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Periodically varying sensitivity to melatonin in a mammalian circadian system

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In this communication we examine the effect of the pineal hormone melatonin on the circadian locomotor activity rhythm of the field mouse *Mus booduga*. Phase shifts in the circadian rhythm of locomotor activity were measured and a phase response curve was constructed following a single dose of 2 µl melatonin (1 mg/kg) at various circadian times. Melatonin administered from circadian time 4 (CT4) to CT16 induced delay phase shifts whereas at phases CT18 to CT2 advance phase shifts were evoked. The magnitude and direction of the phase shifts evoked were a function of the time of the melatonin injections. The results suggest that the circadian timing system controlling the locomotor activity rhythm in the *M. booduga* is responsive to melatonin and also that the daily endogenous rhythm of melatonin may be involved in phasing or entraining the circadian system of mice.

THE vertebrate circadian system has the melatonin secreting pineal gland as one of its most important components[1]. Periodic administration of exogenous melatonin was found to entrain the activity rhythm in some vertebrates[2–5]. Timed administration of melatonin to some rodents also facilitated re-entrainment[6–9] and caused phase advances in the circadian activity rhythms[10,11]. The reported effects of melatonin on circadian rhythms and the presence of melatonin receptors in the suprachiasmatic nucleus (SCN) suggest a direct action of the hormone on the pacemaker[12,13].

Although in the last two decades the literature on PRCs has become abundant, that for melatonin has been very limited[3,14–16]. Recent work in our laboratory (and other laboratories) emphasizes the need to examine a variety of species to phase-shifting effects of exogenous melatonin administration. Furthermore, to confirm the hypothesis that the pineal plays an important role in phasing the circadian oscillator in a mammalian system, the present study was designed to describe the phase response curve (PRC) for melatonin for the locomotor rhythms in the field mouse *Mus booduga*.

Adult male mice *M. booduga* were captured from the fields near the University campus (9°58′N 78°10′E) and were raised under light/dark (LD) 12 : 12 for two weeks. The body weight of these animals ranged from 8 g to 12 g. Melatonin was procured from Sigma (USA), which was dissolved in 50% dimethyl sulphoxide (DMSO)[17].

The locomotor activity rhythms of individual mice ($n = 65$) was monitored by running wheel, connected to an Esterline Angus event recorder (Model A 620 X). The activity wheels (16 cm diameter) were attached to a plexiglass cage ($h \times l \times b = 6 \times 11 \times 8.5$ cm). The running wheels with mice were kept in light-tight experimental cubicles. Temperature (30 ± 1°C) and humidity (70 ± 5%) of the experimental cubicle were constant throughout the experiment. Food (grains) and water were available *ad libitum*.

The mice were maintained in continuous darkness (DD) throughout the duration of the experiment. After attainment of a steady state free-run, the animals' free-running period (τ) was calculated using linear regression. Activity onset was taken as phase reference point and is referred as CT 12. The phase and the magnitude of phase shifts are expressed in circadian hours. Mice were administered with 2 µl of single injections consisting of vehicle (50% DMSO) or melatonin (1 mg/kg)[14,21] at various circadian times (CTs: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22). Injections were given intraperitoneally in constant darkness under a dim red light of wavelength 640 nm. Mice received a maximum of 3 treatments with an interval of three weeks between each treatment. Each mouse was treated at different CTs.

The phase shifts induced by melatonin (experimental) and vehicle injections (control) were calculated as the differences in time between the linear regression carried out for two steady states prior to and after the administration of melatonin or 50% DMSO. The phase shift values were considered negative (delays) when the onset occurred later than the expected time and positive (advances) when they occurred before. The various phases

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at which melatonin and vehicle were administered were plotted on the abscissa and the phase shifts in circadian hours on the ordinate.

The direction and magnitude of phase shifts induced by a single melatonin injection (1 mg/kg) were observed in *M. booduga* ($n = 65$) and a PRC was constructed. The effects of vehicle injections (50% DMSO) were also estimated at various phases of the circadian cycle.

Melatonin resets the phases of the circadian rhythm of locomotor activity in the field mouse *M. booduga*. The resulting phase shifts depend on the phases at which melatonin was injected. Melatonin injected from CT4 to CT16 induces delay phase shifts (phase delays in 31 of 40 mice) (Figure 1) whereas at phases from CT18 to CT2 causes phase advances (advance phase shifts in 24 of 25 mice) (Figure 2). However, the control animals treated with 50% DMSO did not exhibit any phase shifts (except 2 of 38 mice have exhibited small phase shifts, i.e. < 0.10 h). The maximum advance and delay phase shifts were found to occur at phases CT22 and

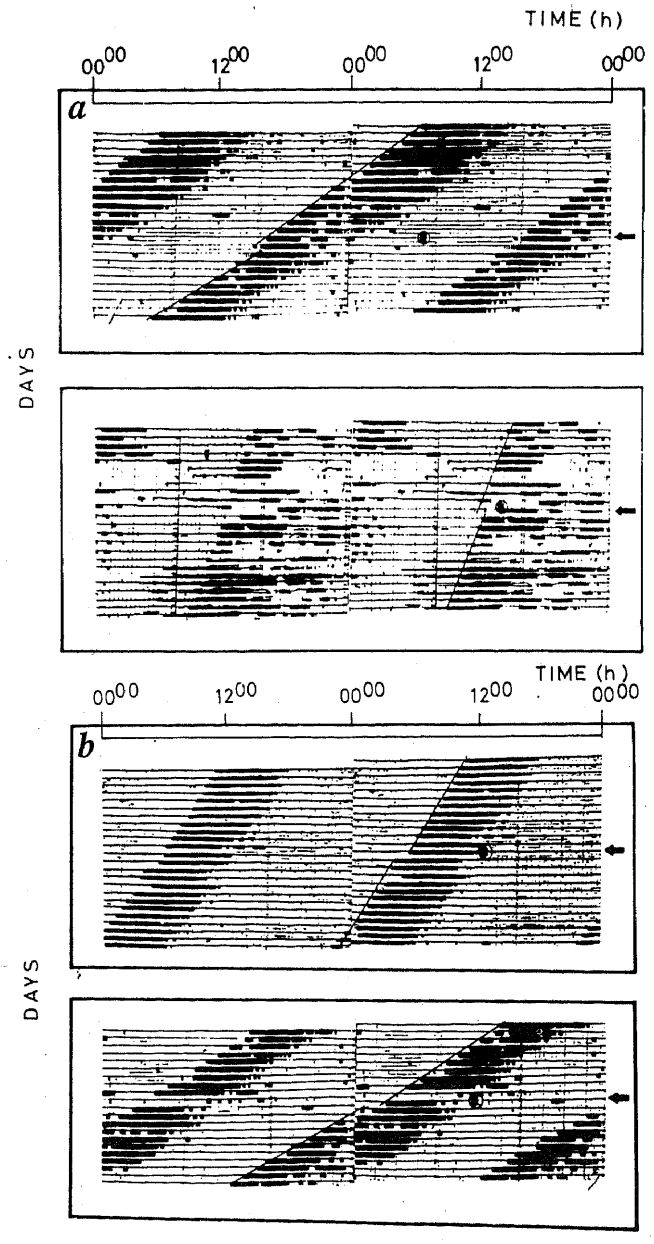

Figure 1. Double-plot actogram illustrating the delay phase shifts evoked at (*a*) CT4 (upper panel) and CT14 (lower panel); (*b*) CT8 (upper panel) and CT10 (lower panel) by the single administration of 2 µl of melatonin (1 mg/kg) on wheel-running activity rhythm in the field mouse *M. booduga*. The day and phase at which melatonin was administered are indicated by solid arrow and star mark within a circle respectively.

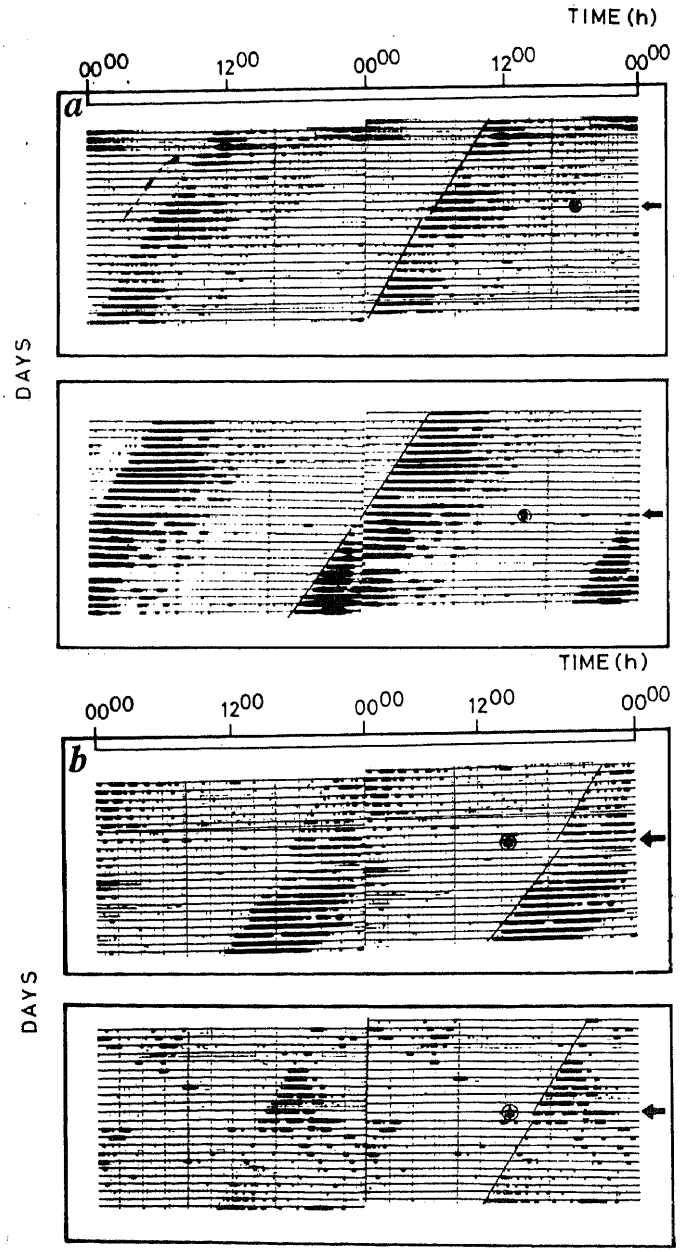

Figure 2. Double-plot actogram showing the advance phase shifts evoked at (*a*) CT0 (upper panel) and CT2 (lower panel); (*b*) CT18 (upper panel) and CT20 (lower panel) by a single administration of 2 µl of melatonin (1 mg/kg) on wheel running activity rhythm in the field mouse *M. booduga*. The solid arrow and star mark within a circle indicate the day and time of treatment, respectively.

CT4 respectively. There exists no region under the PRC which can be referred to as true 'dead zone' as seen in light pulse PRCs of several nocturnal rodents[18].

Exogenous administration of melatonin phase shifts the circadian activity rhythm in the *M. booduga* according to distinct phase response curve. In mice, a single dose of melatonin (1 mg/kg) tended to advance circadian activity rhythms when administered between CT18 and CT2 and to delay circadian activity rhythms when administered between CT4 and CT16. The sensitivity of the circadian pacemaker to melatonin varies periodically, giving rise to a PRC. The PRC due to melatonin appears to be type I (delay to advance transition is gradual) with a maximum phase delay at CT4 and the maximum phase advance at CT22 (Figure 3). In several photoperiodic rodents, there are two windows of sensitivity within the circadian day to the reproductive effects of melatonin: late in the subjective day, followed by a second brief period late in the subjective night[19]. It initially appeared that the circadian effects were restricted to one time of day. At the level of the SCN, however, there is clear evidence of bimodal sensitivity to melatonin. Cassone *et al.*[20] reported that melatonin has a bimodal effect on metabolic activity in the SCN, with the direction

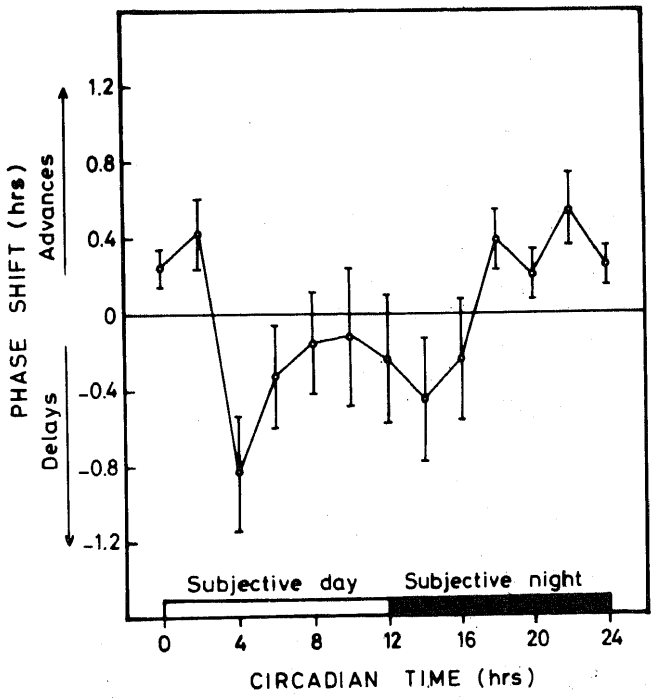

Figure 3. Phase shifts of circadian locomotor activity rhythms induced by administration of a single dose of 2 µl melatonin (1 mg/kg) in the field mice *M. booduga*. The phase shifts caused by melatonin administration are plotted as the mean ± SD for 2 h intervals. Advance phase shifts are plotted as positive (+) and delay phase shifts as negative (–). The open and filled bars denote the subjective day and the subjective night, respectively, with the beginning of subjective night (CT12) determined by the onset of running-wheel activity. Error bars are plotted to represent 95% confidence interval about the means for visual hypothesis testing.

of the response depending on the CT of administration.

Sensitivity to melatonin administration at both dusk and dawn has been observed across species. In rats, a single dose of melatonin is known to advance the phase of locomotor activity rhythms when administered at CT10 but shows no effect at dawn (CT22 to CT2)[14,11]. In the Iguanid lizard, melatonin (10 µg) also phase shifts circadian rhythms, with a phase response curve showing two periods of sensitivity, i.e. phase advances in the late subjective day and phase delays in the late subjective night[3]. Taken together, these results suggest species differences in sensitivity to the dose of melatonin with different responses across species in the period of sensitivity. This might be due to the fact that melatonin-entraining action is limited to a critical phase in the circadian cycle and the target organs are receptive to melatonin only at the certain time of the day[2]. In *M. booduga*, the PRC constructed for melatonin is not similar to the PRCs reported in lizards[3], rats[14], humans[15] and C3H/HeN mice[16]. Such differences in the PRCs might have arisen due to the differences in the mechanism of melatonin action on the circadian pacemaker. Different circadian responses to melatonin may also occur between rat strains. Circadian effects of melatonin in hamsters are diverse and vary with strain, developmental age and method of administration[21]. Characteristics of melatonin-binding sites within the SCN vary both between and within species, as do profiles of endogenous melatonin rhythms. These differences may explain the variations in circadian responses to melatonin[21].

The phase shifts in circadian activity rhythms in *M. booduga* induced by melatonin as compared to those induced by vehicle probably are mediated through a direct action on the circadian timing system. A study by Hastings *et al.*[22] suggests that, in Syrian hamsters, arousal caused by the subcutaneous injection procedure, rather than effects of the hormone, mediates phase advances. In our study, a single injection of vehicle induced phase shifts in only 2 of 38 mice, none of which occurred at times sensitive to melatonin administration.

In the present study, circadian locomotor rhythm remains sensitive to the administration of melatonin at other phases as well. Unlike the case of Armstrong[14], there exists no 'melatonin refractory zone' in melatonin PRC of *M. booduga*. The results suggest that the circadian variation in functional responses to melatonin may be mediated by circadian changes in the sensitivity of melatonin receptors in the SCN[16]. Circadian and diurnal variations in the specific binding of 2-[^{125}I] iodo melatonin with the peak binding at either dusk or dawn occur in the SCN of various species (rat[23], mouse[24], chick[25]).

A substantial body of evidence suggests that melatonin shifts circadian rhythms in mammals through the activation of melatonin receptors in the SCN[16]. The entraining

effects of melatonin are prevented by SCN lesions, and administration of melatonin alters metabolic activity and inhibits cell firing in the SCN of the rat and the Djungarian hamster[5,10,20,26]. *In vitro*, melatonin phase shifts the circadian rhythm of electrical activity in the rat SCN slices[27,28]. Thus these results suggest that melatonin induces phase shifts of circadian activity rhythms in the field mouse *M. booduga* by acting on melatonin receptors in the circadian oscillators.

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Seismic hazard analysis: An artificial neural network approach

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An artificial neural network (ANN) approach is applied for the estimation of seismic hazard in a region. The seismicity rhythm is recognized by means of an ANN approach. The seismicity cycle may be divided into four stages, viz. energy accumulation, increasing release in energy, intense release and the remnant release of seismic energy. The seismicity data from the earthquake catalogue (1790–1990) for the Arakan Yoma and Naga Thrust belt in NE India have been used. Future seismicity for the region is predicted up to the year 2040. The results show that the intense energy release cycle will start somewhere in the year 2030 and will continue up to 2040. The successful operation of ANN and its application to predict seismicity cycle in the selected region shows that the approach may be applied to other areas also for the seismic hazard evaluation.

THE seismic hazard in a region may be defined as the probability of occurrence of earthquake given in units of a certain level of vibrations expected in a particular region within a certain period of time. The final result of the seismic hazard analysis is the determination of the probability of occurrence of natural phenomenon (intensity, magnitude, accelerations, etc.). For such analysis, statistical characteristics of seismic hazard are generally applied. The observations from the past (seismicity) records are used to model the future activity. A variety of statistical procedures may be applied at various stages of the formulation and evaluation of proposed models which, besides the stationary Poisson process, include: Baysian methods facilitating the incorporation of diverse elements of uncertainty and combining estimates by different models; semi-Markov process applied to linear zones; stochastic models and/or clustering for cyclic fluctuation and other trends; models based on precursory phenomena, which consider the probability distribution for magnitude, location and time of occurrence of predicted earthquakes[1]. These methods may be appropriate to reveal some statistical characteristics of seismic hazard, but may not provide a direct quantitative evaluation. In practice, an appropriate assessment usually needs further comprehensive judgements based on experience.

New methods are, therefore, to be introduced to overcome the limitations of conventional methods. Artificial neural network (ANN) approach has recently been touted as having enormous potential for a variety of problems in various fields such as image and signal processing,

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