Short Communication

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Melatonin Accelerates Reentrainment of Circadian Locomotor Activity Rhythms to New Light–Dark Cycles in the Rat

Nobuyuki MARUMOTO, Noboru MURAKAMI, Haruto KURODA, and Takayuki MURAKAMI*

Department of Veterinary Physiology and *Department of Veterinary Anatomy, Faculty of Agriculture, Miyazaki University, Miyazaki, 889–21 Japan

Summary: We examined whether continuous melatonin administration through subcutaneously implanted silastic tubing accelerates reentrainment of circadian rhythms of locomotor activity to shifted illumination cycles. We found that rats required less time to reentrain to an advanced or delayed phase shift of a light–dark cycle while carrying a silastic implant filled with melatonin than while carrying an empty implant. These results suggest that continuous administration of melatonin accelerates the reentrainment of the circadian locomotor activity rhythm to a new light–dark cycle. [Japanses Journal of Physiology, 46, 347–351, 1996]

Key words: circadian rhythm, locomotor activity, melatonin.

The avian pineal gland, as one of the circadian oscillators, plays an important role in regulation of the circadian rhythms of behavior and physiology [1–3]. The suprachiasmatic nucleus, however, is the predominant oscillator in circadian organizations in mammals [4, 5]. The mammalian pineal gland is not generally considered to be involved in circadian organization, since pinealectomy has little effect on the circadian rhythms of behavior and physiology [6]. On the other hand, several observations indicate that melatonin may be involved in regulation of circadian rhythms in mammals as well as in birds. Daily injections of melatonin in pharmacological doses entrain locomotor activity rhythms in rats [7, 8]. Pinealectomy facilitates splitting of the rhythm in hamsters maintained under constant light [9, 10]. The melatonin-binding site is concentrated in the SCN in mammals [11]. We also previously reported that continuous application of melatonin to the SCN accelerates the reentrainment of the circadian adrenocortical rhythm to inverted illumination cycles [12]. This result raised the possibility that melatonin would be therapeutically useful for the reentrainment of circadian rhythms to a rapid shift in the light–dark cycle. It has been shown that melatonin is effective in alleviating jet lag, improving sleep quality and speeding up the resynchronization of plasma melatonin and cortisol rhythms in humans [13, 14]. However, these facilitating effects of melatonin on the reentrainment of circadian rhythms were detected with discontinuous measurements of rhythms, such as those of adrenocorticosterone and melatonin levels [12, 15]. In this study, therefore, we examined whether melatonin facilitates reentrainment of the circadian rhythm to a new light–dark cycle (advanced, delayed and inverted illumination cycles) by measuring locomotor activity rhythms in the rat.

Male Sprague-Dawley rats (about 3 months old) were kept under 12L : 12D condition (light on at 0700 h) in a temperature-controlled room (23±2°C). All illumination was supplied by fluorescent tubes, and the light intensity was about 200 lx at the level of the rat cage. Food and water were given ad libitum.

In the first experiment, 30 rats were pinealectomized under pentobarbital anesthesia, and were implanted subcutaneously with a silastic tube (2.5 mm ID, 3 mm OD, 5.5 cm length) containing melatonin. Another 30 sham-operated rats were implanted with an empty tube of the same size, as controls. All rats

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Correspondence should be addressed to: Noboru Murakami, Department of Veterinary Physiology, Faculty of Agriculture, Miyazaki University, Miyazaki, 889–21 Japan. Tel: +81–985–58–2811 ext. 3705, Fax: +81–985–58–2884

were transferred to individual rat cages (30×20×12 cm) equipped with an apparatus for measuring spontaneous locomotor activity by methods described previously [16]. All movements of each animal in its cage were converted into counts, and total activity counts were collected every 30 min by computer. After the activity rhythms had been recorded for about 2 weeks under the LD conditions, each batch of rats was divided into 3 groups of ten. The first and second groups were subjected to a 5 h advance and a 6 h delay in the LD cycle by advancing and delaying the time of lights-off, respectively. The third group was subjected to an inverted illumination cycle by delaying the lights-off time for 12 h.

In the second experiment, 44 rats were pinealectomized under pentobarbital anesthesia and divided into 2 groups. One group contained 22 rats which were then implanted subcutaneously with the same silastic tubes containing melatonin as those in the first experiment and remaing group of 22 rats were implanted with empty tubes, as controls. Ten days after implantation of the tubes, 7 and 8 rats from each group of 22 rats were subjected to a 8 h advance and a 5 h delay, respectively, in the LD cycle by advancing and delaying the time of lights-off. The remaining 7 rats from each group were subjected to blood sampling. One milliliter blood was collected by heart puncture under ether anesthesia at 1200 h every 3 d for 24 d beginning on day 2 after implantation. Plasma melatonin levels were determined by melatonin assay kit (Bühlmann Lab., Switzerland).

In individual rats, mean±SD of the acrophase in the activity rhythm was calculated using 1 week records before LD change. Then the time taken for reentrainment to the shifted LD cycle was defined as the estimated number of days needed for the acrophase of activity rhythm to shifted hours (plus or minus SD) relative to the preceding acrophase. The acrophase of activity rhythm was obtained by a least square spectrum analysis.

In the first experiment, when sham-operated rats implanted with an empty tube were subjected to a delay in the LD cycle, it took 4.2±0.5 d (mean±SE, $n=10$) for the activity rhythm to reentrain to the new LD cycle (two examples are shown in Fig. 1A and B). On the other hand, it took 2.1±0.4 d ($n=10$) for pinealectomized rats with the melatonin implant to reentrain to the delayed LD cycle (Fig. 1E and F). As shown in Fig. 1E, it appeared to be the activity-onset time rather than the activity-offset time that resynchronized rapidly. When the LD cycle was advanced, it took 7.1±0.6 d for sham-operated rats with the empty tube, and 3.2±0.6 d for pinealectomized rats

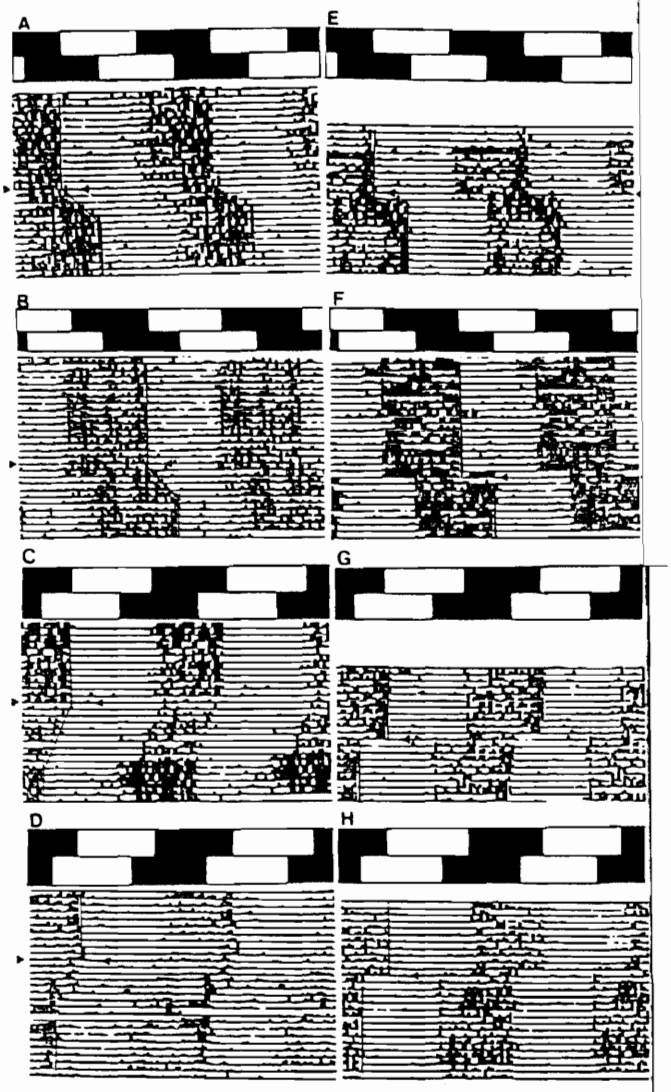

Fig. 1. Examples of double-plotted locomotor activity records in sham-operated rats implanted with empty tubes (A, B, C, D) and in pinealectomized rats implanted with melatonin-containing tubes (E, F, G, H). Rats were subjected to a 6 h delay in the LD cycle (A, B, E, F) or a 5 h advance (C, D, G, H) on the day shown by the triangle.

with the melatonin tube, to reentrain. Two examples from each group are shown in Fig. 1 (C, D and G, H, respectively). With the advanced LD cycle, activity-offset time was generally synchronized more rapidly than activity-offset time by melatonin treatment. When the LD cycle was inverted, reentrainment of the activity rhythm required 6.6 and 4.0 d (mean, $n=10$) in control rats and melatonin-treated rats, respectively. As shown in Fig. 2, it seems likely that melatonin speeded up the reentrainment of activity-onset time rather more than the activity-offset time to the inverted LD cycle.

In the second experiment, when pinealectomized rats implanted with an empty tube were subjected to a delay in the LD cycle, it took 5.4±0.4 d (mean±SE, $n=8$) for the activity rhythm to reentrain to the new

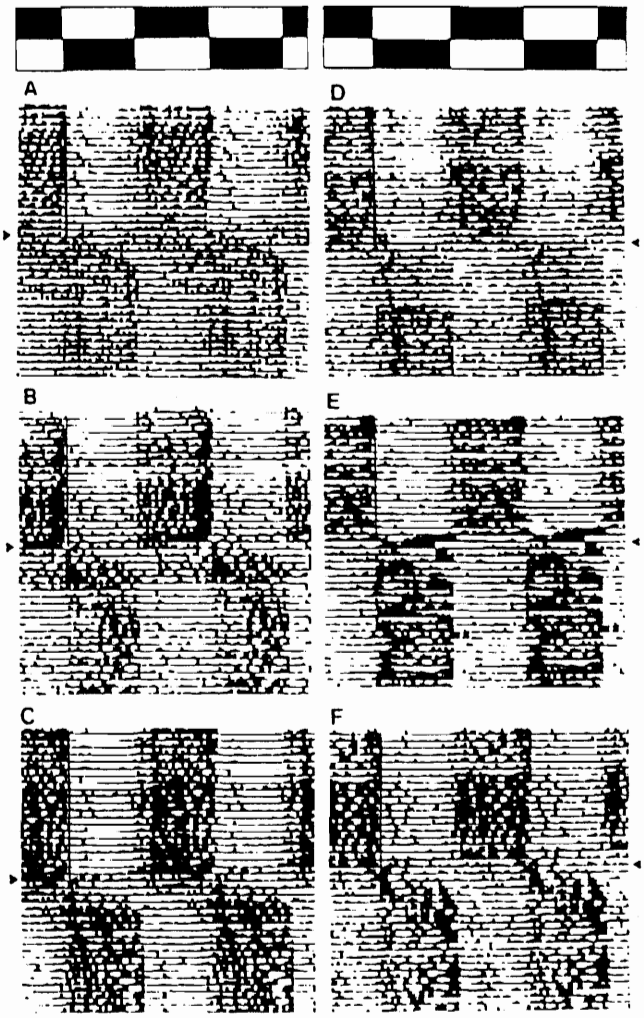

Fig. 2. Examples of double-plotted locomotor activity records in sham-operated rats with implanted empty tubes (A, B, C) and in pinealectomized rats implanted with melatonin-containing tubes (D, E, F). Rats were subjected to an inverted illumination cycle on the day shown by the triangle.

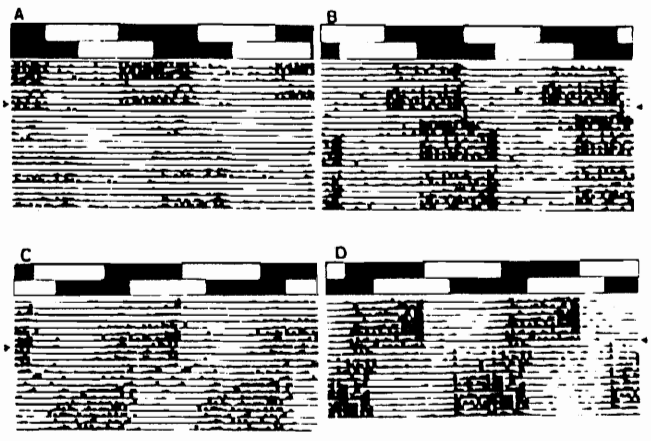

Fig. 3. Examples of double-plotted locomotor activity records in pinealectomized rats implanted with empty tubes (A, C) and melatonin-containing tubes (B, D). Rats were subjected to a 5 h delay in the LD cycle (A, B) or a 8 h advance (C, D) on the day shown by the triangle.

LD cycle (Fig. 3A). On the other hand, it took 2.3 ± 0.6 d ($n=8$) for pinealectomized rats with the melatonin implant to reentrain to the delayed LD cycle (Fig. 3B). When the LD cycle was advanced by 8 h, it took 7.7 ± 0.6 d for pinealectomized rats with the empty tube (Fig. 3C), and 3.9 ± 0.5 d for pinealectomized rats with the melatonin tube, to reentrain (Fig. 3D).

Plasma melatonin levels were measured every 3 d for 24 d after implantation of tubes filled with or without melatonin. Only the first plasma samples which were collected 2 d after implantation of the melatonin tube showed significantly high concentrations of melatonin (820 ± 126 pg/ml; mean$\pm$SE), compared with those collected on the other days. The levels of plasma melatonin were maintained almost constantly at 448 ± 95 pg/ml (upper level: 548 pg/ml; lower level 379 pg/ml) for 22 d in pinealectomized rats implanted with the melatonin tube. On the other hand, in pinealectomized rats implanted with the empty tube, melatonin levels were below 5 pg/ml in all samples. In our rat colony, day and night melatonin mean levels are 10.5 (1200 h) and 215 (2400 h) pg/ml.

The present study, together with previous observations [15, 12], indicates that melatonin accelerates reentrainment of the circadian rhythm to a new light–dark cycle. The action of this continuous administration of melatonin on the circadian rhythm seems to differ from the acute effect reported by others [17–20]. The acute effects of melatonin are dependent on the phase of circadian oscillators, such as phase-dependent phase shift and uptake of 2-deoxyglucose [17–19]. Entrainment by daily melatonin injections takes place only when the activity onset time corresponds to the time of injection [7, 8]. Recently it has been shown that administration of melatonin via a silastic tube both facilitates synchronization of sparrow circadian rhythms to light with very low intensity [21] and accelerates resynchronization following phase shifts of a light–dark cycle in house sparrows [22]. This similarity suggests that there may be common mechanisms involved in the melatonin action on entrainment of circadian rhythm to light–dark cycles in avian species and mammals.

Although the precise mechanism was not determined in this study, such an effect of continual administration of melatonin may be involved in the following mechanisms. First, we previously reported that when the LD cycles were advanced or delayed for 8 h, the circadian clock itself reentranted very rapidly (i.e., within 3 d) and sooner than we expected. However, overt rhythms of locomotor activity took many cycles. The long transient cycles shown by activity records

may be due to the long time-lag for coupling between the circadian clock and the hands of clock. That is, the time-lag for reentrainment between the circadian clock and the hands of clock may be due to the time needed for the overt rhythms coupled with slave oscillators to reach a steady state with the circadian clock [23]. If continuous administration of melatonin facilitates the coupling between the slave oscillator regulating activity rhythm and the circadian clock after a shift of the LD cycle, reentrainment of circadian locomotor activity rhythm may be accelerated. Secondly, continuous administration of melatonin may enhance the responsiveness (sensitivity) of the circadian system to light–dark stimuli: there is evidence that melatonin enhances retinal sensitivity to light [24, 25]. If so, the enhanced response is transferred directly to the SCN via the retino-hypothalamic tract. Further, the SCN has many binding sites for melatonin [11]. Therefore, melatonin may enhance the responsiveness of the SCN to the photic signal.

Melatonin accelerated reentrainment of activity onset more rapidly than its offset in response to a delayed LD cycle. On the other hand, when the rats were subjected to the advanced LD cycle following melatonin treatment, activity-offset was adjusted more rapidly to the new LD cycle than activity onset time. Similar results have been reported in reentrainment of the pineal *N*-acetyltransferase (NAT) activity rhythm to the advanced LD cycle [15]. The reason why activity-onset and offset accelerated to reentrain more rapidly depending on the delayed and the advanced LD cycle, respectively, is not clear. Illnerová *et al.* have discussed this in terms of a two-component (E-M) pacemaker controlling the NAT rhythm (NAT rise reflects the E-component, while NAT decline reflects the M-component). They hypothesized that entrainment of each of the two components into a new light–dark cycle is discrete during transient cycles, and that phase-jump may be involved [26]. Therefore, melatonin may facilitate the reentrainment of each two-component (E and M) pacemaker into a new light–dark cycle by rapid phase-jump. However, the possibility that melatonin accelerated the masking effect of light and dark signals on the locomotor activity during transient cycles remains.

In conclusion, the present study together with others [2, 3, 11, 12, 16, 22] indicates that melatonin accelerates the reentrainment of circadian rhythms following phase shifts of a light–dark cycle in birds, rats and humans.

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