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Chronobiotic effect of melatonin following phase shift of light/dark cycles in the field mouse *Mus booduga*

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Abstract. The objective of this study was to assess whether melatonin accelerates the re-entrainment of locomotor activity after 6 h of advance and delay phase shifts following exposure to LD 12:12 cycle (simulating jet-lag/shift work). An experimental group of adult male field mice *Mus booduga* were subjected to melatonin (1 mg/kg) through i.p. and the control group were treated with 50 % DMSO. The injections were administered on three consecutive days following 6h of phase advance and delay, at the expected time of "lights off'. The results show that melatonin accelerates the re-entrainment after phase advance (29%) when compared with control mice. In the 6 h phase delay study, the experimental mice (melatonin administered) take more cycles for re-entrainment (51%) than the control. Further, the results suggest that though melatonin may be useful for the treatment of jet-lag caused by eastward flight (phase advance) it may not be useful for westward flight (phase delay) jet-lag.

Keywords. Melatonin; re-entrainment; circadian rhythm; locomotor activity; *Mus booduga.*

1. Introduction

The phase relations between entrained circadian rhythms of animals and their entraining periodic signals are determined by the properties of the organism's circadian system and by those of the Zeitgeber (Aschoff 1965; Hoffmann 1969). After a sudden shift of the zeitgeber' s phase, it usually takes several periods for the organisms to become re-entrained and to again reach a constant phase-angle difference. The time taken for re-entrainment and its direction are determined by the phase-angle at which the shifted signal occurs with respect to the animals endogenous rhythms, the strength of the entraining signal and the intrinsic characteristics of the circadian system (Aschoff *et al* 1975). In most species, including man a phase shift in environmental light/dark cycle results, after a transient lag period, in a corresponding phase shift of their circadian rhythms (Aschoff 1969; Pittendrigh 1981; Moore-Ede *et al* 1982). Because resynchronization does not occur immediately, the existence of an endogenous oscillator for circadian rhythms has been postulated.

There are two situations in which the disruption of circadian rhythm is believed to be responsible for health problems, namely shift working (Czeisler *et al* 1982; Turek 1986) and air travel involving rapid crossing of time zones (Ehret and Scanlon 1983; Wever 1985). Synchronization to a new environmental lighting schedule is usually attained after several days; during the transient period, a number of physiological alterations take place (Golombek and Cardinali 1993).

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It might be possible to treat patients with abnormally phased circadian rhythms by administering phase shift inducing drugs. For instance, an administration of the pineal hormone 'melatonin' shifts human circadian rhythms (Lewy *et al* 1992; Zaidan *et al* 1994) and ameliorates disruption of mood and sleep due to both endogenous (blindness, aging, delayed sleep phase syndrome) and exogenous (jet lag, shift work) causes (Folkard *et al* 1993; Petrie *et al* 1993; Oldani *et al* 1994; Garfinkel *et al* 1995). Furthermore, administration of melatonin is known to synchronize free running rhythms in European starlings *Sturnus vulgaris* (Gwinner and Benzinger 1978), rats (Redman *et al* 1983: Warren *et al* 1993) and accelerate re-entrainment after phase shifts in light/dark (LD) schedules (Armstrong and Redman 1985; Golombek and Cardinali 1993).

The objective of our study was to find out the effect of melatonin on the re-entrainment of locomotor activity rhythm in field mice *Mus booduga* following a shift in light/dark cycle (both advances and delays-simulating jet-lag/shift work).

2. Materials and methods

2.1 *Animals*

Adult male mice *Mus booduga* were captured from the fields at Madurai (9° 58′N 78° 10′E) and were maintained under light/dark (LD) 12:12 for about 15 days. Food and water were made available *ad libitum.* Animals were then housed individually in running wheels (wheel diameter 16 cm) with an attached cage ($l \times b \times h$=10cm × 8cm × 14 cm).

2.2 *Drugs*

Melatonin was procured from Sigma Chemicals Co., St. Louis, MO USA and was dissolved in 50% dimethyl sulphoxide (DMSO). Injections (2 µl) were made intraperitonially (i.p.).

2.3 *Monitoring of locomotor activity*

An eccentrically placed magnet in the running wheel facilitated the recording of wheel-running activity by completing and breaking the electric reed-relay circuit which in turn was picked up by an Esterline Angus Event recorder. Strip charts describing running wheel activities for 24 h were pasted one below the other chronologically for the construction of actogram. Onset of activity was used as the reference phase for the determination of phase, period, phase-shifts, etc. (Chandrashekaran *et al* 1983). Onset of activity was estimated by both eye-fitted and regression lines.

2.4 *Experimental procedure*

After a baseline recording of the locomotor activity rhythm in the mice (10 to 15 days) an advance of LD schedule by 6 h was evoked by shortening of the light time and a 6 h delay of LD schedule was evoked by lengthening of the light time.

Experimental (n = 7) and control (n = 6) groups of animals were subjected to the following treatments: (i) 50% DMSO (2 µl) administered on the first three consecutive days of the new LD schedule which constituted the control group, (ii) The experimental

group was injected with melatonin 2 µl (1 mg/kg) for three successive days starting from the next day onwards after LD shift. Both the groups of animals were injected at the expected time of "lights off" in the altered LD cycle. Wheel running activity was monitored until resynchronization was achieved.

2.5 *Statistical analysis*

Results were statistically analysed using Students' *t* test.

3. Results

Figures 1 and 2 depict the locomotor activity pattern of the control (vehicle: 50% DMSO treated) and the experimental (melatonin treated) animals respectively. Results show that melatonin treated animals take fewer cycles (10·2 ± 0·86) for re-entrainment compared to the DMSO injected animals (14·3 ± 0·76) after an advance shift of 6 h in the LD cycle. Melatonin shortens the rate of re-entrainment significantly (P « 0·0001) by 29% compared to the control (figure 5).

Figures 3 and 4 depict the effect of melatonin administration following a phase delay of 6 h in the LD 12:12 cycle. The number of cycles taken for re-entrainment by the experimental animals (4·8 ± 0·64) following 6 h phase delay was found to be significantly higher than the control animals (3·2 ± 0·44). In this case melatonin lengthens the rate of re-entrainment significantly (P « 0·0005) by 51% compared to the control (figure 5).

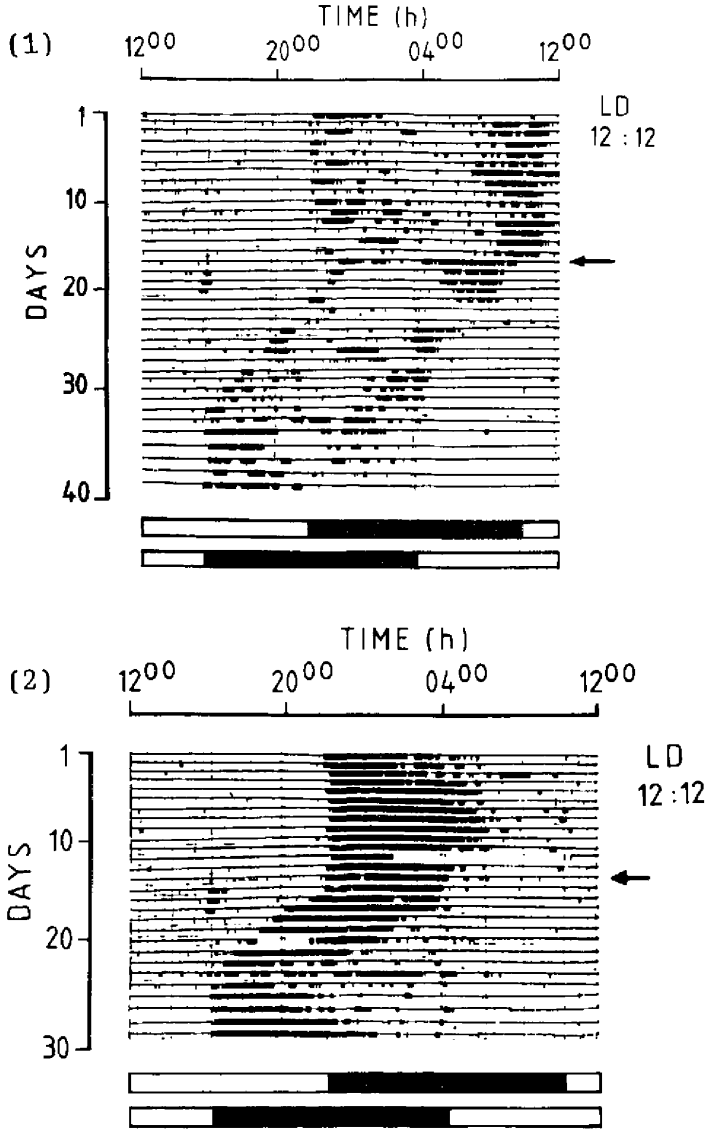
4. Discussion

The results of our experiment (figures 1–5) seem to favour compensatory effect of melatonin (accelerating for phase advance and decelerating for phase delay) on resynchronization of locomotor activity in field mice *Mus booduga* (figure 5). Although reports of the accelerating effects of melatonin on re-entrainment already exist (Golombek and Cardinali 1993), our contribution regarding melatonin treatment after phase delays provides additional information. Unlike previous reports of the short-acting drugs like Benzodiazepine (BZP) triazolam (Turek and Losee-Olsen 1986; Turek and Van Reeth 1988), melatonin differed significantly in accelerating/decelerating the re-entrainment kinetics. Short-acting drugs accelerated resynchronization bringing about acute instantaneous phase resetting of the locomotor activity rhythm (Golombek and Cardinali 1993). On the contrary, the data obtained from our experiment show that melatonin modifies the rate of re-synchronization without causing acute phase advance/delay of the locomotor activity rhythm.

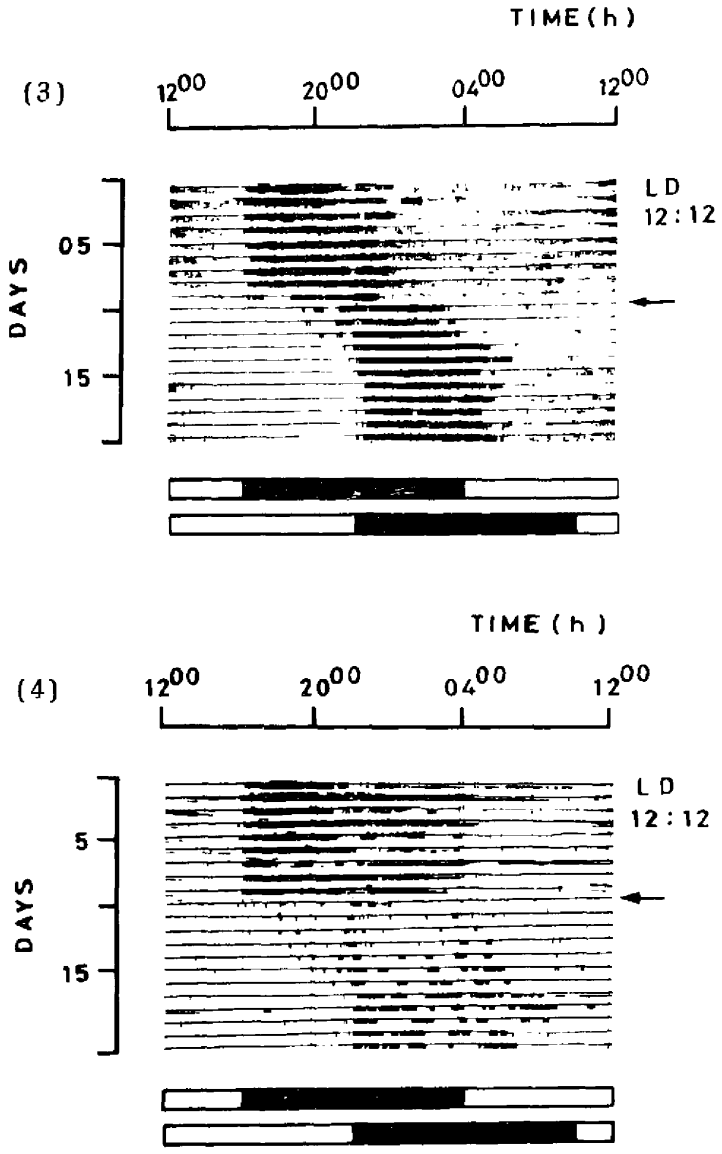
Melatonin administration at new dark onset was found to be more effective in accelerating the re-entrainment of N-acetyltransferase (NAT) rhythm (Illnerova *et al* 1989). On the contrary, daily administration of melatonin to rats exposed to 5 h advance of the LD cycle at the old dark onset accelerated the re-entrainment of locomotor activity rhythms (Redman and Armstrong 1988). Melatonin was also found to be effective for re-entrainment in *Mus booduga,* given just before the new dark onset. But unlike previous report, it accelerated/decelerated the rate of re-entrainment after phase advance/delay by 6 h in the LD cycle (figure 5). Melatonin might act by causing

a phase jump in the pacemaker (Illnerova *et al* 1989) into the advance dark period rather than by advancing the rhythm step by step. Such an argument seems to be legitimate when melatonin is given at the new dark onset i.e., 6 h before the old-dark onset.

According to Golombek and Cardinali (1993) acceleration of the rate of re-entrainment by melatonin after phase advances in the LD cycle suggests three distinct hypothetical interpretations: (i) melatonin may affect the circadian clock by modifying its oscillatory behaviour to induce small phase advances in endogenous cyclicity; (ii) melatonin may modify the communicating pathway between the clock and the overt rhythms (in this case the pathways connecting the Suprachiasmatic nucleus with the



Figures 1 and 2.



Figures 3 and 4.

Figures 1-4. Locomotor activity rhythms in a field mouse *Mus booduga* treated with 50% DMSO (2 µl) and melatonin (1 mg/kg) respectively. After a baseline recording of the rhythms, a 6 h phase advance (**1,2**) and delay (**3,4**) of the LD cycle was achieved (arrow mark). From the next day onwards three consecutive days of injections were given through i.p. at the time of expected "lights off" after the phase shift.

areas of the brain effecting locomotor control); (iii) melatonin may alter the photoreceptive processes participating in entrainment of circadian rhythms.

Several experiments concerning the effect of melatonin on entrainment (Cassone *et al* 1986; Shibata *et al* 1989; Stehle *et al* 1989) suggests that the pineal hormone has

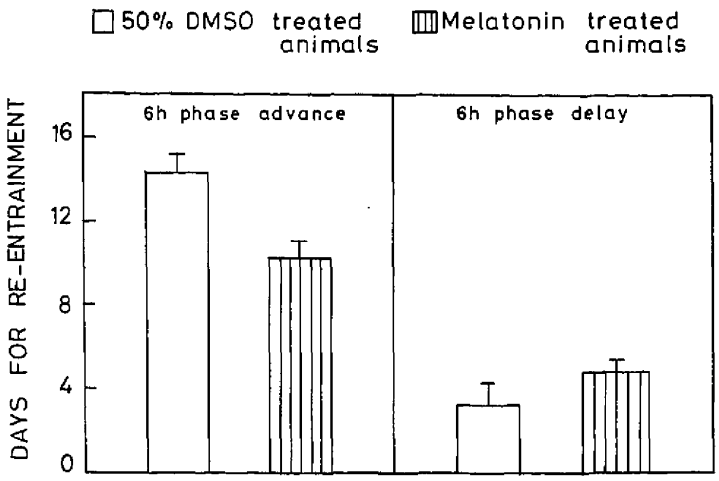


Figure 5. Number of days (± SD) required for the circadian rhyhtm of locomotor activity to be resynchronized after 6h advance and delay in light/dark cycle in the field mouse *Mus booduga.* Injections of vehicle (2 µl DMSO) and melatonin were administered after the phase shifts, at the time of expected "lights of".

direct influence on the circadian clock, allowing faster adaptation of rhythms to a phase advance.

Armstrong (1989) reported that the re-entrainment after a west-ward flight is easier because a westward flight causes phase delays and the human circadian system has the propensity to delay. Therefore, it is worth emphasizing that melatonin combats the effect of jet-lag as a nature's chronobiotic. In our experiment, we have selected 'new dark onset' or 'new light offset' as the phase for melatonin administration. We conclude that while melatonin accelerates re-entrainment after a phase advance of 6 h, it can also slow down the same after a 6 h phase delay. It would be rather interesting to explore the possibility of melatonin accelerating re-entrainment after phase delays by injecting the animals at other circadian times.

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