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Melatonin as a chronobiotic: PROS and CONS

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Abstract. The pineal gland is a major component in the regulation of photoperiodic responses and hence, saisonality. All functions which appear to be controlled by the daylength can be influenced by modifying the output signal of the pineal gland, i.e. melatonin. An important property of the melatonin signal is that the duration of melatonin synthesis and release varies proportionally with the length of the night suggesting that the duration of elevated melatonin is the most important signal conveying the photoperiodic message. In addition to the temporal coordination of saisonal phenomena, melatonin appears to be involved in the control of circadian rhythms in mammals. Activity-rest cycles of rats can be entrained by infusion or injection of melatonin at appropriate times of the daily cycle. Rhythms of neuronal activity of the suprachiasmatic nuclei (SCN) of the hypothalamus can be inhibited and phase-shifted by application of melatonin. The firing rate of SCN neurones of rats can be inhibited by iontophoretic application of melatonin. These data are in good agreement with the high density of melatonin receptors found in the SCN of most - but not all - mammalian species. On the other hand, activity-rest cycles appear to be perfectly normal in animals which lack a daily melatonin rhythm. This, however, might be a laboratory artefact, and this system will probably fail when challenged in a complex situation.

Review

Key words: circadian rhythm, melatonin, pineal gland, photoperiodism, suprachiasmatic nuclei

INTRODUCTION

The earth's rotation around the sun and around itself causes oscillations in the environment on a seasonal as well as on a daily basis. These oscillations include abiotic factors such as ambient illumination or temperature and biotic factors such as activity of predators or sexual partners. In order to cope with these rhythms in the outside world any organism needs a similar rhythmicity in the internal organization of its physiology or behaviour. Several excellent studies have discussed the survival value of endogenous self-sustained - circadian or circannual - rhythms (Aschoff 1964, Moore-Ede and Sulzman 1981, Daan and Aschoff 1982). The major advantage might be that endogenous rhythms enable the organisms to anticipate cycling events in the environment, such as seasonal changes in temperature and food availability or daily variations in the probability to encounter predators. It is also apparently advantageous to separate in time mutually incompatible physiological processes.

In mammals, the dominant circadian pacemaker responsible for the generation of several overt rhythms is located in the suprachiasmatic nuclei (SCN) of the hypothalamus (Rusak and Zucker 1979). The activity of the SCN is synchronized to the 24 h periodicity of environmental light *via* the retino-hypothalamic tract (RHT), a neuronal pathway from the retinae to the SCN. In addition, the SCN is an important relay station between the retinae and the pineal gland. The pineal gland functions as a neurochemical transducer (Axelrod 1974) converting neuronal signals of light perception into an endocrine signal, namely melatonin. Phyllogenetically, melatonin is a very ancient biologically active molecule. It appears to be present already in unicellular algi (e.g. *Gonyaulax polyedra*, Balzer and Hardeland 1991). It has been detected in insects, crustaceans and mollusks (Vivien-Roels and Pévet 1986) and in all vertebrates, and it always seems to be functionally associated with photoreception in general (Gern et al. 1986). Melatonin is synthesized in the pineal gland exclusively during the dark phase of the daily light cycle. Light exposure during the night has a dual effect: (1) it activates the SCN which in turn inhibits the sympathetic innervation to the pineal gland, leading to an acute inhibition of melatonin synthesis, and (2) it induces phase-shifts in the circadian activity cycle of the SCN. Hence in all vertebrates, irrespective of whether they show a diurnal, nocturnal, or crepuscular pattern of locomotor activity, the melatonin level in the pineal gland is increased during the night and low during the day. Because of this exclusive nightly production melatonin has been dubbed "the chemical expression of darkness" (Reiter 1991a). Melatonin is a small, lipophilic molecule and, therefore, it easily crosses cell membranes. Because of this and because melatonin is not stored within the pineal gland, blood levels of melatonin - and melatonin levels of virtually all other body fluids - parallel its pineal production rate (Arendt 1985, Pang 1985). Due to these unique features melatonin appears to be an ideal candidate for a chronobiotic. In its original definition a chronobiotic is "a substance which is capable of therapeutically re-entraining short-term dissociated or long-term desynchronized circadian rhyhms, or prophylactically preventing their disruption following an environmental insult" (Short and Armstrong 1984, cited after Armstrong 1989). In the context of this short review, however, I want to use the term chronobiotic in a more general way and define it as a substance with a clock function which can impart time of day information to all organ systems, and which also conveys a calendar function informing the organism about the time of year. The therapeutic use of melatonin in circadian rhythm disorders and following phase shifts will be discussed in a companion review (Skene et al. 1996). Due to the limited space I also will concentrate on the findings in mammals because in contrast to the situation in birds the mammalian pineal gland is commonly thought to be important for seasonal adjustments only and to be rather unimportant in the circadian organization.

MELATONIN, THE INTERNAL SIGNAL?

A neuroendocrine loop model for the avian circadian system has been suggested by Cassone and

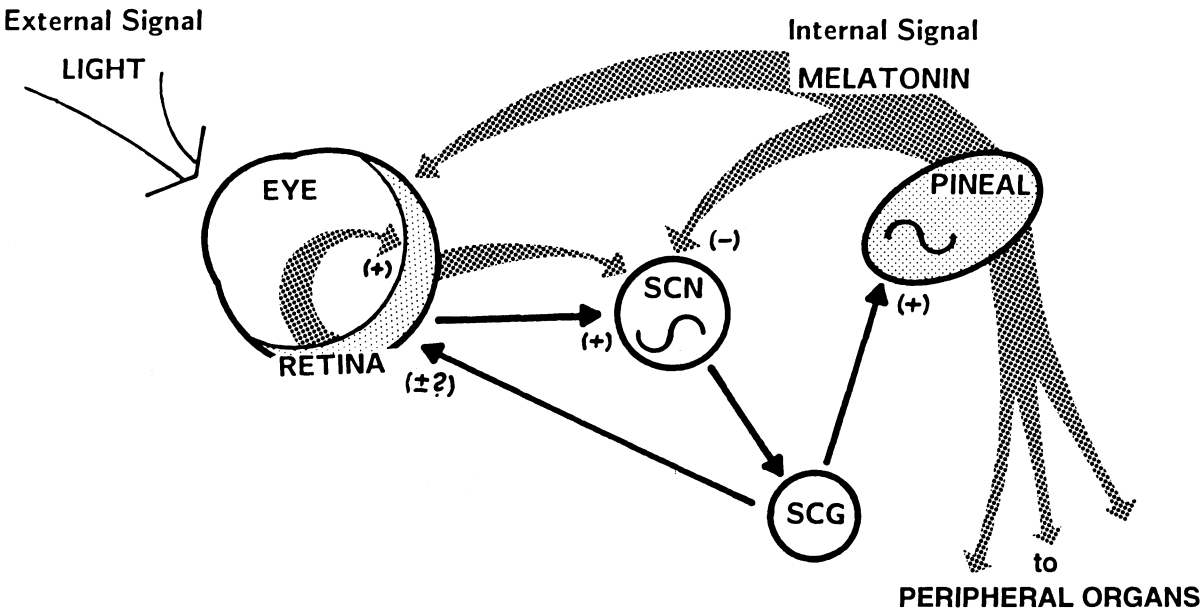

Fig. 1. Neuroendocrine loop model for the mammalian circadian system. Arrows: neuronal connections; thick, shaded arrows: endocrine connections. For further explanations see text (redrawn and modified from Steinlechner 1991).

Menaker (1984) and was recently modified by Lu and Cassone (1993). I have adapted this model to fit our present knowledge of the situation in mammals (Fig. 1). In this model, light is the external signal (zeitgeber) which entrains both the retinal melatonin production as well as the central pacemaker located in the SCN. The SCN may exert feedback control on its visual input. Since a direct neuronal connection from the SCN to the eye is unknown, the most likely pathway is *via* the superior cervical ganglia (SCG) of the sympathetic nervous system. Postganglionic fibres of the SCG innervate both the eyes and the pineal gland. In the eyes these fibres can affect the retinal response in two ways: indirectly by dilating the pupil, and directly by imposing a circadian rhythm of sensitivity to the retina itself (Bobbert and Brandenburg 1982, Groos 1982). The sympathetic control of the pineal gland activity is well established. Upon release of norepinephrine from the sympathetic nerve terminals in the pineal gland at night, melatonin synthesis and release is activated. Melatonin, in turn, acts as an internal signal feeding back on the SCN and possibly also on the eyes. Several studies have shown that melatonin has an inhibitory effect on SCN neuronal activity and that it can phase shift its activity rhythm (Shibata et al. 1989, Stehle et al. 1989, McArthur et al. 1991). In addition to the central sites of action, melatonin diffuses into all cells of the body and can exert its ubiquitous functions in every cell and organ which can read the message, i.e. which has melatonin receptors or some other mechanism for receiving the signal. In this way each of these cells or organs "knows" whether it is day (low melatonin) or night (high melatonin), and it "knows" how long each phase of the 24 h day is.

Several experimental approaches have been used to study the effects of melatonin and the importance of its rhythmic synthesis and release. In all mammals studied so far the pineal gland is the major source for blood levels of melatonin. Therefore, by surgically removing or denervating the pineal gland, or by destroying the SCN, practically all of the circulating melatonin can be removed. Similarly, pineal melatonin production is completely blocked by exposing the animals to constant light (LL). On the other hand, melatonin quite easily can be replenished by implants and/or melatonin rhythms can be restored by daily injections or timed infusions. Especially this latter approach has played

a pivotal role in elucidating melatonin's functions (for a review see Bartness et al. 1993).

SEASONAL ADJUSTMENTS: MELATONIN'S CALENDAR FUNCTION

Annual cycles in reproductive functions are well known in many mammalian species (Aschoff 1955, Sadleir 1969). These seasonal adjustments are necessary to ensure that the young are born and raised during favourable climatic conditions. In small mammals with short gestation time (e.g. voles, mice, hamsters) mating and birth usually takes place in spring and summer. Accordingly, in larger mammals with longer gestation time (e.g. sheep, goats, deer) or in mammals with delayed implantation (e.g. bats, mink, badgers) mating occurs in fall or winter with birth the following spring. It has long been known that the photoperiod, i.e. the seasonal change in duration of the daily light period, is the prime environmental cue regulating the annual cycle of reproduction. But not only the reproductive system shows seasonal cycles. The whole physiology, morphology and behaviour of an animal is more or less obviously subject to seasonal adjustments. This includes for example body weight, pelage (colour and quality), and the entire thermoregulatory system. All these functions are controlled by the photoperiod, ensuring that the animal can adjust its physiology well in advance to the specific demands of each season. The close link between this photoperiodism and mammalian pineal function is well established and has been reviewed many times (Stetson and Watson-Whitmyre 1984, Goldman and Elliott 1988, Reiter 1991b, Steinlechner and Niklowitz 1992). Therefore, suffice it here to briefly mention the main commonly accepted points and then to elaborate a little more on those findings which still leave some questions open or which do not concur with the common knowledge.

All photoperiodic manipulations that have been shown to influence seasonal adjustments, such as gonadal activity, also change the daily pattern of pineal melatonin production and melatonin levels in the circulation. Similarly, all functions which appear to be controlled by the photoperiod can be influenced by modifying the output signal of the pineal gland, i.e. melatonin. If the daily melatonin rhythm is destroyed the animal becomes "blind" towards changes in the photoperiod and hence, all seasonal cycles are abolished if the pineal gland is removed or denervated. On the other hand, melatonin, when properly administered, is capable of inducing seasonal cycles even if the animal is maintained in constant photoperiods. Just as the effect of short photoperiod is different at different times of the year, the effects of melatonin administration vary with the seasons. At times of the year when the animal is refractory towards short photoperiod it is also insensitive towards melatonin. These and similar experiments clearly show that melatonin conveys the photoperiodic message to neuroendocrine regulatory centres. It is neither stimulatory nor inhibitory in itself; it just informs the body about the time of the year, and the animal adjusts its physiology according to the specific requirements of the different seasons. We have good experimental evidence that the duration of the daily melatonin signal is the critical feature for triggering photoperiodic responses. When the length of the night increases the nocturnal melatonin peak is prolonged and conversely, when the length of the night decreases the nocturnal melatonin signal is shortened. This has been shown many times for a large number of species, including humans (Wehr 1991). The most convincing evidence that the duration is the critical parameter comes from experiments using the timed infusion paradigm to deliver melatonin to pinealectomized animals, usually once daily, for a specific number of hours (Bartness et al. 1993). This method was employed for the first time by Carter and Goldman (1983a,b) who demonstrated that infusion of melatonin into pinealectomized Djungarian hamsters leads to short photoperiod responses (here: gonadal involution) if the duration of melatonin infusion is more than 8 h per 24 h. Infusion of melatonin for only 6 h daily or less stimulates gonadal growth (long day response). There appears to be a "critical duration" of the me-

latonin infusion of about 7 h which separates long day from short day responses. This is strongly reminiscent of the "critical photoperiod" and, in fact, if we replot the data by Carter and Goldman (1983a) to match the format of the graphical representation of the critical photoperiod for Djungarian hamsters

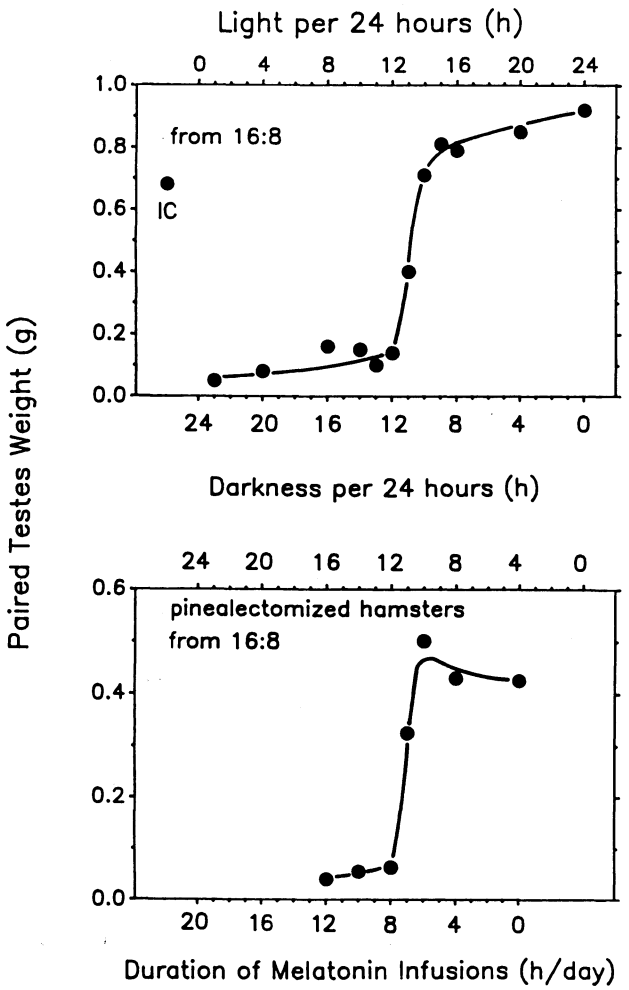

Fig. 2. Upper panel: effect of exposure of juvenile Djungarian hamsters to different photoperiods for 45 days. 13 h of light (= 11 h of darkness) appear to be the "critical photoperiod" dividing long day response (in this case large testes) from short day response (regressed testes) (redrawn after Hoffmann 1982). Lower panel: effect of melatonin infusions of various durations on testes weight of pinealectomized, juvenile Djungarian hamsters. Smaller testes in these hamsters are due to age of animals (30 days vs. 80 days of hamsters in upper panel). A melatonin infusion of 8 h duration appears to be equivalent to a 12 h period of darkness (redrawn after data from Carter and Goldman 1983).

(Hoffmann 1982) then the similarity of the two curves for testicular development is remarkable (Fig. 2, compare upper with lower panel). It appears that daily melatonin infusions for 7 h are equivalent to 11 h of darkness (13 h of light) per day. There are several reports, especially from studies in humans which attribute photoperiodic effects also to the amplitude of the daily melatonin profile. And in fact, changes in the amplitude of the melatonin peak can be quite often observed following transitions from one photoperiod to another, or in a natural photoperiod in the course of a year, or when animals are exposed to a lower ambient temperature (a treatment which can accellerate photoperiodic responses, Pévet et al. 1989, Stieglitz et al. 1994). These changes in amplitude, however, show a high degree of inconsistency and large individual variations which are not correlated with the photoperiodic response. In addition, a pronounced attenuation of the amplitude with age occurs in Syrian hamsters (Reiter et al. 1980) without a concommittant loss of photoperiodic responses (Reiter et al. 1982).

The strongest argument against the duration hypothesis is derived from the fact that the information about the duration *per se* is not sufficient for unequivocal seasonal adjustments. This is so because similar photoperiods, and consequently similar pulse durations, are experienced by the animal twice a year, i.e. prior to and after the summer solstice. Hoffmann and Illnerová (1986) have shown that in Djungarian hamsters the same photoperiod (e.g. L:D 14:10) may be "read" as long or short, depending on the previous light conditions. Despite the oposite physiological responses to the same photoperiod the pineal melatonin profiles were identical in both groups of hamsters (Hoffmann et al. 1986). Apparently, the photoperiodic response depends on the direction and the change in duration of the nocturnal melatonin pulse rather than on the absolute duration of the pulse. It was therefore hypothesized that there must be an internal calendar keeping track of the changes and its direction (Bartness and Goldman 1989).

Initial findings in animals which exhibit strong circannual rhythms led to the conclusion that the pi-

neal gland and melatonin are not important for seasonal adjustments. Harlow and coworkers reported that following pinealectomy the initiation and the progress of hibernation in adult *Spermophilus lateralis* was unaffected (Harlow et al. 1980). Similarly, Sharp et al. (1979) showed that denervation of the pineal gland in horses produced no significant effects during the first reproductive season. Long-term observations, however, reveiled that in all these cases the annual cycle was phase-shifted in the pinealectomized animals as compared to the control groups (Phillips and Harlow 1982). Thus in animals with endogenous circannual rhythms the melatonin signal seems not to be involved in generating the rhythm itself but rather to synchronize the *circa* rhythm to the calendar year (Zucker 1985).

DAILY ADJUSTMENTS: MELATONIN'S CLOCK FUNCTION

Just as is the case with the generation of circannual rhythms, the pineal gland and melatonin appear not to be involved in generating a circadian rhythmicity in mammals. In no instance has removal of the pineal gland disrupted the circadian rhythmicity of a mammal (Rusak and Zucker 1979). Neither in nocturnal nor in diurnal mammals did pinealectomy significantly affect the period of locomotor activity nor the phase response curve to light stimuli (Aschoff et al. 1982, Martinet and Zucker 1985). These and similar results have led to the conclusion that the pineal gland plays no significant role in the regulation of circadian rhythms in mammals. Nevertheless, there is now growing experimental evidence that this might be a prejudgement (Armstrong 1989). Melatonin may indeed play a role in the synchronization of rat circadian rhythms as suggested by entraining effects of daily melatonin injections. Free-running locomotor activity rhythms of laboratory rats were entrained by melatonin injected daily at approximately circadian time 10-12, i.e. shortly before or coincident with activity onset (Redman et al. 1983). This effect is blocked by SCN lesions (Cassone et al. 1986) indicating that the SCN is the target site of melatonin's action. The localization of high affinity melatonin receptors in the SCN of rats and hamsters (Vanecek et al. 1987, Bittman 1993) as well as the effect of melatonin on the rhythmic neuronal firing *in vitro* (Shibata et al. 1989, Stehle et al. 1989, Mason and Rusak 1990) has corroborated this hypothesis. Moreover, McArthur et al. (1991) could phase advance the rhythmic neuronal activity in rat SCN slices by melatonin application, provided that melatonin was applied between circadian time 10 and 14. This is in excellent agreement with the phase when exogenous melatonin can entrain activity rhythms in rats.

Davies and Mannion (1988) have reported that hamster pups' circadian rhythms could be entrained by prenatal melatonin injections to the SCN-lesioned mother (i.e. the mother's circadian clock and melatonin rhythm had been destroyed). Apparently, under normal circumstances the foetus' clock is set and synchronized *in utero* to the mother's rhythm *via* melatonin which is rhythmically synthesized in her pineal gland and transported *via* the blood circulation and placenta to the foetus.

In addition the therapeutic use and proven value of melatonin in humans to alliviate symptoms of jet-lag and other circadian rhythm disorders clearly show that melatonin has some inpact on the circadian system of mammals (see Skene et al. 1996). Taken together these findings suggest that while in mammals the pineal gland and melatonin might not be involved in generating circadian rhythms (as is certainly the case in some birds), melatonin probably feeds back on the SCN and possibly other brain structures to modulate and synchronize their activities (see concluding remarks).

However, even though this might be a very attractive hypothesis, we have to be aware that only a very limited number of mammalian species have been tested for synchronizing effects of melatonin and in which melatonin binding sites in the brain have been identified. It is especially confounding that in mustelid brains no melatonin receptors were detected. In the western spotted skunk (*Spilogale putorius latifrons*), 2-[^{125}I]iodomelatonin binding

sites were localized exclusively in the parts tuberalis (Duncan and Mead 1992), and in ferrets *Mustela putorius f. furo*) only in pars tuberalis and pars distalis (Weaver and Reppert 1990). It certainly needs to be tested whether in these species melatonin can entrain circadian rhythms. Another argument against melatonin being important for control of circadian rhythms comes from work by Ebihara et al. (1986) who showed that several inbred strains of house mice (*Mus musculus*, e.g. C57Bl) lack pineal melatonin due to a genetic defect. Despite of the absence of rhythmic melatonin none of these mouse strains exhibits obvious abnormalities in their circadian behaviour.

CONCLUDING REMARKS

The 24 h cycle of light and darkness is certainly the strongest and a virtually universal zeitgeber for circadian rhythms (Pittendrigh 1981), but it is not the only one. There are additional environmental signals which can act as zeitgeber, such as ambient temperature, food availability, social stimuli etc. However, light is the most reliable, because most noise-free, environmental cue that conveys information about time of day with astronomical precision. Similarly the daylength, or rather its reciprocal, the night length is the most reliable environmental cue to inform animals about the time of year. The predictability of its seasonal change makes anticipatory programming a viable strategy. In nature, night length is defined as the longest period of darkness uninterrupted by light within a 24 h day. Daylight can be interrupted by darkness if, for example, an animal retreats into its borrow underground. Darkness of the night in a natural world is never interrupted by light (not counting flashes of lightning or moon light). Hence, the duration of an uninterrupted pulse of melatonin will be equivalent to the length of the night: melatonin is the endocrine signal for the night length (Reiter 1991a, Steinlechner 1992). Just as light certainly is not the only external signal for entrainment of circadian rhythms, melatonin might not be the only internal signal. Other metabilites of pineal origin or substances released from other oscillators could have similar functions as chronobiotics. To date, however, we have no other "hot" candidate.

In conclusion, there remains little doubt that melatonin can act as a chronobiotic. The few experiments that speak against such an action are either not convincing or possibly relate to other as yet unknown mechanisms of melatonin's action. The question remains then, why would we need a chronobiotic? Animals in which the circadian rhythm of melatonin production has been destroyed by bilateral lesions of the SCN, by pinealectomy or simply by keeping them in constant light, are generally quite healthy and survive. We have to keep in mind, however, that they survive in the stable, safe and simplyfied environment of a laboratory. They probably could not survive - or at least would barely be

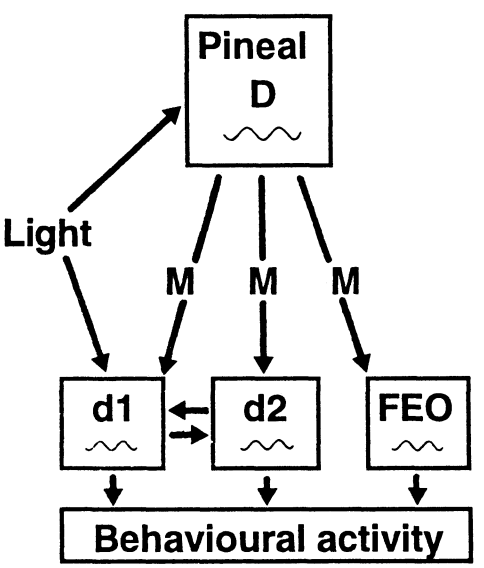

Fig. 3. Gwinner's (1978) model of the avian circadian system modified for the situation in mammals. The pineal (D) oscillator *via* its rhythmic release of melatonin (M) drives several self-sustaining oscillators (d1, d2) that are more or less strongly coupled to each other. In different species the strength of the coupling between the d oscillators varies. The d oscillators could be represented by the SCN which has access to light. The food-entrainable oscillator (FEO) which is neither coupled to the d oscillators nor has access to the light information is kept in synchrony with the other oscillators *via* the chronobiotic melatonin (redrawn and modified after Armstrong 1989).

competitive - in the complex and challenging outside wilderness. Eskes (1982) has elegantly demonstrated that the sexual performance of arrhythmic golden hamsters is impaired, but only when competing with a rhythmic rival for a rhythmic mate. Another reason for survival of animals in which the SCN is destroyed is that the circadian system seems to be quite redundant, i.e. there are probably other oscillators outside the SCN such as the food-entrainable oscillator (Stephan et al. 1979) or the eyes (Remé et al. 1991). In addition, other organs are able to synthesize melatonin in a rhythmic pattern and possibly can compensate for the loss of pineal melatonin (Reiter et al. 1983, Steinlechner et al. 1987). This redundancy of the system gives rise to yet another possible function of the chronobiotic melatonin, i.e. to synchronize the different clocks or self-sustained oscillators of the organism. The underlying model which was first proposed by Gwinner (1978) and later modified by Armstrong (1989) is shown in Fig. 3. This model is especially attractive because it allows to explain interspecies differences by a more or less strong coupling between individual oscillators. If coupling is strong, pinealectomy has no effect on behavioural rhythmicity. If coupling is weak, pinealectomy results in loss of synchronicity and behavioural arrhythmia. The d oscillators could be represented by the SCN. I propose here, that not all the oscillators have access to light, but that there are other oscillators which are not entrained by light, e.g. the food-entrainable oscillator (FEO). These, in particular, need the chronobiotic melatonin to remain synchronized with the other oscillators.

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