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Locomotor Activity Rhythm in the Field Mouse *Mus booduga* Phase-shifts to Melatonin Injections in a Dose-dependent Manner

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ABSTRACT

Melatonin is known to shift the phase of the locomotor activity rhythm in the field mouse *Mus booduga* in accordance with a type-I phase response curve (PRC), with phase delays during the subjective day and phase advances during late subjective night and the early subjective day. At CT4 (circadian time 4; i.e. 16 hr. after activity onset) and CT22 of the circadian cycle, a single dose of melatonin (1 mg/kg) is known to evoke maximum delay and maximum advance phase-shifts, respectively. We investigated the dose-dependent responses of the circadian pacemaker of these mice to a single dose of melatonin at the times for maximum delay and maximum advance. The circadian pacemaker responsible for the locomotor activity rhythm in these mice responded to various doses of melatonin in a dose-dependent manner with the magnitude of phase shifts increasing with dose.

KEYWORDS: circadian rhythm, locomotor activity, mouse, melatonin, phase response curve, dose response curve.

INTRODUCTION

The circadian pacemaker of the field mouse *Mus booduga* has been demonstrated to respond to light (Sharma et al., 1997) and melatonin (Singaravel et al., 1998) in a phase-dependent manner. When the animals maintained in constant darkness (DD) were administered a single intraperitoneal injection of melatonin (1 mg/kg)

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at various phases of their circadian cycle, they responded with phase shifts according to a phase response curve (PRC) of type-I, with phase delays during the subjective day and phase advances during the late subjective night and early subjective day. At CT4 (circadian time 4) and CT22 of the circadian cycle, the melatonin PRC shows maximum delay and maximum (advance) phase shifts respectively (Singaravel et al., 1998). On the other hand, in the course of the light-induced PRC for these mice, a single light pulse administered during early subjective night evokes phase delays whereas during late subjective night it evokes phase advances (Sharma et al., 1997). A comparison of both PRCs shows that the melatonin PRC is out of phase with the light PRC and, moreover, appears to have a different time course and waveform than the light PRC (Sharma et al., 1997; Singaravel et al., 1998).

Melatonin is known to shift the phase of circadian rhythms in lizards (Underwood, 1986), in rats (Armstrong et al., 1989), in C3H/HeN mice (Benloucif & Dubocovich, 1996) and in humans (Deacon & Arendt, 1995; Lewy et al., 1998). Interestingly, in rats, melatonin has very little effect on the circadian timing system during time of day other than the onset of activity (Armstrong et al., 1989) During melatonin entrainment, activity onset continued to be phase-locked to daily melatonin administrations until the injection regime was halted, when rats free-ran, exhibiting their endogenous period. This effect was found to be dose-dependent (Cassone et al., 1986). Humans (Deacon & Arendt, 1995) and pinealectomized rats were also observed to respond to melatonin injections in a dose-dependent manner. The purpose of the present study was to determine the dose response curve for the phase advancing and phase delaying effects of melatonin on the circadian pacemaker, regulating the locomotor activity rhythm, in the field mouse *M. booduga.* These mice were used in the present study because their phase-resetting behaviour to light and melatonin is well known (Sharma, 1996; Sharma & Chandrashekaran, 1997; Sharma et al., 1997). Furthermore, the activity pattern of these mice has a precise, robust, easily measured onset of activity.

MATERIALS AND METHODS

Male field mice (n=90) *M. booduga*, were captured from cultivated fields near the Madurai Kamaraj University campus (9°58′ N lat., 78°10′ E long.) and maintained in the laboratory for about 15 days before being used in experiments. Adult mice (age ≈ 90 days; weighing 8–13 g) were entrained to LD cycles of 12:12 hr (lights on at 06.00 hr and lights off at 18.00 hr) for 15 days and then released into continuous darkness (DD) for the duration of the experiments wherein their locomotor activity was monitored using an activity running wheel (diameter ≈ 20 cm) attached to a transparent plexiglass cage of dimensions $0.07 \times 0.11 \times 0.09$ m, with

a small opening of 0.02 m diameter. Fluorescent daylight (Philips, 20W, 6500 K) was used as the source of light during the LD cycles. Reed-relays attached to the wheels activated the writing stylets of an Esterline Angus A620X Event Recorder. The activity patterns of 18 mice in separate running wheels, placed on open shelves in each experimental room, could be monitored concurrently. The room was protected from any type of external interference. The temperature inside the experimental rooms ($3.05 \times 2.44 \times 4.01$ m), which did not have windows but were gently ventilated, remained constant at 25 ± 1°C and the relative humidity was 75 ± 5%. Food (millet and grain) and water were available *ad libitum*. The rooms were entered at irregular intervals, on an average once in two days, for cleaning of cages, replenishing food and water, administering melatonin injections, etc. Care was taken that the animals were not disturbed except for these inevitable entries which seldom lasted beyond 5–10 mins. Red light of λ>640 nm, obtained with a combination of red and orange filters fitted to a battery-operated torchlight, was used inside the cubicles.

CT12 denotes the onset of activity and hence the onset of subjective night. All phases and phase-shifts given in the text are expressed in hours of circadian time (CT) obtained by multiplying absolute (real time) phases by $24/\tau$. After the attainment of a steady state free-run, τ and phase-shifts were calculated using linear regression analysis. A minimum of 10 days free-run was taken into account for such estimations. The average τ of the animals used in the present experiment was 23.77 ± 0.13 hr. The majority of the mice ($n = 65$) kept in DD were then administered single 2 µl injections of melatonin in various doses (0.1, 1, 10, 100, 1000 µg/kg) at CT4 and CT22. At these times the mice were known to respond to melatonin injections with maximum delay and maximum advance phase-shifts respectively (Singaravel et al., 1998). Control animals ($n = 25$) also maintained under DD, were administered 2 µl of the vehicle (50% dimethyl sulfoxide (DMSO)) at the two tested CTs in order to estimate possible effects of DMSO, disturbances associated with handling, transfer, and human interference, etc. Melatonin was procured from Sigma Chemicals Co., St Louis, M.O., USA. It was dissolved in 50% DMSO. Golombek and Cardinali's (1993) protocol for preparation and dosage of melatonin was followed. The phase-shift data were subjected to a two-way mixed model analysis of variance (ANOVA) treating phase as a fixed factor and intensity as a random factor. Multiple comparisons were performed using *t*-test.

RESULTS

At the phase of maximum advance shifts, CT22, the phase-shifts in the onset of locomotor activity of the field mouse *M. booduga* were not consistent for the lowest selected dose of melatonin (0.1µg/kg), and only two out of seven animals

registered non-zero phase-shifts. However, at the phase of maximum delay shifts, CT4, the same dose of melatonin shifted the phase of the locomotor activity rhythm and four out of seven animals responded with non-zero phase-shifts. The circadian pacemaker responsible for the locomotor activity rhythm in these mice responded to the various doses of melatonin (0.1 μg/kg, 1 μg/kg, 10 μg/kg, 100 μg/kg, and 1000 μg/kg) in a dose-dependent manner with the magnitude of phase shifts at CT4 and CT22 increasing with increase in dose strength (Fig.1). Moreover, the directions of the phase-shifts at both the CTs were consistent (and unam-

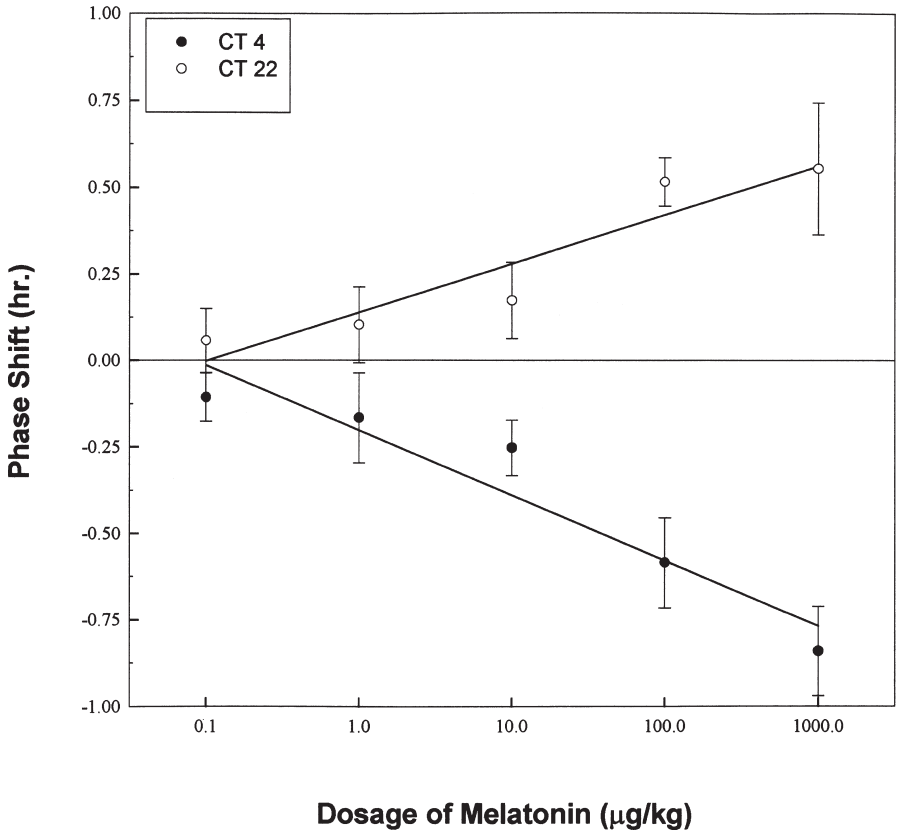

Fig. 1. Dose response curve (DRC) for the locomotor activity of the field mouse *M. booduga.* At the phases of maximum advance (CT22) and delay (CT4) various doses of melatonin were injected and the induced phase-shifts were measured. Strength of dose in log (μg/kg) are plotted along the abscissa and the phase-shifts (advances and delays) are plotted along the ordinate. Error bars about the mean phase-shifts for various doses of melatonin represent the 95% confidence intervals.

biguous) for all doses of melatonin. Animals injected with only the vehicle however, did not show any detectable phase-shift at CT22. The ANOVA showed a significant main effect of phase ($F_{1,50} = 6.89$, $p < 0.025$). The ANOVA also revealed significant effects of dose ($F_{4,50} = 5.31$, $p < 0.005$), as well as of the dose × phase interaction ($F_{4,50} = 7.36$, $p < 0.001$)

Multiple comparisons among the various intensities using two-tailed *t*-tests revealed that the phase-shifts evoked by melatonin of doses between 100 (g/kg and 1000 μg/kg were significantly different from the phase-shifts evoked by a melatonin injection of 0.1 μg/kg dose ($p < 0.001$). No consistent change in the period of the rhythm was observed following injections of melatonin. Figs. 2 and 3 give examples of actograms illustrating phase shifts evoked by various doses of melatonin 0.1 μg/kg, 1 μg/kg, 10 μg/kg, 100 μg/kg, and 1000 μg/kg at the phases of maximum delays (CT4) and maximum advances (CT22), respectively.

DISCUSSION

Previous studies on the circadian locomotor activity rhythm in the field mouse *M. booduga* (Singaravel et al., 1998) have demonstrated that a single dose of melatonin shifts the onset of activity in a phase-dependent manner. The PRC constructed for melatonin had regions of phase advances and phase delays in response to a single dose of melatonin at CT22 and CT4 of the circadian cycle, evoking maximum advance and delay phase shifts, respectively. In the present study, we demonstrate a dose-dependent phase shift due to single doses of melatonin in the field mice *M. booduga.* The dose response curve of melatonin reveals that although single doses of melatonin of 0.1 and 1 μg/kg given at the phases of maximum advance (CT22) and maximum delay (CT4) could evoke small phase-shifts, these shifts were not significantly greater than zero. Only two to four out of seven animals responded with non-zero phase-shifts. However, a single dose of 10 μg/kg of melatonin is sufficient to evoke a significant phase-shift in most of the animals (five to seven out of seven) (Fig.1).

The circadian pacemaker of the field mouse *M. booduga* responded to a single dose of melatonin with a PRC exhibiting both advance (at CT22) and delay phase shifts (at CT4) (Singaravel et al., 1998). The locomotor activity rhythm of these mice free-running in constant darkness exhibited an average τ of 23.73 ± 0.23 hr (mean ± s.d.). Therefore in order to effect entrainment of the locomotor activity rhythm in these mice, a single recurrent daily dose of melatonin causing phase delay of ca. 0.23 hr would be needed. As can be read from the melatonin PRC for these mice (Singaravel et al., 1998) a single dose of melatonin administered during the early subjective day could eventually evoke such phase-shifts. This supports the fact that melatonin may also function as a potential internal zeitgeber

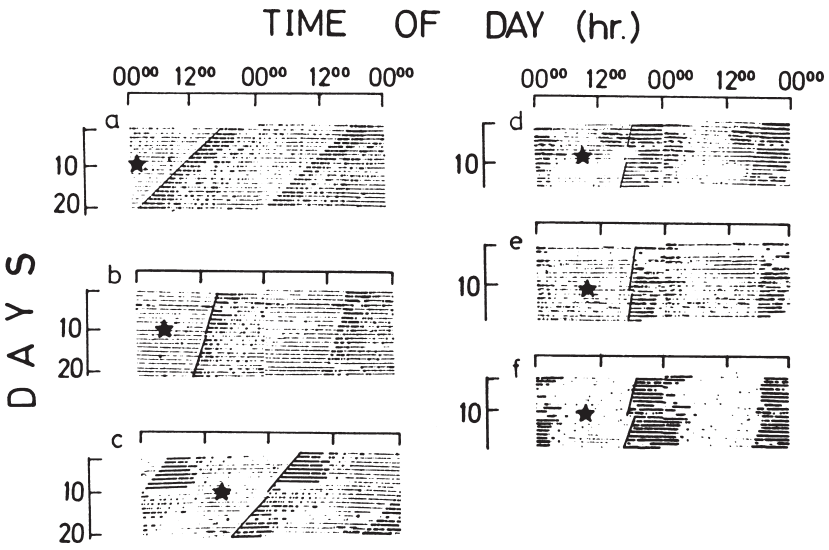

Fig. 2. One example each (from a total of 30 experiments) of the recordings of the wheel-running activity rhythm of adult male mice which were administered various doses of melatonin at the phase of maximum delay (CT4). (a) 0.1 μg/kg; (b) 1 μg/kg; (c) 10 μg/kg; (d) 100 μg/kg; (e)1000 μg/kg. Thick bands indicate intense activity and traces resting (sleep). Delay phase shifts set in without overt transients. Abscissa: time of day (hr); Ordinate: Calendar days.

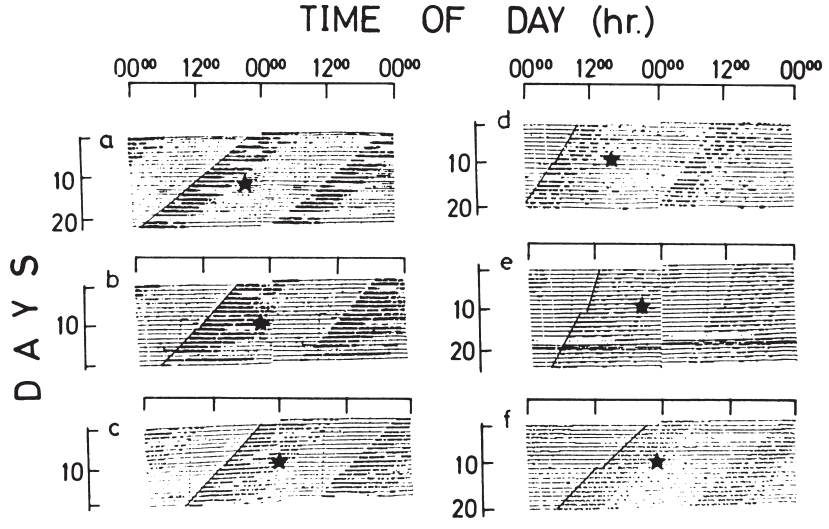

Fig. 3. One example each (from a total of 35 experiments) of the recordings of wheel-running activity rhythm of adult male mice which were administered various doses of melatonin at the phase of maximum advance (CT22). (a) 0.1 μg/kg; (b) 1 μg/kg; (c) 10 μg/kg; (d) 100 μg/kg; (e)1000 μg/kg. Thick bands indicate intense activity. Delay phase shifts sets in without overt transients. Abscissa: time of day (hr); Ordinate: Calendar days.

for the circadian timing system of the field mice *M. booduga*. The fact that there exist two melatonin-sensitive periods supports the argument that endogenous melatonin may have a dual role to play in the circadian timing system. First, as an internal zeitgeber, melatonin may act to maintain coupling between individual oscillators and appropriate phase relationships between the various bodily rhythms (Armstrong, 1989). Second, endogenous melatonin may modulate the photic sensitivity of the SCN circadian pacemaker, facilitating or inhibiting its responsiveness to changes in light intensity.

It is known that circadian systems can be entrained both by photic as well as nonphotic time cues. Feeding a rat at restricted intervals each day makes it possible to entrain some activity rhythms leaving others to free-run (Boulos & Terman, 1980; Coleman et al., 1982). However, in hamsters a periodic opportunity to hoard (Rusak et al., 1988) or periodic arousal through social interaction or cage changing (Mrosovsky, 1988) entrains all components of rhyhtmic activity. Taken together the effects of melatonin on its circadian timing system, its role as an additional non-photic zeitgeber cannot be ignored.

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