Relationship between the daily rhythms of locomotor activity and body temperature in eight mammalian species

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Refinetti, Roberto. Relationship between the daily rhythms of locomotor activity and body temperature in eight mammalian species. Am. J. Physiol. 277 (Regulatory Integrative Comp. Physiol. 46): R1493–R1500, 1999.—The relationship between the daily rhythms of locomotor activity and body temperature was studied by telemetry in four nocturnal and four diurnal mammalian species. The results showed that the two rhythms are very closely synchronized, as they 1) ascend past the daily mean at the same time, 2) reach the daily acrophase at the same time, and 3) are best correlated at time lags approaching zero. The rhythms of nocturnal animals crossed the daily mean at the transition between the light and dark phases of the light-dark cycle and reached their acrophases during the dark phase, whereas the rhythms of diurnal animals crossed the daily mean at the transition between the dark and light phases and reached their acrophases during the light phase. Despite the close synchrony of the two rhythms, the results indicate that the temperature rhythm is not a byproduct of the activity rhythm, as body temperature during the active phase of the daily cycle was higher than body temperature during the inactive phase in all species irrespective of the activity level prevailing during each phase.

MATERIALS AND METHODS

Subjects. The experimental subjects were three flying squirrels (Glaucomys volans, 63–85 g), eight golden hamsters (Mesocricetus auratus, 132–168 g), eight fat-tailed gerbils (Pachyuromys duprasi, 55–113 g), four Mongolian gerbils (Meriones unguiculatus, 60–65 g), four Chilean degus (Octodon degus, 212–251 g), seven thirteen-lined ground squirrels (Spermophilus tridecemlineatus, 148–192 g), and three tree shrews (Tupaia belangeri, 160–221 g). All animals, except for three fat-tailed gerbils and two thirteen-lined ground squirrels, were male. All were young adults. The golden hamsters and Mongolian gerbils were purchased from Charles River Laboratories (Wilmington, MA). The fat-tailed gerbils and the three species of squirrels were purchased from Flyers Specialty Pets (Bullock, TX). The degus were generously provided by Dr. Theresa Lee (University of Michigan), and the tree shrews were obtained from the primate colony at Duke University (Durham, NC).

All animals were housed individually in plastic cages (25 x 46 x 20 cm) lined with wood shavings and kept inside a large ventilated incubator. Food (Prolab rodent chow or Purina cat chow) and water were available ad libitum. To avoid the

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effects of circannual rhythms in heterothermic animals, all squirrels were studied during the summer. Regardless of season, a summer photoperiod (14 h of light and 10 h of darkness per day) and constant ambient temperature of 24°C were maintained throughout the study. Body temperature data from some of the animals used in this study (but not locomotor activity data or their relationship) have been reported previously (22, 24).

Animals were cared for and experimented on in accordance with the guidelines set out by the National Institutes of Health's Guide for the Care and Use of Laboratory Animals.

Data acquisition. Radio sensor-transmitters for the monitoring of body temperature and locomotor activity (models VM-FH and XM-FH, Mini-Mitter, Sunriver, OR) were implanted intraperitoneally several days before the beginning of the study. The transmitters generated temperature-dependent signals with an accuracy better than 0.1°C. Detection of locomotor activity was based on variations in radio signal strength due to linear displacement of the radio transmitter in relation to the radio receiver. Consequently, it was independent of temperature and was sensitive to body movements but not to stationary activity such as grooming, tremor, or respiration. The animals were anesthetized with pentobarbital sodium, and a 2-cm incision was made in the abdominal wall under sterile conditions. The transmitter was placed inside the abdominal cavity, and the muscle wall was sutured. The skin incision was closed with wound clips. Body temperature was monitored with a rectal probe during recovery from anesthesia.

The animal cages were placed on top of radio-receiver boards (model RA-1010, Mini-Mitter), which were attached to a computerized data acquisition system (Dataquest III, Data Sciences, St. Paul, MN). After a variable period of recovery from surgery, data were collected continuously for at least 1 wk from each animal and saved to disk in 6-min bins. Because all animals had a relatively small body mass and activity counts were accumulated over each 6-min bin while body temperature was recorded at the end of each bin, the data collection procedure was sensitive to the potential influence of changes in activity level on body temperature. Studies of rapid changes in body temperature caused by experimenter-induced stressors have shown that asymptotic temperature levels are reached with a latency of 5–12 min (3, 20, 29, 30).

Data analysis. Three methods were used to analyze the phase relationship between the rhythms of body temperature and locomotor activity. The ascendance method involved the determination of the time at which the level of body temperature or locomotor activity crossed the mean level during its daily rise. For each animal, 7 days of records were “educed” into a single day by averaging each bin over the 7 days. The time at which the educed rhythm crossed the 7-day mean was taken as the ascendance phase-reference point for each animal (see Fig. 2).

The acrophase method involved the fitting of a cosine wave to each educed rhythm. A family of cosine waves was generated by the equation \( T_t = M + A \cdot \cos(\theta_t) \), where \( M \) is the mesor (mean level) of the rhythm, \( A \) is the amplitude (half the range of excursion), and \( \theta_t \) is the trigonometric angle (in degrees) corresponding to time \( t \). The values of \( M \) and \( A \) were taken from the actual records of each animal, and \( \theta_t \) was systematically varied from 0 to 360°, corresponding to 0000–2400. The best fit was determined by the principle of the least squares. The time corresponding to the peak of the best-fitting wave was taken as the acrophase reference point (see Fig. 3).

The serial correlation method involved the calculation of coefficients of correlation (by the principle of least squares) between the body temperature and activity rhythms of each animal for 240 distinct phase lags (corresponding to the 240 bins in a day). The lag that resulted in the largest correlation was considered as an index of phase relationship (with a lag of zero signifying perfect synchrony and a lag of 12 h signifying perfect antiphase). To implement the phase lags, data points from the first 6 days of the records of locomotor activity were kept constant, whereas data points from the records of body temperature were serially displaced by one bin to cover the full 7 days. Once the best phase lag had been determined for each animal, additional calculations were conducted to determine the correlations for the dark and light phases of the light-dark cycle separately.

The robustness (strength) of the rhythms of body temperature and locomotor activity was determined by the \( \chi^2 \) periodogram procedure (32) applied to the raw data (7 days of continuous records, or 1,680 data points) of each animal. Robustness was numerically defined as the value of the \( Q_p \) statistic for \( P = 24.0 \) h (240 bins).

For the analysis of the effect of locomotor activity on body temperature, the maximal activity level (the highest activity count recorded at least 4 times during the 7 days) was first determined for the raw data sets of each animal. The data sets were then separated into day and night segments. In each segment, the mean body temperature in temporal bins with different levels of activity (0, 10, 20,..., 100% of maximal activity) was determined. Because activity levels at or above 80% were rarely recorded during the night in diurnal animals or during the day in nocturnal animals, comparisons were restricted to the 0–70% range (see Fig. 8).

Statistical analysis of group means was conducted by factorial ANOVA followed by post hoc comparisons using Tukey's honest significant difference test (12).

RESULTS

A representative record of the body temperature and activity rhythms of a flying squirrel is shown in Fig. 1.

![Fig. 1. Records of body temperature (A) and locomotor activity (B) of a flying squirrel maintained under a 24-h light-dark cycle with 14 h of light and 10 h of darkness (indicated by open and solid bars at top). Data were recorded and are plotted in 6-min bins.](http://ajpregu.physiology.org/Downloadedfrom)
Both variables exhibit clear daily rhythmicity. In this nocturnal species, body temperature and activity level are higher during the dark phase of the light-dark cycle. The two variables oscillate closely together, and transient increases in activity are often accompanied by corresponding increases in body temperature (for instance, the brief increase in activity in the middle of the first light phase in Fig. 1).

Examples of educed rhythms of body temperature and activity of a thirteen-lined ground squirrel are shown in Fig. 2A. In this diurnal animal, body temperature shows a slight gradual increase during the hours preceding the beginning of the light phase, but an abrupt elevation (leading to the crossing of the daily mean) is not observed until ~1 h after lights-on. The body temperature and activity rhythms ascend simultaneously. In the case of this particular animal, the ascendance phase-reference points were 0630 and 0618 for the body temperature and activity rhythms, respectively.

Shown in Fig. 2B are the educed rhythms of body temperature and activity of a golden hamster. In this nocturnal animal, the rhythms ascend and cross the daily mean ~1 h before the beginning of the dark phase. In the case of the particular animal whose data are depicted in Fig. 2, the ascendance phase-reference points were 1806 and 1754 for the body temperature and activity rhythms, respectively.

Educed rhythms of body temperature and corresponding best-fit cosine waves for a Richardson ground squirrel, a fat-tailed gerbil, and a tree shrew are shown in Fig. 3. The Richardson squirrel’s rhythm is shaped similarly to a cosine wave, and, consequently, the best-fitting cosine wave approximates the shape of the animal’s rhythm reasonably well. In this diurnal animal, the acrophase is situated in the light phase of the light-dark cycle. In contrast, the acrophase of the rhythm of the nocturnal fat-tailed gerbil is situated in the dark phase of the light-dark cycle. The shape of the fat-tailed gerbil’s rhythm, as well as that of the tree shrew’s rhythm, is closer to a square wave than to a
cosine wave, however. Consequently, the calculated acrophases do not necessarily correspond to the peak of the rhythm, which is evident in the case of the tree shrew (where the computed acrophase is situated midway between the two daily peaks).

Figure 4 shows the mean phase-reference points of the body temperature and locomotor activity rhythms, as determined by the ascendance method, for the eight species. In all species, the two rhythms cross their respective means at almost exactly the same time. For three of the nocturnal species (flying squirrel, golden hamster, and fat-tailed gerbil), this time is the transition between light and darkness; for the fourth species (Mongolian gerbil), the rhythms ascend past the mean ~4 h before darkness. For three of the diurnal species (degu, thirteen-lined ground squirrel, and tree shrew), the mean is crossed at the time of the transition between darkness and light; for the fourth species (Richardson ground squirrel), ascendance occurs ~3 h later, although the transition between darkness and light is within the 99% confidence limit for the group. Factorial ANOVA indicated a significant species effect \( [F(7,34) = 270.86, \ P < 0.01] \) but no significant difference between the body temperature and activity rhythms \( [F(1,34) = 3.26, \ P > 0.05] \) and no interaction of the two factors \( [F(7,34) = 1.30, \ P > 0.05] \). Post hoc comparisons indicated that ascendance was significantly earlier for the Mongolian gerbil than for the other nocturnal species and significantly later for the Richardson ground squirrel than for the other diurnal species (Tukey's test, \( P < 0.01 \)).

The mean acrophases for the eight species are shown in Fig. 5. Here again, the rhythms of body temperature and locomotor activity have very similar phase-reference points. For three of the nocturnal species (flying squirrel, golden hamster, and fat-tailed gerbil), the rhythms peak at about the middle of the dark phase (5 h after lights-off), whereas the rhythms peak during the light phase in the diurnal species. Factorial ANOVA indicated a significant species effect \( [F(7,34) = 24.89, \ P < 0.01] \) but no significant difference between the body temperature and activity rhythms \( [F(1,34) = 3.57, \ P > 0.05] \). A significant interaction effect was found \( [F(7,34) = 3.31, \ P < 0.05] \), which resulted from the unusual data from the Mongolian gerbil. While the acrophase of the body temperature rhythm of the Mongolian gerbil occurred during the dark phase of the light-dark cycle, the acrophase of the activity rhythm took place in the middle of the light phase.

Analysis of rhythm robustness revealed that the dissociation of acrophases in the Mongolian gerbil was an artifact of the acrophase method. As shown in Fig. 6, the robustness of the activity rhythm of the Mongolian gerbil was lower than the significance line, meaning...
that the activity pattern was not actually rhythmic and, therefore, could not have an actual acrophase. Although only the mean results are shown in the figure, absence of circadian rhythmicity was observed in individual activity records of all Mongolian gerbils. Robust rhythmicity was present in the body temperature and activity data of all other species, although significant interspecies differences were observed \[ F(7,34) = 13.01, P < 0.01 \]. In all cases, the body temperature rhythm was more robust than the activity rhythm \[ F(1,34) = 250.80, P < 0.01 \]. An interaction effect was also found \[ F(7,34) = 3.18, P < 0.01 \], which means that the difference in robustness between the two rhythms varies from species to species.

Data analysis by the serial correlation method produced results in accordance with the other two methods. The best correlation for each individual animal was always statistically significant \( P < 0.01 \) and averaged 0.48 ± 0.03 (SE). The lag times associated with the best correlations averaged 1.8 ± 0.2 bins (or ~10 ± 1 min). This small mean lag time indicates close synchronization of the rhythms overall, although there were significant interspecies differences \[ F(7,34) = 7.28, P < 0.01 \]. Pairwise comparisons by Tukey's test revealed a significantly larger phase lag (24 min) in the Richardson ground squirrels than in all other species (8 min).

Separate computations of correlation coefficients for the dark and the light phases of the light-dark cycle are shown in Fig. 7. Because of the reduction in sample size and range of variation, the coefficients are generally smaller than those computed for full days. Nevertheless, because the number of data points is still relatively large, coefficients as small as 0.2 are statistically significant \( P < 0.01 \). The data shown in Fig. 7 indicate that the correlation between activity and temperature is higher during the dark phase in some species and during the light phase in others, irrespective of their diurnal or nocturnal habits. However, factorial ANOVA revealed no significant effect of species \[ F(7,34) = 1.71, P > 0.10 \], phase \[ F(1,34) = 1.40, P > 0.10 \] or their interaction \[ F(7,34) = 1.61, P > 0.10 \].

In all species, body temperature during the active phase was higher than body temperature during the inactive phase, and this was true even when the different levels of locomotor activity were controlled for, as shown in Fig. 8. ANOVAs conducted separately for each species confirmed that nocturnal body temperatures were higher than diurnal body temperatures in nocturnal species and, conversely, that nocturnal body temperatures were lower than diurnal body temperatures in diurnal species \( e.g., \) for degus, \[ F(1,60) = 13.26, P < 0.01 \]. Also, in all species but flying squirrels and Richardson ground squirrels, higher activity levels were associated with higher body temperatures \( e.g., \) in golden hamsters, \[ F(7,105) = 22.78, P < 0.01 \]. However, in no species was there a significant interaction effect of the two variables. The absence of an interaction effect means that the degree of association of activity and temperature did not differ between day and night.

DISCUSSION

The results showed that the daily rhythms of body temperature and locomotor activity are very closely synchronized in four diurnal and four nocturnal mammalian species. The two rhythms were found to ascend past the daily mean and to reach the acrophase at the same (statistically indistinguishable) time in each spe-

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**Fig. 6.** Mean rhythm robustness for locomotor activity and body temperature rhythms of 8 species. Vertical dashed line indicates 5% significance level. Error bars indicate SE.

**Fig. 7.** Mean correlation coefficients for association between locomotor activity and body temperature during light and dark phases of light-dark cycle for 8 species. Error bars indicate SE.
cies. In the only case in which this was not true (acrophases for the Mongolian gerbil), the phase discrepancy between the two rhythms was due to an analytic artifact resulting from the absence of significant rhythmicity in locomotor activity. Also, in all species, the best correlation between temperature and activity was obtained for time lags close to zero (1.8 bins on average). Although other markers of phase relationship than those used in this study might reveal a partial dissociation between the rhythms of temperature and activity, I believe that the present results clearly demonstrate a close synchrony of the two rhythms.

In a recent study in mice, Weinert and Waterhouse (36) found that the correlation between temperature and activity was significantly better during the light phase than during the dark phase. In the present study, the results suggested that the direction of this imbalance varies from species to species, although no significant overall effect was found. Greater correlation is suggestive of greater causal effect, and it is reasonable to expect that activity will have a greater effect on body temperature during the inactive phase than during the active phase. This is so because body temperature is already close to its homeostatic upper limit during the active phase and, therefore, will not rise as much in response to increased activity as during the inactive phase.

Although the phase relationship between the rhythms of body temperature and locomotor activity was uniform across species, the exact timing of the rhythms differed from one species to another. Generally, the rhythms of nocturnal animals crossed the daily mean at the time of the transition between the light and dark phases of the light-dark cycle and reached their acrophases during the dark phase. The rhythms of diurnal animals crossed the daily mean at the time of the transition between the dark and light phases and reached their acrophases during the light phase. The rhythms were advanced by a few hours in the Mongolian gerbil and delayed by a few hours in the Richard-son ground squirrel. It should be pointed out that the timing of the rhythms in this study was investigated only under a summer photoperiod and may differ under a winter photoperiod.

The synchrony of the rhythms of body temperature and locomotor activity observed here is consistent with
the results of previous studies in rats (23) and degus (14) and with informal observations in other species (4, 10, 25). However, they contrast with results from human studies, in which the body temperature rhythm was consistently reported to phase-lead the activity rhythm (1, 13, 37, 38). Inspection of the reported data from studies in both nocturnal (9) and diurnal (18, 34, 39) nonhuman primates reveals that, although body temperature starts to rise several hours before wake time, the daily mean is crossed within 1 h of the transition in the light-dark cycle (which is consistent with the results from the present study). It would seem, therefore, that the phase relationship between the two rhythms observed in humans is unique among mammals. However, closer inspection of the literature on humans indicates that the difference may be semantic rather than factual. All studies report that body temperature starts rising before wake time and peaks 6–10 h later (1, 7, 13, 15, 28, 33, 37, 38), which is not inconsistent with the data from the present study (e.g., the body temperature of the thirteen-lined ground squirrel depicted in Fig. 2 starts a slow ascent 5 h before lights on and peaks many hours later). Yet body temperature in entrained human subjects crosses the daily mean 1–3 h after, not before, wake time (1, 13, 15, 28, 33, 38). Although detailed records of activity are rarely provided in these studies, it is reasonable to expect that activity will increase during the first few hours after wake time, which would result in its crossing the daily mean at about the same time as body temperature does. Therefore, studies in both human and nonhuman animals agree that body temperature starts a slow ascent several hours before wake time but undergoes a more abrupt rise at the same time as locomotor activity also undergoes an abrupt rise.

In agreement with previous studies in rats and golden hamsters (2, 11, 21), the present results showed that body temperature during the active phase was higher than body temperature during the inactive phase, irrespective of the activity level prevailing during each of the phases. Because the equivalence of activity levels was determined in 6-min bins, parameters of thermal inertia might have distorted the results. However, as referred to in MATERIALS AND METHODS, studies of rapid changes in body temperature caused by experimenter-induced stress in rodents have shown that asymptotic temperature levels are reached with a latency of 5–12 min (3, 20, 29, 30). Thus it is appropriate to assume that increases in activity in this study were reflected in increases in body temperature within the same 6-min bin (or in the immediately subsequent bin), although a greater delay likely occurred between a reduction in activity and a fall in body temperature. This assumption is reinforced by the finding that the best correlation between activity and temperature was obtained for time lags of one or two bins.

The finding that body temperature during the active phase is higher than body temperature during the inactive phase, irrespective of the activity level prevailing during each of the phases, is consistent with human studies showing that the rhythmicity in body temperature persists independently of rhythmicity in activity (6, 16, 19, 31). It is also consistent with the observation of circadian rhythmicity of body temperature in hibernating (motionless) animals (5, 8, 17). Thus it seems justified to conclude that the existence of the body temperature rhythm is not dependent on the activity rhythm.

That increases in activity can accentuate the daily oscillation in body temperature was confirmed in this study by the results from all species, except flying squirrels and Richardson ground squirrels. On the other hand, the absence of an interaction between phase of the light-dark cycle and level of activity indicated that the association of activity and body temperature did not differ between night and day. Thus, unless thermal inertia oscillates in such a manner as to counteract a putative oscillation in the thermogenic action of activity, one can infer that the effect of activity on body temperature does not differ between night and day. Therefore, the close synchrony of the two rhythms is likely to result not from the effect of activity on temperature but from a common signal generated by the circadian pacemaker. Although research on human subjects has suggested the existence of separate pacemakers for the body temperature and activity rhythms, research on other mammalian species has consistently produced results supporting the notion of a single circadian pacemaker (26). The mechanism by which this pacemaker controls the phase relationship of the various bodily rhythms is still unknown.

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