

Hibernation and Circadian Rhythms of Body Temperature in Free-Living Arctic Ground Squirrels

Cory T. Williams^{1,*}

Brian M. Barnes²

Melanie Richter^{1,2}

C. Loren Buck¹

¹Department of Biological Sciences, University of Alaska, Anchorage, Alaska 99508; ²Institute of Arctic Biology, University of Alaska, Fairbanks, Alaska 99775

Accepted 4/29/2012; Electronically Published 6/8/2012

ABSTRACT

In mammals, the circadian master clock generates daily rhythms of body temperature (T_b) that act to entrain rhythms in peripheral circadian oscillators. The persistence and function of circadian rhythms during mammalian hibernation is contentious, and the factors that contribute to the reestablishment of rhythms after hibernation are unclear. We collected regular measures of core T_b (every 34 min) and ambient light conditions (every 30 s) before, during, and following hibernation in free-living male arctic ground squirrels. Free-running circadian T_b rhythms at euthermic levels of T_b persisted for up to 10 d in constant darkness after animals became sequestered in their hibernacula in fall. During steady state torpor, T_b was constant and arrhythmic for up to 13 d (within the 0.19°C resolution of loggers). In spring, males ended heterothermy but remained in their burrows at euthermic levels of T_b for 22–26 d; patterns of T_b were arrhythmic for the first 10 d of euthermia. One of four squirrels exhibited a significant free-running T_b rhythm ($\tau = 22.1$ h) before emergence; this squirrel had been briefly exposed to low-amplitude light before emergence. In all animals, diurnal T_b rhythms were immediately reestablished coincident with emergence to the surface and the resumption of surface activity. Our results support the hypothesis that clock function is inhibited during hibernation and reactivated by exposure to light, although resumption of extended surface activity does not appear to be necessary to reinitiate T_b cycles.

Introduction

Circadian clocks provide organisms with an internal anticipatory system that permits the coordination of physiological and metabolic processes and facilitates the exploitation of favorable conditions for daily behaviors. In mammals, the master circadian clock within the suprachiasmatic nuclei (SCN) of the hypothalamus creates this temporal coordination by transmitting its rhythmic information to oscillators in other brain regions and peripheral organs via a variety of outputs, including through the generation of body temperature (T_b) rhythms (Buhr et al. 2010; Dibner et al. 2010). The adaptive value of circadian clocks is supported by their ubiquitous nature, although the function of such clocks in effectively constant environments is debated (Woefle et al. 2004; Yerushalmi and Green 2009). For example, in Svalbard reindeer *Rangifer tarandus platyrhynchus* and ptarmigan *Lagopus mutus hyperboreus*, species indigenous to the high Arctic, patterns of activity and feeding become semicontinuous and arrhythmic during seasonal intervals of constant light or constant dark (Reierth and Stokkan 1998; van Oort et al. 2005), a characteristic that has been proposed to be common to all polar vertebrates (van Oort 2005). However, daily organization of activity and physiology is maintained throughout the arctic summer in a variety of invertebrates (Syrjamaki 1968; Stelzer and Chittka 2010) and vertebrates (fish: Müller 1973; migratory birds: Hau et al. 2002; mammals: Swade and Pittendrigh 1967). Similarly, we have found that arctic ground squirrels *Urocitellus parryii* exhibit persistent circadian or diurnal rhythms of activity and T_b during summer intervals of constant sun at midarctic latitudes (Long et al. 2005; Williams et al. 2012).

During the constant darkness experienced by hibernating ground squirrels, very low-amplitude circadian T_b rhythms have also been reported during steady state torpor in captive golden-mantled ground squirrels *Callospermophilus lateralis*, and it has been hypothesized that the circadian clock plays a functional role in the timing of periodic arousals from torpor (Daan 1973; Heller and Ruby 2004; Malan 2010). However, using implanted temperature loggers, we were unable to detect circadian rhythms in T_b in arctic ground squirrels hibernating under natural conditions (Williams et al. 2012). Furthermore, resumption of euthermic levels of T_b in spring did not trigger a return to rhythmicity, as pronounced diurnal T_b rhythms take 15–25 d to appear in free-living adult males (Sheriff et al. 2011). After ending torpor, these adult males remain belowground for several weeks, consuming a food cache as they undergo testicular growth and maturation (Barnes 1996) and recuperate their body condition (Buck and Barnes 1999b). The delay in a return to rhythmicity following the resumption of euthermia may in-

* Corresponding author. Present address: Department of Biological Sciences, University of Alberta, Edmonton, Alberta T6G 2R3, Canada; e-mail: cw3@ualberta.ca.

dicates that circadian cycles within the SCN are disrupted during hibernation. Field observations suggest that diurnal T_b rhythms resume about the time animals are first seen on the surface (Williams et al. 2011b), which may indicate that exposure to light is necessary to reinitiate circadian cycles or to resynchronize individual oscillators within the SCN. Hut et al. (2002), however, report spontaneous resumption of circadian T_b rhythms about a week after the return to euthermy in captive European ground squirrels *Spermophilus citellus* held under continuous dim light conditions. If rhythmicity does return in the absence of an external trigger, this would suggest that oscillations in the SCN might persist but be disconnected from the output pathways that influence T_b rhythms.

To better understand the interrelationships among the expression of circadian T_b rhythms, hibernation, and exposure of animals to light under natural conditions, we recorded T_b in free-living squirrels (every 34 min) using implanted data loggers and simultaneously deployed light loggers (recording each 30 s) affixed to collars to record when individuals were exposed to sunlight through transitions in and out of their burrows. Although various external factors can play a role in synchronization and entrainment of circadian rhythms, light is the dominant effector (zeitgeber), and we anticipated that transitions from arrhythmia would coincide with exposure to light.

Material and Methods

Study Species

As the farthest-north hibernating small mammal in North America, the arctic ground squirrel is exposed to profound seasonal changes in photoperiod and ambient temperature (T_a ; Buck and Barnes 1999b; Williams et al. 2011a). Their annual cycle includes a short active season with individuals limiting aboveground activity to 3–5 mo (Carl 1971; Buck and Barnes 1999a). Animals spend the remainder of the year sequestered in frozen burrow systems (hibernacula) alternating between long (2–3 wk) bouts of continuous torpor ($T_b \geq -2.9^\circ\text{C}$; Barnes 1989) interrupted by brief (10–20 h) intervals of euthermic T_b ($35^\circ\text{--}37^\circ\text{C}$) during interbout arousal episodes (Buck et al. 2008). While males terminate heterothermy and feed from cached food for 2–3 wk before first emerging to the surface (Barnes 1996), females are not thought to cache food (Gillis et al. 2005) and typically emerge from their burrows and resume daily activity within 4 d of terminating heterothermy (Sheriff et al. 2011).

Data Collection

Arctic ground squirrels were studied near the University of Alaska Fairbanks's Toolik Field Station ($68^\circ38'\text{N}$, $149^\circ38'\text{W}$) on the North Slope of Alaska. In spring of 2010, we implanted nine adult male ground squirrels (mass: 710 ± 91 g [SD]) with abdominal T_b loggers (modified TidBit Stowaway model TB-ICU32-05+44, coated with Elvax, package weight 14–15 g, Onset Computer, Cape Cod, MA) programmed to record T_b ($\pm 0.19^\circ\text{C}$) at 34-min intervals for up to 24 mo using the meth-

ods described in Long et al. (2007); we also implanted one juvenile squirrel (mass: 593 g) on August 9 with a smaller abdominal T_b logger (iButton model DS1922L, package weight 5 g, Maxim Integrated Products, Sunnydale, CA) programmed to record core T_b ($\pm 0.5^\circ\text{C}$) at 210-min intervals for up to 24 mo. In September 2010, each squirrel was recaptured, anesthetized using isoflurane (2%–5% with oxygen), and outfitted with a temperature and light logger affixed to a neck collar made from zip ties with shrink tubing used to prevent abrasion (GeoLT, package weight 8 g, Earth and Ocean Technologies, Kiel, Germany); mass of the juvenile was 810 g on September 20, the day he was collared, and mass of adults was 915 ± 47 g. Geolocational data loggers, which utilize a miniature luxmeter for ambient light measurement, have been developed to record time of dawn and dusk experienced by migrating birds to determine the latitudes of their changing locations (e.g., Bachler et al. 2010). Here, we use these light loggers to record when ground squirrels transition in and out of their burrows and thus their daily aboveground activity patterns as well as their exposure to light relative to entrainment of circadian rhythms. Sensitivity of the photodiode was 0.1 lux with the resolution changing over the measurement range (0.1–30,000 lux) from 4.5% to $<0.025\%$ of actual reading, providing highest resolution (down to ca. 0.001 lux) toward lower light levels. Loggers affixed to collars were set to record light exposure every 30 s, and therefore light transitions that occur and reverse within 30 s may not be detected. At this sampling interval, light exposure data can be collected for up to 1 yr.

In spring 2011, we successfully recaptured nine of 10 squirrels (one adult was not recaptured despite extensive trapping effort). Of the recaptured squirrels, one had lost its collar, one light logger failed to record, and the implanted T_b logger of a third squirrel failed. Altogether, we obtained concurrent T_b and light exposure data from six squirrels during fall entry into hibernation and heterothermy and, because the batteries of two light loggers failed during midhibernation, from four squirrels during the termination of heterothermy and spring emergence.

Statistical Analysis

We tested for the significance ($\alpha = 0.05$) and periodicity of circadian or diurnal rhythms in T_b data using Lomb-Scargle periodogram analysis (Ruf 1999) in Clocklab software (Actimetrics, Evanston, IL). Our analysis was performed to test for the presence and significance of any rhythm with a period between 5 and 30 h. Periodogram analysis was performed on 10-d blocks of T_b data (1) during the active interval before entering the hibernacula in fall, (2) during belowground euthermy before initiating torpor, (3) during the first bout of torpor after soils had frozen, (4) immediately after individuals first became euthermic following completion of heterothermy, (5) immediately before emergence, and (6) following emergence.

To empirically identify significant change points in time series of T_b for the four males for which we had both light and T_b data during emergence, we applied nonlinear regression

models in SAS statistical software (SAS Institute, Cary, NC) that allowed for different mean T_b (different intercepts) before versus after putative emergence and for a circadian pattern postemergence (a model consisting of cosine curves with a known period of 24 h; Williams et al. 2011b). For each squirrel, we used every day within 10 d of observed emergence as a hypothesized change point and selected the most parsimonious model (most likely change point) among all models using the Akaike Information Criterion (AIC; Akaike 1973).

Results

In fall, before they entered hibernation, core T_b patterns in free-living male ground squirrels showed robust diurnal rhythms of 2°–4°C amplitude that remained diurnal and entrained to 24.0–24.2-h periods ($P < 0.01$). When neck collars with light loggers were deployed, results showed that animals typically emerged from their burrows and became exposed to light after an anticipatory increase in T_b that varied from 1° to 2°C (fig. 1). Daily decreases in T_b were associated with intervals when animals returned to their dark burrows.

Of the six adult male ground squirrels from which we recovered simultaneous T_b and light exposure data in fall, five maintained diurnal activity patterns for 3–9 d before sequestering themselves in their hibernacula between September 22 and September 30 (average September 27). These animals subsequently first entered torpor ($T_b < 30^\circ\text{C}$) 6.6 d later (range 2–13 d; fig. 2). One squirrel (169) entered his burrow on September 29 and remained at euthermic levels of T_b for 22 d during which he occasionally made brief forays aboveground and was regularly exposed to low-intensity light (fig. 2); despite the limited time spent aboveground, he maintained 24.0-h T_b rhythms. In all animals, daily peaks in T_b were attenuated in amplitude, and mean T_b was reduced when animals remained in their burrows and had no aboveground activity as indicated by continuous records of darkness from the light loggers. Two males (nos. 161 and 165) remained at euthermic levels of T_b in constant darkness for ≥ 10 d before first torpor; during this

interval they displayed significant ($P < 0.01$) free-running T_b rhythms of 24.3-h and 23.9-h period length, respectively.

Minimum T_b during torpor steadily decreased from October until late December when squirrels began defending a thermal gradient between T_b (average -1.3°C) and the surrounding colder soil (data not shown). During steady state torpor, T_b was constant and arrhythmic within the resolution of the data loggers ($\pm 0.19^\circ\text{C}$) for as long as 13 d; T_b of the juvenile squirrel (161) was constant during torpor within the 0.5°C resolution of his data logger for as long as 19 d (fig. 3). Light was never detected by animal-borne light loggers during heterothermy. Collar temperature closely tracked T_b during torpor (within 1°C) but deviated frequently from T_b during intervals of belowground euthermia (data not shown); the thermistor was not directly against their skin and was thus influenced by exposure to ambient conditions when squirrels were not curled in a ball within their nests.

In spring, after ending heterothermy, male arctic ground squirrels remained belowground for 22–26 d before commencing daily activity on the surface as evidenced by regular exposure to light (fig. 4). During the first 10 d of euthermia, we did not detect significant rhythms in any of the four squirrels ($P > 0.05$). Three of four squirrels (160, 169, and 161) exposed themselves to very low-amplitude light 4–23 d before they initiated daily aboveground activity (fig. 4). Squirrel 160 experienced two consecutive light readings (30 s apart) of < 1.0 lux 4 d before emergence. Squirrel 169 experienced three consecutive recordings of < 2.0 lux 1 d after terminating heterothermy (23 d preceding emergence). Squirrel 161 experienced four consecutive readings of < 10 lux 4 d before emerging, two consecutive readings of < 2.0 lux 2 d before emerging, and two non-consecutive readings (23 and 163 lux) the day before emergence. During the 10 d preceding their return to regular surface activity, squirrels 160 and 169 exhibited significant ($P < 0.01$) T_b rhythms with a periodicity of 22.1 and 21.4 h, respectively. However, the significance of the rhythm for squirrel 169 may have been driven by the presence of outlier peaks

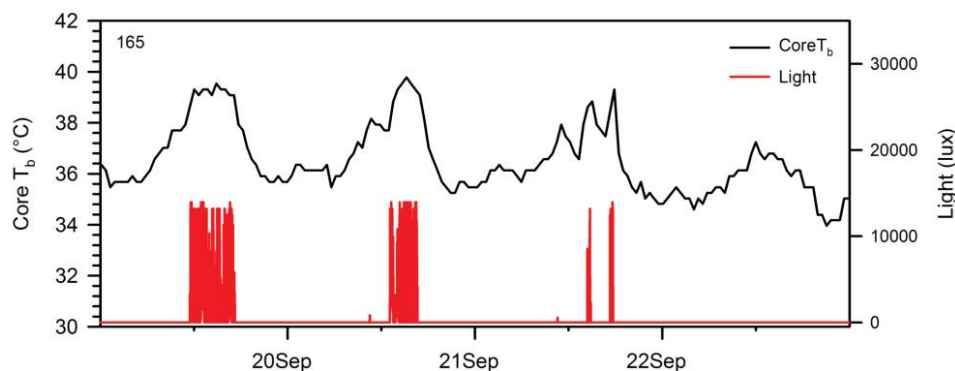


Figure 1. Core body temperature (T_b ; black line) recorded each 34 min and exposure to light (red line) recorded each 30 s versus calendar date of a free-living male arctic ground squirrel before entering the hibernacula in fall. Note that T_b begins to rise before first exposure to light, and the rhythm exhibits attenuated amplitude on the day the squirrel failed to emerge.

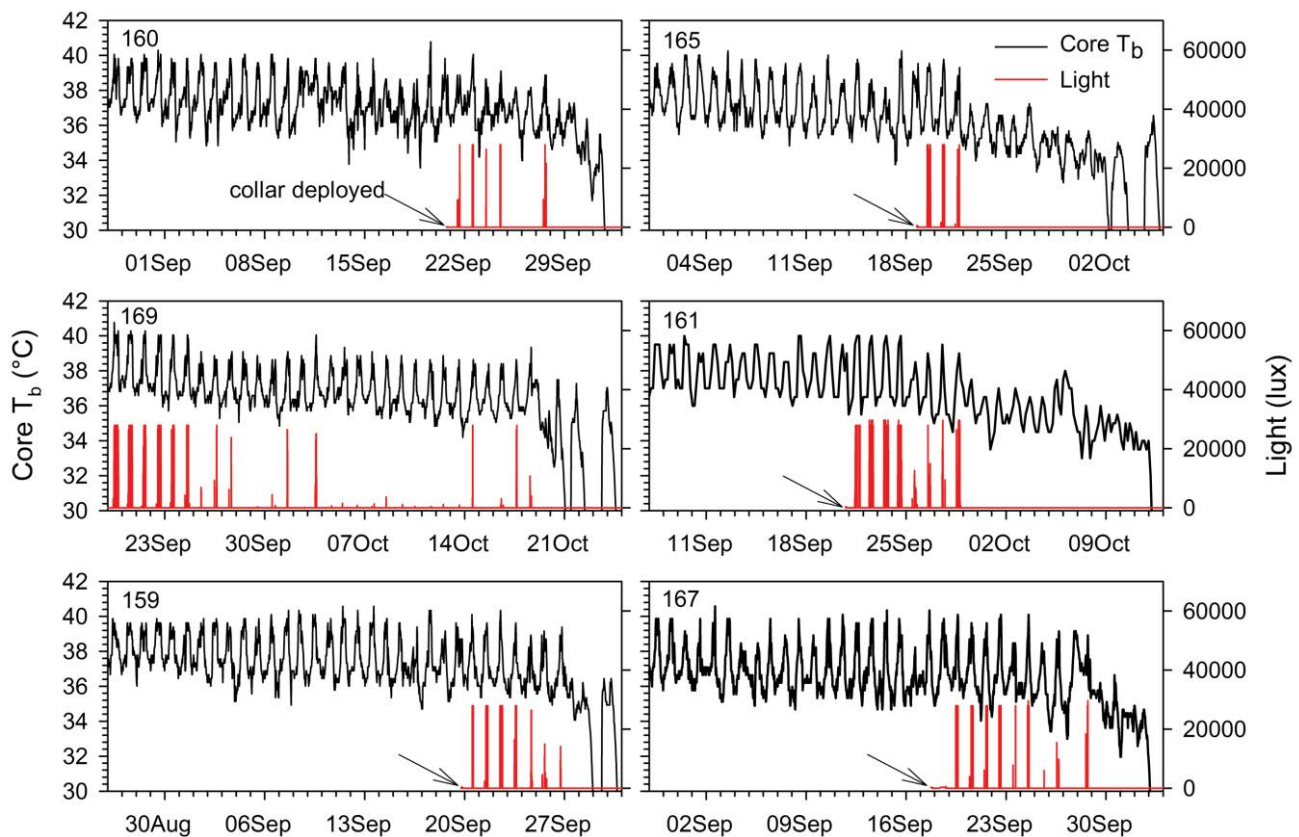


Figure 2. Core body temperature (T_b ; black line) and exposure to light (red line) versus calendar date of six free-living male arctic ground squirrels during entrance into the hibernacula in fall and subsequent initiation of heterothermy. Black arrows indicate the date light loggers were deployed. The two individuals (165, 161) that remained sequestered in their hibernacula for ≥ 10 d before initiating heterothermy maintained significant circadian T_b rhythms (with free-running periods of 23.9 and 24.3 h, respectively). The T_b decreases below 30°C as animals enter torpor.

in T_b during the final 1–2 d belowground, as we could not visually identify a T_b rhythm, and no significant rhythm ($P > 0.05$) was detectable when we excluded the final day spent belowground.

Nonlinear regression models that allowed for different mean T_b before versus after putative emergence and for a circadian pattern postemergence were consistent with a preemergence rhythm in one of four squirrels; the most parsimonious model (lowest AIC) for squirrel 160 identified a change point occurring 9 d before emergence. For two squirrels (161 and 169), the most parsimonious model successfully identified a change point that coincided with their first day spent aboveground (lux $> 20,000$), whereas the second day aboveground was identified as the mostly likely change point for squirrel 165. During the 10 d following emergence, T_b rhythms were diurnal in all four squirrels (23.7–24 h; $P < 0.01$).

Discussion

Circadian rhythms of T_b , in addition to direct timing cues such as humoral and neuronal signals, are generated by the master clock located within the SCN to synchronize peripheral oscil-

lators found throughout the body (Buhr et al. 2010; Dibner et al. 2010). We show that these T_b rhythms either disappear or are dampened to the extent that they are undetectable within the 0.19°C resolution of our T_b loggers during the deep torpor associated with hibernation in arctic ground squirrels. Further, we found that T_b rhythms are not immediately reestablished in spring when squirrels return to euthermic levels of T_b following completion of heterothermy. Circadian T_b rhythms resumed in one squirrel before resumption of surface activity (160), although he was exposed to short intervals of low-intensity light during the belowground euthermic phase. It is currently unclear whether resumption of circadian rhythms is possible without an external light cue. Overall, however, our results are consistent with the hypothesis that circadian function is inhibited during hibernation, including extended intervals of preemergence euthermia, possibly because of a lack of gene expression and protein translation at torpid T_b and/or to desynchronization of cellular cycles within the SCN.

The persistence and potential function of circadian oscillators during hibernation is contentious (reviewed in Larkin et al. 2002). Very low-amplitude (0.1°–0.2°C) circadian T_b rhythms

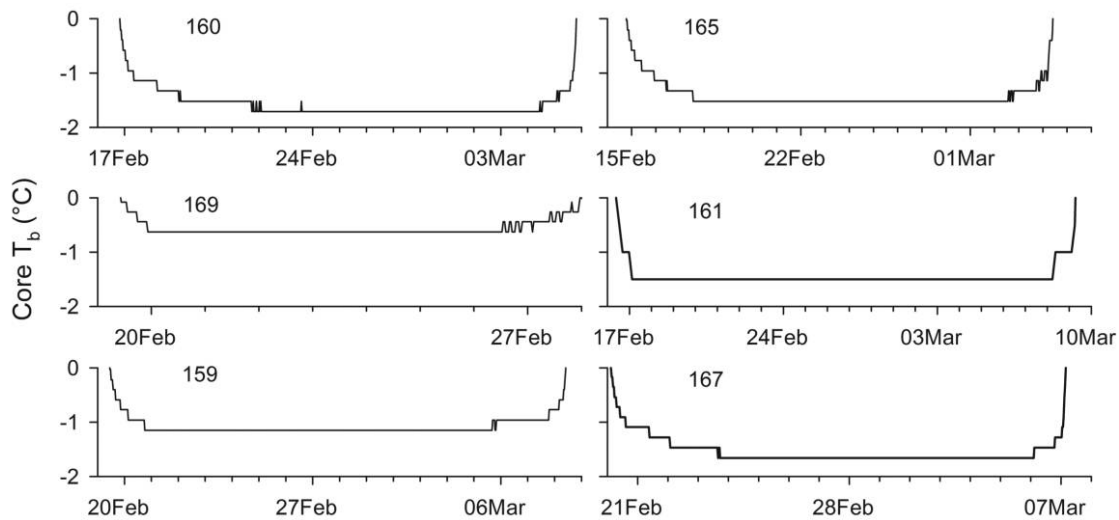


Figure 3. Core body temperature (T_b) versus time of six free-living male arctic ground squirrels during a torpor bout commencing in mid- to late February. The T_b decreased from $>30^\circ\text{C}$ as animals entered the torpor bout and then returned to $>30^\circ\text{C}$ at the end of the bout.

with a broad range of period lengths have been detected during deep torpor in hibernators held under constant dark in captive conditions (Grahn et al. 1994; Florant et al. 2000; Larkin et al. 2002; Ruby et al. 2002), whereas others have failed to detect persistent rhythms in either captive or free-living animals (Florant et al. 2000; Hut et al. 2002; Gür et al. 2009; Tøien et al. 2011; Williams et al. 2012). Florant et al. (2000) found that T_b rhythms were due to oscillations in T_a within the chamber, whereas Ruby et al. (2002) concluded that the T_b oscillations were independent of T_a . Heller and Ruby (2004) postulate that the circadian clock continues to oscillate during torpor and functions in the timing of arousal episodes; this idea is supported by studies where successive entries and arousals from torpor have been observed to occur at the same phase of the circadian cycle (Daan 1973; French 1977; Zervanos et al. 2009) and the finding that ablation of the SCN in hibernating captive golden-mantled ground squirrels eliminates the circadian T_b rhythms that occur in hibernating control animals (Ruby et al. 2002). To date, the only study to directly measure clock-gene expression within the SCN during hibernation, however, found no oscillations in gene expression of the clock genes *Per1*, *Per2*, and *Bmal 1* during multiday torpor in the European hamster *Cricetus cricetus*, and these authors concluded that the molecular circadian clock is arrested during hibernation (Revel et al. 2007). Herwig et al. (2006) found that gene expression within the SCN continued to oscillate during daily torpor in Djungarian hamsters *Phodopus sungorus*, although T_b only dropped to 18°C , which in ground squirrels is the critical temperature below which polysome disaggregation begins and translation of genes is inhibited (Van Breukelen and Martin 2001).

It is well accepted that both transcription and translation are generally suppressed during deep torpor (reviewed in Storey and Storey 2004). Translation of select mRNA transcripts during torpor can continue in brown adipose tissue, however, rais-

ing the interesting possibility for the existence of tissue-specific mechanisms for translational control of subsets of genes that are physiologically relevant to survival during hibernation (Hittel and Storey 2002). This may be the case for clock genes within the SCN given the relatively high metabolic activity of this region, based on ^{14}C -2-deoxyglucose uptake, during hibernation (Kilduff et al. 1990; but see Frerichs et al. 1995). Our current results, however, are consistent with inhibition of clock function during deep torpor. We found no evidence for rhythmicity during torpor because T_b remained constant within the 0.19°C resolution of our data loggers, although this resolution is likely to be insufficient to detect rhythmicity at low T_b (Grahn et al. 1994). However, we found that once ground squirrels returned to euthermia, wherein preheterothermy circadian rhythms have amplitudes of $1^\circ\text{--}2^\circ\text{C}$ (fig. 2), T_b remained arrhythmic for at least 10 d. Absence of T_b rhythmicity following the return to euthermia suggests that the generation of circadian T_b rhythms ceases at some point during heterothermy. Exposure to an external light cue may be necessary to restore rhythmicity, although we did find that T_b rhythms recommenced in one of four animals before resumption of aboveground activity and exposure to solar cycles. The period of this rhythm was shorter (~ 22 h) than the more evident diurnal (24 h) rhythms that were restored once animals resumed aboveground activity (fig. 4).

We detected exposure to low-amplitude light pulses (<10 lux) for three squirrels during the period of euthermia that occurs before they resumed regular aboveground activity. Although we lack data on snow cover during this time frame, snow cover at this site is intermittent and patchy during the winter months (see Atigun River snow camera: <http://toolik.alaska.edu/edc>), and squirrels may have been exposed to light through their burrow entrances, which they leave unblocked. Additionally, light intensity can be as high as 4 lux below 25

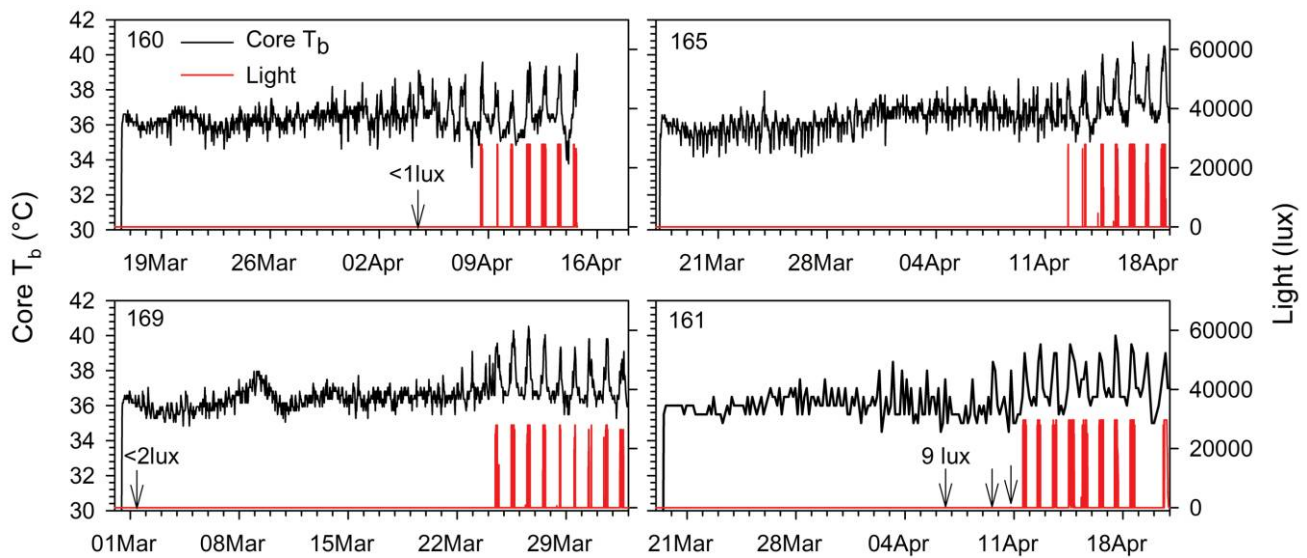


Figure 4. Core body temperature (T_b ; black line) and exposure to light (red line) versus date of four free-living male arctic ground squirrels after their final arousal from torpor and return to euthermia and through the first several days following their emergence and resumption of daily aboveground activity. Black downward-facing arrows indicate very low-intensity light pulses. The most parsimonious model indicated that a change point (transition to a sinusoidal temperature rhythm with a periodicity of 24 h) occurred following emergence for three of four squirrels; the most likely change point for squirrel 160 occurred 9 d before emergence. All four individuals exhibited obvious diurnal rhythms following emergence.

cm of snow (Potvin and Bovet 1975), and squirrels may have been exposed to light even if their burrow entrances were snow covered.

It is possible that some external trigger (such as light) is necessary to resynchronize individual oscillators within the SCN that are no longer in phase with one another following hibernation. If the low T_b associated with torpor prevents RNA transcription and translation within the SCN, then we anticipate the master clock would go through 14–16 stop-start cycles (13–15 arousal episodes) as animals rewarm during the spontaneous arousal episodes over the hibernation season, depending on sex and age (Buck et al. 2008). Expression of several clock-associated genes increase in the hypothalamus and in peripheral tissues early in the euthermic phase of arousal-torpor cycles in arctic ground squirrels (Yan et al. 2008). In the absence of an external zeitgeber to entrain circadian rhythms, such start-stop cycles might lead to desynchrony of individual oscillators within the master clock. We cannot discount the possibility, however, that oscillators within the SCN are synchronized and rhythmic throughout hibernation and that instead it is the output pathways responsible for the generation of T_b rhythms that are inhibited for some time following completion of heterothermy. Likewise, our results are not inconsistent with the recent hypothesis of Malan (2010), who proposes torpor-arousal cycles are controlled by a nontemperature-compensated circadian clock located in a region other than the SCN, as such a clock would not be involved in the generation of circadian T_b rhythms.

The recent finding for the persistence of circadian redox cycles in nonnucleated red blood cells indicates that some cir-

cadian cellular oscillators can act independently of transcription (O'Neill and Reddy 2011). This raises the interesting possibility that circadian rhythms could be maintained in hibernating animals even if transcription were to be completely arrested during deep torpor. However, the persistence of these circadian oscillations has yet to be demonstrated at temperatures below 32°C, and the functional significance and molecular underpinnings of nontranscriptional clocks remain unclear.

Regardless of the mechanisms responsible for disruptions in T_b rhythms during hibernation, our results corroborate our previous findings with respect to using T_b patterns to assess timing of fall entrance into and spring emergence from the hibernacula in free-living ground squirrels (Sheriff et al. 2011; Williams et al. 2011b). Entrance into the hibernacula in fall coincided with a decline in mean T_b and dampened amplitude of the daily oscillation in T_b (fig. 2), whereas spring emergence coincided with the reappearance of obvious diurnal rhythms (fig. 4). Obtaining these types of precise measures of phenology is critical to understanding how animals organize their annual cycles in seasonal environments and to determining their phenological response to climate-driven changes in the seasonal timing of resource availability (Visser and Both 2005).

Conclusions

The circadian rhythm of T_b is lost during heterothermy in hibernating arctic ground squirrels and is not immediately reestablished when males become euthermic again in spring. However, significant rhythms of <24 h were detectable before spring emergence in at least one individual. Whether resumption of

rhythms occurs spontaneously or requires an external cue remains unclear, however, as some individuals were exposed to very low-amplitude light during the preemergence euthermic phase. Predictable shifts in T_b patterns associated with entry into and emergence from the hibernacula support the use of T_b loggers to record the phenology of annually recurring life-cycle events.

Acknowledgments

We thank Stormy Haught and Franziska Kohl for data and field management. This study was supported by funding from the National Science Foundation to B.M.B. (EF-0732763) and C.L.B. (EF-0732755) and awards from the U.S. Army Medical Research and Materiel Command (05178001) to B.M.B. All procedures were approved by the University of Alaska Fairbanks Institutional Animal Care and Use Committee, and work was conducted under appropriate state permits.

Literature Cited

- Akaike H. 1973. Information theory and an extension of the maximum likelihood principle. Pp. 267–281 in B.N. Petrov and F. Csaki, eds. Second international symposium on information theory. Akademia Kiado, Budapest.
- Bachler E., S. Hahn, M. Schaub, R. Arlettaz, L. Jenni, J.W. Fox, V. Afanasyev, and F. Liechti. 2010. Year-round tracking of small trans-Saharan migrants using light-level geolocators. *PLoS ONE* 5:e9566.
- Barnes B.M. 1989. Freeze avoidance in a mammal: body temperatures below 0°C in an arctic hibernator. *Science* 244: 1593–1595.
- . 1996. Relationships between hibernation and reproduction in male ground squirrels. Pp. 71–80 in F. Geiser, A.J. Hulbert, and S.C. Nicol, eds. Adaptations to the cold. University of New England Press, Armidale, Australia.
- Buck C.L. and B.M. Barnes. 1999a. Annual cycle of body composition and hibernation in free-living arctic ground squirrels. *J Mammal* 80:430–442.
- . 1999b. Temperatures of hibernacula and changes in body composition of arctic ground squirrels over winter. *J Mammal* 80:1264–1276.
- Buck C.L., A. Breton, F. Kohl, Ø. Tøien, and B.M. Barnes. 2008. Overwinter body temperature patterns in free-living arctic squirrels (*Spermophilus parryii*). Pp. 317–326 in B.G. Lovegrove and A.E. McKechnie, eds. Hypometabolism in animals: hibernation, torpor and cryobiology. Interpak, Pietermaritzburg, South Africa.
- Buhr E.D., S.H. Yoo, and J.S. Takahashi. 2010. Temperature as a universal resetting cue for mammalian circadian oscillators. *Science* 330:379–385.
- Carl E.A. 1971. Population control in arctic ground squirrels. *Ecology* 52:395–413.
- Daan S. 1973. Periodicity of heterothermy in the garden dormouse, *Eliomys quercinus* (L.) *Neth J Physiol* 23:237–265.
- Dibner C., U. Schibler, and U. Albrecht. 2010. The mammalian circadian timing system: organization and coordination of central and peripheral clocks. *Ann Rev Physiol* 72:517–549.
- Florant G.L., V. Hill, and M.D. Ogilvie. 2000. Circadian rhythms of body temperature in laboratory and field marmots (*Marmota flaviventris*). Pp. 223–231 in G. Heldmaier and M. Klingenspor, eds. Life in the cold: eleventh international hibernation symposium. Springer, Berlin.
- French A.R. 1977. Periodicity of recurrent hypothermia during hibernation in the pocket mouse, *Perognathus longimembris*. *J Comp Physiol A* 115:87–100.
- Frerichs K.U., G.A. Diemel, N.F. Cruz, L. Sokolof, and J.M. Hallenbeck. 1995. Rates of glucose utilization in brain of active and hibernating ground squirrels. *Am J Physiol* 268: R445–R453.
- Gillis E.A., S.F. Morrisson, G.D. Zazula, and D.S. Hik. 2005. Evidence for selective caching by arctic ground squirrels living in alpine meadows in the Yukon. *Arctic* 58:354–360.
- Grahn D.A., J.D. Miller, V.S. Hough, and H.C. Heller. 1994. Persistence of circadian rhythmicity in hibernating ground squirrels. *Am J Physiol* 266:R1251–R1258.
- Gür M.K., R. Refinetti, and Gür, H. 2009. Daily rhythmicity and hibernation in the Anatolian ground squirrel under natural and laboratory conditions. *J Comp Physiol B* 179:155–164.
- Hau M., L.M. Romero, J.D. Brawn, and T.J. Van't Hof. 2002. Effect of polar day on plasma profiles of melatonin, testosterone, and estradiol in high-arctic Lapland longspurs. *Gen Comp Endocrinol* 126:101–112.
- Heller H.C. and N.F. Ruby. 2004. Sleep and circadian rhythms in mammalian torpor. *Annu Rev Physiol* 66:275–289.
- Herwig A., F. Revel, M. Saboureaux, P. Pévet, and S. Steinlechner. 2006. Daily torpor alters multiple gene expression in the suprachiasmatic nucleus and pineal gland of the Djungarian hamster (*Phodopus sungorus*). *Chronobiol Int* 23:269–276.
- Hittel D. and K.B. Storey. 2002. The translation state of differentially expressed mRNAs in the hibernating 13-lined ground squirrel (*Spermophilus tridecemlineatus*). *Arch Biochem Biophys* 401:244–254.
- Hut R., E. Van der Zee, K. Jansen, M. Gerkema, and S. Daan. 2002. Gradual reappearance of post-hibernation circadian rhythmicity correlates with numbers of vasopressin-containing neurons in the suprachiasmatic nuclei of European ground squirrels. *J Comp Physiol B* 172:59–70.
- Kilduff T.S., J.D. Miller, C.M. Radeke, F.R. Sharp, and H.C. Heller. 1990. 14C-2-deoxyglucose uptake in the ground squirrel brain during entrance to and arousal from hibernation. *J Neurosci* 10:2463–2475.
- Larkin J., P. Franken, and H.C. Heller. 2002. Loss of circadian organization of sleep and wakefulness during hibernation. *Am J Physiol* 282:R1086–R1095.
- Long R.A., R.A. Hut, and B.M. Barnes. 2007. Simultaneous collection of body temperature and activity data in burrowing mammals: a new technique. *J Wildl Manag* 71:1375–1379.
- Long R.A., T.J. Martin, and B.M. Barnes. 2005. Body temper-

- ature and activity patterns in free-living arctic ground squirrels. *J Mammal* 86:314–322.
- Malan A. 2010. Is the torpor-arousal cycle of hibernation controlled by a non-temperature compensated circadian clock? *J Biol Rhythms* 25:166–175.
- Müller K. 1973. Seasonal phase shift and the duration of activity time in the burbot, *Lota lota* (L.) (Pisces, Gadidae). *J Comp Physiol* 84:357–359.
- O'Neill J.S. and A.B. Reddy. 2011. Circadian clocks in human red blood cells. *Nature* 469:498–503.
- Potvin C.L. and J. Bovet. 1975. Annual cycle of patterns of activity rhythms in beaver colonies (*Castor canadensis*). *J Comp Physiol* 98:243–256.
- Reierth E. and K.A. Stokkan. 1998. Activity rhythm in high arctic Svalbard ptarmigan (*Lagopus mutus hyperboreus*). *Can J Zool* 76:2031–2039.
- Revel F.G., A. Herwig, M.L. Garidou, H. Dardente, J.S. Menet, M. Masson-Pévet, V. Simonneaux, M. Saboureau, and P. Pévet. 2007. The circadian clock stops ticking during deep hibernation in the European hamster. *Proc Natl Acad Sci USA* 104:13816–13820.
- Ruby N.F., J. Dark, D.E. Burns, H.C. Heller, and I. Zucker. 2002. The suprachiasmatic nucleus is essential for circadian body temperature rhythms in hibernating ground squirrels. *J Neurosci* 22:357–364.
- Ruf T. 1999. The Lomb-Scargle periodogram in biological rhythm research: analysis of incomplete and unequally spaced timeseries. *Biol Rhythm Res* 30:178–201.
- Sheriff M.J., G.J. Kenagy, M. Richter, T. Lee, Ø. Tøien, F. Kohl, C.L. Buck, and B.M. Barnes. 2011. Phenological plasticity: variation in annual timing of hibernation and breeding in two nearby populations of arctic ground squirrels. *Proc R Soc B* 278:2369–2375.
- Stelzer R.J. and L. Chittka. 2010. Bumblebee foraging rhythms under the midnight sun measured with radiofrequency identification. *BMC Biol* 8:93.
- Storey K.B. and J.M. Storey. 2004. Metabolic rate depression in animals: transcriptional and translational controls. *Biol Rev* 79:207–233.
- Swade R.H. and C.S. Pittendrigh. 1967. Circadian locomotor rhythms of rodents in the Arctic. *Am Nat* 101:431–466.
- Syrjamäki J. 1968. Diel patterns of swarming and other activities of two arctic dipterans (Chironomidae and Trichoceridae) on Spitsbergen. *Oikos* 19:250–258.
- Tøien Ø., J. Blake, D.M. Edgar, D.A. Grahn, H.C. Heller, and B.M. Barnes. 2011. Hibernation in black bears: independence of metabolic suppression from body temperature. *Science* 331:906–909.
- Van Breukelen F. and S. Martin. 2001. Translational initiation is uncoupled from elongation at 18°C during mammalian hibernation. *Am J Physiol* 281:R1374–R1379.
- van Oort B.E.H., N.J.C. Tyler, M.P. Gerkema, L. Folkow, A.S. Blix, and K.A. Stokkan. 2005. Circadian organization in reindeer. *Nature* 438:1095–1096.
- Visser M.E. and C. Both. 2005. Shifts in phenology due to global climate change: the need for a yardstick. *Proc R Soc B* 272:2561–2569.
- Williams C.T., B.M. Barnes, and C.L. Buck. 2012. Body temperature rhythms persist under the midnight sun but are absent during hibernation in free-living arctic ground squirrels. *Biol Lett* 8:31–34.
- Williams C.T., A.V. Goropashnaya, C.L. Buck, V.B. Fedorov, F. Kohl, T.N. Lee, and B.M. Barnes. 2011a. Hibernating above the permafrost: effects of ambient temperature and season on expression of metabolic genes in liver and brown adipose tissue of arctic ground squirrels. *J Exp Biol* 214:1300–1306.
- Williams C.T., M.J. Sheriff, J.A. Schmutz, F. Kohl, Ø. Tøien, C.L. Buck, and B.M. Barnes. 2011b. Data logging of body temperatures provides precise information on phenology of reproductive events in a free-living arctic hibernator. *J Comp Physiol B* 181:1101–1109.
- Woelfle M.A., Y. Ouyang, K. Phanvijhitsiri, and C.H. Johnson. 2004. The adaptive value of circadian clocks: an experimental assessment in cyanobacteria. *Curr Biol* 14:1481–1486.
- Yan J., B.M. Barnes, F. Kohl, and T.G. Marr. 2008. Modulation of gene expression in hibernating arctic ground squirrels. *Physiol Genomics* 32:170–181.
- Yerushalmi S. and R.M. Green. 2009. Evidence for the adaptive significance of circadian rhythms. *Ecol Lett* 12:970–981.
- Zervanos S.M., C.M. Salsbury, and J.K. Brown. 2009. Maintenance of biological rhythms during hibernation in eastern woodchucks (*Marmota monax*). *J Comp Physiol B* 179:411–418.