Daily hypothermia and torpor in a tropical primate: synchronization by 24-h light-dark cycle

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Perret, M., and F. Aujard. Daily hypothermia and torpor in a tropical primate: synchronization by 24-h light-dark cycle. Am J Physiol Regulatory Integrative Comp Physiol 281: R1925–R1933, 2001.—To study the temporal organization of daily hypothermia and torpor in a nocturnal Malagasy primate, the gray mouse lemur, body temperature (Tb) and locomotor activity were recorded using telemetry on 39 males held in 24-h light-dark cycles of different photoperiods. Under free-running condition, the circadian Tb and locomotor activity rhythms had a period shorter than 24 h. Circadian daily hypothermia started by a rapid drop in Tb (0.24°C/10 min) at the end of subjective night (13 h 25 ± 10 min) and was characterized by minimal Tb values 3 h 20 ± 5 min later. Spontaneous arousal from daily hypothermia occurred at a fixed time (6 h 05 ± 15 min, n = 7) after the beginning of subjective day. In animals exposed to 24-h light-dark cycles with night duration varying from 10 to 14 h, locomotor activity was strictly restricted to dark time, but the temporal organization of daily hypothermia was not modified, although changes in amplitude of Tb rhythm were observed. Daily hypothermia was directly induced by light and lasted 5 h 10 ± 10 min, with minimal Tb values 3 h 30 ± 30 min (n = 28) after lights on, on condition that nighttime did not exceed the duration of subjective night. However, in animals exposed to 24-h light-dark cycles with night duration varying from 10 to 5 h, the limit of induction of daily hypothermia by light was ~9 h after the beginning of night. Finally, under short days (14:10-h light-dark cycle), long bouts (6 h 50 ± 40 min) of actual torpor (minimum Tb 27.6 ± 0.9°C) were observed and would involve mechanisms depending on physiological changes induced by short day exposure.

circadian rhythm; body temperature; locomotor activity; light response; mouse lemur

The maintenance of a constant body temperature (Tb) is energetically expensive. To save substantial energy, many small mammals enter a state of torpor, during which Tb and metabolic rate are significantly reduced. The ability to use torpor has been considered a primitive mammalian trait and an expression of incomplete normothermic regulation. Torpor is present in many ancestral groups of mammals, and recent studies strongly suggest that it reflects a more general adaptive energy-saving strategy to face fluctuating food supply (1, 7). Torpor is thus commonly found in many small body-sized species living in contrasted climatic conditions or exposed to unpredictable environments (4, 7, 9, 19).

The two distinct patterns of torpor, not mutually exclusive, hibernation and daily torpor, differ typically in the duration of torpor bouts and in their relation to the circadian system (9, 17). Hibernation is characterized by prolonged torpor bouts, highly reduced metabolic rate, and very low Tb. This process provides high energy benefits and occurs in anticipation, before the occurrence of unfavorable environmental conditions, under the control of a seasonal timekeeping mechanism (1, 5, 8, 15, 17, 27). In contrast to hibernation, daily torpor is characterized by a short-duration bout that does not exceed 24 h, so that animals exhibit an active state each day between two torpor episodes. Daily torpor occurs at a distinct time of the day and/or year, and although it confers less energetic advantages than hibernation, it is likely that daily drop in body temperature reduces energetic costs, allowing greater independence in relation to food resources.

Within nonhuman primates, seasonal and daily torpor has been observed only in small body-sized Malagasy prosimians such as mouse lemurs and fat-tailed dwarf lemurs (3, 20, 21, 31, 32). The gray mouse lemur (Microcebus murinus), one of the smallest primes (60–100 g body wt), is considered to be representative of ancestral primate stock. This nocturnal and arboreal species, inhabiting various western Malagasy biotopes, feeds primarily on fruits and insects but also consumes insect secretions, gums, and flowers. To cope with extreme seasonal variations in food availability, mouse lemurs exhibit adaptive seasonal biological rhythms. The breeding period is restricted to hot and rainy summer months (day length >12 h), when food availability is high, and is associated with sustained behavioral and physiological activities. In contrast, during the dry and cold winter (day length <12 h), when food resources remain scarce, both sexes enter complete sexual rest and undergo a pronounced fattening associated with reduced behavioral and physiological activities, gregarious behaviors, and torporous state.

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Seasonal rhythms of mouse lemurs have been demonstrated to be dependent on the photoperiod and can thus be maintained in captivity under artificial light conditions (23, 24). Thermoregulatory abilities were also demonstrated to be triggered by seasonal variations in photoperiod (3). Studies on daily hypothermia in gray mouse lemurs have also been carried out in wild or captive animals by use of telemetry (3, 30, 31). However, daily changes in Tb have mainly been described in an ecological framework, rather than from a chronobiological perspective.

The gray mouse lemur presents a particularly accurate entrainment pattern of daily locomotor activity characterized by a high plasticity in the response to photic conditions (29). In contrast to locomotor activity, which is strongly suppressed by light, the Tb rhythm reflects regulation by the circadian clock in its external (to the light-dark cycle) and internal (to other circadian rhythms) phase relations. The present study aims to explore how seasonal variations in physiological constraints and circadian entrainment of the clock simultaneously regulate the expression of the Tb rhythm. Our results characterize the temporal organization of daily hypothermia in the gray mouse lemur and its 24-h light-dark regulation under controlled conditions, showing that daily hypothermia proceeds from an endogenous circadian function and that its onset is induced by light within certain limits toward the beginning of the night.

MATERIALS AND METHODS

Animals and animal care. The 39 male lesser mouse lemurs (M. murinus, Cheirogaleidae, primates) used in this study were born in a laboratory breeding colony at Brunoy, France (Agreement 962773) from stock originally caught 30 years ago on the southwest coast of Madagascar. All were 2- to 5-yr-old adults. General conditions of captivity were maintained constant with respect to ambient temperature (24–26°C), relative humidity (55%), and ad libitum food availability. In the breeding colony, to ensure highly synchronized changes in biological rhythms within individuals, animals were exposed to an artificial photoperiodic regimen consisting of 3 mo of Malagasy winterlike short day length (10:14 h light-darkness) and 5 mo of Malagasy summerlike long day length (14:10 h light-darkness). In such conditions, breeding or resting state is reached within 4 wk after the change in day length (23).

Light-dark experiments. To study the temporal organization of daily Tb rhythm, males were held in 24-h light-dark cycles of different photoperiods. In all experiments, light was provided by cool fluorescent lamps (250–350 lux), and a dim red light (20 µW/cm², equivalent to 0.002 lux) was provided during the dark time. To minimize social influences during the experiments, animals were housed individually in cages (0.4 × 0.4 × 0.6 m) provided with nest and supports and separated from each other by wooden partitions, ensuring complete visual isolation. To determine endogenous circadian rhythms under free-running condition, seven males previously maintained on a 24-h light-dark cycle (12:12 h light-darkness) for 4 wk were exposed to constant dim red light (RR). To avoid the possibility of entrainment of circadian rhythms by external cues, food was distributed three times a week at various times of the day. Free-running condition under constant light (LL) has been tested on four males, but because of the strong inhibitory effect of light on general activity, these nocturnal animals did not feed. This experiment was thus terminated after 4 days because of the loss of body mass in these animals. For assessing seasonal changes in Tb rhythm, males routinely maintained under summer (14:10 h light-darkness, n = 10) or winter (10:14 h light-darkness, n = 10) light-dark cycle were tested after 2 mo under the given day length. Finally, to determine to which limit the daily drop of Tb can be entrained by light, four males previously maintained on a 24-h light-dark cycle (10:14 h light-darkness) for 4 wk were exposed to a weekly reduction in the dark time from 10 to 9, 8, 7, 6, and 5 h per 24 h.

Recordings of Tb and locomotor activity. Recordings of Tb and locomotor activity were obtained using telemetry at a constant ambient temperature of 25°C. A small telemetric transmitter weighing 3.2 g (model TA107A-F20, Data-scientific) was implanted into the visceral cavity under ketamine anesthesia (1malgene, 100 mg/kg ip). After surgery, animals were returned to their home cage and allowed to recover for 1 mo before continuous recordings of Tb and locomotor activity. Two receiver boards positioned on opposite sides of the cage collected radio-frequency signals; Tb was recorded for 10 s every 5 min. Locomotor activity was recorded continuously, and pulses were generated whenever the animal changed its position relative to the two antennas inside the boards. To filter out noise, a threshold at 2.5 arbitrary units (AU) per 10 min was used, a value corresponding to minor changes in resting position of the animals in their nest. Rhythm profiles for Tb and locomotor activity were obtained by smoothing data for each animal by a 10-min moving-average filter of the signals transferred to the computer (Dataquest Lab Pro).

For experiments under 24-h light-dark cycles, zeitgeber time 12 was referred to as the beginning of the dark time corresponding to the onset of nocturnal activity. In RR free-running conditions, the beginning of subjective night delineated by the spontaneous activity onset was referred to as circadian time 12. To be able to compare animals with different free-running periods, we normalized data by dividing by the individual period lengths to give proportional measures.

On a 15-day-period recording basis, different parameters were calculated for each individual: mean Tb during the dark time or subjective night (Tb Night), mean Tb during the light time or subjective day (Tb Day), minimal and maximal Tb values, giving the range of the Tb rhythm, and their temporal occurrence dating from the beginning of the dark time or subjective night. In the mouse lemur, the daily or circadian drop in Tb was defined as daily hypothermia when minimal Tb was >33°C and as actual torpor when minimal Tb dropped below 33°C. The threshold of 33°C has been chosen according to the obvious bimodal distribution of minimal Tb values. The duration of daily hypothermia and torpor was defined as the time during which Tb was lower than Tb Day. The days during which a torpor bout was observed were treated separately. The average total activity performed by each animal was calculated for dark and light times (or subjective night and day, respectively), and the length of activity was defined as the time between activity onset and activity offset. Finally, animals were weighed before and after the experimental period. Depending on the 24-h light-dark cycle to which the males were exposed, body mass varied from 75 to 110 g (Table 1). Because exposure to long day lengths (>12 h light/day) led to breeding state, the body mass of males exposed to 14:10 h light-darkness or to continuous light was decreased.
RESULTS

Circadian rhythms of Tb and locomotor activity. In the free-running RR condition, Tb and locomotor activity demonstrated robust circadian periodicity (Fig. 1A), with similar dominant periods of 22 h 55 ± 10 min and 22 h 45 ± 20 min (n = 7), respectively. Subjective night and day could be easily delineated by clear onset and offset of locomotor activity. Subjective night lasted, on average, 13 h 25 ± 20 min, and subjective day lasted 9 h 30 ± 20 min. The circadian rhythm of Tb was characterized by significantly higher Tb values during subjective night and lower values during subjective day (F = 13.8, df = 1/12, P < 0.003). A drop in Tb began immediately at the end of subjective night at a linear 0.24 ± 0.04°C/10 min over the 1st h, and minimal Tb values were observed 3 h 20 ± 10 min later (Fig. 1B).

Duration of daily hypothermia, defined as the time during which Tb was lower than mean Tb Day, lasted 4 h 40 ± 20 min, and arousal from daily hypothermia occurred typically 6 h 05 ± 15 min (n = 7) after the beginning of subjective day, with Tb increasing steadily until the onset of activity. During subjective night, activity pulses (0.9 ± 0.2 AU/10 min) were regularly distributed, with a pronounced increase before the spontaneous drop in Tb. Cross-correlation analysis demonstrated that all changes in nocturnal activity were reflected in Tb with a 10-min delay (r = 0.531).

Activity pulses during subjective day (0.9 ± 0.2 AU/10 min) corresponded to changes in resting position of the animals in their nest and represented <7% of the total circadian activity pulses.

Although it is impossible to maintain animals for extended periods of time under constant light because of its strong inhibitory effect on behavioral activities, recordings of Tb and activity in four animals demonstrated that Tb maintained a circadian rhythm (Fig. 2) while very few activity pulses were irregularly distributed (0.6 ± 0.2 AU/10 min) without any significant circadian rhythmicity. The period of Tb circadian rhythm calculated from the first 3 days averaged 25 h 30 ± 15 min (n = 4), a value significantly longer than...
that observed in free-running animals under the RR condition ($F = 77$, df = 1/9, $P < 0.001$). However, the $T_b$ rhythm profile showed a clear free-running rhythm. Even though animals did not exhibit circadian activity rhythm, the beginning of subjective night showed a clear increase in $T_b$ and ended with a decrease in $T_b$ at a linear 0.20 ± 0.02°C/10 min, a pattern identical to that observed under the RR condition. During subjective night (mean duration 12 h 35 ± 10 min, $n = 4$), $T_b$ values were significantly reduced compared with the RR condition ($F = 4.96$, df = 1/9, $P < 0.05$) because of the strong inhibition of activity by constant light (Table 1). In contrast, no significant difference was found for mean $T_b$ during subjective day between LL and RR conditions ($F = 0.4$, df = 1/9, $P = 0.5$). The daily drop in $T_b$ under the LL condition was identical to that observed under the RR condition with minimal $T_b$ 3 h 45 ± 35 min after the onset of $T_b$ decrease and a mean daily hypothermia duration of 5 h 00 ± 20 min (Table 1).

$T_b$ and locomotor activity rhythms under different seasonal 24-h light-dark cycles. In males exposed to 24-h light-dark cycles of different duration from winterlike short days (10:14 h light-darkness) to intermediate (12:12 h light-darkness) and summerlike long days (14:10 h light-darkness), significant changes occurred in $T_b$ parameters according to photocycle (Table 1). Mean $T_b_{\text{Night}}$ and $T_b_{\text{Day}}$ were significantly higher in males exposed to the summer photocycle ($F = 4.1$, df = 2/25, $P < 0.03$ and $F = 4.6$, df = 2/25, $P < 0.02$, respectively) than in those exposed to short and intermediate photocycles within which no differences were recorded ($F = 1.5$, df = 1/16, $P = 0.2$ and $F = 1.1$, df = 1/16, $P = 0.2$ for $T_b_{\text{Night}}$ and $T_b_{\text{Day}}$, respectively). Similarly, males exposed to long photoperiod exhibited higher minimal $T_b$ values during diurnal rest than other males ($F = 3.6$, df = 2/25, $P < 0.04$). In fact, the amplitude of the $T_b$ rhythm remained unchanged: 3.10 ± 0.08°C ($n = 28$). Whereas total activity pulses during the light time did not differ within groups, a significant increase in total nocturnal locomotor activity was shown in animals exposed to long photoperiod ($F = 7.6$, df = 2/25, $P < 0.001$; Table 1). This increase was not related to maximal $T_b$ or $T_b_{\text{Night}}$ ($r = -0.154$ and $r = -0.298$, $n = 28$). Finally, locomotor activity was strictly restricted to the dark time, and temporal distribution was clustered around the time of activity onset and offset. As in animals under the RR condition, changes in nocturnal activity were reflected in $T_b$ with a 0- to 10-min delay according to photocycle ($r = 0.703$ to 0.661).

Although the long photoperiod had a significant effect on the $T_b$ and locomotor activity values, the daily pattern of $T_b$ rhythm and the temporal organization of daily hypothermia demonstrated similar characteristics regardless of light cycle (Fig. 3). The duration of daily hypothermia was not influenced by the type of 24-h light-dark cycle or free running condition ($F = 0.96$, df = 4/34, $P = 0.4$) and averaged 5 h 10 ± 10 min ($n = 39$; Table 1). Likewise, the time required to reach the daily minimum $T_b$ was independent of light condition ($F = 0.5$, df = 4/34, $P = 0.7$). It occurred 3 h 30 ± 10 min ($n = 39$) after the beginning of the drop in $T_b$, a value that did not differ from that of males exposed to free-running conditions ($F = 0.26$, df = 4/34, $P = 0.7$). The relative rate of decrease in $T_b$ (0.24 ± 0.01°C/10 min) was the same regardless of light cycle ($F = 1.4$, df = 4/34, $P = 0.2$), but the correlation of its onset with
the beginning of the light phase disappeared in 10:14 h light-darkness. In this case of short days, the onset of decrease in Tb started before the onset of light. Finally, arousal from daily hypothermia with rewarming to higher Tb occurred typically 6 h 10 ± 10 min (n = 39) after the beginning of the diurnal period or subjective day, independent of light condition (F = 0.63, df = 4/34, P = 0.6).

Besides the significant effect of long day exposure on Tb and activity levels, another seasonal effect concerned the occurrence of actual torpor in animals exposed to winterlike photoperiod (14:10 h light-darkness). Torpor bouts (minimal Tb < 33°C) have been observed one to three times per week in 8 of 10 animals exposed to short days at a constant ambient temperature of 25°C (Fig. 4). In such cases, daily minimum Tb averaged 27.6 ± 0.9°C (n = 8), a value just above the ambient temperature. When torpor occurred, the shape of the daily Tb rhythm was significantly modified. The decrease in Tb occurred earlier during the nighttime: on average, 9 h 20 ± 40 min after the beginning of night compared with ~13 h 30 min for daily hypothermia in animals exposed to short periods of daylight (F = 51.8, df = 1/16, P < 0.001). However, the rate of decrease in Tb remained unchanged (0.24 ± 0.05°C/10 min). Owing to the early decrease in Tb, torpor bouts were significantly longer than daily hypothermia (F = 5.34, df = 1/16, P < 0.03), and all Tb parameters were significantly decreased (Table 2). Nevertheless, the occurrence of minimal Tb and end of torpor remained synchronous with that observed for daily hypothermia (F = 1.38 and 1.65, df = 1/16, P = 0.2, respectively).

With regard to locomotor activity, no significant difference in total nocturnal activity was recorded, regardless of the presence of torpor (F = 1.31, df = 1/16, P = 0.3). The only difference consisted of the distribution of activity that was clustered in the first part of the night in the case of torpor.

**Synchronization and limits of induction of daily hypothermia by light.** In this nocturnal primate, light has clearly a strong inhibitory effect on locomotor activity, as evidenced in animals exposed to continuous light. In free-running animals under the RR condition, the length of activity exceeded the time of inactivity and covered ~58% of the circadian period. This day-to-night ratio is close to that imposed by the 12:12- and 10:14-h light-dark cycles. We were interested in studying the effect of shorter night duration on Tb and activity rhythms to delineate the limit of induction of hypothermia by light. Under exposure to 24-h light-dark cycles with night duration varying from 10 to 5 h (Fig. 5), activity pulses were restricted to dark time regardless of the photocycle, and thus the length of activity was strictly correlated to the nighttime duration (r = 0.999, n = 6). Dependent on the duration of the light phase, the amount of activity during the dark time significantly varied (F = 9.32, df = 9/53, P < 0.001). Exposure to shorter night duration did not induce a compensatory increase in locomotor activity; nocturnal activity progressively decreased as soon as the night duration dropped below 9 h, until it was totally inhibited under continuous light (Fig. 6).

An earlier light onset led to a rapid decrease in Tb, followed by a plateau that lasted longer the greater the day length (Fig. 5). This first part of the decrease in Tb after light onset corresponded to the drop in locomotor activity, because Tb remained, on average, at 36.2 ± 0.1°C (n = 20), a value significantly different from that recorded during the light period (F = 4.7, df = 1/38, P = 0.03) but close to that observed during subjective night in light-inhibited animals under the LL condition. The second part, delineated by an inflexion point in the Tb decrease, was accompanied by a significant burst of activity compared with diurnal resting activity (9.2 ± 0.7 AU/10 min, F = 98.9, df = 1/38, P < 0.001), indicating that animals were moving into their nest before entering sleep. If time of occurrence of this point is considered from the beginning of the dark time, it was synchronous with the drop in Tb under 14:10 h light-darkness, and minimal Tb was reached after 3 h 40 ± 15 min, a latency identical to that recorded for 24-h light-dark cycles with night duration >10 h, including the RR condition (F = 3.2, df = 9/53, P = 0.4). Likewise, in all 24-h light-dark cycles with night duration ≤10 h, arousal from daily hypothermia occurred at a fixed time in relation to phase of the light-dark cycle, i.e., 16 h 10 ± 15 min after the beginning of the dark phase, independent of night duration (F = 0.7, df =

Table 2. Tb parameters and duration of daily hypothermia in animals exposed to short days when they exhibited daily hypothermia or torpor

<table>
<thead>
<tr>
<th></th>
<th>Daily Hypothermia</th>
<th>Torpor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tb Night, °C</td>
<td>36.9 ± 0.1</td>
<td>35.8 ± 0.2*</td>
</tr>
<tr>
<td>Tb Day, °C</td>
<td>35.4 ± 0.1</td>
<td>32.0 ± 0.6*</td>
</tr>
<tr>
<td>Minimal Tb, °C</td>
<td>34.7 ± 0.1</td>
<td>27.6 ± 0.9*</td>
</tr>
<tr>
<td>Amplitude, °C</td>
<td>3.1 ± 0.1</td>
<td>10.0 ± 0.8*</td>
</tr>
<tr>
<td>Duration, min</td>
<td>294 ± 20</td>
<td>410 ± 40*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Animals were exposed to 10:14 h light-darkness. *Significant differences at P < 0.001.

Fig. 4. Tb and locomotor activity daily rhythms when actual torpor bouts (minimal Tb < 33°C) were observed in animals held in 10:14 h light-darkness. Stippled area, nighttime. For comparison, the thin line recalls the daily pattern of Tb rhythm with only daily hypothermia. Values are means ± SE (n = 8).

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In contrast to other 24-h light-dark cycles under which the level of activity during the night did not modify minimal $T_b$ values ($r = 0.1960$), minimal $T_b$ tended to decrease with the decrease in the duration of the dark time to $<9$ h ($r = 0.957, P < 0.01$). Whereas the onset of daily hypothermia was directly induced by light when night duration was reduced from 14 to 10 h (Fig. 7), temporal organization of daily hypothermia (entry into and arousal from) remained similar to that observed under 15:9 h light-darkness when night duration was $<9$ h, demonstrating that the limit of induction of daily hypothermia by light would be $\sim 9$ h after the beginning of night.

Fig. 5. $T_b$ and locomotor activity daily rhythms in animals held in 24-h light-dark cycles with night duration (stippled area) varying from 10 to 5 h. Values are means $\pm$ SE ($n = 4$). L, light; D, darkness.

Fig. 6. Total locomotor activity units during a 24-h period in animals held in 24-h light-dark cycles with night duration varying from 14 to 5 h or during a circadian period in animals held under RR or LL condition. Values are means $\pm$ SE. a–d, Significant pairwise differences between groups ($P < 0.05$, Tukey’s post hoc test).

Fig. 7. Duration of daily hypothermia cumulative to its onset dating from the beginning of subjective night in free-running condition (LL and RR) or from the beginning of the dark time in animals held in 24-h light-dark cycles with night duration varying from 14 to 5 h. Onset of daily hypothermia was entrained by light on until night duration was reduced to 9 h. No significant difference in duration of daily hypothermia was observed, regardless of photoperiod condition.
DISCUSSION

Under constant conditions of ambient temperature, the gray mouse lemur exhibits a robust rhythm of $T_b$ and locomotor activity typical of a nocturnal species (25). These daily variations are driven by an endogenous circadian rhythm expressed in a free-running condition. The circadian period of $T_b$ in mouse lemurs maintained in constant dim light at 25°C (RR condition) averaged 29 h, a value close to that found for the circadian rest-activity rhythm (29). With free running under constant light, the circadian period of $T_b$ lengthened to 25 h 30 min, a result in agreement with Aschoff’s rules for nocturnal animals.

In mouse lemurs, light is a potent zeitgeber for locomotor activity, and responses to light manipulations occurred with no aftereffect and no changes in velocity, suggesting a very accurate entrainment capacity in this species (29). In our study, locomotor activity is strictly restricted to the dark time, regardless of the duration of the light phase, and is almost totally absent in the LL condition, showing that light exerts a strong inhibitory effect on locomotor activity. Under the RR condition, a clear circadian rhythm is expressed, and locomotor activity length averaged 13–14 h. In contrast, the dramatic masking effect of light on locomotor activity was evidenced under the LL condition, under which no circadian locomotor activity rhythm could be detected. The total amount of locomotor activity remains constant under exposure to short day length (<12 h/day or RR condition), while animals exhibit a winter resting state marked by high body mass. In contrast, under summerlike day lengths, the total amount of activity was greatly increased, despite the reduction of nighttime duration, reflecting the well-known changes in behaviors and physiological functions induced by long photoperiod in mouse lemurs (for review see Ref. 23). Nevertheless, total activity decreased under abnormally long day lengths, even if animals remained in an active breeding state, as exemplified by their low body mass. Under the RR condition or 24-h light-dark cycles, changes in activity level during the nighttime were reflected in $T_b$ values with a 10-min delay, whereas a total inhibition of activity under the LL condition led to a reduction by ~1°C of $T_b$, showing the effect of activity on $T_b$ values.

$T_b$ rhythm in mouse lemurs is characterized by a high amplitude due to the presence of a daily hypothermia proceeding from an endogenous circadian function. At a constant ambient temperature of 25°C, daily hypothermia starts by a rapid and linear drop in $T_b$, leading to minimal $T_b$ after 3 h followed by a spontaneous rewarming to normothermic values 3 h later. Although constant in its pattern, the onset of daily hypothermia is triggered by the onset of light in photoperiods from 10:14 h to 15:9 h light-darkness. Such an induction by light has been demonstrated in rodents, bats, and marsupials (4, 8, 12, 15, 16, 33, 35) and disappears after suppression of the suprachiasmatic nuclei, the main endogenous pacemaker (26). In mouse lemurs, the upper limit is represented by the free-running condition and by extension of the short day condition, where the onset of daily hypothermia occurs spontaneously 13–14 h after the beginning of the nocturnal activity. When night duration is ≤14 h, the entry into daily hypothermia is triggered by light. Telemetric experiments conducted with night duration <10 h indicated that the lower limit of induction of daily hypothermia by light is ~9–10 h after the beginning of subjective night. It is close to that proposed for the human circadian pacemaker, i.e., ~4 h before minimum $T_b$ (14).

The temporal pattern of daily hypothermia was found identical in the free-running condition or 24-h light-dark cycles of different duration and is highly similar to those found in several daily heterotherms (9, 13, 17, 19, 22). Previous studies in wild mouse lemurs have suggested that entry into and arousal from daily hypothermia were dependent on changes in ambient temperature occurring early in the morning and in midday, respectively (21, 31). Clearly, our data give evidence that the daily drop in $T_b$ is generated by an endogenous circadian rhythm and is not primarily linked to changes in ambient temperature, as has also been stressed in other heterothermic mammals (22).

In mouse lemurs, daily hypothermia starts with a rapid drop in $T_b$, reflecting a rapid decrease in metabolic rate (21, 31, 32). Despite significant changes in body mass, sex hormones, and thyroxine and cortisol levels triggered by seasonal photoperiodic variations (11, 23), the rate of $T_b$ decrease remains constant, suggesting that the daily drop in $T_b$ is not dependent on hormones. In contrast, actual torpor bouts were observed only in animals maintained in short photoperiod at 25°C ambient temperature and with food available ad libitum. Frequency of torpor was low (0–3 times/wk), and minimum $T_b$ reached a value always 1–2°C higher than ambient temperature. In hibernators, torpor bouts are found during short days, and their frequency and amplitude increase with decreasing ambient temperature or food restriction (8, 33). In mouse lemurs, frequency of actual daily torpor can be enhanced by diet restriction (10) and probably, although not yet tested, by decreasing ambient temperature, since in captive and wild animals, torpor bouts with minimal $T_b$ of ~11–15°C have been observed when external temperature was ~10°C (3, 31). Contrary to daily hypothermia, torpor would be dependent on the photoperiodic state or on changes in hormones induced by short day exposure, especially daily melatonin secretion that was dependent on photoperiod in mouse lemurs (2) and of which the effect on $T_b$ is well known (6, 28). Torpor bouts would thus be induced by homeostatic factors. Even if the timing of onset of torpor is different from the timing of daily hypothermia, minimal $T_b$ and arousal from torpor and daily hypothermia occur at the same time during the light time, suggesting that it proceeds from the same circadian endogenous process. In wild mouse lemurs (31, 32), arousal from torpor was clustered within a narrow time window. This reinforces the idea that the onsets of daily hypothermia and torpor are dependent on exter-
nal (to light) and internal (to physiological constraints) synchronism and that the following pattern of torpor bout is strictly determined by an endogenous mechanism.

Although it was found to have a pleisiomorphic origin (7, 18), daily hypothermia in small mammals living in contrasted conditions would represent an adaptive energy-saving strategy. A daily decrease in Tb for a duration equal to 25% of the daytime gives regular energetic benefits that have been estimated to save 20–40% of daily energy expenditure in mouse lemurs. Daily hypothermia observed in mouse lemurs has also been described in other small Malagasy mammals (35), and it would represent an adaptive endogenous mechanism that would have evolved to face high-resources seasonality and high levels of summer rainfall variability as a consequence of El Niño southern oscillation that periodically affects Madagascar.

Perspectives

Most properties of the circadian clock are based on locomotor activity responses to light pulses. In the gray mouse lemur, the Tb rhythm is characterized by a phase of daily hypothermia of great amplitude that reflects an expression of one endogenous component of the clock and that delinates the end of nocturnal activity, regardless of locomotor activity pattern. Owing to the strong inhibitory effect of light on the locomotor activity of mouse lemurs, further studies on the plasticity of the circadian clock need to be based on Tb profiles. Finally, this study introduces the possibility that the precise relationship between locomotor activity and Tb rhythms and their control by the circadian clock can be determined.

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REFERENCES


