhaps through the SCN. The suppressive and entraining effects of light therefore determine when melatonin is synthesized (the phase of the melatonin rhythm) and how long synthesis will continue, thus providing information about the external light-dark cycle.

Tailoring of the melatonin profile by photoperiod is readily observed in species in which melatonin is elevated for the greater part of the night (Fig. 2A). Melatonin concentrations in the pineal (Siberian hamster) (*19*), cerebrospinal fluid (monkey) (*20*), and blood (monkey and sheep) (*18, 20*) increase with the onset of darkness and remain elevated until the onset of light. In general, variations in the duration of elevated melatonin accurately reflect changes in the duration of darkness over most of the range of natural day lengths.

A second means by which the pattern of melatonin production can be varied is typified by the rat and Syrian hamster, in which pineal melatonin begins to increase 3 hours or more after the onset of darkness (Fig. 2B) (*19*). These species are extremely sensitive to light during the early period of darkness, such that even a brief exposure to light 2 to 4 hours after the onset of darkness results in a further delay or total inhibition of the increase in melatonin (*15*). More important, the daily elevation in melatonin is restricted to the latter part of the dark period and usually lasts 6 to 8 hours, regardless of the duration of darkness; however, the timing of the onset of these elevations shifts with changes in the light-dark cycle (*21*). In contrast to the sheep, monkey, and Siberian hamster, then, the rat and Syrian hamster may monitor shifts in the phase of peak melatonin secretion to interpret day length.

Summary. There is a daily rhythm in the production of the pineal hormone melatonin in all mammalian species. Production is stimulated by darkness and inhibited by light. This provides a signal reflecting the changing environmental lighting cycle. In seasonally breeding mammals that use changes in the photoperiod to time their reproductive cycles, temporal signals to the reproductive system are controlled by the daily rhythm in melatonin production.

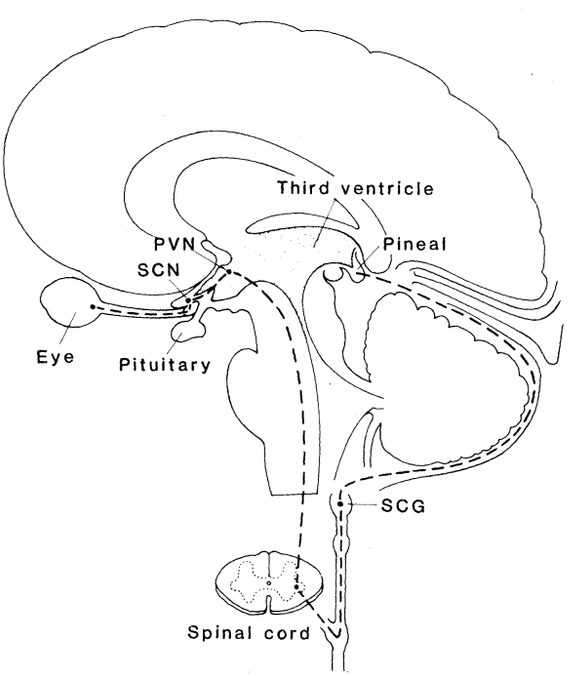

Fig. 1. Diagram of the human brain (midsaggital section), showing the neural pathway (dashed line) from the eye to the pineal gland. Abbreviations: SCN, suprachiasmatic nuclei; PVN, paraventricular nuclei; and SCG, superior cervical ganglia.

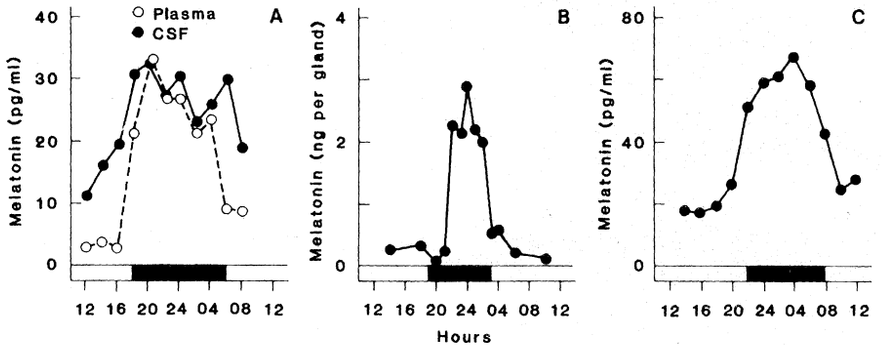

Fig. 2. Daily melatonin profile in (A) rhesus monkey plasma and cerebrospinal fluid (CSF), (B) rat pineal, and (C) human plasma. The dark bars represent the daily period of darkness (*19, 20*).

The melatonin profile in humans is shown in Fig. 2C. The effect of exposing humans to various durations of light and darkness has yet to be determined. However, some studies indicate that modulation of the amplitude of the melatonin signal may be a third important variable (*22, 23*).

In summary, the mammalian pineal generates a daily melatonin signal that can be altered by light in its duration, phase, and amplitude. A signal that can be modulated in these ways has the potential for synchronizing complex physiological processes in which temporal coordination is crucial. Recent data suggest that the pineal coordinates seasonal changes in body weight, coat color, and torpor in rodents independently of the reproductive system (*24*), but the temporal coordination of reproduction has been most extensively studied.

Seasonal Control of Reproduction

Many mammalian species from temperate and polar latitudes display annual cycles of fertility and infertility. These seasonal breeding cycles ensure that offspring are produced at a time that is optimal for survival. To time their breeding, mammals have adapted their physiology to respond to cues from the environment. The most frequently used cue is the annual change in day length or photoperiod. Seasonal mammals are generally classified as "long-day breeders" (for example, ferrets and some rodents) or "short-day breeders" (sheep and deer) depending on whether their gonads are activated as day lengths increase or decrease. The particular strategy is partly determined by the gestation period of each species, other aspects of their physiology, and the environment (*25, 26*).

The ability to mimic long and short photoperiods in the laboratory has helped our understanding of the endocrine and neural events underlying seasonal reproduction. It has been found that the endocrine events preceding gonadal activation are similar in long- and short-day breeders. Within a few days of exposure to a gonad-stimulating photoperiod, a series of events is initiated that results in decreased sensitivity to the negative feedback effects of gonadal steroids, followed by a gradual "turning on" of the pituitary and gonads. On the other hand, exposure to a gonad-inhibiting photoperiod results in increased sensitivity to negative feedback, followed by a reduction in pituitary activity and gonadal involution (*27*).

Experiments on the Syrian hamster, sheep, and mink indicate that there is a circadian basis to measurement of day length by mammals (*28, 29*). Of special relevance is the finding that lesions of the hypothalamic SCN render hamsters and sheep unable to measure day length so as to coordinate their breeding activity with the seasons (*30*).

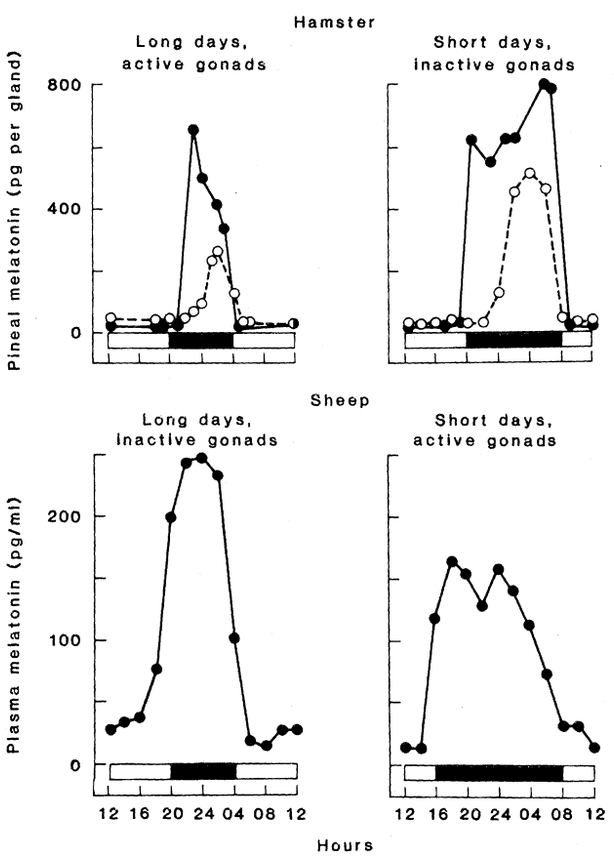

Fig. 3. Daily pineal melatonin profiles of Syrian hamsters (○) and Siberian hamsters (●) in short days and long days and daily plasma melatonin profiles of sheep in short days and long days (*19, 21*).

Role of Melatonin in Reproduction

We know that the daily melatonin rhythm closely reflects the lighting cycle and has the potential of imparting temporal cues to the body. We also know that the hypothalamic-pituitary-gonadal (HPG) axis varies in activity, showing temporally coordinated short- and long-term cycles. If the pineal serves as an interface between the environmental clock and the HPG axis, one would expect reproductive events to be accompanied by changes in the melatonin rhythm. Indeed, modulation of the amplitude of the melatonin signal may be a factor in regulating the ovarian cycle. The nocturnal peak of pineal melatonin in the rat is lowest on the day of estrus, and a nadir in morning plasma melatonin has been reported in women during the periovulatory period (*22, 31, 32*). [However, no change in the daily melatonin

profile has been observed during the ovarian cycles of hamsters or sheep (*33–35*).]

Are there seasonal changes in the daily melatonin profile? As discussed earlier, the duration, amplitude, and phase of the melatonin signal may change with exposure to different photoperiods. In some species, such as Siberian hamsters and sheep, melatonin is elevated for greater lengths of time during short days than on long days (Fig. 3) (*19, 34*). In one breed of sheep the total amount of melatonin secreted under long and short days is similar, with higher levels secreted at night under the former photoperiod (*36*). The Syrian hamster, on the other hand, shows a change in amplitude as well as a phase shift in its pineal melatonin profile when transferred from long to short days (Fig. 3) (*19, 21*). It is therefore difficult to say which features of the melatonin profile code for effects of photoperiod on reproduction. It is important to note, however, that patterns of melatonin production can be similar in short- and long-day breeders. Hence, it is unlikely that melatonin is an exclusively antigonadal hormone in seasonal breeders—an early claim that was based on the assumption that long-day breeders undergo gonadal regression during short days as a result of prolonged melatonin secretion; corresponding events in short-day breeders were not considered (*37*).

Two sets of data on sheep hint at the subtle way in which the melatonin profile influences reproduction. One experiment (*38*) provided evidence that the generation of circadian rhythms of melatonin may be required for measurement of day length by the reproductive system: normal gonadal responses were obtained only when the plasma melatonin rhythm was entrained to the light-dark cycle. The other experiment (*29*) showed that gonadal refractoriness to long and short days was accompanied by a loss in the temporal coordination between release of melatonin and the lighting cycle. The pattern of melatonin production thus appears to relay information on day length to the HPG axis.

The clearest evidence of a role for melatonin in the coordination of reproductive events comes from experiments in which parameters of the melatonin rhythm are altered. For example, melatonin rhythms have been abolished, masked, or changed in duration, amplitude, or phase.

In intact Syrian hamsters a single morning injection of melatonin given 2 hours after the onset of light for 8 weeks during long days had no effect on reproductive function, whereas in a similar study melatonin injected 4 hours before the onset of dark caused gonadal regression (*39, 40*). These findings suggest that melatonin administered at a sensitive time of day may provide a gonadal inhibitory signal similar to that induced by exposure to short days (*39, 40*).

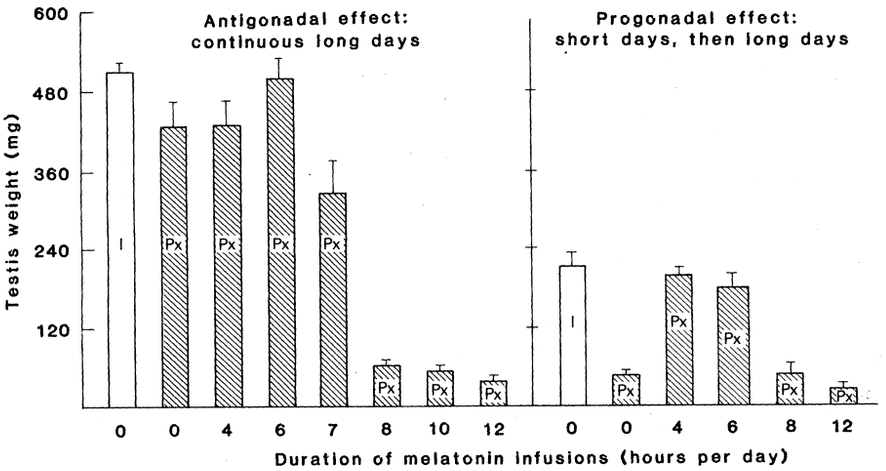

Fig. 4. Weights of testes from intact (I) or pinealectomized (Px) immature Siberian hamsters infused with melatonin for various durations. Antigonadal effects of 8- to 12-hour daily infusions were observed in animals held continuously in long days and progonadal effects of 4- and 6-hour daily infusions were observed in animals transferred from short days to long days (*42, 43*).

The above might suggest a critical time of sensitivity to melatonin; however, single daily injections of melatonin given to pinealectomized hamsters 4 hours before "lights off" failed to induce gonadal regression, suggesting that the exogenous melatonin in some way synergized or summated with the endogenous melatonin rhythm (*40*). A later study (*41*) in which three daily injections of melatonin spaced over 6 hours induced gonadal involution in pinealectomized animals suggested that the important feature might be the duration of elevated concentrations of melatonin in the circulation, since the response could be obtained independently of the lighting cycle or the time of day.

The last observation led Carter and Goldman (*42, 43*) to test whether the duration of elevated melatonin relays photoperiodic information to the reproductive system of the Siberian hamster (puberty in this species can be delayed by exposure to photoperiodic cycles with less than 8 hours of light per day). Pinealectomized juvenile hamsters were implanted with subcutaneous catheters through which melatonin could be infused for various periods. When melatonin was infused continuously for 8 hours or more each day, gonadal development was inhibited even though the animals were kept under a long photoperiod (*42*) (Fig. 4). The inverse experiment was also performed, in which gonadal development was stimulated by melatonin (a progonadal effect) in pinealectomized hamsters previously exposed to short days. In this study vehicle infusion during exposure to long days did not stimulate the gonads, whereas 4- or 6-hour infusions of melatonin did (Fig. 4). The effects of the melatonin infusions were obtained regardless of the time of day at which they were started or the doses used (*43*).

Support for the idea that durational changes in melatonin are the key to gonadal responses to photoperiod has also come from studies of sheep. Pinealectomized and ovariectomized ewes implanted with estradiol showed decreased sensitivity to the negative feedback effects of the steroid when infused with melatonin for 8 hours during exposure to otherwise gonad-inhibiting long days. Conversely, 16-hour infusions of melatonin during otherwise gonad-stimulating short days resulted in an increased sensitivity to the negative feedback effects of the steroid (*44*). Recently, estrous cyclicity was advanced in intact ewes held under natural day lengths and administered melatonin orally in their feed a few hours before the onset of darkness so as to extend the daily signal of melatonin (*45, 46*) (Fig. 5). Thus durational changes in melatonin may be sufficient to drive seasonal changes in reproduction.

Pinealectomy, Ganglionectomy, and Melatonin Implants

Appreciation that durational changes in the daily rhythm of melatonin secretion are critical in transducing the environmental lighting cycle were inferred

from studies in which this daily rhythm was eliminated. One approach has been pinealectomy, which removes melatonin from the circulation (*47*). When Syrian hamsters maintained under long days are pinealectomized and then exposed to short days, they fail to show gonadal regression (*48*). This result, and the observation that pinealectomy prevents the gonad-inhibiting effects of blinding or exposure to constant darkness, led to the concept that the pineal has solely antigonadal effects (*39*). The shortcomings of this interpretation were suggested when it was found that, under short days, hamsters first undergo gonadal regression but then show spontaneous gonadal recrudescence and are refractory to further exposure to short days. Exposure to long days is necessary to resensitize the animal to the gonad-inhibiting effect of short days (*39*). Thus, in pinealectomized hamsters, the gonads remain permanently active because of the lack of a melatonin signal to convey information about changes in day length.

A markedly different reproductive effect of pinealectomy is seen in the Turkish hamster, another long-day breeder. Pinealectomy performed during long or short days results in involution of the gonads, indicating that the pineal might be progonadal (*49*).

The effects of pinealectomy in other species are often more subtle. For example, pineal removal in ferrets does not produce constant ovarian cyclicity. Instead, individual ferrets followed for up to 2 years showed periods of estrus at infrequent intervals, estrus not being synchronized within the group or with any phase of the annual photoperiodic cycle. These experiments led to the conclusion that the pineal is necessary for coordinating the reproductive system with the photoperiod (*50*).

Early experiments in female sheep indicated that pinealectomy does not alter estrus cyclicity; however, the ewes were kept alongside intact rams, which could have indirectly provided photoperiodic synchrony through pheromones (*51*). Indeed, later studies have shown that, as in the ferret, pinealectomized sheep lose coordination between estrus cyclicity and photoperiod in the second year after pinealectomy (during the first year normal cyclicity occurs as though under the "inertia" of the preoperative exposure to long days (*52*). Rams respond differently to pinealectomy in that their gonads recrudesce and then remain permanently active (*53, 54*). The underlying cause of the different responses of rams and ewes to pinealectomy may be that the energetic limits on reproduction are usually imposed by the female of the species. On the other hand, the species differences in response to pinealectomy (permanently active ovaries in the Syrian hamster versus periodically active ovaries in ferrets and sheep) may reflect different reproductive strategies required by species having different life-spans (*50*).

These experiments all point to the importance of the pineal in the response of the HPG axis to changes in day length. Pinealectomy does not have any marked effects on nonphotoperiodic species, such as the laboratory rat (*55, 56*). Similarly, pinealectomy is apparently ineffective during photoindependent phases of the reproductive cycle of otherwise photoperiodic species. For example, puberty, which is photoindependent in the Syrian hamster, is unaltered by pinealectomy, whereas photodependent puberty in the Siberian hamster is delayed by pinealectomy (*57*).

Pinealectomy completely eliminates detectable levels of melatonin, while interruption of the neural input to the pineal by superior cervical ganglionectomy abolishes periodic melatonin secretion (*58*). In sheep, melatonin release still occurs but is no longer coordinated with the lighting cycle, and elevations occur randomly during both light and dark phases of the day, providing no consistent melatonin signal (*36*). The reproductive consequence of ganglionectomy in rams is like that of pinealectomy: permanent gonadal recrudescence that cannot be reversed by exposure to long days (*54*). The same procedure, when performed in male lambs exposed to natural day lengths, results in early testicular regression, followed by premature recrudescence (*59*). The observations in ganglionectomized male sheep suggest that these animals are unable to entrain their melatonin production to the environmental lighting cycle, rendering them unresponsive to photoperiodic challenges.

In early attempts to prove that melatonin is the active pineal compound inhibiting reproduction in long-day breeders, animals were treated with constant-release capsules (*25, 39*). Such treatment provided animals with more melatonin, but these supraphysiological levels of melatonin masked circadian changes in circulating melatonin. The results of the experiments were equivocal. For example, weasels implanted with melatonin underwent gonadal regression during stimulatory day lengths (*60*). However, in Syrian hamsters implanted with melatonin capsules and exposed to long days gonadal regression was induced in some studies but not in others (*25, 61, 62*). One consistent observation in this species is that melatonin implants block the gonad-inhibiting effects of short days (*62*).

Similar experiments on Siberian hamsters at first showed the implants to induce gonadal regression during long days and to delay gonadal recrudescence on transfer from short to long days. It was then noted that these animals had experienced the naturally decreasing day lengths of autumn before they received the implants and that the season in which implants were placed could influence the outcome of the experiment. Thus, melatonin implants placed during a period of maximum gonadal activity had no effect, and melatonin implants placed during exposure to short days prevented gonadal regression in a manner similar to that seen in the Syrian hamster (*25, 62*).

The results of the experiments described suggest neither a pro- nor an antigonadal role for the pineal and its melatonin. Rather, they indicate that in order to correctly interpret day length, animals must experience a daily rhythm in melatonin. The almost equivalent effects of pinealectomy, ganglionectomy, and melatonin implants on seasonal reproductive cycles stem from the fact that these procedures eliminate transduction of the external lighting cycle.

Melatonin and Puberty

Changes in the pattern of melatonin production around puberty have long been sought to prove Marburg's hypothesis that the pineal regulates the onset of that event. Data on melatonin levels during development in animals have failed to show a consistent correlation between the ontogeny of the daily melatonin rhythm and the onset of sexual development (*63–65*). Although many investigations of the relation between melatonin secretion and puberty have been conducted in humans, the data are still equivocal.

Single blood samples taken from prepubertal boys in the daytime have been reported to contain higher concentrations of melatonin than samples from prepubertal girls and adolescents; mean daytime concentrations greatly exceeded nocturnal levels found in adults (*66, 67*). In a recent study blood samples drawn from children at different stages of sexual development and from adults showed no significant differences in daytime concentrations of melatonin; however, nighttime concentrations in both sexes showed a significant inverse relation with sexual maturation and secretion of luteinizing hormone (*68*). These findings

do not demonstrate a causal relation, only a correlation, between melatonin concentrations and reproductive activity. In contrast, we did not find any significant differences between the 24-hour melatonin profiles (samples were collected at 2- or 3-hour intervals) of prepubertal, pubertal, and adult subjects (*67*).

Noninvasive techniques have also been used to estimate changes in melatonin secretion during development. Measurements of urinary melatonin have produced conflicting results (*69*), probably because less than 5 percent of the total melatonin secreted each day is excreted as nonmetabolized melatonin (*70*). The latter problem was overcome by assaying the principal metabolite of melatonin, conjugated 6-hydroxymelatonin—which accounts for more than 90 percent of daily melatonin excretion—in overnight urine samples. With this approach no significant changes were found in the concentrations of this metabolite in children between the ages of 3 and 16, except during breast development in girls, when there was a small increase (*71*). To date, clinical studies have focused on changes in the amount of melatonin secreted during sexual maturation, not on possible temporal changes in the melatonin rhythm. Consequently, the question of whether melatonin rhythms are altered during reproductive development remains open.

Sites and Mechanisms of Melatonin's Action on Reproduction

The experiments reviewed above provide convincing evidence that there is a cause-and-effect relation between pineal function and the coordination of reproduction in adults, at least in seasonal animals. But the target tissues involved and the means by which melatonin exerts its effect have yet to be identified.

Since melatonin is found in both cerebrospinal fluid and peripheral blood, it may exert its effects on central nervous system (CNS) or peripheral (nonneural) structures or both. Uptake of systemically administered tritiated melatonin in vivo indicates that the hormone might indeed act at both kinds of sites, since it was concentrated in the hypothalmus, hippocampus, anterior pituitary, gonads, reproductive tract, and even the pineal itself (*72*). Immunocytochemistry should allow a more precise demarcation of melatonin binding areas; for example, the SCN were shown by this method to concentrate more melatonin than any other hypothalamic area (*73*).

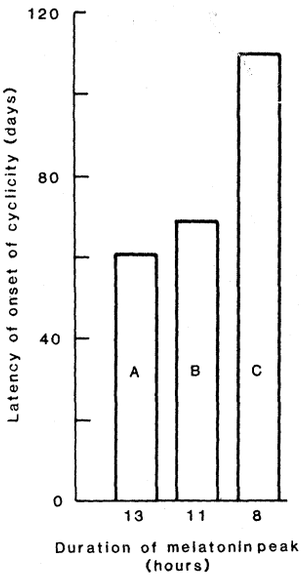

Fig. 5. Onset of estrous cycles in ewes kept in (A) long days and given melatonin in the afternoon, (B) short days, and (C) a natural lighting cycle. Duration of elevated plasma melatonin was estimated from the interval between the half-heights of the nocturnal peak (*46*).

Attempts have been made to quantify the number of melatonin receptors in various tissues by techniques in which labeled and unlabeled melatonin compete for putative binding sites. The results have been equivocal, and no method has been routinely used to investigate melatonin receptors. Demonstration of a melatonin receptor awaits the development of an appropriate high-affinity ligand.

The observed uptake of melatonin by peripheral tissues has led investigators to search for direct actions of melatonin on reproductive tissues. Effects of melatonin on steroidogenesis (*74*) and steroid receptors in uterine tissues have been reported (*75*). The mechanisms underlying these actions are not known. Another peripheral effect of melatonin is on anterior pituitary function in the developing rat. The response of rat gonadotrophs to stimulation by gonadotropin-releasing hormone (GnRH) was found to be blunted by treatment with melatonin in vivo and in vitro, suggesting that melatonin might alter the action of GnRH on gonadotrophs (*76*). Similar studies in other species, however, have not shown this effect (*77*).

Several lines of evidence suggest that the reproductive effects of melatonin are mediated by CNS sites. For example, increased sensitivity of the hypothalamus and pituitary to the feedback effects of gonadal steroids can be induced with melatonin in the absence of the gonads (*44*); melatonin induces a reduction in estrogen receptors in the brains of ovariectomized rats (*78*); manipulations that result in alterations in the melatonin profile (pinealectomy, superior cervical ganglionectomy, and melatonin replacement and supplementation) result in changes in the secretion of luteinizing and follicle-stimulating hormones that are controlled at the CNS level (*36, 79*); and changes in pituitary secretion of prolactin, which is also under hypothalamic control, accompanies changes in the concentration of circulating melatonin (*79–81*).

When melatonin is injected into the cerebral ventricles or implanted into the hypothalamus of the white-footed mouse in amounts small enough to ensure minimal diffusion, there is a modulation of gonadotropin secretion and gonadal activity (*82*). The possibility that these effects result from direct action of melatonin on the release of GnRH is supported by the finding that melatonin stimulates GnRH secretion from perfused hypothalami in vitro (*83*). Proposed mechanisms by which melatonin might modify the activity of GnRH-containing neurons include (i) alterations of the electrical activity of the neuron; (ii) impairment of contractile processes involved in the axonal transport of GnRH-containing granules; and (iii) changes in the synthesis and secretion of the catecholamines, monoamines, and prostaglandins, all of which are believed to regulate release of GnRH (*84*).

The central actions of melatonin on reproduction may be mediated through the hypothalamic SCN, which, as mentioned before, serve as a biological clock for a number of circadian rhythms. This is suggested by (i) a study in which gonadal regression in the white-footed mouse was greatest when crystalline melatonin was implanted in the vicinity of the SCN (*82*) and (ii) a study in which daily intrahypothalamic injections of melatonin induced gonadal regression in the white-footed mouse when administered in the afternoon but not in the morning (*85*).

The significance of [^{3}H]melatonin uptake by the pineal is unclear. Such uptake might be a regulatory feedback mechanism. Melatonin injections in ferrets alter the pineal content of serotonin, a precursor of melatonin (*50*) [but injections of melatonin in the Syrian hamster do not alter pineal melatonin (*86*)]. Several peptides that appear to modulate gonadotropin secretion in vivo have been isolated from the pineal, and it has been suggested that melatonin might serve as a local hormone regulating the release of one or more of these (*87*).

The traditional endocrinological view that hormones have either a stimulatory or an inhibitory role does not seem to apply to melatonin. Indeed, expectations of such clear-cut effects has impeded understanding of the role of melatonin in reproduction for nearly two decades. Melatonin cannot be considered pro- or antigonadal; rather, it relays temporal information to the HPG axis, thereby coordinating reproductive activities.

A role for melatonin in reproductive physiology is now established; however, its site and mechanism of action remain unknown. Any proposed mechanism of the action of melatonin must take into account its periodic appearance in the circulation—leading to the question of how periodic signals are processed. The mechanism by which the information from the daily signal is summated and processed over periods of weeks and months also remains to be answered.

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