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Oklejewicz, M; Daan, S; Strijkstra, AM; Heldmaier, G.

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M. Oklejewicz · S. Daan · A.M. Strijkstra

Temporal organisation of hibernation in wild-type and *tau* mutant Syrian hamsters

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Abstract The temporal pattern of hibernation was studied in three genotypes of Syrian hamsters with different circadian periodicity to assess a potential circadian control of alternating torpor and euthermy. We recorded the pattern of hibernation by measuring activity in continuous dim light and constant environmental temperature (6 ± 1 °C). In spite of differences in the endogenous circadian period of three genotypes (*tau* +/+: ≅24 h, *tau* +/–: ≅22 h, and *tau* –/–: ≅20 h) torpor bout duration was statistically indistinguishable (*tau* +/+: 86.9 ± 5.3 h; *tau* +/–: 94.2 ± 3.3 h; *tau* –/–: 88.8 ± 6.2 h). The time between two consecutive arousals from torpor showed unimodal distributions not significantly different between genotypes. The first entry into torpor occurred within the active phase of the circadian cycle in all genotypes whereas the first arousal from torpor appeared to be timed randomly with respect to the prior circadian cycle. The amplitude of the activity rhythm was lower after hibernation compared with the amplitude before hibernation. The results suggest that in the Syrian hamster the circadian system does not control periodicity of torpor and arousal onsets in prolonged hibernation at 6 °C.

Keywords Circadian rhythm · Hibernation · Syrian hamster · *Tau* mutation · Torpor bout

Abbreviations *CT* circadian time · *DD* constant darkness · *LD* light-dark cycle · T_a ambient temperature · *TB* torpor bout

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M. Oklejewicz (✉) · S. Daan · A.M. Strijkstra
Zoological Laboratory, University of Groningen,
P.O. Box 14, 9750 AA Haren, The Netherlands
E-mail: m.oklejewicz@biol.rug.nl
Tel.: +31-50-3632026
Fax: +31-50-3632148

Introduction

Hibernation greatly reduces energy expenditure in mammals during periods of extended low environmental temperatures and food shortage (Geiser 1988; McKee and Andrews 1992). Hibernation consists of prolonged torpor bouts (TBs) with body temperatures approaching the ambient temperature (T_a), interrupted by periodic bouts with euthermic body temperatures. The length of the TBs varies between species (Geiser and Ruf 1995) and depends on several factors, such as time of a year (Daan 1973; Pohl 1981; French 1985), and environmental temperature (French 1977; Strijkstra and Daan 1997).

A recurring question in the hibernation literature is the involvement of the endogenous biological clock in the timing of entrance into and arousal from torpor. The main issue is whether the circadian pacemaker functions at low body temperatures. If its oscillation continues and retains control over activity patterns, it may either be vastly decelerated (Berger et al. 1993) or run at temperature-compensated circadian periods close to 24 h and trigger arousal every second, third, or subsequent cycle. There is some evidence that the circadian system is indeed involved in controlling the timing of torpor and arousal. In light-dark conditions, several species (Syrian hamsters, Pohl 1961; European hamsters, Canguilhem et al. 1994; Wollnik and Schmidt 1995) enter torpor within their nocturnal active phase but arouse from torpor at random times of day. In other studies (e.g. garden dormouse, Daan 1973; big brown bat, Twente and Twente 1987), both entry into torpor and arousal were restricted to certain phases of the daily cycle.

Limited information is available from studies performed in conditions of constant illumination. Garden dormice in constant darkness show circadian rhythmicity in arousal-to-arousal intervals (Daan 1973). In golden-mantled ground squirrels, *Spermophilus lateralis,* free-running rhythms of arousal from hibernation could not be detected in constant conditions (Twente and

Twente 1965). It is possible that these differences are related to body temperatures during torpor. Rather high T_as (12 °C in Daan's 1973 study) lead to shorter TBs (French 1985). Circadian control of short TBs may be more conspicuous for several reasons: (i) circadian timing of shorter intervals is statistically more easily distinguishable from random noise, (ii) the controlling pacemaker gradually damps out in prolonged torpor, or (iii) the pacemaker persists with higher amplitude at the elevated body temperatures characteristic for short TBs.

A possible way to investigate the circadian control of TBs is provided by hibernators with deviating circadian cycles. The *tau*-mutant Syrian hamster (Ralph and Menaker 1988) provides this opportunity. The present study explores if the *tau* mutation indeed affects the temporal organisation of hibernation. If bouts of hibernation are an extension of circadian cycles due to a deceleration of the circadian pacemaker at low body temperature (Heller et al. 1989; Ruby et al. 1989), *tau* mutant hamsters, with a free-running period of about 20 h, are expected to have a proportionally shorter torpor duration compared with wild-type hamsters.

Materials and methods

36 male Syrian hamsters (*Mesocricetus auratus*), age 9-months-old were derived from a breeding colony established at the Zoological Laboratory, Haren (Oklejewicz et al. 1997). Wild-type (*tau* +/+), heterozygote (*tau* +/–), and homozygote *tau* (*tau* –/–) mutant hamsters (F2 generation) were obtained by crossing heterozygous parents. The phenotype was determined by monitoring wheel-running activity under constant dim red light. The average circadian period for *tau* +/+ genotype was 24.2 ± 0.2 h (SEM; $n = 12$), for *tau* +/– it was 22.5 ± 0.1 h ($n = 12$), and for *tau* –/– 20.3 ± 0.1 h ($n = 12$).

The breeding colony was maintained at light-dark (LD) 14:10 throughout and showed no signs of seasonality. To elicit hibernation behaviour, hamsters (age circa 40 weeks) were first group-housed and maintained under short day photoperiod, LD 8:16 and $T_a = 21 \pm 2$ °C. After 4 weeks hamsters were transferred to a temperature-controlled experimental room (6 ± 1 °C). The persistence of a small amplitude fluctuation with a 24-h period in T_a should be noted (Fig. 1). This room was continuously illuminated by dim red incandescent light with light intensities of less than 0.5 lux (constant darkness, DD). Here, all hamsters were individually housed in open-top Plexiglas cages (l×w×h: 25×25×40 cm) with externally attached Plexiglas nest boxes (l×w×h: 12.5×12.5×9.5 cm). Throughout the experiment, the animals had access to food and water ad libitum, replenished at weekly intervals.

Recordings

Nest box temperature sensors and passive infrared (PIR) motion detectors were used to monitor activity and the pattern of hibernation. On the bottom of each nest box a temperature sensor was placed following the method described by Daan (1973). The nest box temperature was recorded continuously at 1-min intervals. The recordings allowed us to distinguish between torpor and the euthermic state in those animals that actually used the nest box for hibernation ($n = 8$). The beginning of a TB was defined as the first continuous slow decrease of the nest box temperature followed by a gradual monotonic decline towards prolonged hibernation temperatures. The end of a TB was defined as the first increase in a gradual monotonic rise in nest box temperature (Fig. 1).

PIR sensors (Wonderex FX-35, Optex, Torrance, Calif.) fixed on the top of the cages monitored gross activity within the main cage. Data were collected in 2-min bins by a PC-based event recording system. Some hamsters did not use the nest box for torpor episodes. In these animals, we used the first and last 2-min. interval of activity in each bout as indicators of torpor and arousal onset. Two out of 12 *tau* +/+ hamsters used in the experiment did not hibernate, all 12 *tau* +/– hibernated, and 2 out of 12 *tau* –/– did not hibernate, while 3 *tau* –/– died in the course of the experiment. Two other *tau* –/– were omitted from the analysis because one had only three TBs and the other irregular torpor. The number of individual records available for torpor analysis was therefore 10 *tau* +/+, 12 *tau* –/+, and 5 *tau* –/– hamsters.

Among these 27 hamsters, eight used their nest boxes as hibernaculum. Data collected on these individuals allowed calculating the TB duration on the basis of both nest-box temperature recordings and activity data (Fig. 1, Table 1). TBs calculated from activity data were on average 1.6 h (± 0.5 h) longer than TBs calculated from the nest-box temperature data. This was caused by the fact that the increase in body temperature precedes body movements as detected by the infrared sensors. Since there was no difference between the three genotypes in the average TB duration

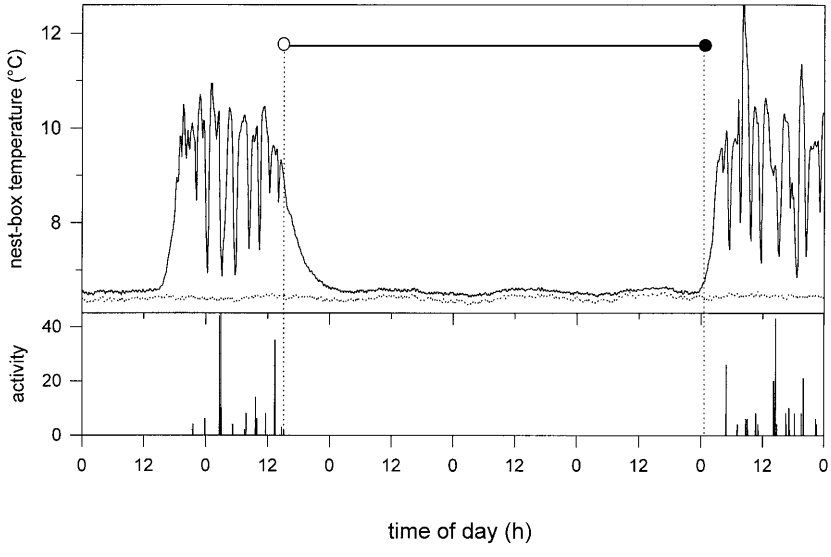

Fig. 1 Example of simultaneous nest-box temperature and activity recording. The *horizontal line* indicates the definition of the torpor bout (TB) with torpor onset (○) and arousal (●) marked. Room temperature is presented as a *dotted line*

Table 1 The maximum torpor bout (TB) length (TB ± SEM; h) for subset of hamsters that hibernated in the nest-box calculated for two recording methods: the nest-box temperature and activity counts (n = number of TBs)

ID	n	$TB_{nest\ box}$	$TB_{activity}$
tau +/+ (no. 2)	12	108.9(3.5)	111.9(3.3)
tau +/+ (no. 5)	7	68.8(5.7)	69.5(5.9)
tau +/+ (no. 6)	9	51.6(4.4)	52.2(4.5)
tau +/– (no. 7)	15	93.4(3.0)	93.8(2.9)
tau +/– (no. 32)	13	85.3(3.5)	87.0(3.9)
tau –/– (no. 24)	12	60.5(3.2)	64.1(3.4)
tau –/– (no. 27)	9	67.2(3.6)	70.2(3.9)
tau –/– (no. 36)	10	76.1(2.6)	76.1(2.7)

based on both types of measurements, we used the overall activity data for all presented parameters.

Statistics

Differences between the three genotypes were tested for statistical significance by one-way analysis of variance and post-hoc Tukey test. Differences within individuals were tested by paired t-test (Sokal and Rohlf 1995). The angular-angular statistic (Zar 1999) was applied on the circular data of circadian timing. For correlation between times of entry into and termination of torpor, a modified angular-angular correlation was used (Hut et al. 2001a). Raleigh's test was applied for testing the uniformity of circular data, the parametric paired Hotelling test for comparison between circadian times, the Watson-Williams test for testing differences in circadian timing between genotypes. All data are presented as means ± SE unless mentioned otherwise. Significance was accepted at $P < 0.05$ (two-tailed).

Results

Pre-hibernation period and hibernation season

The pre-hibernation period was defined as the number of days between first exposure to the cold environment and the onset of the first TB. Wild-type hamsters had a slightly longer pre-hibernation period than *tau* +/– and *tau* –/–hamsters (Table 2), yet genotype did not significantly contribute to the explained variance in pre-hibernation period (ANOVA, $P = 0.5$). The duration of hibernation in days between the first and last day in torpor was not significantly associated with genotype (ANOVA, $P = 0.5$; Table 2). The same applies when duration of hibernation is expressed in number of circadian cycles (*tau* +/+: average 59 cycles; *tau* +/–: 72 cycles; and *tau* –/– 82 cycles; ANOVA, $P = 0.09$).

Body weight during hibernation

Before hibernation, *tau* +/+ hamsters were heavier (157.8 ± 6.6 g) than *tau* +/– (129.8 ± 6.0 g) and *tau* –/– (128.4 ± 4.4 g) hamsters (ANOVA, $P = 0.005$) and this difference persisted after hibernation (ANOVA, $P = 0.004$). Weight loss during hibernation (19.2 ± 5.7 g in *tau* +/+; 4.5 ± 5.0 g in *tau* +/–; 15.6 ± 5.6 g in *tau* –/–) was not significantly associated with genotype (ANOVA, $P = 0.1$).

Table 2 Characteristics of temporal organisation of hibernation (means ± SEM) presented for three genotypes of hamsters

	tau +/+	*tau* +/–	*tau* –/–
Pre-hibernation period (days)	47.3 (3.3)	41.1 (3.8)	40.2 (9.8)
Hibernation season (days)	58.8 (6.0)	66.0 (4.6)	68.4 (8.9)
TB duration, TB_{max} (h)	86.9 (5.3)	94.2 (3.3)	88.8 (6.2)
Arousal-to-arousal interval (h)	113.3 (3.9)	116.2 (2.9)	114.2 (4.1)
Arousal-to-arousal interval (cycle)	4.7 (0.2)	5.3 (0.1)	5.7 (0.2)
CT of the first torpor onset	20.5 (0.7)	20.2 (0.8)	18.6 (1.5)
Amplitude of the activity rhythm:			
Before hibernation	1.79 (0.20)	2.39 (0.13)	2.34 (0.39)
After hibernation days 0–5	1.25 (0.14)	1.54 (0.13)	1.82 (0.32)
After hibernation days 5–10	2.48 (0.53)	1.92 (0.25)	1.77 (0.21)

TB duration

TB duration for *tau* +/+ hamsters varied from 29 h to 130 h, for *tau* +/– hamsters from 29 h to 117 h, and for *tau* –/– from 39 h to 111 h. The TB duration increased steadily from the first short TB, reached a 'plateau' level during mid-hibernation, and decreased towards the end of hibernation. Hamsters from all three genotypes followed the same pattern, except one individual (no. 33 - *tau* +/–, Fig. 2) which did not show apparent changes in TB duration. The relation between the sequence and duration of TB was described by fitting a quadratic regression. We determined the maximum TB (TB_{max}) for each individual from the fitted curve, as illustrated in Fig. 2.

Genotype did not contribute significantly to the variance in TB_{max} (ANOVA, $P = 0.5$; Table 2). The average number of TBs during hibernation was smaller in *tau* +/+ hamsters (12.6 ± 0.9) than in *tau* +/– (16.2 ± 0.7) and *tau* –/– hamsters (15.8 ± 1.8; ANOVA, $P = 0.02$). This maybe related to the fact that *tau* +/+ hamsters had a slightly shorter (though not significantly so) mean duration of hibernation.

Arousal-to-arousal interval

Frequency distributions of arousal-to-arousal intervals are shown in Fig. 3. For none of the three genotypes and individual data is the distribution convincingly multimodal. As with TBs, arousal-to-arousal intervals changed in the course of hibernation. The first and the last intervals were more variable than in the middle of hib-

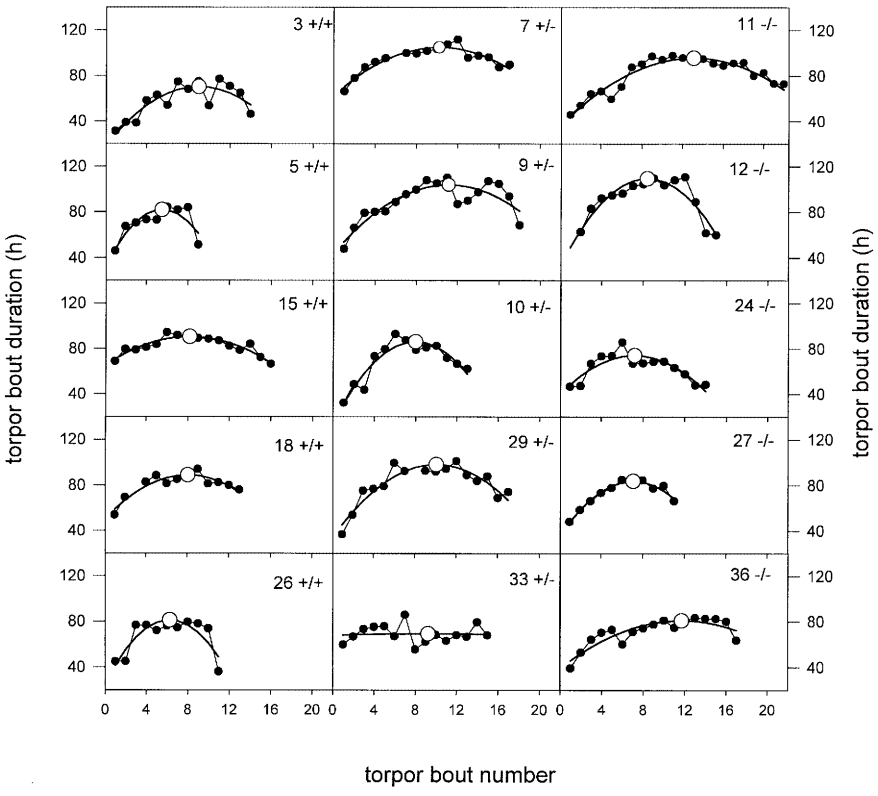

Fig. 2 Examples of the relationship between TB duration and sequence of TBs in the course of hibernation for five individuals from each genotype: wild-type (+/+), heterozygote (+/–), and homozygote *tau* mutants (–/–). The fitted quadratic function is shown as a *line* and the maxima as *open circles*

ernation (examples in Fig. 5). For each individual, the average arousal-to-arousal interval was calculated as the average of the five longest intervals. Between the three genotypes, arousal-to-arousal intervals did not differ significantly (ANOVA, $P=0.8$; Table 2). When expressed as the average number of completed circadian cycles between two consecutive arousals, the arousal-to-arousal interval differed significantly between genotypes (ANOVA, $P=0.002$) with a smaller number of cycles for *tau* +/+ hamsters compared with *tau* +/– and *tau* –/– hamsters (Table 2).

Onset of torpor and arousal

The first TB may be of particular significance for the question of circadian control of torpor since they are typically shorter (Fig. 2) and body temperature often does not drop as far as during the middle of hibernation (Hut et al. 2001a). We therefore analysed the timing of the first two bouts. Circadian time (CT) for the onset of the first TB was calculated for 6 *tau* +/+ hamsters, 9 *tau* +/–, and 5 *tau* –/– hamsters. The remaining individuals had fewer than five activity onsets in DD before hibernation onset available to establish the circadian time. The activity onset (CT 12) was defined as the upward crossing between a short and long running mean (6 h and a 24 h for *tau* +/+, 5.5 h and 22 h for *tau* +/–, and 5 h and 20 h for *tau* –/–, respectively) of the original activity counts (Meerlo et al. 1997). Circadian time of torpor and arousal onset was determined from the number of circadian hours after the last activity onset at CT 12.

All individuals entered their first torpor during the subjective night (between CT 12 and CT 24; Fig. 4). The average circadian time of first torpor onset did not vary between genotypes (Watson-Williams test; $F_{(2,17)}=1.496$; $P=0.25$). The onset of first arousal was not restricted to a particular circadian phase for any of the genotypes (Fig. 4A). There was no significant correlation between onset of the first TB and arousal for any of the three genotypes ($r=0.07$ for *tau* +/+, $r=0.11$ for *tau* +/–, and $r=-0.20$ for *tau* –/–; $P>0.3$).

The timing of the second TB showed more variation with respect to phase of the circadian cycle than entry into the first TB (Fig. 4B), although CT for onsets into first and second TBs did not differ significantly neither in *tau* +/+ hamsters [$F_{(2,4)}=1.95$, $P=0.3$, Hotelling test], nor in *tau* +/– and *tau* –/– hamsters [$F_{(2,4)}=1.75$, $P=0.2$; $F_{(2,4)}=8.40$, $P=0.06$].

The circadian rhythm before and after hibernation

The amplitude of the activity rhythm before and after hibernation is presented in Table 2. Before hibernation, hamsters had higher activity counts than after hibernation (Fig. 5). The amplitude of the activity rhythm was calculated as the difference between maximum and minimum of the short running mean and divided by the long running mean of activity counts. Wild-type hamsters had a higher amplitude of activity rhythm before

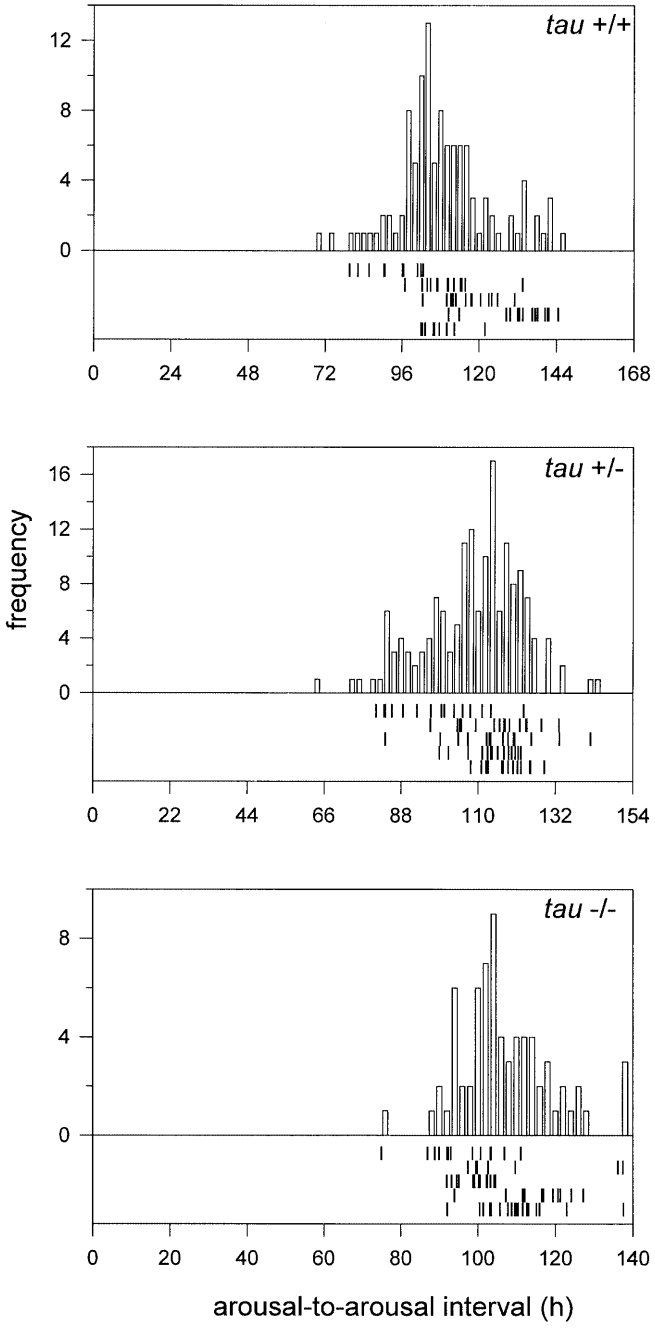

Fig. 3 Frequency distribution (in 2-h bins) of arousal-to-arousal intervals for three genotypes of hamsters. Width of the abscissa is 7 circadian cycles in each case. For each genotype all data for five individuals with the highest number of hibernation bouts are presented

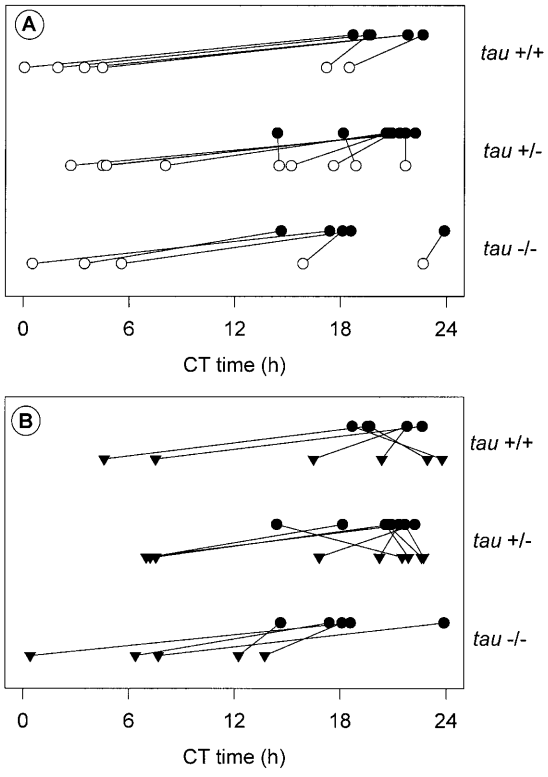

Fig. 4 **A** The circadian time (*CT*) of onsets of the first TB (●) and first arousals (○). **B** CT of onsets of the second TB (▼) in relation to onset of the first for individuals from three genotypes (wild-type *tau* +/+, heterozygote mutants *tau* +/+, and homozygote mutants *tau* –/–)

hibernation compared with the first 5 days after hibernation ($n=7$; $t=3.39$, $P=0.02$; Table 2). The amplitude after day 5 post-hibernation was not significantly different from pre-hibernation values (paired *t*-test, $t=1.11$; $P=0.3$). *Tau* +/– hamsters showed a similar pattern: lower amplitude during the 1st 5 days after hibernation ($n=9$; $t=6.74$; $P=0.0001$) and restoration of the amplitude between day 5 and day 10 (paired *t*-test, $t=1.81$; $P=0.1$). *Tau* –/– hamsters showed a similar change in amplitude, although this decline did not reach significance ($n=5$; $t=0.85$; $P=0.4$).

One individual from each genotype did not restore rhythmicity 10 days after hibernation. For the rhythmic animals, the post-hibernation free-running circadian period did not differ from the pre-hibernation period (example Fig. 5).

Discussion

Hibernation pattern

All three genotypes of hamsters are capable of hibernation in captivity. They showed a regular pattern of TB duration, with a steady increase towards the middle of hibernation and a subsequent decrease at the end of hibernation. Similar patterns have been described for other hibernators, including ground squirrels (Grahn et al. 1994), European hamsters (Canguilhem et al. 1994; Wollnik and Schmidt 1995), and dormice (Daan 1973).

Testicular regression, presumably elicited in our experiments by the initial exposure to short days, is essential for the induction and maintenance of hibernation in Syrian hamsters (Frehn and Liu 1970; Janský

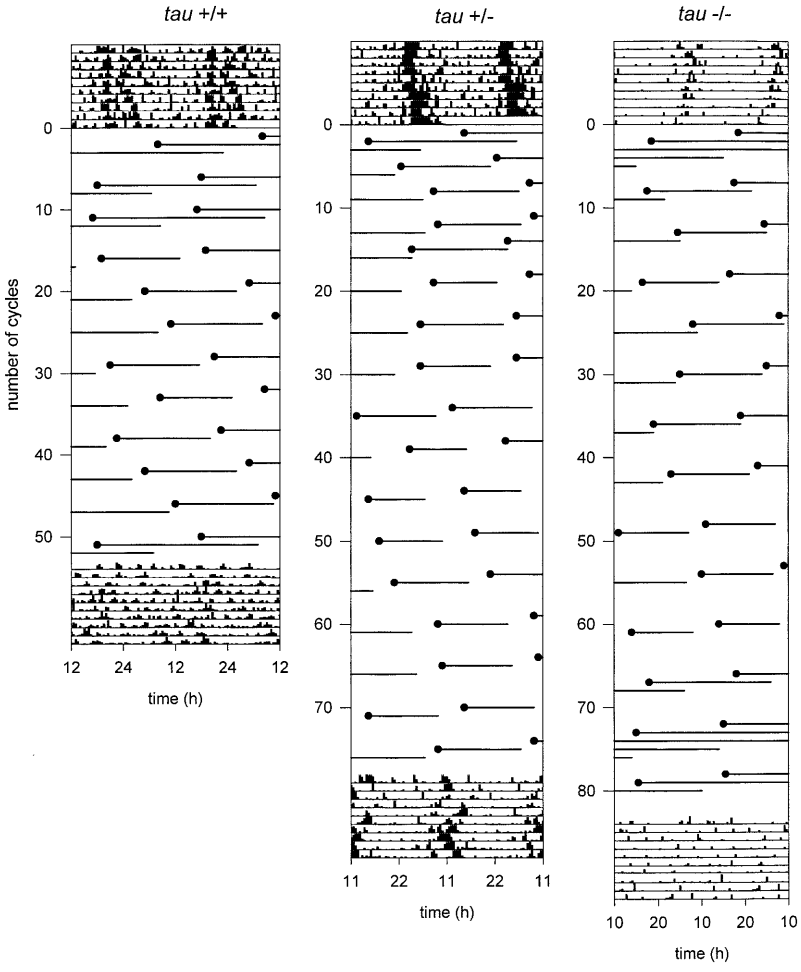

Fig. 5 Three representative double plots of euthermic arousals and of activity records during ten cycles before and after hibernation for wild-type (*tau* +/+), heterozygote (*tau* +/–), and homozygote *tau* mutant hamsters (*tau* –/–). *Dots* indicate each onset, and *lines* indicate the duration of euthermic episodes during hibernation

et al. 1984). Mutant hamsters complete testicular regression about 1.4 weeks earlier than wild-type hamsters (Loudon et al. 1998). Thus, we might expect mutant hamsters to initiate hibernation earlier than wild-type hamsters. Although on average *tau* –/– started to hibernate 7 days earlier than *tau* +/+ hamsters, this difference was not significant. The pre-hibernation period exceeded 43 days for all genotypes, which corresponds reasonably with 40–50 days (Janský et al. 1984) and with 57 days (Lyman 1954) in wild-type Syrian hamsters.

The spontaneous duration of hibernation did not differ between genotypes and lasted on average 64 days, considerably shorter than the 95 days reported by Lyman (1954) for hamsters held at 5 °C in a light-dark cycle. In the closely related Turkish hamster, *Mesocricetus brandti*, hibernation even exceeded 149 days in constant dim light and 8 °C (Pohl 1981).

Timing of TBs

Hamsters of all genotypes entered their first torpor within the active phase and aroused from this first torpor randomly across the circadian cycle. The onset of the second TB was not clearly concentrated within the active phase, but also not significantly different from the first torpor. The results resemble timing of entry into and arousal from torpor in the Turkish hamster studied under LD conditions (Pohl 1996) and in the European hamster hibernating both in semi-natural and laboratory conditions (Wollnik and Schmidt 1995; Waßmer and Wollnik 1997). Similarly, in Syrian hamsters studied in light-dark conditions (Pohl 1961) about 90% of TB onsets occurred within the dark phase of a cycle whereas onsets of arousal were randomly distributed. In deep hibernators, such as the European ground squirrel, only the onsets of the first and second TBs occurred within the active phase, whereas the onsets of arousal were randomly scattered across the 24-h cycle (Hut et al. 2001a).

Few studies have addressed the timing of torpor in constant light or dark conditions. Twente and Twente (1987) reported arousal from hibernation in the big brown bat at a fixed time of the day. In the golden-mantled ground squirrel, arousal occurred at fixed phase angle of a body temperature cycle (Grahn et al. 1994). We however did not detect in Syrian hamsters any signs of circadian regulation of onset and termination of TBs, other than first onset.

Amplitude of circadian rhythm after hibernation

The low amplitude of the activity rhythm during the 1st days after hibernation suggests that the circadian pacemaker may require a few cycles to restore the pre-hibernation oscillations. Pohl (1981) also observed arrhythmicity after hibernation in the Turkish hamster. In the European ground squirrel, the body temperature rhythm is initially decreased in amplitude after hibernation in both natural and laboratory conditions (Hut et al. 2001a, 2001b). The reduction in rhythm amplitude may be viewed as circumstantial evidence that the circadian pacemaker does not fully function at low body temperatures during hibernation, and requires recovering in euthermic conditions afterwards.

Circadian rhythmicity during hibernation

In golden-mantled ground squirrels, the suprachiasmatic nucleus (SCN) shows a higher metabolic activity during hibernation compared with most other brain areas (Kilduff et al. 1989). Since the SCN is the primary circadian pacemaker in mammals (Ralph et al. 1990), this might suggest that the circadian system is indeed active during hibernation. Although a study in dormice at a temperature of 12 °C and constant light did provide evidence for circadian control of torpor cycles (Daan 1973), the present study did not reveal such evidence in hamsters hibernating at 6 °C. In spite of the difference in circadian period, the three genotypes had essentially similar timing and duration of TBs, consistent with the absence of a circadian contribution to the control of torpor.

Conclusions from previous reports on circadian control of timing of torpor during hibernation are summarized in Table 3. In some cases, data or analysis were inconclusive as indicated by a *question mark*. The majority of studies performed in LD conditions show rhythmicity in the timing of TBs independently of T_a (Table 3, *top panel*). In some physiological variables, e.g. the plasma melatonin concentration in hamsters (Vaněček et al. 1984, 1985) and marmots (Florant et al. 1984), no circadian rhythm could be detected in hibernation. The persistence of rhythmicity in LD and natural photoperiod (NP) conditions may of course be attributed to exogenous variables and provides no evidence for control by the circadian pacemaker.

In studies under constant light conditions, the results are less consistent (Table 3). The detection of rhythmicity in DD is considerably more difficult than in LD since the timing of TBs cannot easily be related to a particular phase of a circadian cycle throughout hibernation. Garden dormice (Daan 1973) and pocket mice (French 1977) showed behavioural rhythmicity in constant conditions. In the dormice TBs were recorded at a rather higher (12 °C) T_a; in the pocket mice the majority of TBs

Table 3 Overview of studies on rhythmicity during hibernation in light-dark (*LD*) or natural photoperiod (*NP*) and constant darkness (*DD*) sorted by the decrease in ambient temperature (T_a); (*NT* natural T_a). Rhythmicity in hibernation was divided into three groups based on parameters measured and analysed: *A*-rhythmicity in entry into hibernation, *B*-rhythmicity in arousal from hibernation, and *C*-circadian oscillations in physiological parameters.(+ persistence of rhythmicity, – absence of rhythmicity, *?* inconclusive)

Species	T_a	Light condition	Rhythmicity			Source
			A	B	C	
Mountain pygmy-possum	NT	NP	+	+		Körtner et al. (1998)
	3–10 °C	LD	+	+		
European hamster	NT	NP	+	+	–	Wollnik and Schmidt (1995)
European hedgehog	NT	NP	+	+	–	Fowler and Racey (1990)
Marmot	5 and 15 °C	LD			–	Florant et al. (1984)
Syrian hamster	15 °C	LD	+	–		Pohl (1961)
Garden dormouse	12 °C	LD	+	+		Daan (1973)
Syrian hamster	11.5 °C	LD			–	Vaněček et al. (1984, 1985)
Turkish hamster	8–10 °C	LD	?	?		Pohl (1987, 1996)
Pocket mouse	8 and 18 °C	LD	+	+		French (1977)
Common dormouse	8 °C	LD	?	?		Pohl (1967)
European hamster	8 °C	LD	+	+		Waßmer and Wollnik (1997)
European hamster	6 °C	LD	+			Canguilhem et al. (1994)
Marmot	5 °C	LD			–	Florant et al. (1984)
California ground squirrel	5–8 °C	LD	+	+		Strumwasser (1959)
Garden dormouse	12 °C	DD	+	+		Daan (1973)
Golden-mantled ground squirrel	11 °C	DD	?	?		Twente and Twente (1965)
	2 °C	DD	?	?		
Golden-mantled ground squirrel	10 °C	DD			+	Grahn et al. (1994)
Little brown bat	9 °C	DD			+	Menaker (1959)
Big brown bat	9 °C	DD			+	
Pocket mouse	8 °C	DD		+		French (1977)
Turkish hamster	8 °C	DD	?	?		Pohl (1981)
Common dormouse	8 °C	DD	?	?		Pohl (1967)
Syrian hamster	7 °C	DD	–	–		This study
European hedgehog	4 °C	DD		+		Kristoffersson and Soivio (1964; 1967)

lasted only 1 day or 2 days. Rhythmicity in short TB duration is more easily distinguished from random noise. In the golden-mantled ground squirrel oscillations in body temperature within a TB have been found (Grahn et al. 1994), but it is unknown whether such rhythmicity affects arousal-to-arousal intervals. For the European hedgehog, we re-analysed data presented by Krisoffersson and Soivio (1964, 1967) and found a multimodal distribution of arousal-to-arousal intervals with peaks roughly every 24 h. This contradicts the conclusion of authors that "the timing of spontaneous arousals and entries into deep hypothermia does not confirm the possible continuance of the circadian rhythm during hibernation period". In contrast to most rodent hibernators, hedgehogs may forage during arousals. Whether the circadian system maintains control over activity during hibernation may well differ between species, as the function of circadian regulation must be related to benefits of circadian timing in natural conditions. In species that forage during periodic euthermy, such as the hedgehog, the pygmy-possum, the European hamster, circadian control over torpor may well be of adaptive significance (Körtner and Geiser 2000).

In Syrian hamsters with different circadian period, we found no evidence for involvement of the circadian system in the temporal organisation of hibernation. Possibly in hamsters hibernating at higher T_as, where TB durations are shorter, circadian rhythmicity might be detectable. Be this as it may, the data presented here appear to definitely rule out the idea that a torpor-arousal cycle is simply a circadian cycle slowed down by low temperature (Berger 1993). The data are inconsistent with the prediction from this hypothesis that *tau* mutant hamsters would have shorter torpor cycle.

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References

Berger RJ (1993) Cooling down to hibernate: sleep and hibernation constitute a physiological continuum of energy conservation. Neurosci Lett 154:213–216

Canguilhem B, Malan A, Masson-Pévet M, Nobelis P, Pévet P, Le Minor J (1994) Search for rhythmicity during hibernation in the European hamster. J Comp Physiol B 163:690–698

Daan S (1973) Periodicity of heterothermy in the garden dormouse, *Eliomys quercinus*. Neth J Zool 23:237–265

Florant GL, Rivera ML, Lawrence AK, Tamarkin L (1984) Plasma melatonin concentrations in hibernating marmots: absence of a plasma melatonin rhythm. Am J Physiol 247:R1062–R1066

Fowler PA, Racey PA (1990) Daily and seasonal cycles of body temperature and aspects of heterothermy in the hedgehog, *Erinaceus europaeus*. J Comp Physiol B 160:299–307

Frehn JL, Liu C (1970) Effects of temperature, photoperiod, and hibernation on the testes of golden hamsters. J Exp Zool 174:317–324

French AR (1977) Periodicity of recurrent hypothermia during hibernation in the Pocket mouse, *Perognathus longimembris*. J Comp Physiol A 115:87–100

French AR (1985) Allometries of the duration of torpid and euthermic intervals during mammalian hibernation: a test of the theory of metabolic control of the timing of changes in body temperature. J Comp Physiol B 156:13–19

Geiser F (1988) Reduction of metabolism during hibernation and daily torpor in mammals and birds: temperature effect or physiological inhibition? J Comp Physiol B 158:25–37

Geiser F, Ruf T (1995) Hibernation versus daily torpor in mammals and birds: physiological variables and classification of torpor patterns. Physiol Zool 68:935–966

Grahn DA, Miller JD, Houng VS, Heller HC (1994) Persistence of circadian rhythmicity in hibernating ground squirrels. Am J Physiol 266:R1251–R1258

Heller HC, Krilowicz BL, Kilduff TS (1989) Neural mechanisms controlling hibernation. In: Malan A, Canguilhem B (eds) Living in the cold. INSERM/J Libbey Eurotext, Paris, pp 447–460

Hut RA, Barnes B, Daan S (2001a) Body temperature patterns before, during, and after natural hibernation in the European ground squirrel. PhD thesis, University of Groningen, pp 47–61

Hut RA, Van der Zee E, Jansen K, Gerkema MP, Daan S (2001b) Gradual reappearance of post-hibernation circadian rhythmicity correlates with numbers of vasopressin containing neurons in the suprachiasmatic nuclei of European ground squirrel. PhD thesis, University of Groningen, pp 63–77

Janský L, Haddad G, Kahlerova Z, Nedoma J (1984) Effect of external factors on hibernation of golden hamsters. J Comp Physiol B 154:427–433

Kilduff TS, Radeke CM, Randall TL, Sharp FR, Heller HC (1989) Suprachiasmatic nucleus: phase dependent activation during the hibernation cycle. Am J Physiol 257:R605–R612

Körtner G, Geiser F (2000) The temporal organization of daily torpor and hibernation: circadian and circannual rhythms. Chronobiol Intern 17:103–128

Körtner G, Song X, Geiser F (1998) Rhythmicity of torpor in a marsupial hibernator, the mountain pygmy-possum (*Burramys parvus*), under natural and laboratory conditions. J Comp Physiol B 168:631–638

Kristofferson R, Soivio A (1964) Hibernation of the hedgehog (*Erinaceus europaeus*). The periodicity of hibernation of undisturbed animals during the winter in a constant ambient temperature. Ann Acad Sci Fenn A 80:1–22

Kristofferson R, Soivio A (1967) A comparative long-term study of hibernation in Finnish and German hedgehogs in a constant ambient temperature. Ann Acad Sci Fenn A 122:1–23

Loudon ASI, Ihara N, Menaker M (1998) Effects of a circadian mutation on seasonality in Syrian hamsters (*Mesocricetus auratus*). Proc R Soc Lond B 265:517–521

Lyman CP (1954) Activity, food consumption and hoarding in hibernators. J Mammal 35:545–552

McKee G, Andrews JF (1992) The costs and savings of hibernation in the golden hamster. J Physiol 446:451

Meerlo P, Van den Hoofdakker RH, Koolhaas JM, Daan S (1997) Stress-induced changes in circadian rhythms of body temperature and activity in rats are not caused by pacemaker changes. J Biol Rhythms 12:80–92

Menaker M (1959) Endogenous rhythms of body temperature in hibernating bats. Nature 184:1251–1252

Oklejewicz M, Hut RA, Daan S, Loudon ASI, Stirland AJ (1997) Metabolic rate changes proportionally to circadian frequency in *tau* mutant Syrian hamsters. J Biol Rhythms 12:413–422

Pohl H (1961) Temperaturregulation und Tagesperiodik des Stoffwechsels bei Winterschläfern. Z Vgl Physiol 45:109–153

Pohl H (1967) Circadian rhythms in hibernation and the influence of light. In: Fisher KC, Dawe AR, Lyman CP, Schonbaum E, South FE (eds) Mammalian hibernation III. Oliver and Boyd, Edinburgh, pp 140–151

Pohl H (1981) Temporal structure of hibernation behaviour of the Turkish hamster, *Mesocricetus brandti*, under controlled laboratory conditions. Acta Univ Carol Biol 9:177–180

Pohl H (1987) Circadian pacemaker does not arrest in deep hibernation. Evidence for desynchronisation from light cycle. Experientia 43:293–294

Pohl H (1996) Circadian and circannual rhythmicity in the Turkish hamster, *Mesocricetus brandti*. In: Geiser F, Hulbert AJ, Nicol SC (eds) Adaptation to the cold. Tenth International Hibernation Symposium. University of New England Press, Armidale, pp 87–93

Ralph MR, Menaker M (1988) A mutation of the circadian system in golden hamsters. Science 241:1225–1227

Ralph MR, Foster RG, Davis FC, Menaker M (1990) Transplanted suprachiasmatic nucleus determines circadian period. Science 247:975–978

Ruby N, Ibuka N, Barnes B, Zucker I (1989) Suprachiasmatic nuclei influence torpor and circadian temperature rhythm in hamsters. Am J Physiol 257:R210–R215

Sokal RR, Rohlf FJ (1995) Biometry. Freeman, San Francisco

Strijkstra AM, Daan S (1997) Ambient temperature during torpor affects NREM sleep EEG during arousal episodes in hibernating European ground squirrels. Neurosci Lett 221:177–180

Strumwasser F (1959) Factors in the pattern, timing and predictability of hibernation in the squirrel, *Citellus beecheyi*. Am J Physiol 196:8–14

Twente JW, Twente JA (1965) Effect of core temperature upon duration of hibernation of *Citellus lateralis*. J Appl Physiol 20:411–416

Twente JW, Twente JA (1987) Biological alarm clock arouses hibernating big brown bats, *Eptesicus foscus*. Can J Zool 65:1668–1674

Vaněček J, Janský L, Illnerova H, Hoffman K (1984) Pineal melatonin in hibernating and aroused Golden hamsters (*Mesocricetus auratus*). Comp Biochem Physiol A 77:759–762

Vaněček J, Janský L, Illnerova H, Hoffman K (1985) Arrest of the circadian pacemaker driving the pineal melatonin rhythm in hibernating Golden hamsters, *Mesocricetus auratus*. Comp Biochem Physiol A 80:21–23

Waßmer T, Wollnik F (1997) Timing of torpor bouts during hibernation in European hamsters (*Cricetus cricetus* L.). J Comp Physiol B 167:270–279

Wollnik F, Schmidt B (1995) Seasonal and daily rhythms of body temperature in the European hamster (*Cricetus cricetus*) under semi-natural conditions. J Comp Physiol B 165:171–182

Zar JH (1999) Biostatistical analysis, 4th edn. Prentice-Hall, London