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# Plasma Melatonin Rhythms in Euthermic Marmots (Marmota flaviventris)

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## ABSTRACT

Plasma melatonin concentrations were measured in marmots (*Marmota flaviventris*) maintained under three short-day (4L:20D; 8L:10D; 10L:14D) and one long-day (14L:10D) photoperiod(s). Each animal had a daily rhythm of plasma melatonin with elevated plasma melatonin levels occurring during the dark period of the lighting cycle. There were no significant differences between any peak values during the night. The mean duration of elevated night melatonin concentrations was significantly different between long-day (16L:8D) and 8L:16D or 4L:20D animals ($P<0.01$). Daytime plasma melatonin levels were not significantly different among the photoperiods. These results characterize plasma melatonin rhythms in a sciurid rodent and demonstrate that this rhythm is modified by photoperiod. Therefore, the plasma melatonin profile could convey information about day length to the animal or, alternatively, the rhythm may be acting as a time-keeping mechanism for other physiological functions.

## INTRODUCTION

In photoperiodic rodents such as the Syrian hamster (*Mesocricetus auratus*), short photoperiods (<12.5 h of light) induce a period of gonadal quiescence, a response mediated by the pineal gland. Removal of the pineal gland in the Syrian hamster abolishes the inhibitory effect of light on the reproductive system (Reiter, 1980). The pineal may regulate reproduction by a transduction of day length information via hormonal signals to the reproductive axis (Carter et al., 1982; Yellon et al., 1982). The hormone responsible for conveying photoperiodic information to the reproductive system may be the pineal product melatonin, since exogenously administered melatonin can mimic the inhibitory effect of short day length on the reproductive system. The timing of these injections, however, is crucial in producing this effect (Tamarkin et al., 1976; Reiter et al., 1976; Goldman et al., 1981).

Under various laboratory lighting schedules, melatonin is synthesized and released from the pineal gland with low levels produced during the day and peak levels occurring during the night. In most rodent species studied, melatonin levels are elevated only during a discrete window of time at night. However, in one rodent species, the Siberian hamster (*Phodopus sungorus*), the daily pineal melatonin rhythm changes as a function of photoperiod. In this species, increasing the length of darkness increases the period of elevated pineal melatonin levels (Yellon et al., 1982), suggesting that changes in the melatonin profile could provide day length information to the reproductive axis.

The above-mentioned studies have focused on rodent species whose reproductive activities are very sensitive to changes in photoperiod. However, some rodents such as golden-mantled ground squirrels (*Spermophilus lateralis*) and woodchucks (*Marmota monax*) appear to be much less sensitive to changes in photoperiod

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(Pengelley and Asmundson, 1974; Davis, 1976), since alterations in photoperiod have little effect on the timing of reproductive, body weight, and hibernation cycles. Unfortunately, little is known about seasonal cycles in marmots (*Marmota flaviventris*).

Recently, the effect of pinealectomy has been noted in adult golden-mantled ground squirrels (Phillips and Harlow, 1982). However, no study has been published describing the daily melatonin rhythm in a sciurid rodent, including marmots. Thus, we wished to determine if a plasma melatonin rhythm is present in the marmot and if it would be altered by changes in day length. The present studies demonstrate that the duration of nocturnal plasma melatonin changes in response to differing amounts of light.

## MATERIALS AND METHODS

The animals used in this study were yellow-bellied marmots purchased from an animal dealer in Colorado or trapped in the Elk Mountains of Colorado. Animals of both sexes were used, and their weights ranged between 3–6 kg. All animals were caged individually and provided with marmot chow (Purina diet), fresh vegetables, sunflower seeds and water, ad libitum. At least 4 animals were maintained on either one of three short-day photoperiods (10L:14D; 8L:16D; 4L:20D) or on a long-day photoperiod (16L:8D), for at least 8 weeks prior to experimentation. The experimental room temperature was 22°C ± 2°C, and during all experiments a red light (40 watt) was on continuously in the room. All animals were implanted with an aortic catheter, as previously described (Florant and Weitzman, 1980), and were allowed to recover from the surgical procedure for at least 2 weeks at room temperature before being used for an experiment.

At the beginning of each experiment, a blood sampling assembly, previously described (Florant and Weitzman, 1980), was attached to the external catheter 1 h prior to taking the first blood sample, allowing the animal time to acclimate to the sampling apparatus and the brief period of handling. In brief, each blood sampling assembly consisted of a polyethylene tube measuring about 200 cm in length, connected to an 18-gauge needle with 2 three-way stopcocks in series fitted with 3 syringes for blood collection. Blood samples were collected in the following manner: the heparinized saline in the line and the first 1 ml of blood were drawn into one syringe: in the second syringe, 2.0 ml of blood were withdrawn as the sample. The contents in the first syringe were slowly infused back into the animal, and the line was flushed using a third syringe containing heparinized saline (15 units/ml). Blood samples were collected every 3 h for 2 consecutive days. Each sample was immediately centrifuged, the plasma was separated from the red blood cells, and the plasma was frozen at −20°C for analysis. The red blood cells were resuspended in sterile saline and reinfused into the animal at the middle of the experiment (after 24 h) and after the experiment was over (48 h). The samples were analyzed in duplicate by radioimmunoassay (Tamarkin et al., 1979), having intra- and interassay coefficients of variation of 10% and 20%, respectively. Occasionally a sample was lost: this produced the sample size variation.

The duration of elevated plasma melatonin concentration during the dark portion of the light-dark schedule was arbitrarily determined by measuring the time interval between 2 points: the midpoints between the data points on either side of 60 pg/ml on the rising and falling phase of melatonin concentration profile. For example, in Fig. 1, the midpoint of the rising phase is 70 pg/ml (19:30) and the falling phase is 110 pg/ml (0.4:30). Thus, the time lapsed between these two points is equal to 9 h. Over this period of time (duration) we assume plasma melatonin concentrations remained above 60 pg/ml. A paired $t$ test or Student's $t$ test was used to analyze day/night differences and differences in duration of elevated plasma melatonin levels between the different photoperiods.

## RESULTS

A daily rhythm of plasma melatonin was observed in marmots kept in short or long photoperiods. Figure 1 depicts the melatonin profile of 4 animals exposed to 10L:14D for 8 weeks. Blood samples were collected from each animal twice, at the indicated times. Daytime plasma melatonin levels were low, ranging from 28 to 54 pg/ml of plasma. Within 2 h after the onset of darkness plasma melatonin levels began to rise and peak levels were observed between 2400 and 0400 h. Plasma levels began to decline prior to lights-on. In this lighting schedule, all four animals studied had similar daily patterns, with a mean duration of elevated levels equal to 9.0 h ± 1.0 h (Table 1).

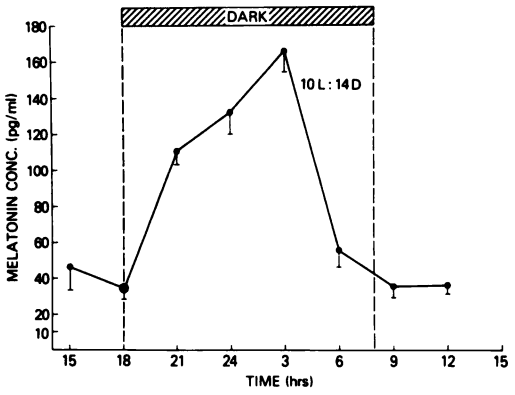


FIG. 1. Daily plasma melatonin concentrations in 4 marmots collected over a 2-day period. Each point represents the mean ± SEM (N=8) of samples collected at the indicated time. *Circled points* are collected in the light. Photoperiod was 10 h of light.

TABLE 1. Plasma melatonin concentration in euthermic marmots (*Marmota flaviventris*).

| | Duration (h) | Amplitude (pg/ml) | Daytime (pg/ml) |
|---|---|---|---|
| Short day | | | |
| 4L:20D | 12.0 ± 1 (8)[a] | 99.3 ± 17 (8) | |
| 8L:16D | 11.5 ± 1 (10) | 120.7 ± 28 (9) | $\bar{X}$=32 ± 3.0[b] |
| 10L:14D | 9.0 ± 1 (8) | 166.8 ± 11 (8) | N=49, range (9–54) |
| Long day | | | |
| 16L:8D | 9.0 ± 1 (8) | 89.3 ± 8.8 (8) | $\bar{X}$=35.5 ± 4.0 N=44, range (16–52) |

[a]Mean ± SEM; numbers in parentheses represents the number of 24-h profiles pooled.

[b]Group mean for all short-day animals; *N* represents the number of samples collected and pooled for a daytime mean.

By extending the dark period by 2 h (8L:16D) the duration of the plasma melatonin rhythm was also increased (Fig. 2). In this photoperiod, plasma melatonin levels began to increase within 2 h after lights-out and began to fall at least 2 h before lights-on. This photoperiod produced a mean elevated melatonin duration of 11.5 h ± 1.0 h in the 5 animals studied. A further 4-h extension of the dark period (Fig. 3: 4L:20D) also increased the duration of this hormonal rhythm. The 4-h increase in darkness resulted in a 3-h increase in the duration of elevated melatonin levels compared to those observed in 10L:14D. Although differences in peak levels were noted between individual animals studied in these photoperiods (Table 1), the peak plasma melatonin levels for an individual over the 2 days of study were similar (Fig. 4).

In comparison to the short photoperiods studied, marmots maintained in a long photoperiod exhibited a somewhat different temporal profile. In the 4 animals studied the nocturnal increase in plasma melatonin occurred late in the dark period. As illustrated in Fig. 5, plasma melatonin levels began to increase within 2 h of the dark period. Within 2 h of lights on, plasma melatonin concentrations were close to baseline values. Although the melatonin rhythm appeared to be less closely coupled to the light-dark and the dark-light transitions, as was observed in the 3 different short photoperiods, the daily increase in plasma melatonin was still occurring primarily at night. In this photoperiod the duration of elevated plasma melatonin levels was 9.0 ± 1.0 h, which is significantly less than ($P<0.01$) the

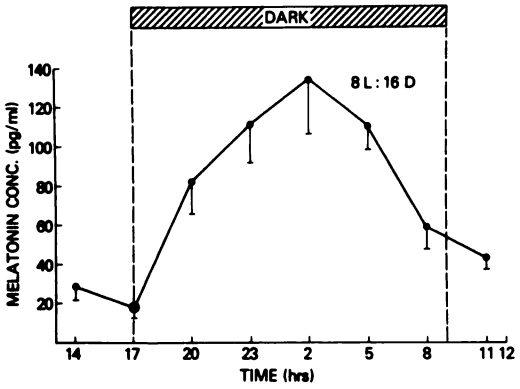


FIG. 2. An illustration of the plasma melatonin concentration over a 2-day period in 5 animals (N=10) maintained under a 8L:16D schedule.

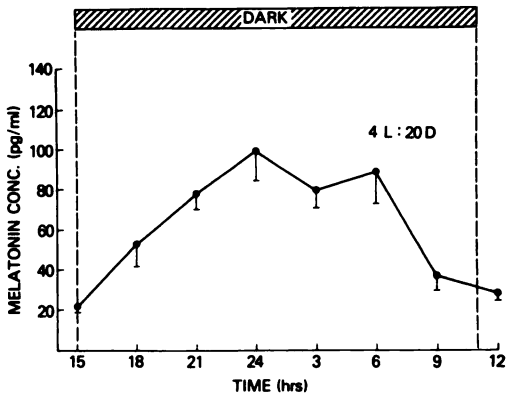


FIG. 3. The daily rhythm of plasma melatonin measured in 4 animals over 2 days kept in a lighting cycle of 4L:20D. Samples taken at 1500 h were collected in the light phase.

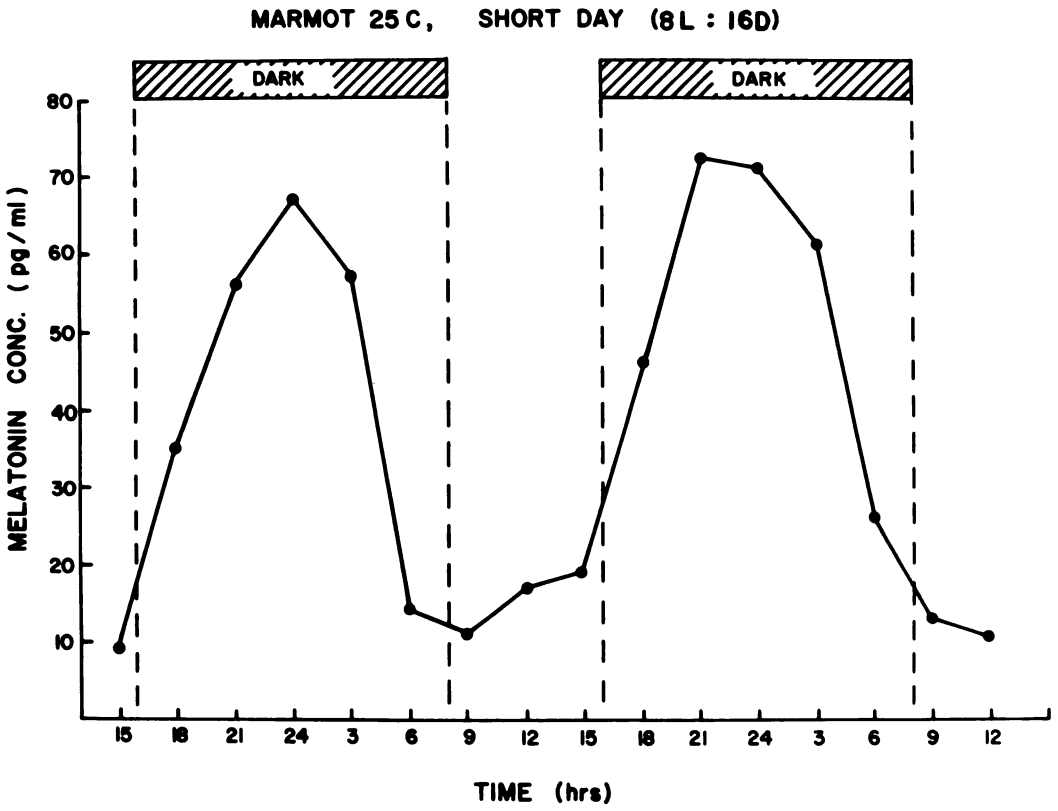


FIG. 4. A typical illustration of plasma melatonin concentration in a marmot continuously sampled over a 2-day period (8L:16D).

mean duration of melatonin in 8L:16D or 4L:20D (Table 1).

## DISCUSSION

This is the first study to characterize the 24-h plasma melatonin profile of a sciurid rodent, the marmot. By employing our method for plasma sampling, samples could be obtained over a 2-day period, allowing us to characterize better the changes in plasma melatonin in an individual animal. Furthermore, the plasma melatonin rhythm in marmots appears to be responsive to changes in photoperiod.

It is well known that marmots are seasonal breeders (Armitage, 1962). It is not known, however, if the daily melatonin rhythm is the hormonal signal regulating reproductive function in this species, as has been observed in other seasonally breeding rodents (Reiter, 1980). If the melatonin rhythm is essential for distinguishing season, then it would be crucial that a change in this pattern occur between photostimulatory and photoinhibitory lighting cycles. We did observe that in extremely short photoperiods (8L:16D and 4L:20D), plasma melatonin levels remained high longer.

No data exist on the sensitivity of the marmot to subtle changes in photoperiod. Marmots spend as much as 8 months of the year under-

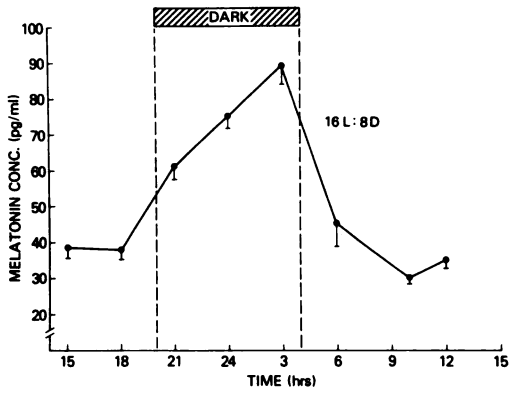


FIG. 5. A plot of the plasma melatonin concentrations measured in 4 marmots maintained under a long day length (16L:8D).

ground (August–April). Given this circumstance they might not see subtle changes in day length. More likely, they experience extremes in photoperiod from the long days of summer to the constant darkness, or close to constant darkness, of the burrow. Thus, in this species the melatonin rhythm might not be sufficiently responsive to initiate changes in reproductive activity.

Previous studies on a closely related species, the woodchuck, suggest that photoperiod may not be as significant an environmental zeitgeber as ambient temperature (Mrosovsky, 1978, for a review). Temperature sensing capabilities are well developed in marmots (Florant and Heller, 1978), and with recent evidence that the pineal gland may influence body temperature regulation (Ralph et al., 1979), it is tempting to speculate that the effect of the pineal on body temperature may be mediated through the daily melatonin secretory profile. Melatonin is also thought to affect the frequency and duration of torpor and hibernation in mice and ground squirrels, respectively (Lynch et al., 1978; Phillips and Harlow, 1982). Our results lead us to suggest that the melatonin rhythm in marmots is certainly capable of timing circadian events as daily torpor and could be involved in timing hibernation.

The daily melatonin rhythm in all animals studied has been shown to be a centrally controlled response which is driven by a central circadian pacemaker (Takahashi and Zatz, 1982). In the case of the daily melatonin rhythm, the central circadian pacemaker receives visual input and transmits that information via a multisynaptic pathway to the superior cervical ganglia which stimulates the pineal gland to synthesize melatonin. In the absence of light-dark cues, the daily melatonin rhythm persists with a period determined by the individual's central circadian pacemaker. This would be the full circadian expression of the melatonin rhythm. In the present study, the relatively small increase in duration noted between 8L:16D and 4L:20D photoperiods indicates that perhaps the full circadian expression of the melatonin rhythm is achieved in 8L:16D, and increasing amounts of darkness do not result in a significantly longer period of elevated melatonin. In addition, the duration of elevated melatonin in 2 animals kept in total darkness was not significantly different from elevated melatonin durations measured under these two short photoperiods (unpublished observations).

In all laboratory-reared rodents, light presented at night induces a "turning-off" of melatonin synthesis. Recent evidence has been presented that clearly demonstrates that this response to light at night is a function of the animal's life history (Reiter et al., 1982). Ground squirrels (*Spermophilus richardsonii*) caught in the wild undergo a suppression in pineal melatonin content only when exposed to very intense light at night, whereas, the same species raised in artificial lighting undergoes pineal melatonin suppression with light intensities equivalent to their artificial room light. In our study, all animals were caught in the wild, and were then exposed to artificial illumination for at least 3 months prior to these studies. We do not know if light presented at night would or would not suppress plasma melatonin in these animals.

The role of the photoperiodically regulated plasma melatonin rhythm in the marmot is unknown. We suspect the rhythm's function may be time-keeping for nonreproductive physiological systems, such as body temperature regulation and hibernation. Reproductive function of the marmots, however, may be under photoperiodic control, and we are currently performing experiments to examine this possibility. In essence, our present data do not allow us to ignore the fact that a melatonin rhythm exists in the marmot and that the rhythm is modified by a photoperiod. As such, we can not rule out the possibility that the pineal gland and its melatonin rhythm could be involved in time-keeping functions and/or acting as a neuroendocrine transducer for day length.

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