Circadian rhythms of body temperature and locomotor activity in the antelope ground squirrel, *Ammospermophilus leucurus*

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A B S T R A C T

We studied circadian rhythms of body temperature and locomotor activity in antelope ground squirrels (*Ammospermophilus leucurus*) under laboratory conditions of a 12L:12D light-dark cycle and in constant darkness. Antelope ground squirrels are diurnally active and, exceptionally among ground squirrels and other closely related members of the squirrel family in general, they do not hibernate. Daily oscillations in body temperature consisted of a rise in temperature during the daytime activity phase of the circadian cycle and a decrease in temperature during the nighttime rest phase. The body temperature rhythms were robust (71% of maximal strength) with a daily range of oscillation of 4.6 °C, a daytime mean of 38.7 °C, and a nighttime mean of 34.1 °C (24-h overall mean 36.4 °C). The body temperature rhythm persisted in continuous darkness with a free-running period of 24.2 h. This pattern is similar to that of hibernating species of ground squirrels but with a wave form more similar to that of non-hibernating rodents. Daily oscillations in body temperature were correlated with individual bouts of activity, but daytime temperatures were higher than nighttime temperatures even when comparing short episodes of nocturnal activity that were as intense as diurnal activity. This suggests that although muscular thermogenesis associated with locomotor activity can modify the level of body temperature, the circadian rhythm of body temperature is not simply a consequence of the circadian rhythm of activity.

1. Introduction

Circadian rhythmicity of body temperature is ubiquitous in mammals and birds. In a thermoneutral environment, the body temperature of some species oscillates less than 1 °C on a daily basis, but as much as 5 °C in others (Aschoff, 1983; Refinetti and Menaker, 1992). In energetically challenging situations, such as low ambient temperature or seasonally limited food availability, some small species of mammals and birds exhibit daily torpor, in which body temperature may decline by 10 °C or more on a daily basis (Ruf and Geiser, 2015).

Ammospermophilus leucurus is particularly interesting from the perspective of thermal biology because, unlike many other Holarctic (Northern Hemisphere) ground squirrels and related members of the squirrel family's Tribe Marmotini (Helgen et al., 2009; Hoffmann et al., 1993), the antelope ground squirrel does not hibernate (Chappell and Bartholomew, 1981; Karasov, 1983; Kenagy and Bartholomew, 1985). It ranges widely across arid environments in western North America from the Great Basin and Mojave Desert southward into the Sonoran Desert and throughout the Baja California Peninsula (Hall, 1981).

Despite the unique thermal physiology of *A. leucurus*, the circadian

rhythmicity of its body temperature has not been studied under controlled laboratory conditions. A hibernator of similar body size, the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*) exhibits a large amplitude in the oscillation of its body temperature while housed in the laboratory under conditions approximating thermal neutrality prior to the first torpor bout of the hibernation season (Refinetti, 1996; Russell et al., 2010). We have undertaken the current comparative study in order to learn whether quantitative characteristics of the body temperature rhythm of the antelope ground squirrel resemble those of hibernators such as the thirteen-lined ground squirrel or those of non-hibernating rodents such as the laboratory rat (Honma and Hiroshige, 1978; Ikeda and Inoué, 1998; Nagashima et al., 2003). We describe here the daily rhythmicity of body temperature and its interrelationship with the rhythmicity of locomotor activity in the antelope ground squirrel housed in the laboratory under a light-dark cycle as well as under constant darkness.

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2. Methods

2.1. Subjects

Antelope ground squirrels were born in the laboratory to dams captured while pregnant in Owyhee County, Idaho (Idaho Department of Fish and Game permit number 160812). Ten animals from four different litters (5 males and 5 females) were used in this study. The squirrels were four months old and weighed about 125 g, typical of adult body weight, at the beginning of the study. They were housed individually in polycarbonate cages with wire tops (36 cm length, 24 cm width, 19 cm height) lined with cellulose bedding (Comfort Bedding, BioFresh, Ferndale, WA). The cages were kept inside light-tight, ventilated individual chambers maintained at $25 \pm 0.5\,^\circ$C with $20 \pm 5\%$ relative humidity. Food (Purina Rodent Diet 5001, Lab Diet, St. Louis, MO) and water were available *ad libitum*.

2.2. Procedure

All procedures were approved by the Boise State University Animal Care and Use Committee under Protocol No. 006-AC16-014.

Body temperature was monitored continually with a temperature-sensitive PIT tag system. Passive integrated transponders (PIT) are radio-frequency microchips housed inside glass ampoules the size of a grain of rice that are widely used for animal identification. LifeChip PIT tags with bio-thermo technology (Destron Fearing, Eagan, MN) are temperature-sensitive tags with resolution better than $0.1\,^\circ$C in the range of 32–42 °C. The PIT tags were injected intraperitoneally with a sterile 12-gauge needle while the animals were under isoflurane anesthesia. The signal from the transponders was monitored by a custom-designed antenna system connected to an RM310 reader and an SM303 multiplexer (Biomark, Boise, ID). Locomotor activity was monitored with Konlen passive infrared motion sensors (Light in the Box, Seattle, WA). Temperature and activity data were continually recorded with a desktop computer and saved in 6-min bins (0.1 h intervals).

The squirrels were studied under the vivarium temperature of $25 \pm 0.5\,^\circ$C. This ambient temperature is slightly below the thermoneutral zone of *A. leucurus* (30–34 °C) as determined by Dawson (1955). Vivarium temperatures are often maintained below the thermoneutral zone for laboratory rodents (David et al., 2013), and, in our particular case, the lower ambient temperature may have led to a slight overestimation of the daily range of oscillation of body temperature. An overestimation is possible because the range of oscillation of body temperature is extended in some species at ambient temperatures below thermoneutrality (Refinetti, 1997).

Illumination was controlled by a programmable electronic timer (ChronTrol XT, ChronTrol Corp., San Diego, CA) that activated white-light-emitting-diode units (Backup Trailer Light, Online-LED-Store, Ontario, CA) generating an illuminance of approximately 600 lx, as measured 8 cm above the cage floor. Data were collected from each animal for three or more weeks under a 12 L:12D light-dark cycle and three or more weeks in constant darkness. The 10 animals were studied simultaneously in separate individual chambers. Data collection was started early in August and completed by mid October 2017.

2.3. Data analysis

Individual time series were analyzed by cosinor rhythmometry (Nelson et al., 1979; Refinetti et al., 2007) for five rhythmic parameters: mesor (mean level), amplitude (half the range of daily excursion), acrophase (time of the daily peak), period (the duration of each cycle), and robustness. Rhythm robustness refers to the strength of rhythmicity and is closely related to the stationarity of the time series. Robustness is independent of amplitude, except at the extreme low end of the range, because a rhythm with zero amplitude also has zero robustness. Rhythm robustness was computed as the percentage of total variance

accounted for by the cosine fit (Refinetti et al., 2007).

For the purpose of standardization, 10-day segments were selected for analysis after stable entrainment was achieved (as defined by at least 20 consecutive days with less than 30-min variability of daily activity onsets). Temperature files were filtered for removal of recording artifacts prior to analysis. A reading was considered an artifact if it registered less than 32 °C or more than 42 °C without being preceded by a lowering or rising trend. Artifactual readings (which were fewer than 2% of the readings in a data set) were replaced by the immediately-preceding temperature reading.

Locomotor activity was quantified as the number of changes in the passive infrared detection signal ("counts") per 6-min bin. For the analysis of the effect of locomotor activity on body temperature, Pearson correlation coefficients were calculated for the activity and temperature data from each animal. For further analysis, the data sets were separated into day and night segments. In each segment, the mean body temperature in temporal bins with different levels of activity was determined. This procedure allowed the computation of separate regressions of temperature on activity for the daytime and the nighttime.

3. Results

All animals exhibited significant daily rhythmicity of body temperature and locomotor activity, as determined by cosinor rhythmometry ($p < 0.00001$). Four-day segments of the records of body temperature of two representative squirrels are shown in Fig. 1. The animal whose records are shown in the top panel (Fig. 1A) exhibited a daily range of oscillation of body temperature of almost 4 °C. Its body temperature typically started to rise at around the time of lights-on, declined briefly by about 2 °C around the middle of the light phase, and finally fell to the nocturnal low around the time of lights-off. The squirrel whose records are shown in the lower panel (Fig. 1B) did not show a decrease in body temperature during the middle of the day, but the general form of its oscillation of body temperature was otherwise similar to that of the first animal.

The remaining eight squirrels had temperature rhythms similar to those shown in Fig. 1. Four of the ten animals exhibited a large temperature decrease (~2 °C) lasting 30–60 min, during the middle of the day, but this did not occur every day, and it did not occur at the same time of day over successive days. Thus, when 10 consecutive days for all 10 squirrels were averaged into one day to yield an educed rhythm, the oscillatory pattern was smooth, with no midday decrease (Fig. 2). This educed rhythm was characterized by a sharp rise in temperature starting an hour before lights-on and a more gradual fall in temperature starting two hours before lights-off and reaching the nighttime trough
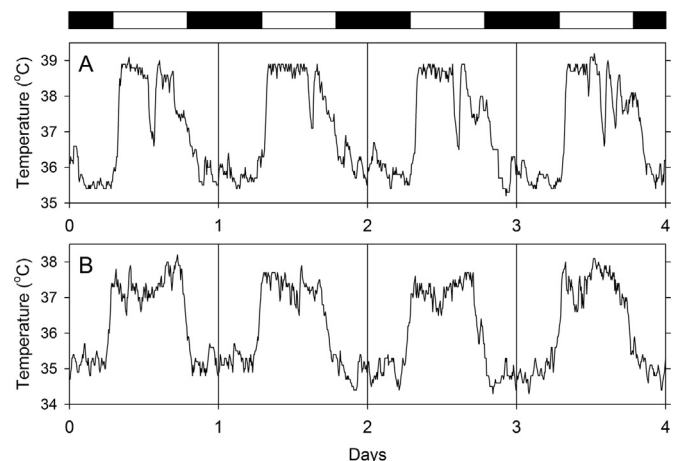

Fig. 1. Four-day segments of body temperature recordings in two representative squirrels. The dark and white horizontal bars above the graphs denote the dark and light phases of the prevailing 24-h light-dark cycle.