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Thomas Waßmer · Franziska Wollnik

Timing of torpor bouts during hibernation in European hamsters (*Cricetus cricetus* L.)

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Abstract Body temperature (T_b) of seven European hamsters maintained at constant ambient temperature (T_a = 8 °C) and constant photoperiod (LD 8:16) was recorded throughout the hibernating season using intraperitoneal temperature-sensitive HF transmitters. The animals spent about 30% of the hibernation season in hypothermia and 70% in inter-bout normothermy. Three types of hypothermia, namely deep hibernation bouts (DHBs), short hibernation bouts (SHBs), and short and shallow hibernation bouts (SSHBs), were distinguished by differences in bout duration and minimal body temperature (T_m). A gradual development of SSHBs from the diel minimum of T_b during normothermy could be seen in individual hamsters, suggesting a stepwise decrease of the homeostatic setpoint of T_b regulation during the early hibernation season. Entry into hibernation followed a 24-h rhythm occurring at preferred times of the day in all three types of hypothermia. DHBs and SHBs were initiated approximately 4 h before SSHBs, indicating a general difference in the physiological initiation of SSHBs on the one hand and DHBs and SHBs on the other. Arousals from SHBs and SSHBs also followed a 24-h rhythm, whereas spontaneous arousals from DHBs were widely scattered across day and night. Statistical analyses of bout length and the interval between arousals revealed evidence for a free-running circadian rhythm underlying the timing of arousals. The results clearly demonstrate that entries into hypothermia are linked to the light/dark-cycle. However, the role of the circadian system in the timing of arousals from DHBs remains unclear.

Key words Telemetry · Laboratory conditions · Body temperature · Entry · Arousal

Abbreviations *CET* central European time · *CNS* central nervous system · *CSD* circular standard deviation · *DD* constant darkness · *DHB* deep hibernation bout · *HB* hibernation bout · *LD* light-dark cycle · *r* mean vector length · *SHB* short hibernation bout · *SSHB* short and shallow hibernation bout · T_a ambient (cold room) temperature · T_b body temperature · T_m minimal body temperature

Introduction

In homeothermic animals of temperate and Arctic latitudes, hibernation is one of the most effective ways to conserve energy during the cold season. Declines in body temperature (T_b) to near ambient temperatures (T_a) can reduce total energy expenditure during the hibernation season to 77% of basal in golden hamsters (McKee and Andrews 1992), to 16% of basal in marmots (Heldmaier et al. 1993), and to 12% of basal in ground squirrels (Wang 1979). During winter, European hamsters (*Cricetus cricetus*), like all other hibernators, run through a rhythmical succession of torpor periods and episodes of normothermy. Without these periodic arousals mammalian hibernators could easily reduce energy expenditure to 3% of euthermic level. In Richardson's ground squirrels, for example, more than 80% of the energy expended over the winter is used for the purpose of returning to normothermic conditions between torpor bouts (Wang 1979). It is still not clear why hibernators do not stay in deep torpor throughout the winter to maximize energy savings, but instead periodically return to normothermy.

Early theories claimed that periodic arousals were necessary to remove waste products accumulated during the preceding bout, or to restore homeostasis in one or more aspects of the cellular milieu (Willis 1982). Several studies have therefore investigated the effect of factors

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T. Waßmer · F. Wollnik
Department of Biology, University of Konstanz, P.O. Box 5560,
D-78434 Konstanz, Germany

F. Wollnik (✉)
Abteilung für Tierphysiologie, Biologisches Institut,
Universität Stuttgart, Postfach 80 11 40,
D-70511 Stuttgart, Germany,
Tel.: +49-711/685-5001, Fax: +49-711/685-5090

that influence metabolism, such as body size (French 1985; Heldmaier 1990), diet (Geiser and Kenagy 1993; Geiser et al. 1994), and T_a (Twente and Twente 1965a; French 1982; Geiser and Kenagy 1988; Geiser and Broome 1993) on torpor bout duration. Today, the "metabolic replenishment theory" has been largely abandoned because it has been difficult to conclusively demonstrate either waste accumulations or disruptions of cellular homeostasis during hibernation. More recent hypotheses state that periodic arousals were due to fluctuating levels of CNS neurochemicals (Wang 1988, 1993) or accumulating sleep deprivation (Daan et al. 1991; Trachsel et al. 1991), but so far support for these hypotheses has been rather scarce.

Based on the orderly pattern of T_b changes during hibernation it has been suggested that the timing of entry into and of arousal from hibernation is controlled in part by the circadian system (Strumwasser et al. 1967; Pohl 1967, 1987; Geiser et al. 1990; Canguilhem et al. 1994; Grahn et al. 1994). A 24-h rhythm in the timing of entry into hibernation under a LD cycle has been clearly demonstrated in various species such as ground squirrels (Strumwasser 1959), big brown bats (Twente and Twente 1987), garden dormice (Daan 1973), pocket mice (French 1977), hedgehogs (Fowler and Racey 1990), and European hamsters (Canguilhem et al. 1994; Wollnik and Schmidt 1995). However, studies on the timing of arousal have given inconclusive results. Twenty-four-hour rhythms in the timing of hibernation bouts under LD conditions were demonstrated in California ground squirrels (Strumwasser 1959), garden dormice (Daan 1973), brown bats (Twente and Twente 1987), and hedgehogs (Fowler and Racey 1990), but most studies found no correlation between arousal and the time of day (Kristoffersson and Soivio 1964; Strumwasser et al. 1967; Twente and Twente 1965b; Pohl 1987; Thomas 1993; Canguilhem et al. 1994; Wollnik and Schmidt 1995). It has, therefore, been suggested that arousals are controlled by a free-running circadian clock, regulating either the duration of hibernation bouts (HB) or the timing of arousal. In the first case, the duration of each bout is a multiple of the circadian period plus a constant remainder. Such a relationship has, so far, only been demonstrated for bats (Menaker 1959) and ground squirrels (Grahn et al. 1994). In the second case, the interval between consecutive arousals is a multiple of the circadian period, and it should thus be possible to detect a free-running rhythm in the timing of consecutive arousals (Pohl 1967). Such a circadian periodicity could be demonstrated in Turkish hamsters despite the fact that the animals were kept under an LD cycle (Pohl 1987, 1996).

For the European hamster, two studies have investigated the timing of hibernation, one under constant laboratory conditions (Canguilhem et al. 1994) and one under semi-natural conditions (Wollnik and Schmidt 1995). Neither of the studies found any evidence for a 24-h rhythm in the timing of arousals from deep hibernation. However, while both studies agreed in proposing that arousals followed a free-running circadian rhythm, they differed completely with respect to the statistical analysis and interpretation of the data. Canguilhem et al. (1994) used a specially developed contingency test to provide evidence for the hypothesis that the duration of individual hibernation bouts is regulated by the circadian system. In contrast, our previous study (Wollnik and Schmidt 1995) demonstrated a free-running period in the timing of arousals. One aim of the present study was to determine the hibernation pattern of the European hamster under constant laboratory conditions so that the results could be compared with those of Canguilhem et al. (1994). Since their study regarded hibernation as an on-off process, it looked only at entries and arousals, but lacked any information on T_b changes during hibernation. Therefore, a second aim of this study was to give a detailed analysis of T_b fluctuations under constant laboratory conditions based on continuously recorded T_b values.

Materials and methods

Animals and housing

The European hamsters used in this study (four adult males in winter 1992/93 and four adult males in winter 1993/94) had been obtained in late spring from a free-ranging population near Strasbourg, France. During the summer, the animals were kept under standard laboratory conditions (long photoperiod LD 16:8; T_a 20 ± 1 °C). In October, the hamsters were transferred into a cold room and subjected to a short photoperiod (LD 8:16, lights on at 0600 hours CET in winter 1992/93 and 0800 hours CET in winter 1993/94) until the end of the experiment (April 93 and April 94, respectively). Temperature-sensitive transmitters were implanted in mid-October, about 2–4 weeks before T_a was gradually lowered to 8 ± 1 °C. In the cold room the animals were housed individually in plastic containers of approximately 1 × 1 × 1 m size. The containers were open at the top but covered with wire mesh. They were filled with 0.2 m of soil to be used as burrowing material and contained various nest boxes made of plastic. Dry hay was offered as nesting material and was replenished once a month. Food pellets (Altromin) and water were given *ad libitum* on a feeding rack and were replenished twice a week at variable times between 0800 and 1600 hours CET. Once a week additional nutritives such as apples, bananas and nuts were given. Once a month the hamsters were weighed and their reproductive status was established by testes palpation under light ether anaesthesia (Ethrane, Abbott GmbH, Wiesbaden, Germany). During the hibernation season this procedure was suspended. Litter change during the hibernation period was not necessary because of the relatively large size of the containers.

Surgery and temperature telemetry

T_b was measured with temperature-sensitive radio transmitters described in detail by Wollnik and Schmidt (1995). The transmitters (cylindrical shape, diameter 1.5 cm, height 2.5 cm, weight approximately 8 g) were implanted into the peritoneal cavity after the hamster had been anaesthetized with intraperitoneal injections of 50 mg Ketamin (Kemint, Alvetra GmbH, Neumünster, Germany) and 10 mg Rompun (Bayer, Leverkusen, Germany) per kg body weight. For a post-operation period of 10–14 days the animals were housed in polyethylene cages (35 × 55 × 20 cm) at 20 °C and were then returned to their housing containers. No increased morbidity, mortality, or other problems resulted from the long-term use of intraperitoneal transmitters.

Statistical analyses

T_a and T_b of four hamsters were continuously monitored with a temporal resolution of 5 min over a period of 4–6 months in winters 1992/93 and 1993/94. All subsequent analyses were based on these 5-min values. Residual data that were outlying due to technical or detection failures were rejected by assigning an upper limit to the value of acceptable rates of temperature change. During normothermic days the upper limit was set to 1 °C per 5 min (12 °C h^{-1}), while during hibernating days the limit was set between 3–5 °C per 5 min (36–60 °C h^{-1}) in order to include the extreme temperature changes observed during arousals from HBs. Since one transmitter failed in winter 1992/93, only seven hamsters could be included in the statistical analyses. The following parameters were calculated from the temperature recordings during hibernation (Fig. 1): (1) time of day, t_1, at the beginning of each HB (entry), given by the first measurement of T_b below 30 °C; (2) time of day, t_m, of each HB when the minimum body temperature (T_m) was reached; (3) time of day, t_4, at the end of each HB (arousal), given by the first measurement above 30 °C; (4) duration of each HB, defined as the time between t_1 and t_4; (5) duration of inter-bout normothermia between consecutive HBs, given as the interval between t_4 and the next t_1; and (6) rate of temperature change during entry into (cooling rate) and arousal from (warming rate) a HB, calculated as (20–30) °C/(t_2–t_1) and (30–20) °C/(t_4–t_3), respectively. If T_m did not drop below 20 °C temperature changes were calculated using t_m instead of t_2 and t_3.

A computer program for repeated measures analyses of variance (Statistica, StatSoft) was used to compare data across animals and years. When appropriate, Sheffé's multiple *t*-test and Tukey's honest-significant-difference (HSD) test were used for post-hoc comparisons. The presence of circadian rhythmicity during normothermic and hypothermic periods was verified using the chi-square periodogram (Sokolove and Bushell 1978). Circular statistics (Batschelet 1981) were used to test the significance of timing of entry into and arousal from hibernation. The times of day of these events were converted into degrees, and mean vector directions and lengths (*r*) as well as CSD (circular standard deviation) were calculated to describe how well the data coincided. The significance of the computed mean vectors was tested using Raleigh's test (*z*-test), while differences between various circular distributions were tested using the Mardia-Watson-Wheeler test.

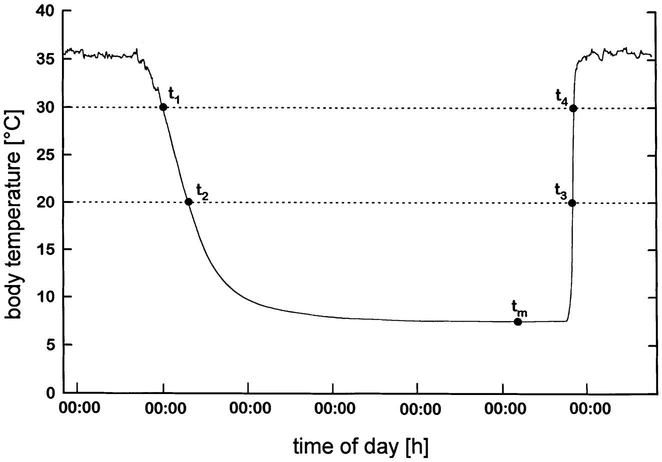

Fig. 1 Time-course of a typical deep hibernation bout (DHB). Entry into torpor was defined as the time of day, t_1, when T_b first dropped below 30 °C. Arousal from torpor was defined as the time of day, t_4, when T_b first raised above 30 °C. Time t_m was given by the time of day when T_b reached its absolute minimum, T_m. Time t_2, when T_b dropped below 20 °C, was used to compute cooling rates ((20–30) °C/(t_2–t_1)), whereas time t_3, when T_b rose above 20 °C, was used to calculate warming rates ((30–20) °C/(t_4–t_3))

Results

Types of hibernating behaviour and time-pattern of hibernation

Three types of hibernal torpor were distinguished (Fig. 2): (1) DHBs with T_m below 20 °C and bout duration longer than 24 h; (2) SHBs with T_m below 20 °C and bout duration shorter than or equal to 24 h; and (3) SSHBs with T_m above 20 °C and bout duration shorter than or equal to 24 h. This definition resulted in the classification of 62 DHBs, 19 SHBs and 115 SSHBs. All seven hamsters showed some type of heterothermy during the recording period. Figure 3 shows representative recordings demonstrating the variety of the hibernation patterns. One hamster (#6) exhibited only SSHBs interrupted by rather long intervals of normothermy. Two animals showed SSHBs and a few additional DHBs (e.g. animal #7), and four hamsters had a regular pattern of DHBs interrupted by episodes of SSHBs as well as normothermy (e.g. animals #5 and #8).

During the second winter all four hamsters showed so-called "test-drops" (Strumwasser 1959) at the beginning of the hibernating season. These are consecutive bouts of hypothermia with a progressive decrease of T_b (SHBs and SSHBs), which gradually develop into DHBs (Figs. 3, 4A,B). During SSHBs, T_m occurred at approximately the same time of day as daily T_m during normothermy (Fig. 4C). Circular statistics revealed furthermore that the timing of T_m during SSHBs (0850

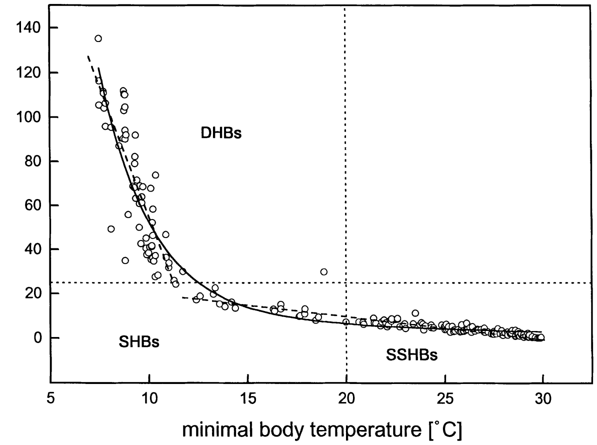

Fig. 2 Definition of three types of torpor bouts in the European hamster: (1) Deep hibernation bouts (DHBs), $T_m < 20$ °C, bout duration > 24 h; (2) short hibernation bouts (SHBs), $T_m < 20$ °C, bout duration < 24 h; and (3) short and shallow hibernation bouts (SSHBs), $T_m \geq 20$ °C, bout duration < 24 h. Note the close relationship between both parameters as indicated by the exponential decay fit (*solid line*, bout duration $= 129.4^{-T_m/2.58} + 13.65^{-T_m/15.11}$, $r = 0.96$, $P < 0.001$). In addition, linear correlations (*broken lines*) were calculated for 1-day torpor bouts (bout duration $= 0.98 \times T_m + 29.66$, $r = -0.95$, $P < 0.001$) and multi-day torpor bouts (bout duration $= -24.05 \times T_m + 293.77$, $r = -0.84$, $P < 0.001$)

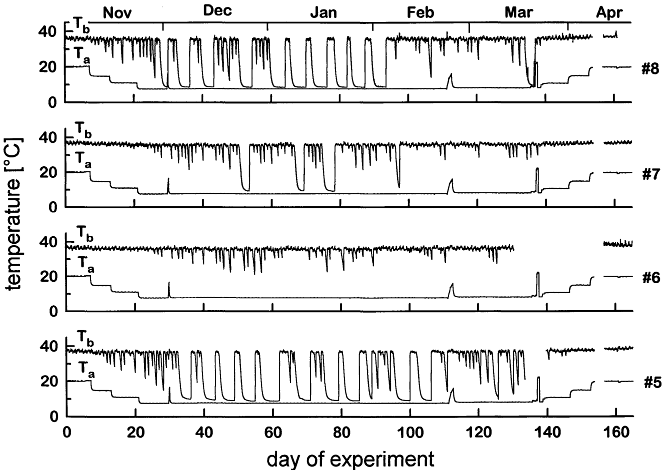

Fig. 3 Individual patterns of heterothermy in four representative hamsters during the winter 1993/94. The *upper line* shows the body temperature (T_b) of the animal, the *lower line* shows the ambient temperature (T_a) of the cold room. Interruptions of temperature measurements were due to technical problems of the recording devices. The temperature regulation of the cold room failed three times around experimental days 30, 112, and 137

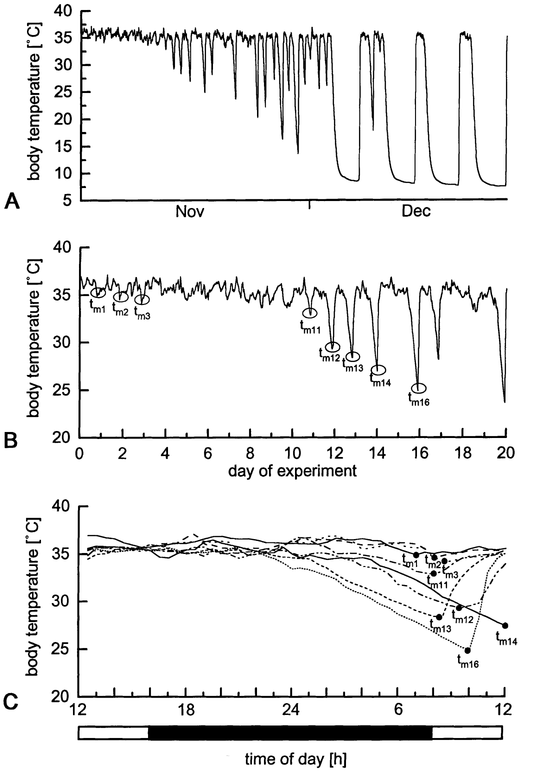

Fig. 4A–C Examples of "test-drops" at the beginning of the hibernation season; **A** body temperature (T_b) of hamster #5, showing normothermic T_b during the first half of November, short and shallow hibernation bouts (SSHBs) and short hibernation bouts (SHBs) in the second half of November, and deep hibernation bouts (DHBs) from December on; **B** T_b of hamster #5 during days 1–20, demonstrating the stepwise decrease of minimal body temperature (T_m) during periods of normothermy (t_{m1} through t_{m3}) and during successive SSHBs (t_{m11} through t_{m16}); **C** overlayed T_b plots of selected days. Note how times t_m (*black circles*) of minimal body temperature were clustered between 0700 and 1200 hours CET

hours CET ± 2.56 h, mean vector length (r) = 0.78, $P < 0.005$) was kept constant throughout the hibernation season and was not significantly different from the timing of normothermic T_m (0833 hours CET ± 1.73 h, $r = 0.90$, $P < 0.001$), indicating a homology of SSHBs and daily minima of normothermic T_b.

Changes of T_b during hibernation

Table 1 lists characteristic parameters of all three types of hibernal torpor. Mean cooling rate during entry into hypothermia was −1.3 ± 0.4 °C h^{-1}. It did not differ between bout types or months, while warming rates differed significantly between bout types ($F_{2,181} = 98.6$, $P < 0.001$) and months ($F_{4,181} = 3.5$, $P < 0.01$). Furthermore, warming rates were correlated with T_m ($r = -0.73$, $P < 0.001$) and bout duration ($r = 0.57$, $P < 0.001$), the latter due to the strong correlation between T_m and bout duration ($r = -0.84$, $P < 0.001$). In DHBs, T_m ($F_{4,58} = 5.12$, $P < 0.005$) differed significantly between months, while no significant difference could be found regarding bout duration. In SSHBs, significant differences between months were found for T_m ($F_{4,110} = 4.37$, $P < 0.005$) and bout duration ($F_{4,110} = 3.62$, $P < 0.01$). Mean duration of the hibernation season was 99.4 ± 20.6 days. About 30% of it was spent in hypothermia (29.1 ± 21.4 days), the rest in interbout normothermy (72.9 ± 19.2 days). There was a strong correlation between T_m and bout duration (Fig. 2A) demonstrated not only by the two linear correlations calculated for 1-day torpor bouts (SHBs and SSHBs, $r = -0.95$, $P < 0.001$) and multi-day torpor bouts (DHBs, $r = -0.84$, $P < 0.001$), but also by a continuous non-linear correlation analysis of all data points using an exponential decay function ($r = 0.96$, $P < 0.001$).

In contrast to SSHBs and SHBs, which were immediately terminated after reaching T_m, DHBs showed prolonged intervals of nearly constant T_b (Fig. 1). The longest such DHB lasted 5.6 days. During those HBs, T_b showed small oscillations of less than 0.2 °C (Fig. 5). Unfortunately, the bout durations were too short to allow for a statistical verification of rhythmic components. However, there was an obvious 24-h rhythm with an amplitude of 0.1 °C in T_a of the cold room that was probably caused by the LD cycle and could be verified by chi-square periodogram analysis. Autocorrelation analysis revealed that the small oscillations of T_b during DHBs simply trailed the fluctuations of the cold-room temperature by 2–3 h. In contrast, all seven animals showed a pronounced 24-h rhythm in T_b during prolonged normothermic episodes between HBs. As shown in Fig. 5, these 24-h fluctuations of normothermic T_b had a higher amplitude and a different phase than the low amplitude oscillations of T_a and were, therefore, independent of the fluctuations of the cold-room.

Table 1 Changes of T_b during entry into (cooling rate) and arousal from (warming rate) hibernation bouts in seven European hamsters, and associated physiological data (means ± standard deviation). Duration of heterothermy is the total time from entry into the first torpor bout until arousal from the last torpor bout of the hibernation season

	Deep hibernation bouts (n = 62)	Short hibernation bouts (n = 19)	Short and shallow hibernation bouts (n = 115)
Cooling rate [°C h^{-1}]	-1.3 ± 0.2^{a}	-1.3 ± 0.2^{a}	-1.2 ± 0.4^{a}
Warming rate [°C h^{-1}]	$18.1 \pm 7.3^{a,\ c}$	19.2 ± 4.6^{a}	8.1 ± 4.4^{b}
T_m during hibernation bouts [°C]	9.6 ± 1.6^{c}	15.9 ± 2.3	26.1 ± 2.6^{c}
Bout duration [h]	66.2 ± 29.61	13.8 ± 4.1	3.9 ± 2.4^{c}
Duration of inter-bout normothermy [h]		68.00 ± 82.55 (range: 12.5–500.5)	
Total time spent in inter-bout normothermy [days]		72.9 ± 19.2	
Duration of heterothermy [days]		99.4 ± 20.6	
Total time spent in torpor [days]		29.1 ± 21.4 (range: 4.1–59.8)	

Significant differences between bout types are indicated by different superscript letters^{a,b} ($P < 0.05$, two-way ANOVA). Significant differences between bout types are not indicated for the variables T_m during hibernation bouts and bout duration, since these variables were used to define the three bout type categories

^{c}indicates significant differences between months ($P < 0.05$, two-way ANOVA and $P < 0.05$, Sheffé-test). The variables T_m during hibernation bouts and bout duration were analysed by a one-way-ANOVA ($P < 0.05$) only with respect to differences between months

Daily rhythms in the timing of HBs

Circular statistics revealed significant mean vectors for the timing of entries into DHBs in all six hamsters showing this type of torpor, whereas no significant mean vector could be found for the timing of arousals (Table 2, Fig. 6). Entries into DHBs occurred predominantly during night hours between 2243 and 0443 hours CET. There were no significant differences in the timing of entries between animals within a year, but a significant difference between years. The mean time of entry into DHBs was 2349 hours CET during the winter 1992/93, and 0200 hours CET during the winter 1993/94 (Fig. 6A). This difference was most likely caused by the different timing of the LD cycle in the winter 1992/93 (lights on at 0600 hours CET) and the winter 1993/94 (lights on at 0800 hours CET). In contrast to the clear 24-h rhythm of entries, arousals from DHBs occurred at all times of day (Fig. 6B).

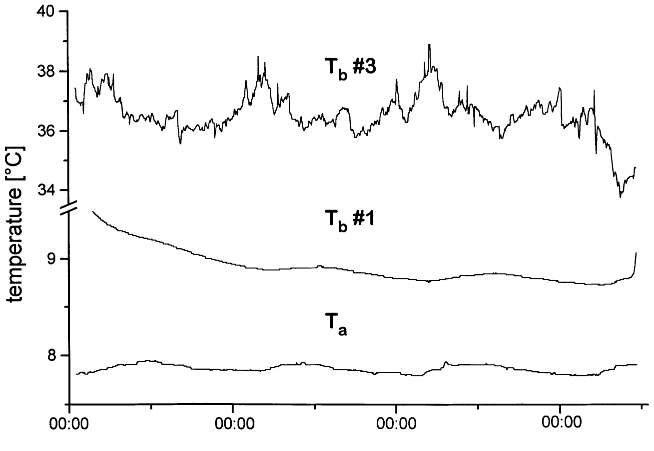

Fig. 5 Fluctutations of body temperature (T_b) during euthermy (hamster #3, *top curve*) and in deep hibernation (hamster #1, *middle curve*) shown together with ambient temperature (T_a) of the cold room (*bottom curve*). In hamster #1, the low amplitude fluctuation (< 0.2 °C) of T_b during DHB trailed the 24-h rhythm of T_a by 2 h 30 min ($r = 0.672$, $P < 0.01$). At the same time, T_b of the normothermic hamster #3 revealed a robust 24-h rhythm with a larger amplitude (> 2 °C) and a different phase

For SHBs and SSHBs, circular statistics produced significant mean vectors for both entries into and arousals from torpor (Table 2, Fig. 6C, D). All five hamsters (#2, #5–8) whose number of SSHBs was sufficient for data analysis entered into torpor around 0608 hours CET and arose from torpor around 0840 hours CET. The two hamsters with SHBs (#5 and #8) entered into torpor around 0118 hours CET and arose from torpor around 1324 hours CET. Timing of entries and arousals of SHBs were significantly different from those of SSHBs ($P < 0.001$), because entries into SHBs were initiated at approximately the same time as entries into DHBs, i.e. 4 h earlier than entries into SSHBs.

Timing of arousals from DHBs

The results presented above do not exclude the possibility that DHBs are controlled by a free-running circadian system. As mentioned before, the circadian system could control the timing of arousal in two ways: the circadian clock could control bout duration, in which case the duration of each hibernation bout would be a multiple of the underlying circadian period plus a constant remainder. Alternatively, the timing of arousal could be controlled by a free-running rhythm, in which case the interval between successive arousals would be a multiple of the underlying circadian period. In order to determine if either one of the two hypotheses was confirmed by the present data, we analysed the frequency distributions of DHB duration and of the intervals between successive arousals. The frequency distribution of DHB duration (Fig. 7A) had some conspicuous peaks. DHBs were terminated more likely 36, 65, 93, or 113 h after torpor onset. However, the intervals between

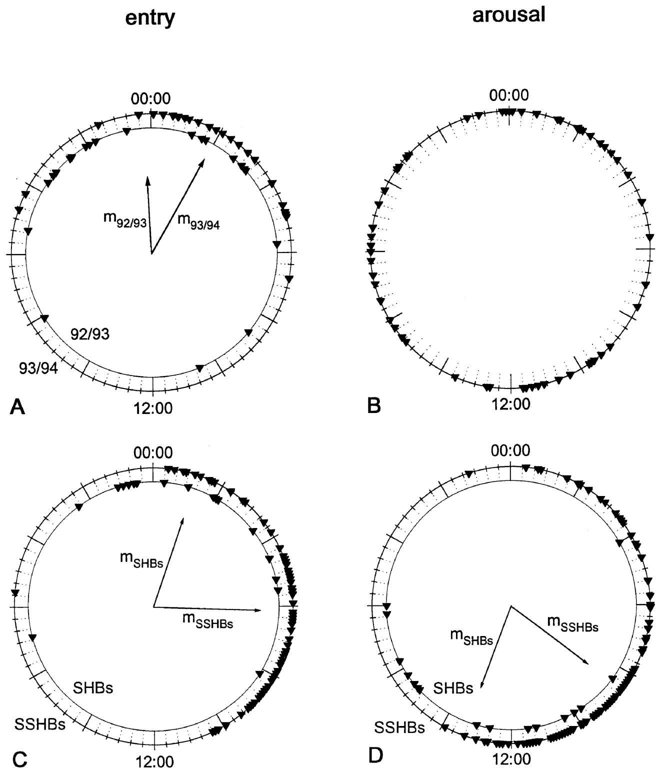

Fig. 6A–D Polar plots of entries into and arousals from deep hibernation bouts (DHBs), short hibernation bouts (SHBs), and short and shallow hibernation bouts (SSHBs). The full circle of 360° represents 24 h in Central European time (CET): **A** daily timing of entries into DHBs during hibernation periods 1992/93 and 1993/94. The mean vector for 1992/93 ($m_{92/93}$; $r = 0.55$, $P < 0.001$) points to 2349 hours CET ± 3.62 h CSD. The mean vector for 1993/94 ($m_{93/94}$, $r = 0.79$, $P < 0.001$) points to 0201 hours CET ± 2.46 h CSD; **B** daily timing of arousals from DHBs during hibernation periods 1992/93 and 1993/94; **C** daily timing of entries into SHBs and SSHBs during hibernation periods 1992/93 and 1993/94. Entries into SHBs occurred predominantly around 0118 hours CET ± 3.08 h CSD ($r = 0.68$, $P < 0.001$). Entries into SSHBs were clustered around a mean vector ($r = 0.76$, $P < 0.001$) pointing to 0608 hours CET ± 2.36 h CSD. The circular distributions of entry times of SHBs and SSHBs were significantly different from each other ($P < 0.001$) with SHBs initiating about 5 h earlier than SSHBs; **D** daily timing of arousals from SHBs and SSHBs. Mean arousal times were 1324 hours CET ± 3.25 CSD ($r = 0.64$, $P < 0.001$) for SHBs and 0840 hours CET ± 2.57 CSD ($r = 0.71$, $P < 0.001$) for SHBs, i.e. SHBs were terminated approximately 4–5 h later than SSHBs. The circular distributions of arousals from SHBs and SSHBs were significantly different from each other ($P < 0.001$)

consecutive peaks of the frequency distribution were not identical as would be expected if bout duration was an integer multiple of a constant circadian period, but ranged from 29 to 19 h. Therefore, the present data provided no evidence for a circadian rhythm in the duration of DHBs.

The frequency distribution of the intervals between successive arousals (Fig. 7B), on the other hand, showed a clear clustering of the data. Intervals of 19 or 24 h were never observed, whereas maximum numbers appeared at 84, 105.6, 127, 146.4, and 168 h. Autocorrelation and periodogram analyses of the frequency distribution re-

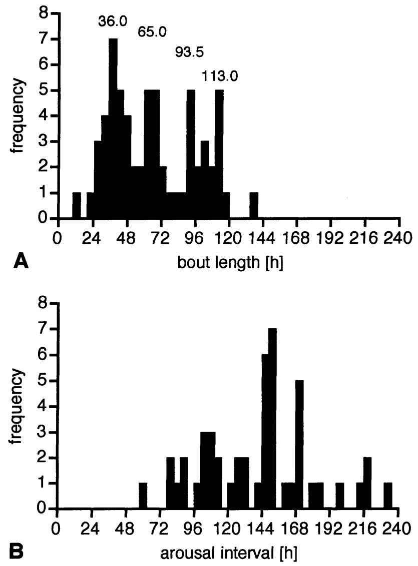

Fig. 7A, B Frequency histograms of (**A**) torpor bout duration and (**B**) the interval between successive arousals. Bin width is 5 h

vealed an overall period length between 21 and 22 h. The limited amount of data, however, precludes a more precise estimate at the present time. Nevertheless, the frequency distribution is clearly far from unimodal, indicating that the timing of successive arousals is controlled by some cyclic process.

Discussion

Characteristics of hibernating behaviour

The present study demonstrated a great variety of individual differences in the pattern of heterothermy. Some animals showed a rather regular pattern of torpor bouts with a steady increase of bout length during midwinter, while others showed only a few and rather short torpor bouts. Similar hibernation patterns have been described for various species of ground squirrels (Strumwasser 1959; Wang 1979; Michener 1992; Barnes and Ritter 1993; Grahn et al. 1994), hedgehogs (Fowler and Racey 1990), dormice (Pohl 1967), pocket mice (French 1977), and European hamsters (Canguilhem et al. 1994; Wollnik and Schmidt 1995) kept under natural or semi-natural as well as laboratory conditions. In most studies of hibernation under natural and semi-natural conditions, 1-day torpor bouts occurred exclusively prior to the first DHB and/or after the last DHB and were thus called "test-drops" (Strumwasser 1959). However, there are also reports of 1-day torpor bouts observed in the middle of the hibernating season. In European hedgehogs, for example, brief periods of shallow torpor were observed between July and April (Fowler and Racey 1990). Although 80% of these short torpor bouts occurred in August and September immediately prior to and at the beginning of the hibernal period, the authors

Table 2 Timing of entries into and arousals from all three types of hibernation bouts for individual hamsters. Data are presented as mean time of day ± CSD (CET), together with length r of the mean vector and the level of significance according to Rayleigh's test describing the degree of coincidence. Individual mean vectors for each type of hibernation bout were calculated if $n \geq 3$

	Deep hibernation bouts		Short hibernation bouts		Short and shallow hibernation bouts	
	Entry	Arousal	Entry	Arousal	Entry	Arousal
Hibernation period 1992/93						
#1	$n = 16$ 00:37 ± 4 h 03 min $r = 0.43$; $P < 0.05$	$n = 17$ 22:49 ± 4 h 55 min $r = 0.17$; n.s.				
#2	$n = 3$ 22:43 ± 1 h 24 min $r = 0.93$; $P < 0.001$	$n = 3$ 04:33 ± 3 h 00 min $r = 0.69$; n.s.			$n = 6$ 06:15 ± 2 h 07 min $r = 0.85$; $P < 0.01$	$n = 6$ 10:30 ± 1 h 54 min $r = 0.88$; $P < 0.005$
#3	$n = 13$ 23:30 ± 3 h 28 min $r = 0.59$; $P < 0.01$	$n = 14$ 07:12 ± 4 h 32 min $r = 0.29$; n.s.				
Hibernation period 1993/94						
#5	$n = 13$ 01:37 ± 2 h 51 min $r = 0.72$; $P < 0.001$	$n = 13$ 16:12 ± 4 h 52 min $r = 0.19$; n.s.	$n = 12$ 01:23 ± 2 h 48 min $r = 0.73$; $P < 0.01$	$n = 12$ 13:19 ± 3 h 29 min $r = 0.58$; $P < 0.01$	$n = 22$ 05:57 ± 2 h 41 min $r = 0.75$; $P < 0.001$	$n = 22$ 09:19 ± 2 h 35 min $r = 0.77$; $P < 0.001$
#6					$n = 21$ 05:29 ± 2 h 51 min $r = 0.72$; $P < 0.001$	$n = 21$ 10:01 ± 1 h 25 min $r = 0.93$; $P < 0.001$
#7	$n = 4$ 04:43 ± 1 h 27 min $r = 0.93$; $P < 0.001$	$n = 4$ 01:31 ± 2 h 04 min $r = 0.85$; n.s.			$n = 35$ 06:34 ± 2 h 17 min $r = 0.82$; $P < 0.001$	$n = 39$ 09:26 ± 2 h 44 min $r = 0.74$; $P < 0.001$
#8	$n = 11$ 01:27 ± 1 h 30 min $r = 0.92$; $P < 0.001$	$n = 11$ 16:45 ± 4 h 15 min $r = 0.38$; n.s.	$n = 5$ 01:02 ± 3 h 40 min $r = 0.54$; n.s.	$n = 5$ 13:33 ± 2 h 38 min $r = 0.76$; $P < 0.05$	$n = 27$ 05:10 ± 2 h 14 min $r = 0.83$; $P < 0.001$	$n = 27$ 09:23 ± 1 h 59 min $r = 0.87$; $P < 0.001$

suggested they be termed "transient shallow torpor" rather than "test-drops", because only 20% of the DHBs were preceded by these short torpor episodes.

In the present study, one of seven animals showed only SSHBs throughout the entire winter (Fig. 3, hamster #6). Similar observations had been made in two recent studies on ground squirrels. Barnes and Ritter (1993) reported that one of 18 arctic ground squirrels living in an outdoor enclosure in Alaska at −7 °C showed only 1-day torpor bouts. In a laboratory study on golden-mantled ground squirrels, one of 21 animals showed only two 1-day torpor bouts and two animals remained normothermic throughout the hibernation season, i.e. from October until April (Grahn et al. 1994). These results suggest that 1-day torpor bouts are not limited to the beginning or the end of the hibernation season, but can be observed all winter long either as the only type of torpor, or interspersed among regular DHBs. On the other hand, the regular occurrence of SSHBs and SHBs observed in the present study under laboratory conditions is clearly at variance with the results of our previous study performed under semi-natural conditions (Wollnik and Schmidt 1995). We can, therefore, not rule out that SSHBs and SHBs in the middle of the hibernating season are an experimental artefact caused by some disturbance of hibernation under laboratory conditions.

The *a priori* definition of three different types of torpor, i.e. SSHBs, SHB, and DHBs, was only partly justified by our results. Plotting of bout duration versus T_m (Fig. 2) revealed a more or less continuous distribution of HBs with a clear distinction between 1-day and multi-day torpor bouts. For this reason, we consider at least DHBs and SSHBs as different types of torpor. This is further supported by the finding that these two bout types differ with respect to the timing of entries into hypothermia. DHBs are initiated around midnight, i.e. about 4 h earlier than SSHBs, which start around 0600 hours CET. As a result of the relatively constant decrease of T_b during entry into hypothermia, hamsters entering into a SSHB in early morning reach T_ms of approximately 25 °C in the middle of the day. The occurrences of T_m during SSHBs fall into a small time window, very similar to the daily occurrences of T_m in euthermic animals (Fig. 4C). In contrast, animals entering into a DHB at midnight are already in deep torpor around midday without showing any 24-h pattern in the timing of T_m. Furthermore, in individual hamsters SSHBs developed from the daily minimum of T_b during normothermy, suggesting a stepwise decrease of the homeostatic setpoint of T_b regulation. SSHBs could thus be explained as extensions of normal daily T_b cycles seen in normothermic individuals (Heller et al. 1989). The nature of the third category, SHBs, is not clear yet. SHBs could either be interpreted as interrupted DHBs, because they were initiated at the same time of day as DHBs, or as single-day torpor episodes of essentially the same type as SSHBs, since they show the same linear correlation between bout duration and T_m (Fig. 2). This latter view is supported by the functional similarity between SHBs and SSHBs expressed in the 24-h rhythm in the timing of arousals. A 24-h rhythm in the timing of arousals from SHBs, however, could also be explained by an external disturbance of the animals during a sensitive period in the beginning of a DHB, leading to a preliminary interruption of the DHB.

Temporal organization of HBs

The aim of the present study was to analyse the hibernation behaviour of European hamsters under standard laboratory conditions without any direct effect of spontaneous fluctuations in T_a, and to compare these results with our previous study performed under semi-natural conditions (Wollnik and Schmidt 1995). The temporal organization of SHBs and SSHBs followed a clear 24-h rhythm. The timing of both entries and arousals occurred at preferred times of the day, leading to a 24-h rhythm in the timing of T_m in the middle of the light phase, i.e. during the period normally occupied by rest and sleep. One-day torpor bouts of dormice (Daan 1973), pocket mice (French 1977), Djungarian hamsters (Ruf et al. 1989), and hedgehogs (Fowler and Racey 1990) follow a similar timing pattern.

In contrast, DHBs showed a 24-h rhythm only in the timing of entry into hypothermia. Entries into DHBs were clustered around midnight (8 h after lights out), whereas arousals from DHBs were scattered across all day and night hours. These findings confirm previous observations made on European hamsters kept under constant laboratory (Canguilhem et al. 1994) and semi-natural conditions (Wollnik and Schmidt 1995). The presence of a 24-h rhythm in the timing of entries under standard laboratory conditions with constant T_a rules out the possibility that entry into DHBs is triggered directly by an external stimulus such as a decrease of T_a during night or early morning hours. Instead, entries into DHBs seemed to be locked to the LD cycle, because they were clustered shortly before midnight (2349 hours CET) in the hibernation season 1992/93 but occurred predominantly around 0200 hours CET in the winter 1993/94. This difference was most likely caused by the 2-h difference between the timing of lights on in the winters 1992/93 (lights on at 0600 hours CET) and 1993/94 (lights on at 0800 hours CET), suggesting a fixed phase angle between the lighting regime and initiation of DHBs (Daan 1973). The fact that we did not detect a similar difference in the timing of SSHBs can only be explained by the small number of SSHBs recorded during the first winter (six SSHBs in hamster #2).

The present study revealed that in the European hamster, entries into DHBs start at an earlier time of day than entries into SSHBs. Similar results have been reported for other species. In hedgehogs, for example, multi-day HBs started at 0141 hours CET, whereas short and shallow HBs started significantly later at 0651 hours CET (Fowler and Racey 1990). In dormice, 1-day topor bouts initiated at the end of the dark period, while multi-day torpor bouts started about 6 h earlier in the middle of the night (Daan 1973). These results suggest that the timing of entry into hypothermia is controlled by a rather complex physiological mechanism that seems to take into account not only the time of day but also the type of torpor bout that is about to be initiated.

Persistence of rhythmicity in hibernation

The functional significance of the circadian system in the temporal organization of hibernation bouts remains one of the most enduring questions in hibernation research. A possible role of the circadian system in the timing of arousals was first suggested by Folk (1957), who claimed that periodic arousals from hibernation may be an extension of rhythmicity seen in the normothermic state. Subsequently, Strumwasser et al. (1967) proposed their "three-factor-theory" of hibernation. According to their model, a circadian clock continues to function during hypothermia, but is below a critical threshold. This threshold decreases as a function of time and temperature. Consequently, arousal from a multi-day bout of hibernation is triggered when the decreasing threshold crosses the level of the circadian oscillation. In addition, a seasonal modulator is responsible for the well-documented change in bout length during the hibernation season (Twente and Twente 1967; Wang 1979).

During deep hibernation, the circadian system could be in either of the following two functional states: it could either remain sensitive for the entraining effect of possible environmental time cues, in which case one would expect to find a 24-h rhythm in the timing of arousal as has been demonstrated for dormice kept in a light-dark cycle (Daan 1973) or for hedgehogs kept under natural environmental conditions (Fowler and Racey 1990). Alternatively, the circadian system could be free-running due to the constant conditions or an insensitivity to available time cues, in which case no preference in the timing of arousals should be expected unless the endogenous period is very close to 24 h. Just as previous studies on European hamsters (Canguilhem et al. 1994; Wollnik and Schmidt 1995), the present study found no correlation between the timing of arousals and time of day, although the animals were kept under a LD cycle. Timing of arousal from DHBs can, therefore, not be controlled by an entrained 24-h rhythm. However, the lack of a clear daily pattern in the timing of arousals does not rule out that periodic arousals from DHBs are controlled in part by a clock with a period other than 24 h. During prolonged hypothermia the circadian system may run free and arousals may thus occur at a preferred phase of the circadian cycle.

Only few studies have produced direct evidence for the persistence of a physiological rhythm during deep hibernation. For example, some of the very early studies on hibernating bats (Menaker 1959), ground squirrels (Pohl 1967), and dormice (Pohl 1961) reported residual daily rhythms of T_b and of respiration in deep torpor. In all three species arousal from hibernation occurred at the same phase of the circadian cycle. Only recently, the first conclusive evidence for a relationship between bout duration and circadian oscillations was provided. Grahn et al. (1994) observed persisting circadian rhythms of T_b in ground squirrels kept under DD in a cold room at 10 °C. The periods of these rhythms were different in individual HBs, but regardless of the period, arousal from an HB always occurred at the same phase of the underlying T_b rhythm. The data obtained in the present study did not allow a similar analysis. Although we did observe rhythmic modulations of T_b during DHBs (Fig. 5), bout durations were too short to allow verification of circadian intrabout rhythms in T_b by standard methods such as periodogram analysis. Furthermore, periodogram analysis of T_a revealed a 24-h temperature cycle of the cold room with an amplitude of 0.1 °C, and the daily cycles observed in T_b during DHBs simply seemed to trail the 24-h rhythm of T_a with a time lag of 2–4 h.

Without the demonstration of a physiological rhythm persisting during deep hibernation, the only other way to provide evidence for an underlying circadian rhythm is a subtle statistical analysis of bout length and/or the interval between successive arousals. A number of statistical approaches have been used in the past to test for periodicity in bout length or timing of arousal (Strumwasser et al. 1967; Pohl 1967; Canguilhem et al. 1994) but most of them appear to have one or the other shortcoming. For example, they may assume that the endogenous period underlying each hibernation bout is temperature compensated and remains constant during winter. In the light of Grahn's (1984) study demonstrating a great variety of periods even within one individual, this seems rather unlikely.

Despite these drawbacks, the present study employed a number of statistical procedures for the analysis of bout length and the interval between successive arousals. They revealed a frequency distribution of bout length with predominant peaks at 36, 65, 93.5 and 113 h. However, the interval between these peaks ranged from 19 to 29 h, which does not support the hypothesis that bout duration is an integer multiple of some circadian period plus a constant remainder. The frequency distribution of the interval between arousals, on the other hand, showed a prominent clustering of peaks every 21 or 22 h, which is in accordance with a second, alternative model for the timing of arousals from HBs (Pohl 1987). This model is based on the assumption that the circadian clock does not regulate bout duration but the timing of arousals. Assuming that the clock runs free during the entire hibernation season, this model implies that it should be possible to detect circadian periodicity in the timing of consecutive arousals. Such a free-running rhythm has indeed been demonstrated in Turkish hamsters (Pohl 1987, 1996) and in European hamsters investigated under semi-natural conditions (Wollnik and Schmidt 1995). Another finding supporting this model is the clustering in the frequency distribution of the arousal-to-arousal interval presented in this study. It should be noted that the clustering was detected in the combined measurements of six animals whose individual values were more or less evenly distributed. The multimodal distribution was, therefore, not simply due to a preference of individual animals for a specific arousal-to-arousal interval.

From our results, we find it conceivable that the timing of entries into and arousals from DHBs is controlled by a multi-oscillatory process. One oscillator is likely to be entrained to the LD-cycle, because the animals are undoubtedly synchronized to the environmental cycle during interbout normothermy. It does not seem unreasonable to assume that another oscillator controlling arousal is insensitive to environmental time cues and thus free-running throughout the hibernation season, because in contrast to the artifcial laboratory conditions of the present study, where the animals were not completely shielded from the environmental lighting conditions, it is rather unlikely that hamsters in their natural hibernacula would be able to perceive information on the light/dark cycle.

In conclusion, the data presented in this study clearly demonstrate the importance of the circadian system for the temporal organisation of HBs in European hamsters. As in other species, there is now considerable evidence that periodic arousals from torpor represent some kind of persistence of the circadian periodicity normally seen in the behaviour of the European hamster. However, the exact nature of the circadian control mechanism regulating torpor bout duration is not clear yet. It seems most likely that both temperature-dependent homeostatic processes building up an arousal threshold, and a circadian component establishing sensitive and insensitive periods for arousals work together synergistically to control arousal time and bout duration during hibernation.

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