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Melatonin and the pineal gland: influence on mammalian seasonal and circadian physiology

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The pineal hormone melatonin is secreted with a marked circadian rhythm. Normally, maximum production occurs during the dark phase of the day and the duration of secretion reflects the duration of the night. The changing profile of secretion as a function of daylength conveys photoperiodic information for the organization of seasonal rhythms in mammals. The role of melatonin in mammalian circadian physiology is less clear. However, exogenous melatonin can phase shift, and in some cases entrain, circadian rhythms in rodents and humans. It can also lower body temperature and induce transient sleepiness. These properties indicate that melatonin can be used therapeutically in circadian rhythm disorder. Successful outcomes have been reported, for example in jet lag and shift work, and with cyclic sleep disorder of some blind subjects. Melatonin receptors of several subtypes are found in the brain, the retina, the pituitary and elsewhere. They are currently under intense investigation. Melatonin agonists and antagonists are under development.

The pineal gland appears to serve the same function in all mammals studied to date. The pattern of secretion of its major hormone, melatonin, conveys information concerning light–dark cycles to the body physiology for the organization of seasonal and circadian rhythms (Arendt, 1995). Recently, much progress has been made in identifying, localizing and characterizing melatonin receptors. This review provides a brief summary of the important features of the production and effects of melatonin that impinge upon its circadian physiology and seasonal functions and that have proved of use in the general study of biological rhythms. Wherever possible, broad reference is made to reviews rather than the original literature. Where no citation is given to publications before 1994, references can be found in Arendt (1995) and other cited reviews.

The pineal gland is part of the visual system and mammalian pinealocytes are derived evolutionarily from the pineal photoreceptors of lower vertebrates. The influence of the pineal gland on the circadian system appears to be more important in lower vertebrates than in mammals. In some reptiles and birds, the pineal appears to act as a central circadian rhythm generator. In house sparrows (*Passer domesticus*), pinealectomy leads to arrhythmicity which can be restored by transplanting a pineal from another bird (Menaker *et al*., 1981). The circadian phase of the donor bird is conveyed to the host with the transplant. It is possible to culture pineal explants and dispersed pineal cells from fish, lizards and birds, and these preparations retain their circadian melatonin production *in vitro*. In contrast, the mammalian pineal does not retain endogenous rhythmicity in culture.

The retinae of lower vertebrates also generate melatonin rhythms in culture and the hamster retina (maintained at low temperature; Tosini and Menaker, 1996) can show the same phenomenon, which suggests that there may be a circadian pacemaker in the mammalian eye.

In contrast to reptiles and birds, pinealectomy in mammals has rather more subtle effects on the circadian system. For example, the rate of resynchronization of rats to a phase shift of the light–dark cycle is faster in pinealectomized than in intact animals, and pinealectomy leads to disrupted circadian rhythms in rats kept in constant light (Armstrong, 1989; Cassone, 1992). The pineal, and indeed melatonin secretion, cannot be regarded as essential in the adult mammalian circadian system given that, in their absence, virtually normal function is maintained.

In mammals, the ability to respond to changing artificial daylength in terms of seasonal functions is abolished by pinealectomy or denervation of the gland. In long-lived species, such as sheep and ferrets, pinealectomy leads to desynchronization of seasonal rhythms in reproductive function from the annual periodicity, which suggests that the pineal essentially synchronizes the endogenous cycle to a yearly periodicity.

Production of melatonin

Melatonin is synthesized within the pineal gland itself, in the retina and possibly in some other sites. However, in mammals, most if not all of the hormone reaching peripheral sites is derived from the pineal, and pinealectomy leads to a great reduction, in most cases to undetectable concentrations, of circulating melatonin. Melatonin is synthesized from tryptophan via 5-hydroxylation by tryptophan-5-hydroxylase to 5-hydroxytryptophan, decarboxylation by aromatic amino-acid decarboxylase to 5-hydroxytryptamine (serotonin), *N*-acetylation by *N*-acetyl transferase to *N*-acetylserotonin (NAT) and *O*-methylation by hydroxyindole-*O*-methyltransferase (HIOMT) to melatonin (*N*-acetyl-5-methoxytryptamine) (Fig. 1). The cardinal feature of this synthetic pathway is its rhythmicity. The activity of the enzyme NAT in particular increases from 30-fold to 70-fold at night and, in most circumstances, is rate-limiting in melatonin synthesis (Klein *et al*., 1997).

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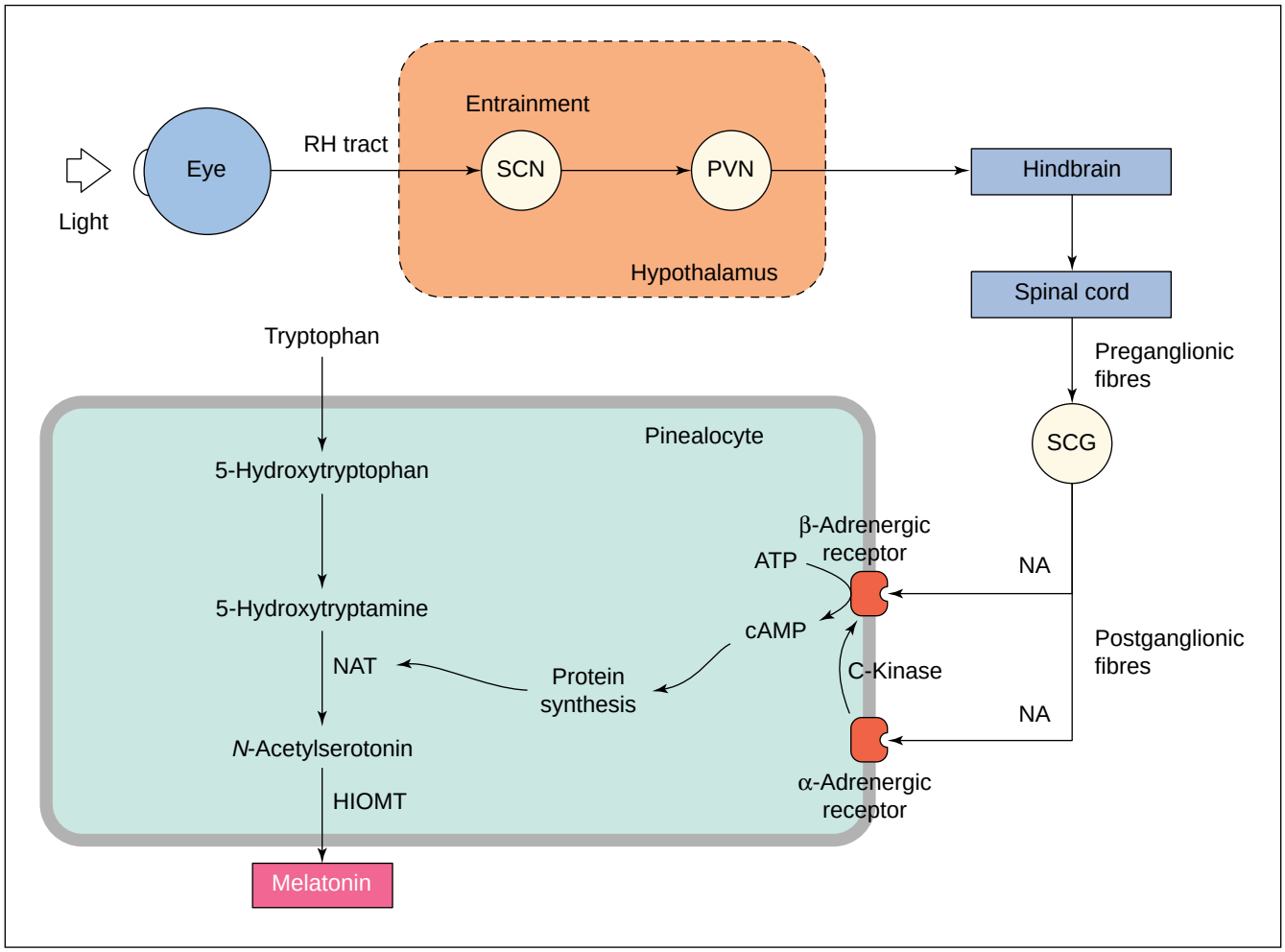


Fig. 1. Diagram of the major controlling mechanisms in melatonin synthesis. The rhythm is generated in the suprachiasmatic nucleus (SCN), entrained by light via the retino–hypothalamic pathway (RH tract). The signal passes via the paraventricular nucleus (PVN, albeit with some controversy), hindbrain, spinal cord, superior cervical ganglion (SCG) to pineal noradrenergic (NA) receptors. Serotonin *N*-acetyltransferase (NAT), in most circumstances the rate-limiting enzyme in melatonin synthesis, increases 30–70-fold during the dark phase through noradrenergic stimulation. HIOMT, hydroxyindole-*O*-methyltransferase.

The pineal is innervated primarily from a peripheral sympathetic tract arising in the superior cervical ganglion, and there is some evidence for direct central innervation. Sectioning the sympathetic nerve supply abolishes melatonin rhythmicity and mimics the effects of pinealectomy. The endogenous circadian rhythm of melatonin, like most other circadian rhythms, is generated in the suprachiasmatic nuclei (SCN) and entrained principally by the light–dark cycle acting via the retino–hypothalamic tract, probably with a contribution from the lateral geniculate nucleus (Fig. 1).

Sympathetic pineal input terminates in adrenoceptors, characterized as beta 1 and alpha 1 in rodents and humans, with a recent report of alpha-2D adrenoceptors being present in rodents and bovines. There is good evidence that the control of melatonin secretion in humans is similar to that of rats. In rats, it is clear that beta-adrenoceptor stimulation of pineal activity is potentiated by concomitant α-adrenoceptor stimulation. The transmitter is noradrenaline. The events surrounding pineal adrenoceptor stimulation have been used extensively to characterize β- and α-adrenoceptor function.

Human melatonin rhythms and reproductive function

In humans, the melatonin rhythm in either plasma or saliva is arguably the best marker of the phase of the endogenous 'biological clock', principally because it is demonstrably controlled by the SCN and because very few 'masking' factors influence its production. Only light suppression grossly masks the rhythm (Fig. 2). Furthermore, the melatonin rhythm is coupled tightly to the core temperature rhythm, known to be a good circadian rhythm marker, with the peak of melatonin secretion corresponding closely to the nadir of temperature (Fig. 2). In the same individual, it is highly reproducible in amplitude, details of profile and in timing in a synchronized environment, almost like a hormonal fingerprint (Fig. 2). This means that small differences can be highly

significant. Between individuals there are large differences in amplitude.

The disadvantage of plasma melatonin is that only 'snapshots' can be taken of evolving circadian status in order to avoid unacceptable blood loss. In humans, the use of saliva avoids this, but brings with it the problem that it can only be collected during the night by waking the subject. In view of the non-invasive nature of sampling, the major urinary melatonin metabolite 6-sulfatoxymelatonin (aMT6s) is especially useful for long term and field studies. aMT6s reflects accurately both the quantitative and qualitative aspects of melatonin secretion, albeit with some loss of detail. Abnormal melatonin profiles have rarely been reported in clinical studies. One problem with many clinical observations is the lack of control of environmental and postural variables, and much work needs reassessment with this in mind.

The onset of a circadian melatonin rhythm with peak values at night is established by 9 months of age in humans. Secretion reaches a lifetime peak between 3 and 5 years, subsequently declining to adult amounts by 15–18 years. Amplitude remains relatively stable until old age, when a marked decline is reported in most studies. Low amplitude in old age may be related to general lack of robustness of the circadian system. Whether or not the declining plasma concentrations during puberty have any causal role in pubertal development remains to be proven. However, evidence is accumulating for a role of melatonin in human reproduction. In precocious puberty, amplitude is high for age, and in delayed puberty, amplitude is low for age. Moreover, hypothalamic amenorrhoea is associated with high melatonin concentrations and studies in Finland have shown high daytime melatonin associated with anovulatory cycles (references in Arendt, 1995). Very large doses of melatonin (80–300 mg) have been shown variously to suppress LH and, in combination with norethistrone (an oral contraceptive minipill), to suppress ovulation when given at night, to increase the amplitude of LH pulses when given in the morning and to potentiate testosterone-induced suppression of LH when given in the late afternoon (references in Arendt, 1995).

Light suppression and entrainment of melatonin secretion

For a given period of darkness, melatonin is produced during the dark phase (Fig. 3) and the duration of its secretion is dictated by the duration of darkness up to a defined duration of secretion which is species dependent. In sheep, the duration of melatonin secretion extends to approximately 16 h in 8 h:16 h light:dark (but does not expand further in dark:dark) and retracts to 8 h in 16 h:8 h light:dark, whereas in hamsters, while the duration of secretion positively reflects the duration of darkness, it occupies rather less of the dark phase than in sheep (Arendt, 1986).

Light of sufficient duration and intensity suppresses nocturnal melatonin production. The amount of light required is dependent on both species and photoperiodic environment. For example, in some laboratory-raised animals less light is needed than in the same species raised in the wild. Humans require 2500 lux for complete suppression, although partial suppression can be observed with as little as 100–300 lux (Fig. 2c).

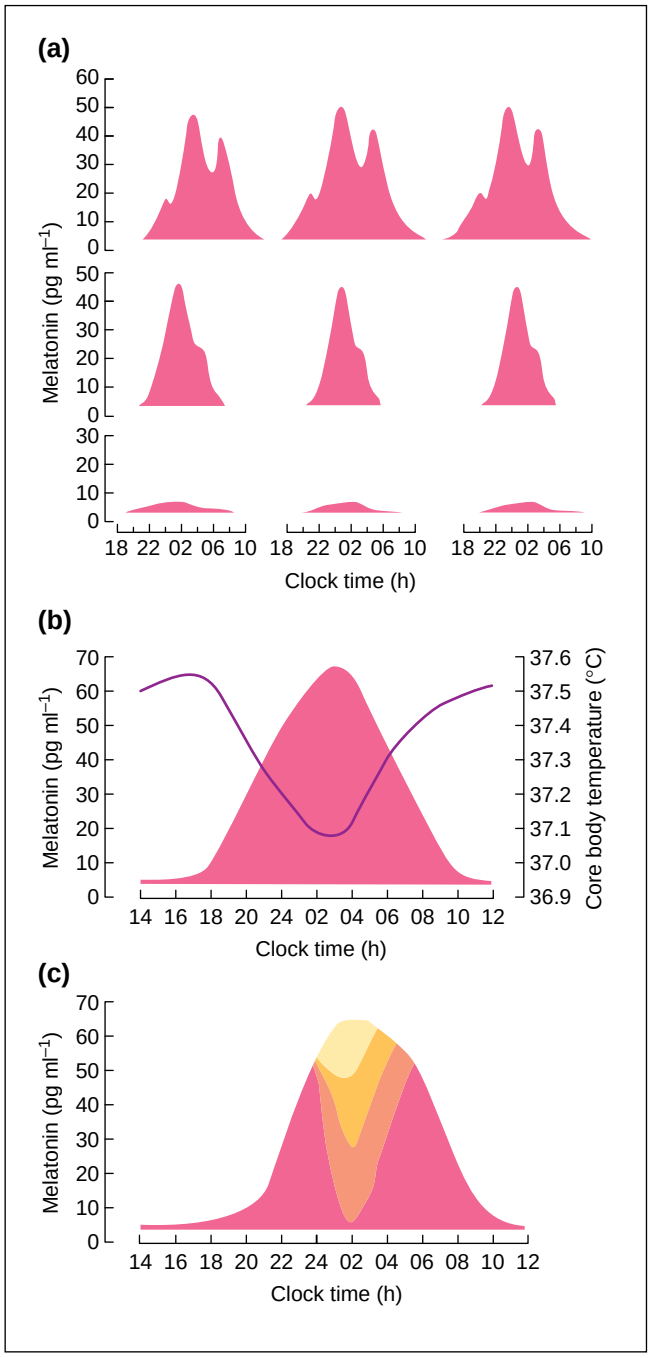


Fig. 2. Diagrammatic representation of some examples of human melatonin secretion profiles (■). (a) two major peaks (top), a peak with a shoulder (middle), a low amplitude unimodal profile (bottom). Note that individual profiles are very reproducible but that there are major individual differences in both amplitude and pattern of secretion. (b) The melatonin and core temperature rhythms (—) are closely coupled, the nadir of core temperature occurs within approximately 1 h of the peak of melatonin. (c) Bright light is required to suppress melatonin completely at night. The effect on plasma melatonin of three increasing light intensities (■, ■, ■) given from 24:00 h to 02:00 h is shown. Full suppression is usually found with 2500 lux white light, partial suppression has been observed with as little as 100 lux, with substantial individual variations in sensitivity.

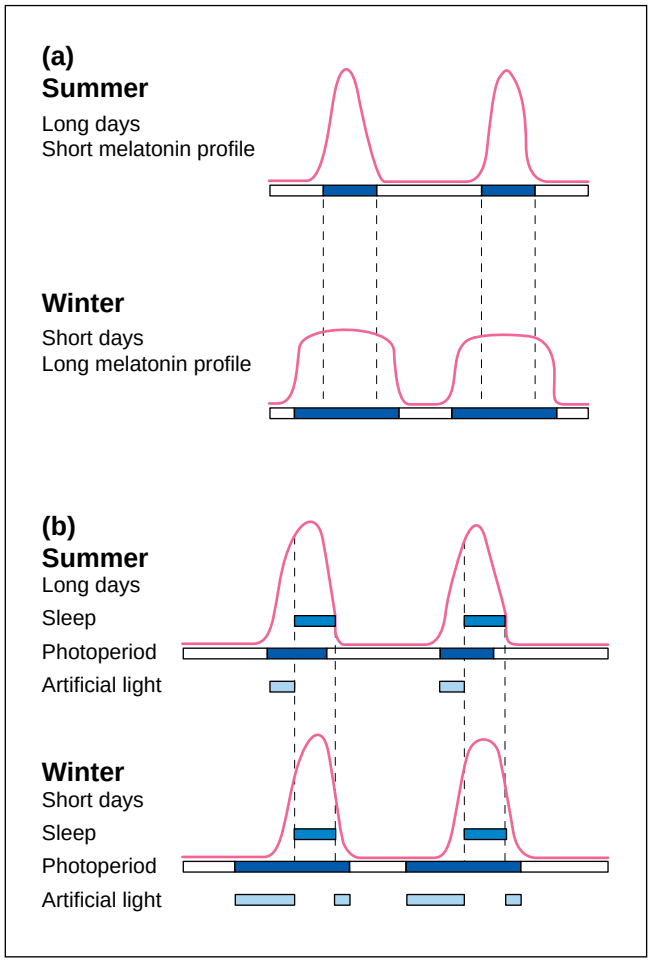


Fig. 3. Duration of melatonin secretion response to photoperiod change in sheep (a) and humans (b). ■, darkness; ■, sleep; ■, artificial light (humans). Note that humans rarely show duration changes unless living in artificial photoperiods with total darkness for long periods. A small phase delay is usually seen in winter.

There are very large individual variations. Elucidating this human requirement for bright light has proved to be of fundamental importance in our understanding of human circadian and photoperiodic physiology.

The phase of the free-running melatonin rhythm can be reset by a single light pulse, with the magnitude and direction of shift being dependent on the circadian time at which light is applied. Phase delays are induced by light exposure in the late subjective day and early subjective night whereas phase advances follow light in the middle-to-late subjective night and early subjective day. The direction and magnitude of shift in response to light is summarized as the phase response curve (PRC). Such light-induced phase shifts are being exploited to aid adaptation to shift work, to time zone change and in pathology (Eastman *et al.*, 1995). It is possible that they depend partly on suppression of melatonin production for their efficiency but this remains to be proven.

The change in duration of melatonin secretion as a function of duration of night is found in the vast majority of species studied, with the possible exception of domestic pigs. Usually, the only seasonal change in human melatonin is in phase, with delayed phase being characteristic of winter in normal healthy individuals. Two daily light pulses of 2500 lux given as a skeleton spring photoperiod in the dim light conditions of winter in the Antarctic will phase-advance the delayed melatonin to a summer phase position (Broadway *et al.*, 1987). Thus, the elements of a photoperiodic response remain in human physiology. If humans are kept for 2 months in 8 h:16 h light: dark, where the 16 h of darkness are completely dark, it is possible to show an increase in the duration of melatonin secretion compared with the same individuals kept for 2 months in 14 h:10 h light: dark (Wehr, 1991).

The clearest abnormalities in melatonin secretion are observed in blind subjects with no light perception (NLP) and in circadian dysrhythmia during adaptation to phase shift. Several groups have described both free running rhythms and entrained but abnormally phased rhythms of melatonin and other variables in the blind (see references in Lockley *et al.*, 1997). Czeisler *et al.* (1995) have shown that, in some patients with no light perception, circadian entrainment is possible and that this is linked to the ability of light to suppress melatonin in these individuals. This phenomenon of 'hypothalamic light perception' is known in animals in which the retinohypothalamic projection is intact but the primary and accessory optic tracts have been sectioned. The nature of the photoreceptors concerned with circadian responses is as yet unknown but is under intense investigation. Free-running blind people attempting to live on a 24 h day suffer periodic sleep disturbance and this occurs mostly when the melatonin rhythm, and indeed other endogenous circadian rhythms, are 180° out of phase with the normal cycle.

Role of melatonin in seasonal cycles

The duration of melatonin secretion is the critical parameter signalling daylength for the organization of seasonal rhythms. Infusion of appropriate duration profiles of physiological concentrations of melatonin to pinealectomized hamsters and sheep has shown the critical role of melatonin duration in signalling daylength. In the short-day breeding sheep, the daily infusion of 'long-duration' melatonin to simulate long nights (short days) is inductive to reproductive activity. In contrast, in the long-day breeding hamster, such infusions are inhibitory and short-duration melatonin is inductive. The frequency of melatonin infusions does not appear to be critical over periodicities ranging from 20 to 26 h but, for an infusion to be read efficiently, it appears that it must be perceived as a single block (although there is some recent controversy on this point). For example, two daily infusions of 4 h and 4 h separated by a 2 h gap in hamsters are read as 4 h not 8 h whereas, if the gap is sufficiently small, the two periods are perceived as 8 h. In addition, photoperiodic history is important in the interpretation of any melatonin duration signal. For example, transferring hamsters from 8 h:16 h light:dark to 12 h:12 h light:dark leads to perception of 12 h:12 h light:dark as a long day, whereas transferring from 16 h:8 h light:dark to 12 h:12 h light:dark leads to perception of 12 h:12 h light:dark as a short day (Hastings *et al.*, 1989).

Karsch's group have provided evidence in pinealectomized sheep that the seasonal breeding cycle is an endogenous annual

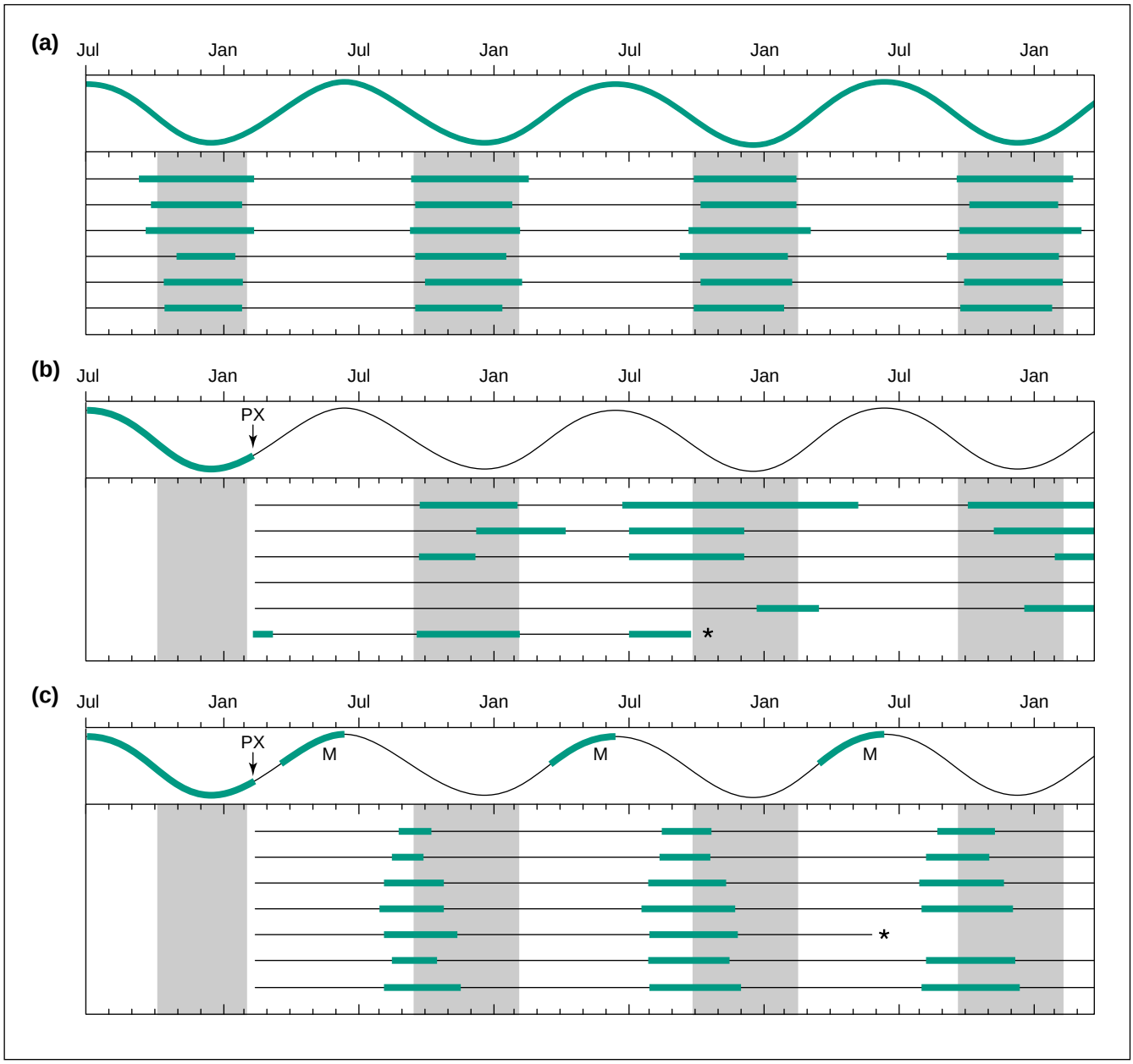


Fig. 4. Synchronization of the breeding cycle in pinealectomized sheep by a 70 day block of daily 8 h infusions (long-day signal) in spring from data published by Woodfill *et al.* (1994), kindly supplied by F. Karsch, University of Michigan. Three groups of sheep are shown: (a) pineal intact controls; (b) pinealectomized animals (note the desynchronization of the breeding season from the annual cycle); (c) pinealectomized and infused daily for 70 days with a long-day melatonin profile (note the synchronization of reproductive activity). ▬, Increase of LH, representative of the normal breeding season. This long-day signal is sufficient to set the whole annual cycle in the absence of other photoperiodic information from melatonin secretion. PX, pinealectomy.

rhythm that desynchronizes from 365 days in the absence of the pineal. It can be resynchronized by a single block of 70 consecutive days of long-day melatonin infusions (Woodfill *et al.*, 1994; Fig. 4). Thus, the long days of spring are presumably both necessary and sufficient to cue the entire annual cycle in the absence of other seasonal time cues. Long days are similarly essential for the appropriate timing of pubertal development in sheep.

In ruminants, it is possible to create a winter duration melatonin profile in animals on a natural or artificial summer photoperiod by feeding 3–5 mg melatonin adsorbed onto a food pellet 5–6 h before onset of darkness (Arendt, 1986). If animals are fed daily from midsummer in this way, they respond as if exposed to winter photoperiod: that is, by early onset of seasonal reproductive function, winter coat growth and the suppression of prolactin secretion as found in short winter days (Arendt,

1986). Melatonin mimics the winter photoperiod. The use of continuous release subcutaneous implants or oral soluble glass bolus preparations (retained in the rumen) leads to increased melatonin concentrations throughout the light–dark cycle. During summer, this is read by body physiology as a 'super short' day and seasonal functions are modified appropriately. For agricultural purposes, this is a practical approach to induction of early breeding: for early lamb production or manipulation of other commercially important animal products such as winter pelage and milk production. However, it is necessary for the animal to experience a period of long days before exogenous melatonin will induce a short-day effect. This phenomenon is known as refractoriness and is common to photoperiodic responses in many species. Refractoriness is broken either by the natural long days of spring or by treatment with artificial long days early in the year.

Commercial preparations of melatonin for use in agriculture for the manipulation of seasonal breeding in sheep have been developed, and at least one is registered and available in a number of countries including Australia and the UK. The long-term results of its use have yet to be fully evaluated.

Effects of melatonin on circadian rhythms

The relationship of the pineal to the circadian system in mammals has already been referred to as modulatory. It is difficult to make a case for a major role of the pineal or melatonin in mammalian circadian control. Nevertheless, there are effects of melatonin on mammalian circadian rhythms that are proving of considerable therapeutic use.

In rats free running in dark:dark, daily melatonin injections (5 μg–1 mg) can resynchronize activity–rest cycles to 24 h when the injection time approaches the time of onset of free-running activity (Armstrong, 1989). In rats exhibiting a persistent phase delay of activity–rest cycles in entrained conditions, melatonin will phase advance the rhythm and, after a forced 8 h advance phase shift, given at the original dark onset, it can dictate the direction of re-entrainment. The PRC of activity–rest to melatonin injections in rats shows only phase advances. Similar effects have been reported in hamsters, although vehicle injections can entrain by 'arousal'. However, there is clear evidence for entrainment of hamster pups by prenatal melatonin treatment.

In humans, both pharmacological and physiological doses of melatonin can induce phase shifts. Early work suggested that 2 mg melatonin given daily in the late afternoon (17:00 h) for a period of one month was sufficient to induce a phase advance of the endogenous melatonin rhythm of 1–3 h (when the endogenous and exogenous components could be distinguished) (Arendt *et al*., 1985). This was accompanied by an earlier onset of evening fatigue or sleep and also an earlier timing of the prolactin rhythm. There were no effects on other anterior pituitary hormones. These observations suggested that melatonin had 'chronobiotic' effects on the human circadian system. An acute sleep-inducing effect and modification of the EEG by pharmacological doses of melatonin has been known since the early 1970s. There is now evidence that melatonin shifts sleep time in delayed sleep phase insomnia, that low dose (5 mg) melatonin increases sleep propensity in a time-dependent manner, with an increase in subjective fatigue after acute administration of physiological amounts of melatonin (< 1 mg) and that there are acute, dose-dependent advances in sleep timing over the dose range 0.05–5.0 mg (for a review see Arendt, 1997).

Melatonin will also induce phase delays in humans when given in circadian early morning, and several human PRCs have been described (Fig. 5). The melatonin PRC approximately mirrors the light PRC, lending further credence to the concept of melatonin as a darkness hormone with respect to the circadian system as well as in photoperiodism. Given the PRCs to light and melatonin, and the general tendency of human circadian rhythms to delay, it is possible that early morning light resets the endogenous clock daily by phase advance, and the evening melatonin increase may reinforce this effect. Thus, it is possible to propose a physiological role for the pineal and melatonin in circadian organization in mammals.

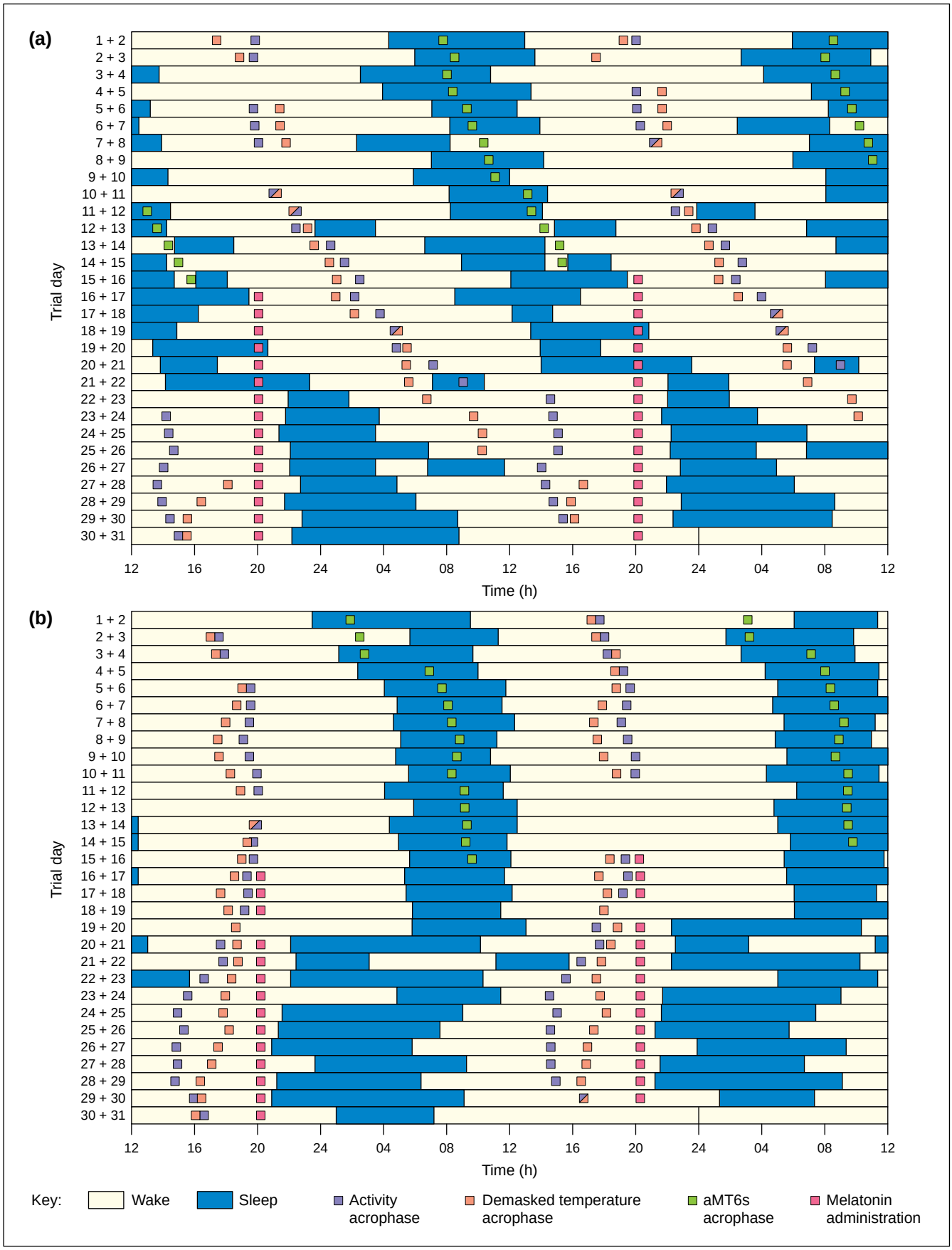
Whether or not melatonin can entrain all free-running rhythms remains doubtful (Middleton *et al*., 1997). In the case of some blind subjects, melatonin clearly stabilizes sleep–wake cycles to 24 h; however, simultaneous entrainment of other rhythms has only been reported in one case. Nevertheless, when melatonin is used in concert with other time cues to hasten adaptation to phase shift, it clearly affects endogenous variables, such as core temperature, and cortisol and endogenous melatonin, as well as the sleep–wake cycle.

In humans, the nadir of core temperature occurs within 1 h of the peak of melatonin together with the peak of fatigue and the trough in ability to perform certain tasks (reflected in high night-time accident rates in night shift workers), and this association may in part be causal. Melatonin (0.5–5.0 mg) acutely suppresses core body temperature and Cagnacci *et al.* (1992) have calculated that possibly 40% of the amplitude of the rhythm in core body temperature may be due to night-time melatonin secretion. The acute suppression induced by a single dose of exogenous melatonin is dose-dependent, is accompanied by decreased alertness, and correlates closely with the acute phase advance induced when given at 17:00 h (Deacon and Arendt, 1995). Importantly, bright light increases body temperature and alertness when given at night, and this can be reversed by melatonin. Whether the changes in core body temperature are relevant to phase-shifting mechanisms remains doubtful.

Target sites of melatonin for the control of seasonal rhythms

The target sites of melatonin are currently under intense investigation. Lesions of the mediobasal hypothalamic area can block photoperiodic effects of melatonin on gonadotrophins (Lincoln, 1992; Maywood and Hastings, 1995). Implants of

Fig. 5. Two individual subjects showing phase delay (a) and phase advance (b) of the sleep–wake cycle after administration of melatonin (5 mg day^{-1}) at 20:00 h from day 16. Placebo (identical capsule with lactose filler) was given at the same time on days 1–15. (Redrawn from Middleton *et al*., 1997, by permission.)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Melatonin administration

melatonin in the hypothalamus suppress LH release in rats. Implants or infusion of melatonin in the mediobasal hypothalamus mimic or block photoperiodic responses in several species (for example, Lincoln, 1992; Maywood and Hastings, 1995). In prepubertal rats, melatonin inhibits LHRH-induced LH release in pituitary cultures at concentrations comparable with those circulating in the blood. There is also evidence that melatonin influences LHRH secretion from the hypothalamus in co-cultures of median eminence and pars tuberalis.

Lincoln and Clarke (1994) have investigated seasonal rhythms in hypothalamic–pituitary-disconnected Soay rams, animals in which the pars tuberalis and pituitary are intact but have no input from hypothalamic neurohormones and transmitters. In such conditions, the tonic inhibitory and presumably dopaminergic control of prolactin secretion is abolished, as is the circadian rhythm. Nevertheless, the well-characterized seasonal variations in prolactin concentrations persist and respond to both changing artificial daylength and melatonin administration. The inference is that melatonin acts at the pituitary for this particular seasonal variation via pars tuberalis melatonin receptors. However, the gonadotrophic hormones do not respond and clearly there is scope for seasonal effects at the hypothalamus as well as at the pituitary.

The development of 2-[^{125}I]iodomelatonin as a high specific activity ligand has permitted the identification of high affinity (K_d value 25–175 pmol l^{-1}), saturable, specific and reversible melatonin binding to cell membranes in the central nervous system, initially in the SCN and the pars tuberalis of the pituitary (for review see Morgan and Williams, 1989). Both peripheral and central high-affinity binding sites for melatonin are found using 2-iodomelatonin as ligand. The most consistent binding site is within the pars tuberalis of the pituitary gland, but with frequent binding in the mediobasal hypothalamus, the retina, the SCN and many other brain sites. There are changes in detectable binding with age; for example, in fetal rats, the first appearance of binding is in the pars distalis and pars tuberalis of the pituitary, with SCN labelling appearing in later gestation. Pars distalis binding is absent in adult rats but persists after birth in the neonate. This suggests that such binding may indeed underlie function since melatonin will inhibit GnRH-induced pituitary LH release in prepuberty but not in adulthood.

Target sites of melatonin influencing circadian rhythms

The most obvious target tissue for the actions of melatonin on the circadian system is the SCN and, in view of its effects on the mechanisms of light transduction (Iuvone and Gan, 1995), receptors in the retina may also be of importance in circadian light perception. SCN-lesioned rats do not respond to melatonin by restoration of activity–rest cycles, although since the SCN itself is essential for generation and expression of activity–rest rhythms this experiment is susceptible to different interpretations.

The SCN shows clear melatonin binding in human post-mortem tissue. The most convincing evidence that the SCN is a target site for circadian effects of melatonin has derived from SCN-containing hypothalamic slice preparations *in vitro*. It is possible to show that the 24 h rhythm of electrical activity persists *in vitro* for several cycles and is phased in relation to the donor's previous light–dark cycle. Physiological concentrations of melatonin added to the cultures at different circadian times induce substantial phase advances according to a phase–response curve resembling that seen in intact animals using activity–rest as a circadian marker rhythm (Gillette and McArthur, 1996).

The amphibian melatonin receptor was cloned in 1994, and cloning of the sheep and human receptors was reported shortly thereafter (Reppert *et al*., 1994) with high structural similarity (80%) between sheep and human clones. Three subtypes are reported: Mel 1a, 1b and 1c. *In situ* hybridization studies in several mammals have shown signals in both the pars tuberalis and the SCN, and the pharmacology of the recombinant receptors is identical to the endogenous G-protein-linked receptor. The Mel 1b receptor does not appear to be necessary for photoperiodic responses in hamsters (Reppert, 1996).

These receptors are members of a new receptor group that is distinct from other G-protein-linked groups. This work opens large new perspectives and approaches not only for the study of the mechanism of action of melatonin but also for the development of new molecules for therapeutic use. Initial studies using 'knockout' mice genetically engineered to be devoid of Mel 1a receptors suggest that phase shifts of the SCN to melatonin *in vitro* are retained but that the ability of melatonin to suppress SCN neural activity is abolished (Liu *et al*., 1997). Thus, Mel 1a is implicated in inhibition of SCN activity with the possibility that Mel 1b (present in barely detectable amounts in these animals) is the phase shifting receptor.

Therapeutic uses of melatonin

Circadian rhythm disorder affects a very large number of people, including many shift workers and individuals suffering from jet lag, blindness, insomnia and other problems of old age, some psychiatric disorders and conditions where natural zeitgebers are very weak (for example dim light in high latitude winters). Circadian rhythm disturbance may also accompany many other conditions as a secondary effect.

The problems of shift work, where individuals are required to work during the low point of performance and high point of fatigue, and to sleep at an inappropriate circadian time are of real economic importance. Moreover, the health problems of shift workers include sleep disorder, gastrointestinal disturbance and increased susceptibility to cardiovascular disease. All of these may be related to rhythm disruption. In some respects shift work and jet lag are similar, in others they are not. For example night shift workers operate throughout their night shift counter to the natural zeitgebers whereas time zone travellers adapt with the help of the environment. The use of increased light intensity at night (> 1000 lux) and specified phase shifting light regimens is proving to be successful as an aid to adaptation and improved efficiency. However, not all circumstances allow the use of bright light: it is expensive to install and maintain and its potential deleterious effects on the eyes remains fully to be assessed. The use of melatonin as a phase shifting mechanism offers a convenient alternative. The combined use of timed melatonin and bright light is likely to provide optimum phase shifting conditions.

Early work in a placebo controlled study indicated that, over an advance phase shift of eight time zones, suitably timed melatonin (5 mg) significantly improved night sleep latency and quality, daytime alertness and hastened the resynchronization of endogenous melatonin and cortisol rhythms. Most subsequent work has confirmed the behavioural effects in field studies and the circadian re-entrainment in simulation studies. The timing of melatonin treatment for westward time zone transitions poses problems over fewer than eight time zones as, according to the PRC, it should be taken in the middle of the night. Moreover, preflight early morning treatment to initiate a phase delay can lead to loss of alertness at a very inappropriate time of day. In the author's experiments, melatonin is not given preflight but is taken at bedtime after westward flight for 4 days. To date, with 474 people taking melatonin and 112 taking placebo, the overall reduction in perceived jetlag (visual analogue scale, 100 = very bad jetlag, 0 = negligible jetlag) over all time zones and in both directions is 50%. Included in these figures are both placebo controlled and uncontrolled studies, between which no difference was observed. Side effects reported more than once are (melatonin–placebo): sleepiness (8.3–1.8%), headache (1.7–2.7%), nausea (0.8–0.9%), giddiness (0.6–0%), and light-headedness (0.8–0%).

Melatonin has been used in two small controlled studies of night shift workers, compared with both placebo and no treatment, and timed to phase delay by administration after the night shift and before daytime sleep (Folkard *et al.*, 1993; Sack *et al.*, 1994). It was successful in improving night shift alertness, sleep duration and quality in one study and improved circadian adaptation assessed by endogenous melatonin production in the other: clearly, more data are required, particularly about the effect of melatonin on work-related performance.

We have developed a means of simulating time-zone transition or shift work without environmental isolation using a combination of timed moderately bright light (1200 lux, 9 h followed by 8 h imposed darkness or sleep) delaying or advancing by 3 h each day followed by 2 days stable treatment at the new phase. In this way, it is possible to induce 9 h phase shifts while maintaining internal synchronization. Subsequently, subjects are abruptly returned to the local time cues, simulating time zone transition or rotating shift systems. After an abrupt phase advance of 9 h, melatonin (5 mg), timed to phase advance, immediately improves sleep quality and duration together with alertness and the ability to perform low and high memory load cancellation tests (Deacon and Arendt, 1996) (Fig. 6). This improvement is evident before any major phase shift has occurred in endogenous markers such as core body temperature. Although resynchronization of endogenous variables is hastened, the acute effects of melatonin appear to be as important as any induced phase shift. In the author's opinion, melatonin reinforces acutely and chronically physiological phenomena connected with darkness, in particular sleep.

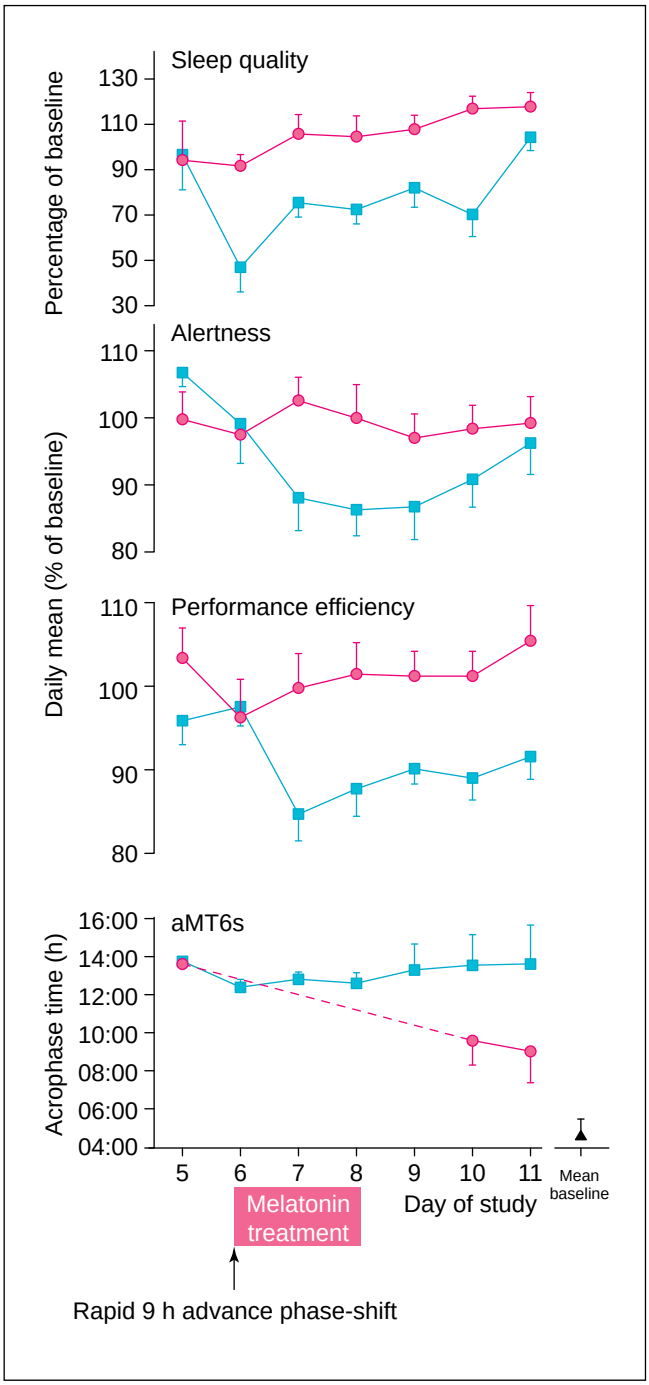

Fig. 6. Melatonin (●) hastens behavioural adaptation to phase shift compared with placebo (■), when taken for 3 days at 23:00 h after an abrupt simulated 9 h phase advance (equivalent of travelling from Los Angeles to Paris); subjective sleep quality (and polysomnographically recorded sleep, not shown), alertness and performance are improved, before and during an increase in the rate of adaptation of the endogenous melatonin rhythm, as assessed by 6-sulfatoxymelatonin (aMT6s) measurement in urine. Note that beneficial effects are seen long before circadian adaptation is complete, indicating a major contribution of acute effects of melatonin. (Redrawn from Arendt and Deacon, 1997, with permission).

Melatonin antagonists and agonists

In view of the very large potential market for melatonin-like effects in both occupational health and pathology, a number of pharmaceutical companies have initiated development of specific formulations of melatonin and of novel analogues. The most interesting napthalenic agonists have similar effects to

melatonin on SCN electrical activity, on *in vivo* rhythm physiology in rodents (for example, Ying *et al*., 1996) and on human circadian rhythms (Cajochem *et al*., 1995). Besides these analogues, many other potentially useful molecules are under investigation. The detailed pharmacology of these new antagonists is eagerly awaited.

Melatonin has provoked the development of a whole new pharmacology of so-called chronobiotic drugs. It is also claimed to be a powerful antioxidant, immunostimulant, anti-ageing factor and general cure-all. The publicity surrounding these claims has been detrimental to serious investigation of its proven therapeutic uses. It is to be hoped that suitable clinical trials will lead to a more rigorous definition of its uses and limitations and, most particularly, to its long-term safety.

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