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ORIGINAL PAPER

Body temperature patterns and use of torpor in an alpine glirid species, woolly dormouse

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Abstract Woolly dormice, *Dryomys laniger* Felten and Storch (Senckenbergiana Biol 49(6):429–435, 1968), are a small (20–30 g), omnivorous (mainly insectivorous), nocturnal glirid species endemic to Turkey. Although woolly dormice have been assumed to hibernate during winter, no information exists on body temperature patterns and use of torpor in the species. In the present study, we aimed to determine body temperature patterns and use of torpor in woolly dormice under controlled laboratory conditions. Accordingly, body temperature (Tb) of woolly dormice was recorded using surgically implanted Thermochron iButtons, small and inexpensive temperature-sensitive data loggers. Woolly dormice exhibited robust, unimodal daily Tb rhythmicity during the euthermic stage before the beginning of hibernation. They displayed short torpor before they began hibernation, although the tendency to enter short torpor was different among individuals. Woolly dormice began hibernation within 1–3 days after exposure to cold and darkness, i.e., on October 22–24, and ended hibernation in the first half of April. Hibernation consisted of a sequence of multiday torpor bouts, interrupted by euthermic intervals. Thus, the patterns of hibernation in woolly dormice were similar to those observed in classical hibernating mammals.

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Introduction

Endotherms have evolved the ability to maintain a high and constant body temperature (Tb) under conditions of highly fluctuating ambient temperatures (Ta) through a high metabolic rate (Körtner and Geiser 2000). Maintaining a high and constant Tb has long been thought to provide endotherms with distinct physiological and ecological benefits (Bennett and Ruben 1979). However, especially for small endotherms, this is highly energetically expensive when the weather is cold and/or when energy reserves and access to these reserves are limited (Lyman et al. 1982; Lovegrove 2000). Thus, under adverse conditions, many small endotherms use torpor, which is a facultative reduction of metabolic rate and Tb to reduce energy expenditure (Geiser and Kenagy 1988; Heldmaier and Ruf 1992). Two common patterns of torpor can generally be distinguished: daily torpor (lasting shorter than 1 day) and hibernation (a sequence of multiday torpor bouts, interrupted by brief periods of high metabolic rate and euthermic Tb, i.e., euthermic intervals; Geiser and Ruf 1995). However, these patterns of torpor vary among and within species and even within an individual (McKechnie and Mzilikazi 2011).

Woolly dormice, *Dryomys laniger* Felten and Storch 1968, are a small (20–30 g), omnivorous (mainly insectivorous), nocturnal glirid species endemic to Turkey. Woolly dormice inhabit rocky and stony habitats in the Taurus Mountains in southern Anatolia and in a few localities in northeastern Anatolia, above 1,500 m, and therefore the geographic distribution of the species is very fragmented (Spitzenberger 1976; Kryštufek and Vohralík 2005; IUCN 2008). Woolly dormice are naturally rare (IUCN 2008) because they have a small geographic distribution, specialized niche requirement, and low local abundance. Woolly dormice have been the subject of many systematic studies (e.g., Felten and Storch 1968; Felten et al. 1973; Mursaloğlu 1973; Kıvanç et al. 1997; Yiğit et al. 2003, 2011), but little information on the biology and ecology of the species has been reported, mainly by Spitzenberger (1976) and Kryštufek and Vohralík (2005). Although woolly dormice have been assumed to hibernate during winter (Kryštufek and Vohralík 2005), no information exists on body temperature patterns and use of torpor in the species.

In the present study, we aimed to determine body temperature patterns and use of torpor in woolly dormice under controlled laboratory conditions. Accordingly, Tb of woolly dormice was recorded using surgically implanted Thermochron iButtons, small and inexpensive temperature-sensitive data loggers, which have made recording Tb of animals much easier (Kart Gür et al. 2009). Following Lovegrove (2009), the data loggers were modified before implantation to reduce their size and weight because they are too large and heavy to implant into woolly dormice. Because of a memory split problem related to modification, the data loggers stopped recording Tb of woolly dormice before the end of hibernation. However, we believe that these data are interesting enough to be presented especially because the data set is one of the most comprehensive data sets available on body temperature patterns and use of torpor in glirid species (Bieber and Ruf 2009; Pretzlaff and Dausmann 2012).

Materials and methods

Woolly dormice and housing

On September 15–16, 2010, three woolly dormice (males) were live-trapped using Sherman live traps from free-ranging populations in Bolkar Mountains (2,350 m in altitude) and Aladağlar (2,950 m in altitude). Immediately upon capture, they were transported from these study areas to a temperature- and photoperiod-controlled laboratory at Hacettepe University, Ankara, at a distance of approximately 380 km. On September 18, two additional woolly dormice (females) trapped from the same free-ranging population in Bolkar Mountains and housed in an uncontrolled laboratory at Hitit University, Çorum, for approximately 1 month were transported to the same laboratory at Hacettepe University, at a distance of approximately 245 km.

Woolly dormice maintained in the laboratory were kept individually in polycarbonate cages (36×24×19 cm) lined with hard wood shavings. Each cage was equipped with a small (6×6×6 cm) open-topped wooden nest box with a circular entrance. Cotton was provided as nesting material. They used these nest boxes for sleeping and hibernating and for nest-building activities. Woolly dormice were given food and water ad libitum throughout the study, including hibernation. The diet consisted of cat chow supplemented with seed mixture (nuts and sunflower seeds) and fresh fruits (apple and pear). They were weighed (±0.1 g) at irregular intervals until the beginning of hibernation. The weighing procedure was suspended to eliminate disturbances during hibernation.

The laboratory was kept at Ta 18±1 °C and light–dark cycle (200–0 lux) 12:12 (lights on at 07:00) to simulate the temperature and photoperiod in natural environments that woolly dormice inhabit. From October 21, 2010 to April 18, 2011, Ta was lowered to 5±1 °C, and lights were kept continuously off. Ta was also monitored by a temperature-sensitive data logger (for details of the data logger, see below). In the laboratory, equipment malfunction caused a fall of Ta to 11 °C between September 30 and October 2 and a brief (6–7 h) rise of Ta to 20 °C on October 20.

Specifications of Thermochron iButton

Thermochron iButton (DS1922L-F5; Maxim Integrated Products, Sunnyvale, CA, USA) is a small and inexpensive temperature-sensitive data logger. The data logger comprises a printed circuit board, which integrates a thermometer, real-time clock, and SRAM memory for storing temperature, time, and date readings, and a non-replaceable 3-V lithium battery. The circuit board and battery of the data logger are encased in a rugged stainless steel case. The data logger has a range of measurement from −40 to 85 °C and records a total of 8,192 8-bit readings with a resolution of 0.5 °C or 4,096 16-bit readings with a resolution of 0.0625 °C, taken at equidistant intervals ranging from 1 s to 273 h.

Modification, programming, and waxing of Thermochron iButtons

Thermochron iButtons were modified before implantation to reduce their size and weight because they are too large and heavy to implant into woolly dormice. For modification, the stainless steel case of the data loggers was opened to remove their components (i.e., the circuit board and battery). After changing the battery, these components were reconstructed without the stainless steel case that accounts for a large proportion of the size and weight of the data loggers (for further details on the modification of Thermochron iButtons, see Lovegrove 2009). After modification, the data loggers were programmed to record up to a total of 8,192 Tb readings with a resolution of 0.5 °C at 50-min intervals throughout the study (see "Results" section). The data loggers were wrapped in a piece of cling wrap and parafilm prior to waxing to prevent the wax from sticking to their components. Then, to improve water resistance and prevent tissue reaction, the data loggers were coated in paraffin-elvax compound (Mini Mitter Company, Oregon, USA) and cold-sterilized in glutaraldehyde solution for 1 day before being implanted into the peritoneal cavity of woolly dormice. Before the study, the wax-coated, modified data loggers were calibrated in an insulated water bath (MGW Lauda C6, Westbury, NY) at water bath temperatures from 10 to 35 °C using a calibrated and certified mercury thermometer (with a range of measurement from −1 to 50 °C and a resolution of 0.1 °C; Allafrance, UMS, Ankara, Turkey).

The modified Thermochron iButtons were launched and downloaded (1-Wire® protocol, USA) using the modified blue dot receptor (for details on the modification of the blue dot receptor, see Lovegrove 2009).

The weight of the unmodified, intact Thermochron iButtons is approximately 2.94 g as supplied. Modification reduced the weight of the data loggers to 1.93±0.09 g (mean ± SD, range = 1.86–2.05, *n*=5), 66 % of their initial weight. The weight of the wax-coated, modified data loggers was 2.48±0.18 g (2.24–2.63, *n*=5), which constituted approximately 12 % of the body mass of woolly dormice at the date of implantation. The data loggers of this weight range appeared not to affect adversely the locomotor performance in woolly dormice (Rojas et al. 2010). Also, no macroscopic sign of injury or inflammation caused by the data loggers was observed at the time of their removal.

Implantation

All surgical operations were conducted under sterile conditions in a laboratory at Hacettepe University. On September 19 and April 26, the wax-coated, modified Thermochron iButtons were surgically implanted into and removed from, respectively, the peritoneal cavity of woolly dormice under general anesthesia. The anesthesia was injected intraperitoneally with 100 mg/kg ketamine (Ketasol® 10 %; Richter Pharma AG, Wels, Austria) and 10 mg/kg xylazine (Alfazyne® 2 %, Alfasan International B. V. Woerden, Netherlands). The lower part of the abdomen was shaved and scrubbed with povidone-iodine solution (Isosol 10 %; Merkez Laboratory, Istanbul, Turkey). A 1-cm midline incision was made in the skin and linea alba in the ventrum beginning approximately 1 cm above the genital. Then, the data loggers were implanted into or removed from the peritoneal cavity of woolly dormice. Any macroscopic sign of injury or inflammation caused by the data loggers was checked at the time of their removal. The abdominal muscles and skin were closed separately with 4–0 polyglycolic acid (Braun, Safil green) and 4–0 polypropylene (Eticon, Prolene) sutures in a simple interrupted pattern, respectively. After implantation or removal of the data loggers, woolly dormice were kept individually in polycarbonate cages in an uncontrolled laboratory for recovery from surgical stress. An open window provided natural temperature and photoperiod fluctuations.

The capture, handling, and care of woolly dormice were approved by the Local Ethical Committee for Animal Care and Use of Hacettepe University School of Medicine, Ankara (protocol no. B.30.2.Hac.0.05.06.00/3) and were consistent with the Guide for the Care and Use of Laboratory Animals (Institute for Laboratory Animal Research-ILAR 2011).

Data analysis

Entry into torpor was defined as the beginning of the phase of a rapid and continuous decrease in Tb (≥3.5 °C within 50 min), and arousal from torpor was defined as the ending of the phase of a rapid and continuous increase in Tb (≥3.5 °C within 50 min). Both entry into and arousal from torpor coincide with major changes in metabolism (Heldmaier and Ruf 1992; Hut et al. 2002). Torpor bouts of different extents were defined using the following terms: (1) short torpor as period of low metabolic rate and Tb lasting shorter than 1 day; (2) prolonged torpor as period of low metabolic rate and Tb lasting longer than 1 day, but shorter than 2 days; and (3) multiday torpor as period of low metabolic rate and Tb lasting longer than 2 days, interrupted by euthermic interval (period of high metabolic rate and euthermic Tb lasting shorter than 1 day; Kobbe et al. 2011).

For the analysis of daily rhythmicity (under Ta 18 °C and light–dark cycle), Tb data before the beginning of hibernation were used. Tb data from the first week after implantation were discarded, as the patterns of the daily oscillation of Tb were clearly irregular while woolly dormice recovered from surgery. Tb data after 1 day before the first short torpor were also discarded, as woolly dormice began heterothermy. Thus, the euthermic stage before the beginning of hibernation used for the analysis of daily Tb rhythm lasted from 5 to 18 days, depending on individuals. For the analysis of circadian rhythmicity (under Ta 5 °C and constant darkness), Tb data from the steady-state part of the longest multiday torpor were used. The steady-state part of the longest multiday torpor lasted from 15 to 19 days, depending on individuals.

The presence of broad rhythmicity (from 1 h to several weeks) was investigated by Fourier analysis with significance testing by Fisher's method (Siegel 1980). The presence of a 24-h rhythmicity was investigated by cosinor rhythmometry (Nelson et al. 1979; Refinetti et al. 2007), with confirmation by the Lomb–Scargle periodogram procedure (Ruf 1999). Cosinor rhythmometry provided estimates of the three main rhythmic parameters: mesor (mean level), amplitude (half the range of excursion), and acrophase (time of the daily peak). Two additional parameters (range of excursion and robustness) were also evaluated. The range of excursion is simply the distance between the lowest and the highest Tb recorded during the interval being analyzed. The robustness (or strength) reflects the stability of the rhythm and is independent of the amplitude of the rhythm (Refinetti 2004). Robustness was computed as ($V_{\mathrm{mod}}/V_{\mathrm{tot}}$) × 100, where V_{mod} is the variance explained by the cosine model in the regression procedure and V_{tot}, the total variance of the data.

The time of day of entries into and arousals from torpor was evaluated by Rayleigh's test (providing an *r* value for significance; Zar 1999) for circular uniformity, with $P<0.05$ indicating that entries into and/or arousals from torpor were nonrandom and occurred at specific times of day. Descriptive statistics (i.e., mean current direction, length of mean vector, variance, and standard deviation) and graph visualization were performed using a data analysis software package (Oriana, Version 3.0; Kovach Computing Services).

Descriptive statistics are presented as mean ± standard deviation (unless otherwise mentioned) and range, and *n* denotes the number of individuals and *N*, the number of observations from individuals.

Results

Body mass

All woolly dormice were given unrestricted access to food throughout the study, including hibernation. Body mass was 20.6±2.5 g (18.0–24.0 g, $n=5$) at the date of implantation (i.e., approximately when they were transported to the laboratory). Woolly dormice gained at least 47 % of their body mass during the month after implantation and therefore weighed 30.2±1.5 g (28.9–32.5 g, $n=5$) shortly before the beginning of hibernation, i.e., on October 20. During hibernation, they did not consume a measurable amount of food, and no food or food remnants were found in their nest boxes. Woolly dormice lost at least 28 % of their body mass during hibernation and therefore weighed 21.7±2.5 g (17.4–23.4 g, $n=5$) shortly after the end of hibernation, i.e., on April 18. Body mass was 28.2±3.0 g (24.8–32.3 g, $n=5$) at the date of explantation.

Body temperature recordings

Four out of the five Thermochron iButtons recorded a total of 4,096 Tb readings by February 7, although they had been programmed to record up to a total of 8,192 Tb readings throughout the study. These four data loggers were reprogrammed in the same way after the study. Then, we noticed that although they have no external data recording functions, such as pressure monitoring (i.e., they function as a temperature logger only), their memories were split in default into two equal sections, each storing 4,096 8–bit readings, for temperature and external data. In this case, "enable sampling" option should have been deactivated for external data. The fifth data logger failed to record any Tb readings. This data logger could not be reprogrammed after the study. Thus, we cannot explain why it failed to record any Tb readings.

Daily Tb rhythm during the euthermic stage before the beginning of hibernation

All woolly dormice exhibited robust, unimodal daily Tb rhythmicity (under Ta 18 °C and light–dark cycle) during the euthermic stage before the beginning of hibernation ($P<0.001$; Figs. 1 and 2). The mean curve of Tb indicates that the daily Tb rise preceded lights-off and the daily Tb fall preceded lights-on by a few hours (Fig. 2). The daily Tb rhythm had a mesor of 36.4±0.3 °C (36.0–36.8 °C, $n=4$), acrophase of 00:11±1.1 h (23:22–01:49, $n=4$), amplitude of 1.6±0.3 °C (1.4–2.0 °C, $n=4$; range of excursion of 5.6±1.3 °C, 4.0–7.0 °C, $n=4$), and robustness of 66±4 % (62–70 %, $n=4$). The acrophase of the daily Tb rhythm indicates that the middle of the plateau of peak Tb was achieved approximately 5 h after lights were off, which is consistent with the mean curve of Tb (Fig. 2).

Short and prolonged torpor bouts before the beginning of hibernation

All woolly dormice entered torpor before they began hibernation on October 22–24. However, two woolly dormice (no. 76 and no. 80) displayed torpor more commonly, while the other two (no. 77 and no. 78) spent an extended period of time euthermic with a few torpor bouts (Fig. 3). Due to interruption of some torpor bouts caused by several reasons (e.g., repair activities in the laboratory, weighing body mass), we could not examine all available torpor bouts for some parameters (see below).

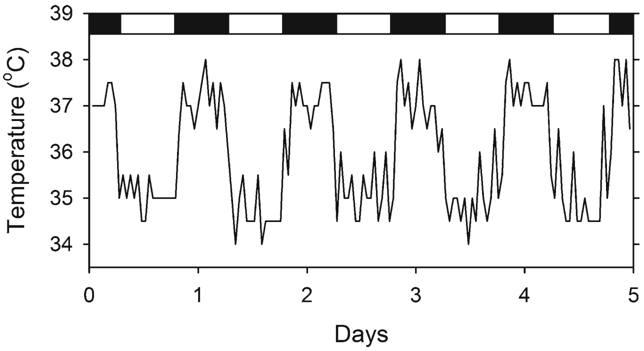


Fig. 1 A five-consecutive-day segment of Tb during the euthermic stage before the beginning of hibernation of a representative woolly dormouse (*D. laniger*; no. 77) maintained in the laboratory at Ta 18± 1 °C and light–dark cycle (200–0 lux) 12:12 (lights were on at 07:00). Data were collected and plotted at 50-min intervals. The *white and black horizontal bars* above the graph indicate the duration of the light and dark phases of the light–dark cycle, respectively

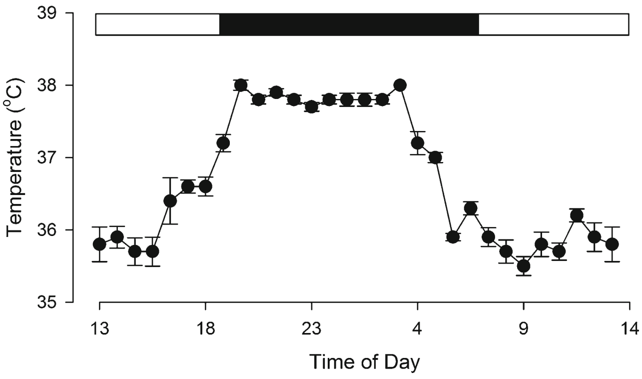
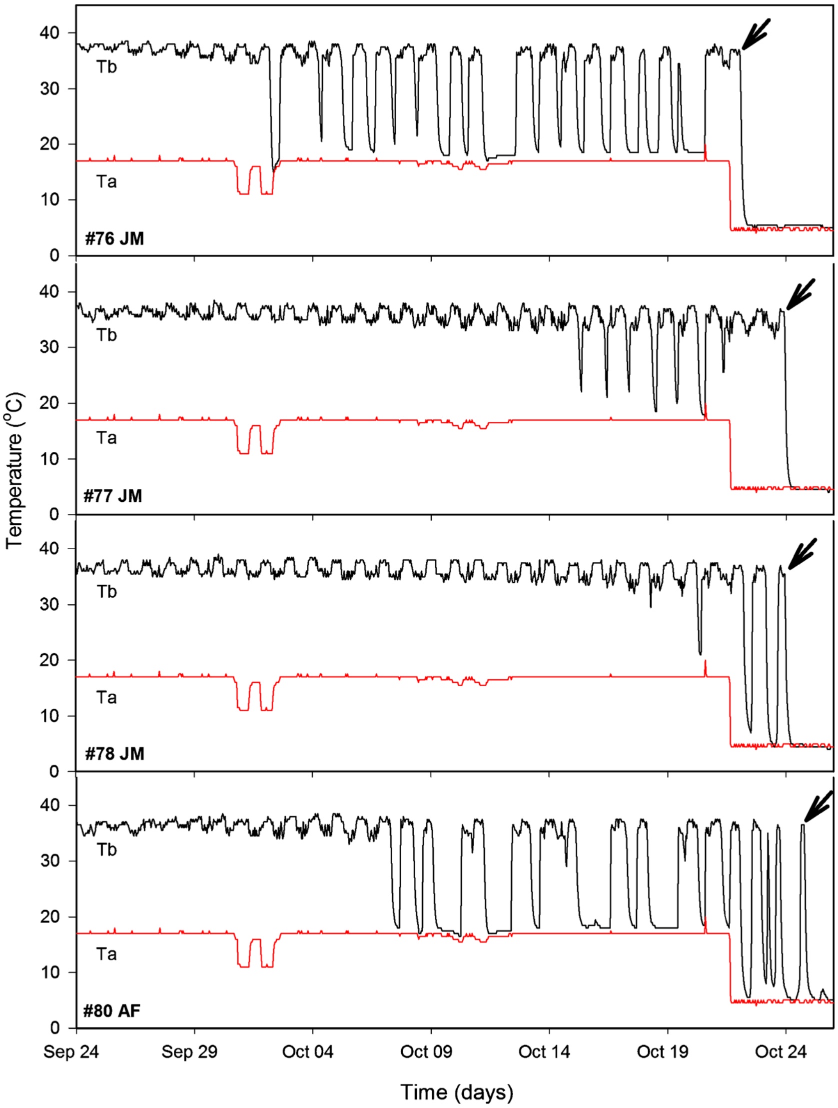


Fig. 2 A mean curve of Tb during the euthermic stage before the beginning of hibernation of woolly dormice (*D. laniger*) maintained in the laboratory at Ta 18±1 °C and light–dark cycle (200–0 lux) 12:12 (lights were on at 07:00). Each data point (mean ± SE) was obtained by averaging over five-consecutive-day segments of woolly dormice. Data were collected and plotted at 50-min intervals. The *white and black horizontal bars* above the graph indicate the duration of the light and dark phases of the light–dark cycle, respectively

All woolly dormice displayed short torpor (period of low metabolic rate and Tb lasting shorter than 1 day) at Ta 18 °C and light–dark cycle, although the tendency to enter short torpor was different among individuals (Fig. 3). The number and duration of these short torpor bouts, respectively, were 7.5±5.4 (2–15, *n*=4, *N*=30) and 8.3±3.9 h (3.3–16.7 h, *n*=4, *N*=24). Short torpor was also displayed by two woolly dormice (no. 78 and no. 80) at Ta 5 °C and darkness, i.e., in the period immediately before the beginning of hibernation (Fig. 3). The number and duration of these short torpor bouts, respectively, were 3.0±1.4 (2–4, *n*=2, *N*=6) and 12.4±5.1 h (7.5–21.7 h, *n*=2, *N*=6).

Two woolly dormice (no. 76 and no. 80) displayed prolonged torpor (period of low metabolic rate and Tb lasting longer than 1 day, but shorter than 2 days) at Ta 18 °C and light–dark cycle (Fig. 3). The number and duration of these prolonged torpor bouts, respectively, were 3.0±1.4 (2–4, *n*=2, *N*=6) and 32.7±3.0 h (29.2–35.8 h, *n*=2, *N*=5). No

Fig. 3 One-month segments of Tb before the beginning of hibernation of woolly dormice (*D. laniger*) maintained in the laboratory. The laboratory was kept at Ta 18±1 °C and light–dark cycle (200–0 lux) 12:12 (lights were on at 07:00). Starting on October 21, Ta was lowered to 5±1 °C, and lights were kept continuously off. The beginning of hibernation is indicated by the *arrows*



prolonged torpor was displayed by woolly dormice at Ta 5 °C and darkness, i.e., in the period immediately before the beginning of hibernation (Fig. 3).

Both entries into and arousals from short torpor at Ta 18 °C and light–dark cycle were nonrandom and occurred at specific times of day (entries: r=0.92, P<0.001; arousals: r=0.70, P<0.001; note that the sample size was not sufficient to examine short torpor bouts at Ta 5 °C and darkness and prolonged torpor bouts in the same way). Entries into short torpor occurred at an angle of 82.3±23.4° (10.8–117.3°, n=4, N=30) or 05:29±1.6 h (00:43–07:49, n=4, N=30), whereas arousals from short torpor occurred at an angle of 202.4±49.1° (142.2–300.8°, n=4, N=24) or 13:29±3.3 h (09:29–20:03, n=4, N=24; Fig. 4).

The lowest Tb during short and/or prolonged torpor bouts closely followed Ta (Fig. 3) and was 19.0±2.1 °C (15.0–25.5 °C, n=4, N=29) at Ta 18 °C and light–dark cycle and 6.2±1.3 °C (4.5–7.5 °C, n=2, N=6) at Ta 5 °C and darkness.

Hibernation

All woolly dormice began hibernation within 1–3 days after exposure to Ta 5 °C and darkness, i.e., on October 22–24, and ended hibernation in the first half of April. Hibernation consisted of a sequence of multiday torpor bouts (periods of low metabolic rate and Tb lasting longer than 2 days), interrupted by euthermic intervals (periods of high metabolic rate and euthermic Tb lasting shorter than 1 day). However, we could not obtain complete Tb readings for the entire hibernation season because all Thermochron iButtons stopped recording the Tb of woolly dormice on February 7 before the end of hibernation (see above). On that day, they had all been in hibernation for 107.2±1.1 days (106.1–108.8 days, n=4) and in torpor for 10.3±6.6 days (0.5–13.8 days, n=4; Fig. 5). Thus, we could not examine hibernation parameters (e.g., the number and duration of multiday torpor bouts) for the entire hibernation season.

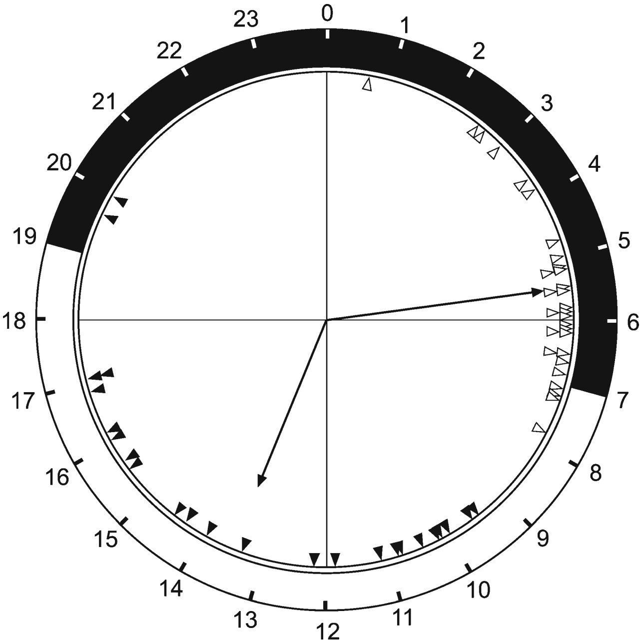


Fig. 4 The time of day of entries (*open triangles*) into and arousals (*filled triangles*) from short torpor at Ta 18±1 °C and light–dark cycle (200–0 lux) 12:12 (lights were on at 07:00). The direction of the *arrows* indicates the mean time and the length of the *arrows*, the concentration (r). The *white and black circular bars* indicate the duration of the light and dark phases of the light–dark cycle, respectively

Entries into the first multiday torpor (i.e., hibernation) were distributed in the evening hours (22:15±2.6 h; 18:15–01:23, n=4), whereas arousals from the first multiday torpor were evenly distributed in day hours (23:28±5.1 h; range = 00:25–23:23, n=4; note that the sample size was not sufficient for statistical analysis). Hibernation started with moderately long multiday torpor bouts (about 7 days), and the duration of multiday torpor bouts increased as the hibernation season progressed and appeared to start decreasing before the time when the data loggers stopped recording Tb. Thus, the longest multiday torpor bouts occurred in December and January (Fig. 5) and lasted 18.2±1.7 days (16.8–20.7 days, n=4). The number and duration of multiday torpor bouts, respectively, were 7.8±1.5 (7–10, n=4, N=31) and 12.2±4.2 days (3.6–20.7 days, n=4, N=31; note that an incomplete torpor of each individual was excluded from these statistics). Short torpor was observed in two woolly dormice (no. 77 and no. 78; number: 2.5±2.1, 1–4 and duration: 12.0±3.2 h, 8.3–16.7 h, n=2, N=5), whereas no prolonged torpor was observed in woolly dormice (Fig. 5). The number and duration of euthermic intervals, respectively, were 9.0±3.4 (7–14, n=4, N=36) and 5.4±3.2 h (1.7–19.2 h, n=4, N=36). Thus, woolly dormice were in torpor for 98.1±1.0 % (96.7–99.1 %, n=4) of the time when they were in hibernation.

Tb during euthermic intervals was 35.8±0.8 °C (33.0–37.0 °C, n=4, N=199). The lowest Tb during multiday torpor bouts closely followed Ta (Fig. 5) and was 4.5±0.5 °C (4.0–5.5 °C, n=4, N=29). During the steady-state part of the longest multiday torpor, Tb was constant and arrhythmic within the resolution of the data loggers (0.5 °C) in three woolly dormice (no. 76, no. 77, and no. 80; robustness ≤2 %). However, Tb exhibited significant 24–h rhythm in one woolly dormouse (no. 78; robustness = 15 %, P<0.001), which is most likely produced by Ta or uncontrolled changes in the laboratory (Fig. 6).

Discussion

The present study provided one of the most comprehensive data sets available on body temperature patterns and use of torpor in glirid species (Bieber and Ruf 2009; Pretzlaff and Dausmann 2012), even with small sample sizes and incomplete data sets.

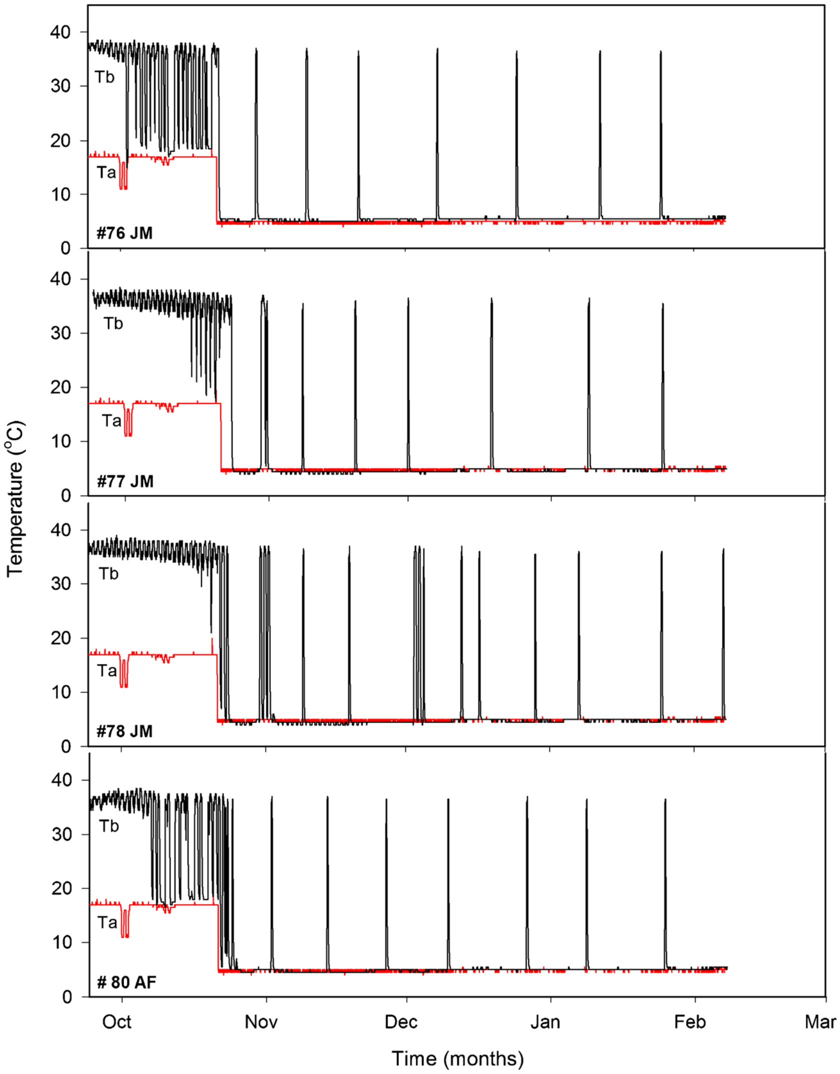


Fig. 5 Tb before and during hibernation of woolly dormice (*D. laniger*) maintained in the laboratory. The laboratory was kept at Ta 18±1 °C and light–dark cycle (200–0 lux) 12:12 (lights were on at 07:00). Starting on October 21, Ta was lowered to 5±1 °C, and lights were kept continuously off

Body mass

Pre-hibernation fattening is under hypothalamic control and facilitated mainly by two mechanisms: an increase in food intake and a decrease in motor activity (Mrosovsky 1971). There is also evidence that use of torpor before the beginning of hibernation facilitates and accelerates pre-hibernation fattening by reducing the cost of euthermy in some hibernating mammals (Krzanowski 1961; Scott et al. 1974; Speakman and Rowland 1999). Woolly dormice increased their body mass before the beginning of hibernation, as typical for fat-storing hibernating mammals (Cranford 1978; Florant et al. 2010). Although we could not evaluate the role of use of torpor before the beginning of hibernation in regulating pre-hibernation body mass gain, the timing of torpor during the day did not interfere with foraging in this nocturnal species, so that a positive energy balance can be maintained during pre-hibernation fattening.

Some glirid species prepare for hibernation by combining pronounced fattening with extensive food hoarding and may forage from food stores at euthermic intervals during hibernation (Humphries et al. 2001; Kryštufek and Vohralík 2005; Mzilikazi et al. 2012) so as to supplement body fat reserves (Geiser 2001). However, woolly dormice appeared to rely mainly on body fat reserves to fuel energy demands during hibernation. This conclusion was based mainly on the fact that, during hibernation, they did not consume a measurable amount of food, and no food or food remnants were found in their nest boxes. Moreover, as is the case for woolly dormice (Spitzenberger 1976; Kryštufek and Vohralík 2005), for a species with a strong tendency towards insectivorous diet, it is unlikely to avoid food fluctuations by food hoarding (Geiser and Baudinette 1988).

Body temperature recordings

Thermochron iButtons, small and inexpensive temperature-sensitive data loggers, have made recording the Tb of animals much easier (Kart Gür et al. 2009). However, the data loggers should be modified before implantation to reduce

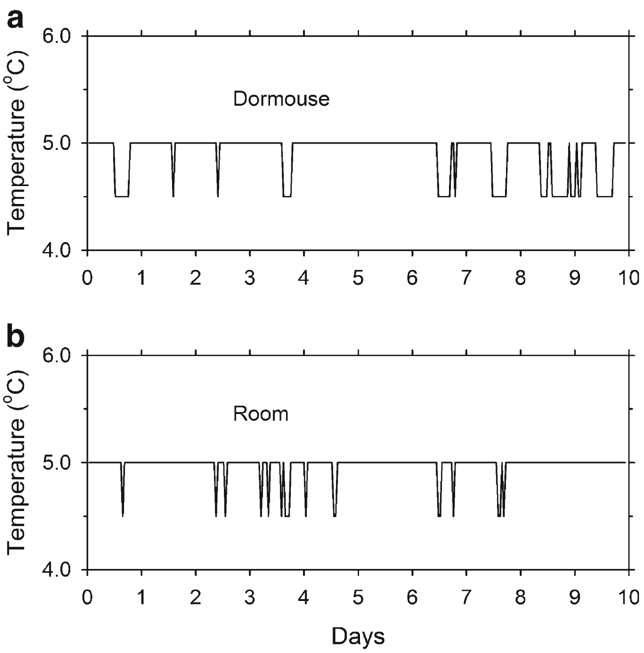


Fig. 6 **a** A 10-consecutive-day segment of Tb during the steady-state part of the longest multiday torpor of a woolly dormouse (*D. laniger*; no. 78), in which Tb exhibited a significant 24-h rhythm, maintained in the laboratory at Ta 5±1 °C and darkness. **b** Ta in the laboratory on the same days

their size and weight if they are too large and heavy to implant into an animal, as is the case for woolly dormice. Thus, following Lovegrove (2009), the data loggers were modified in the present study. Because of a memory split problem related to modification, the data loggers stopped recording the Tb of woolly dormice before the end of hibernation. This memory split may be due to the fact that the data loggers were not continuously powered while the battery was replaced during modification and/or an unknown reason concerning modification. Thus, if the battery needs to be replaced during modification, in order to ensure that they are functioning properly, the data loggers should at least be continuously powered during this process. Also, after modification, the researcher should make sure that specifications of the data loggers were not changed.

Daily Tb rhythm during the euthermic stage before the beginning of hibernation

The circadian Tb rhythm is generated by an endogenous pacemaker located in suprachiasmatic nuclei of the hypothalamus and entrained by a periodic external signal, mainly by light–dark cycle (Daan and Aschoff 2001; Ruby et al. 2002). Woolly dormice exhibited robust, unimodal daily Tb rhythmicity with an amplitude of 1.6 °C (under Ta 18 °C and light–dark cycle) during the euthermic stage before the beginning of hibernation, which confirms and expands the list of mammals that exhibit daily Tb rhythm (Refinetti and Menaker 1992; Kart Gür et al. 2009; Williams et al. 2012). The robustness of the daily Tb rhythm was 66 %, which is similar to the robustness (50–80 %) of the daily Tb rhythm in small mammals maintained under controlled laboratory conditions (Refinetti 1998). The acrophase of the daily Tb rhythm was during the dark phase of light–dark cycle (approximately 5 h after lights were off). This nocturnal acrophase is in agreement with diurnal acrophase in diurnal mammals and nocturnal acrophase in nocturnal mammals (Refinetti 1996, 1999), although some exceptions are available (e.g., nocturnal acrophase in diurnal mammals: Fluxman and Haim 1993; Haim et al. 1997; Keckler et al. 2010; Piccione et al. 2002). To our knowledge, no much information exists on the circadian and/or daily Tb rhythm of glirid species maintained under controlled laboratory conditions, which prevents a detailed comparison with our results. Also, because we maintained woolly dormice only under light–dark cycle before the beginning of hibernation, our data do not allow us to identify the endogenous free-running circadian Tb rhythm whose demonstration is traditionally considered for proving the endogenous origin of circadian oscillations (Mumm et al. 1989).

Short and prolonged torpor bouts before the beginning of hibernation

Some hibernating mammals display preliminary torpor (e.g., test drop, short torpor) before the beginning of hibernation, which is considered to indicate physiological or biochemical preparations for hibernation (Strumwasser 1958, 1960; Heller and Colliver 1974; Russell et al. 2010). However, it appears that these preliminary torpor bouts are not a prerequisite for hibernation since some species or some individuals of a species do not display preliminary torpor (Kart Gür 2008; Kart Gür et al. 2009; Sheriff et al. 2012). The use of preliminary torpor by a glirid species was first reported in the present study. All woolly dormice displayed preliminary torpor (short and/or prolonged torpor) before the beginning of hibernation while they were maintained in the laboratory at Ta 18 °C and light–dark cycle. However, there was a great deal of individual variation in the tendency to enter preliminary torpor and the time spent in this torpor. More studies on both free and captive individuals are clearly needed to further elucidate this complex behavior in different hibernating mammals.

The time of day of entries into and arousals from short torpor was entrained by the light–dark cycle in woolly dormice. Entries into and arousals from short torpor occurred toward the morning and during the early afternoon, respectively, and therefore short torpor was mostly restricted to the day, i.e., the woolly dormice's resting phase. In mammals, entries into torpor are restricted to the resting phase (Körtner and Geiser 2000). Arousals from torpor, on the other hand, occur apparently at random or is free-running under the light–dark cycle, showing that many heterotherms do not perceive or use light stimuli when torpid (Körtner et al. 1998). In the present study, it is difficult to explain how the light–dark cycle could affect the time of day of arousals from short torpor in woolly dormice. All woolly dormice constructed a nest of cotton material in the wooden nest box, curled up with their heads tucked in and eyes close to the bottom of the nest, the tail bent over their heads, and covered their backs with additional cotton material. However, it seems likely that the circadian system was not disturbed by Tbs of ~18 °C as was reached during short torpor and was still capable of keeping time.

Hibernation

All woolly dormice began hibernation within a few days after exposure to cold and darkness. Hibernation consisted of a sequence of multiday torpor bouts, interrupted by euthermic intervals. Thus, the patterns of hibernation in woolly dormice were similar to those observed in classical hibernating mammals (Pengelley and Fisher 1961; Heller and Poulson 1970). In addition to multiple multiday torpor bouts, a few brief torpor bouts (i.e., short torpor lasting shorter than 1 day) were also observed during hibernation. Although these torpor bouts are believed not to be important functionally (Geiser 2001), they should clearly increase the energetic cost of hibernation. The duration of multiday torpor bouts increased until the longest multiday torpor as the hibernation season progressed, as typical for hibernating mammals in both field and laboratory conditions (Wang 1979; French 1985, 1988; Young 1990; Michener 1992; Kart Gür et al. 2009). Because we could not obtain complete Tb readings for the entire hibernation season, it is impossible to know how the duration of multiday torpor bouts changed throughout the rest of the hibernation season.

Woolly dormice had multiday torpor bouts lasting on average 12 days, with euthermic intervals on average of 5 h. Thus, they were in torpor for an average of 98 % of the time when they were in hibernation. Although euthermic intervals account for approximately 1 % of the total time budget during winter, over 70 % of the energy used during hibernation is consumed during these euthermic intervals (Wang 1979). Thus, because small fat-storing hibernating mammals are more constrained energetically than large ones, they should spend a smaller proportion of their time at high Tb than the large ones (French 1985). In the present study, the duration of euthermic intervals in woolly dormice was short, which is in agreement with the allometric predictions described for other fat-storing hibernating mammals of similar body size (French 1985; Humphries et al. 2001).

It has long been controversial whether the circadian Tb rhythm persists during hibernation. Whereas some hibernating mammals maintain circadian rhythmicity in Tb during deep hibernation under constant laboratory conditions (Menaker 1961; Grahn et al. 1994), others lose circadian rhythmicity in Tb (Jansky et al. 1989; Fowler and Racey 1990; Wollnik and Schmidt 1995; Revel et al. 2007). One explanation for the variability in maintenance of the circadian rhythmicity in Tb during hibernation is that the sensitivity of Tb to small fluctuations in Ta can lead to false-positive conclusions regarding rhythm persistence (Wollnik and Schmidt 1995; Florant et al. 2010; Oklejewicz et al. 2001; Ruby 2003). We did not observe the circadian Tb rhythm during the steady-state part of the longest multiday torpor in woolly dormice, either because the circadian Tb rhythm was absent or because the range of excursion was smaller than the resolution of Thermochron iButtons (0.5 °C), except for one individual in which Tb exhibited significant 24-h rhythm, most likely produced by Ta or uncontrolled changes in the laboratory.

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