

Seasonal changes in the thermoregulation of laboratory golden hamsters during acclimation to seminatural outdoor conditions

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Abstract

Proper adjustments of the thermoregulatory mechanisms ensure survival in the natural environment. In the present study, we tested the hypothesis that laboratory golden hamsters (*Mesocricetus auratus*) housed under seminatural outdoor conditions are able to acclimatize to daily and seasonal changes in the environment despite their long history of breeding in captivity. The animals experienced natural changes in the photoperiod and ambient temperature characteristic for central Poland. During experiments in the thermal gradient system, the daily rhythms of body temperature (measured as the temperature of brown adipose tissue, T_{BAT}), preferred ambient temperature (PT_a) and activity were measured in summer, autumn and spring. We found that mean T_{BAT} was highest in autumn and least in summer, reflecting seasonal changes in the capacity for nonshivering thermogenesis (NST). In summer, T_{BAT} followed the robust daily rhythm with the amplitude of 1.1 ± 0.1 °C. This amplitude was depressed in autumn (0.2 ± 0.1 °C) and partially restored in spring (0.4 ± 0.1 °C). Seasonal changes in the daily amplitude of T_{BAT} recorded during both transitional periods, i.e., in autumn and spring, seem to be associated with hamsters' hibernation. In autumn, mean daily PT_a was lower than in summer and spring, indicating the lowering of a set point for core body temperature (T_b) regulation. Locomotor activity was much higher in spring than in summer and autumn, and it always predominated at night. We conclude that laboratory golden hamsters housed under seminatural conditions express daily and seasonal changes in the thermoregulatory mechanisms that, despite long history of breeding in captivity, enable proper acclimatization to seasonally changing environment and ensure successful hibernation and winter survival.

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1. Introduction

Golden hamster (*Mesocricetus auratus*) is a small nocturnal rodent widely used in laboratory studies. At constant laboratory ambient temperature (T_a), mean amplitude of core body temperature (T_b) rhythm varies between 2.5 and 4 °C (Refinetti, 1996a; DeCoursey et al., 1998; Song and Rusak, 2000), while, in a thermal gradient system, where free choice of preferred ambient temperatures (PT_a) is given, this amplitude is reduced to 1 °C (Refinetti, 1995).

Acclimatization (or seasonal acclimation) to cold and short photoperiod (SP) results in small mammals increasing their nonshivering thermogenesis (NST) capacity (Heldmaier and Lynch, 1986; McElroy and Wade, 1986; Heldmaier et al., 1989). In small mammals, like golden hamsters, NST is a primary source of heat (Janský, 1973). In placental mammals, a major site for NST is brown adipose tissue (BAT). Furthermore, NST plays a fundamental role during arousal from hibernation (Smalley and Dryer, 1963; Hayward and Lyman, 1967; Smalley and Dryer, 1967; Janský, 1973). Golden hamsters are hibernating animals, therefore more efficient NST is obviously beneficial for their survival.

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Present distribution area of wild golden hamsters lies in the agricultural Aleppinian plateau in Syria and seems to be limited with natural barriers: mountains in the north and west and river in the east (Gattermann et al., 2001). The climate of this area is continental, with large daily and seasonal variations in ambient temperatures: summer is hot and dry, while winter is cold and wet (Taborska, 1990; Gattermann et al., 2001). Under such environmental conditions, hamsters should exhibit seasonal changes in thermoregulation to cope with the seasonal changes in the environment. However, we know nothing about these changes in thermoregulatory mechanisms in wild golden hamsters inhabiting natural environments. Until 1997, golden hamsters had not been found in the natural environment since 1930. Laboratory populations of hamsters stem all from a single lineage that originated about 70 years ago (Taborska, 1990; Weinert et al., 2001). When individuals were found again in their natural environment, the activity rhythms of wild and laboratory populations were compared. It was found that activity patterns were similar in both laboratory and wild hamsters, although interindividual differences were much higher in wild animals (Weinert et al., 2001).

The aim of present study was to investigate daily and seasonal changes in body temperature (measured as BAT temperature, T_{BAT}), PT_a and activity that occur during preparation to winter inactivity and during emergence from it during spring. In laboratory experiments, environmental conditions are usually constant or change rapidly, and animals cannot experience gradual and complex changes that occur in the natural environment. Seasonal rhythms in golden hamsters living in natural environment have not been studied. Up to now, there are no data on thermoregulation in wild golden hamsters or animals housed under seminatural conditions. In the present experiments, we used golden hamsters originating from commercial breeding colony and housed them under laboratory outdoor conditions to ensure exposure to complex changes in the environment throughout the year. Although climate in Poland differs from climate in

Syria, our animals experienced seasonal variations in T_a similar to the variations observed in the natural environment of golden hamsters (Table 1). Experiments were done in three seasons: summer—activity time, late autumn—final phase of preparation to hibernation season and spring—termination of hibernation and recommence of activity period. We hypothesized that, despite their long history of breeding in captivity, golden hamsters exposed to the seasonal changes in the environment express gradual seasonal changes in thermoregulatory mechanisms and behavior due to acclimatization. We predicted that mean T_{BAT} would increase in autumn due to increased capacity for NST (Jefimow et al., 2004). However, as during hibernation circadian rhythm of T_b disappears (Janský, 1989), we predicted that daily rhythm of T_{BAT} would be dampened during both transitional periods (autumn and spring), leading to disappearance of the rhythm towards winter and its reestablishment in spring. In addition, as the daily rhythm of PT_a is correlated with the daily rhythm of T_b (Gordon, 1994; Refinetti, 1995), we predicted that seasonal changes in T_{BAT} would be accompanied by seasonal changes in PT_a , leading to the lowering of PT_a in autumn. Thus, both rhythms would be adjusted to natural changes in photoperiod and ambient temperature.

2. Material and methods

2.1. Animals and housing

This research took place at N. Copernicus University (Toruń, Poland; 53°00' N, 18°56' E). Experiments were done on six adult male golden hamsters (*Mesocricetus auratus*) housed under laboratory outdoor conditions. Animals (5 to 7 months old) were transferred from the laboratory into separate outdoor enclosures located on the campus in the end of April (Fig. 1). Each enclosure consisted of a wooden box (1.0×1.0×1.0 m) with a plastic cage (67×67×67 cm) inside it. The plastic cage was filled

Table 1

Mean, minimum and maximum values of air temperature, soil temperature and mean duration of light phase of day from May 2002 to April 2003

	Air temperature (°C)			Soil temperature (°C)			Light (h)
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean
V	17.6 (22.0)	4.4 (13.0)	31.1 (29.0)	21.0	16.7	24.8	16.2
VI	17.6 (26.0)	8.2 (18.0)	33.9 (34.0)	21.4	18.0	25.6	17.3
VII	20.4 (29.0)	10.8 (22.0)	34.3 (36.0)	23.6	20.3	26.4	16.8
VIII	21.6 (28.0)	10.5 (21.0)	33.3 (36.0)	25.5	24.6	27.2	15.5
IX	13.9 (25.0)	−1.0 (18.0)	30.1 (32.0)	21.1	16.3	25.3	12.4
X	7.4 (20.0)	−1.5 (12.0)	18.6 (26.0)	13.7	11.0	17.5	10.0
XI	4.0 (12.0)	−3.9 (16.0)	14.6 (18.0)	9.0	7.0	11.3	8.4
XII	−3.7 (7.0)	−16.5 (3.0)	3.5 (12.0)	3.7	0.2	9.1	7.7
I	−2.0 (6.0)	−21.0 (2.0)	7.7 (10.0)	1.7	−0.8	4.1	8.2
II	−3.0 (7.0)	−17.6 (2.0)	7.7 (12.0)	0.8	−0.1	2.4	9.7
III	2.7 (10.0)	−9.1 (4.0)	17.1 (16.0)	3.6	0.8	7.7	11.9
IV	4.6 (15.0)	−2.9 (8.0)	18.9 (22.0)	6.6	4.7	9.9	13.5

In parentheses, values for Aleppo, Syria are given (source: www.climate-zone.com/climate/syria/celsius/aleppo.htm).

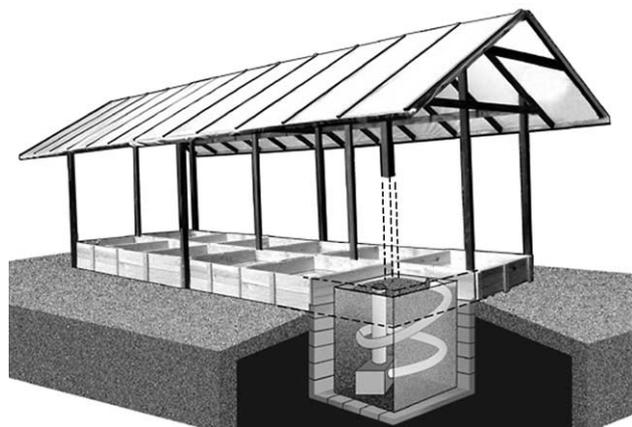


Fig. 1. Outdoor enclosures for golden hamsters used in the present experiments. The animals were housed there from April 2002 to May next year. Details are given in the Material and methods section.

with sand and sawdust. The small wooden box—“hamster’s house” (25×25×20 cm) was buried at the bottom of the plastic cage. A 1.5-m long aluminium corrugated tube (7 cm in diameter) connected this box with the surface of the cage. An additional tube (filled with Styrofoam) was attached vertically to “hamster’s house” and enabled us to take hamster out from the enclosure for experiments. The area between box and cage was filled with soil and sand to ensure better insulation because, in the natural environment, hamsters usually do not experience temperatures below 0 °C. The topside of each enclosure was covered with wire netting. A transparent roof was set up about 2 m above all enclosures to protect the animals from rain, but it still ensured natural photoperiod and temperature. Animals were provided with sawdust and hay as bedding material. They had free access to water and food (seeds, dried vegetables, oat flakes and dried bread). Fresh vegetables or fruits (lettuce, carrot or apple) were supplied every week (in summer—twice a week). In autumn, the amount of sunflower seeds given to the hamsters was increased to mimic natural variations in a diet composition of hibernators (Hill and Florant, 1999).

Environmental conditions were monitored continuously using DataHog 2 Logger (Skye Instruments, UK). Ambient temperature (T_a ; 10 cm above the ground and at the level of 2 m), soil temperature (T_s ; outside the “hamster’s house,” i.e., at a depth of about 60 cm) and light intensity (above the enclosures) were recorded every 1 h.

Animals were weighed before being transferred to outdoor enclosures and then before each series of experiments. At least 6 days before experiment, we implanted a polyethylene cannula (0.8 mm in diameter; 5–7 cm in length depending on the size of the animal; Sims Portex, England) in each hamster under Sevoflurane anaesthesia (Abbott Laboratories, UK). The cannula was inserted under the skin through a small incision (3 mm) at the back of the neck and then fixed with surgical thread and adhesive to the skin. During the experiments, the cannula served as a guide for a

copper–constantan thermocouple (0.6 mm in diameter, W-TW-36 P2; Physitemp Instruments, USA).

After surgery, animals were transferred back to their enclosures.

2.2. Experimental design

Experiments were done in summer (13.07–07.08.2002), late autumn (12.11–07.12.2002) and spring (28.03–18.04.2003). To ensure successful undisturbed hibernation of hamsters, we omitted the winter series of experiments.

About half an hour before each experiment, hamster was removed from its enclosure and transferred to the laboratory where it was tested individually in the thermal gradient system that allowed for continuous and simultaneous measurements of preferred ambient temperature (PT_a), body temperature [measured as brown adipose tissue (BAT) temperature, T_{BAT}] and activity. T_{BAT} was measured with the copper–constantan thermocouple wire (0.6 mm in diameter, W-TW-36 P2; Physitemp Instruments) to 0.1 °C. Thermocouple wire was inserted into the cannula at the depth of interscapular region, where large BAT deposits are present, and fixed with a small piece of adhesive tape. Before each experiment, BAT location was examined carefully on the basis of temperature recordings, namely, the highest temperature corresponded to the location of BAT. Thermal gradient system consisted of long aluminium chamber (120 cm length×10 cm height×8 cm width) divided by half-width partitions into 16 compartments of the same size and covered with transparent Perspex to permit light entry. A narrow slit in the lid allowed movement of the thermocouple that was suspended above the gradient by an elastic band. Such design allows hamster to move freely inside the gradient without a load. The system was heated at one end and cooled at the other end, resulting in a range of temperatures increasing linearly from 0 to 45 °C. Infrared photoemitter–photodetector pairs placed in each compartment detected position of animal. Activity was measured using commercial ultrasound motion sensors that register animals’ movements as all or nothing response. Thus, activity is presented as the percent of the time of experiment when animals were active. T_{BAT} , PT_a and activity were recorded at 1-s intervals and saved on disk. The apparatus was set-up in the room with natural photoperiod. Light intensity was measured with calibrated light sensor in arbitrary units.

Each animal was placed in the thermal gradient box for 3 days. On the first day of experiments, the thermocouple wire was inserted into the cannula, and hamster was put into the gradient box. While in the gradient, food and water were offered to the hamster ad libitum.

To avoid the influence of food searching on temperature selection, eight feeders were placed equidistantly along the gradient. If hamsters chewed their thermocouple lead wires, the break was immediately repaired. During lead repair, animals were handled for no more than 15 min.

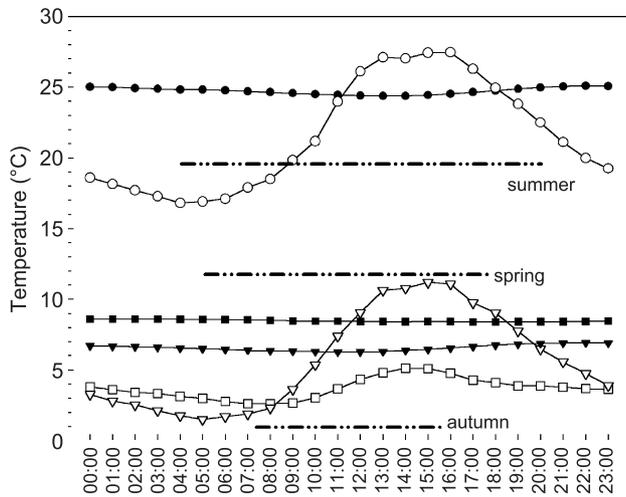


Fig. 2. Mean daily changes in ambient temperature (T_a , open symbols) and soil temperature (T_s , closed symbols) in the outdoor enclosure during the time when experiments in the thermal gradient system were done (about 3 weeks during each season). Circles—summer, squares—autumn, triangles—spring. Horizontal lines indicate duration of the light phase of day.

After the experiment, each hamster was transferred back to its enclosure and remained there until the next series of experiments.

2.3. Statistical analysis

The first day of each experiment was excluded from analysis. If a hamster chewed its lead, data collected for 1 h after the repair were discarded. In each hamster, all data recorded within 48 h were averaged in 1-min intervals, and then mean T_{BAT} , PT_a and activity during the light (day) and dark (night) phase of the day as well as daily mean were calculated. Daily amplitudes of T_{BAT} and PT_a rhythms were calculated in each hamster as the difference between mean values during day and night. Maximum daily variations in T_{BAT} and PT_a rhythms were calculated as the difference between maximum and minimum values, where the maximum and minimum values were defined as the mean of the lowest and the highest readings recorded in each hamster during experiment, respectively. Additionally, changes in T_{BAT} , PT_a and activity at light-to-dark and dark-to-light transitions (mean of recordings from 1 h before and 1 h after the beginning of the light or dark phase) were analysed separately. The influence of season and time of day on T_{BAT} , PT_a and activity, as well as seasonal changes in body mass, were analyzed using repeated measures analysis of variance (ANOVA) followed by Tuckey's post hoc test. In Figs. 4–7, mean values for all six hamsters are presented. In Figs. 4–6, 48-h recordings are presented, and each point is an average of the previous 30 min. In Fig. 7, 2 h of experiment are presented (1 h before and 1 h after the beginning of the dark phase), and each point is an average of the previous

1 min. The Pearson correlation coefficient r was used to test for correspondence between T_{BAT} and PT_a as well as between T_{BAT} or PT_a and activity.

Differences were considered statistically significant if $P < 0.05$. All values are presented as mean \pm S.E.

3. Results

3.1. Environmental conditions

The mean outdoor ambient temperature (T_a , measured 10 cm above the ground) during the time of experiments was 21.6 °C (minimum=11.1; maximum=37.1 °C) in summer, 3.7 °C (minimum=-8.8; maximum=14.6 °C) in autumn and 5.6 °C (minimum=-2.4; maximum=21.9 °C) in spring. Mean T_a measured at the level of 2 m above the ground differed from the above ones by about 0.5 °C. Mean soil temperature (T_s) was always above 0 °C: in summer, 24.8 °C (21.7 to 27.2 °C), in autumn, 8.5 °C (4.6 to 10.1 °C) and, in spring, 6.6 °C (4.7 to 9.9 °C). Mean daily changes in T_a and

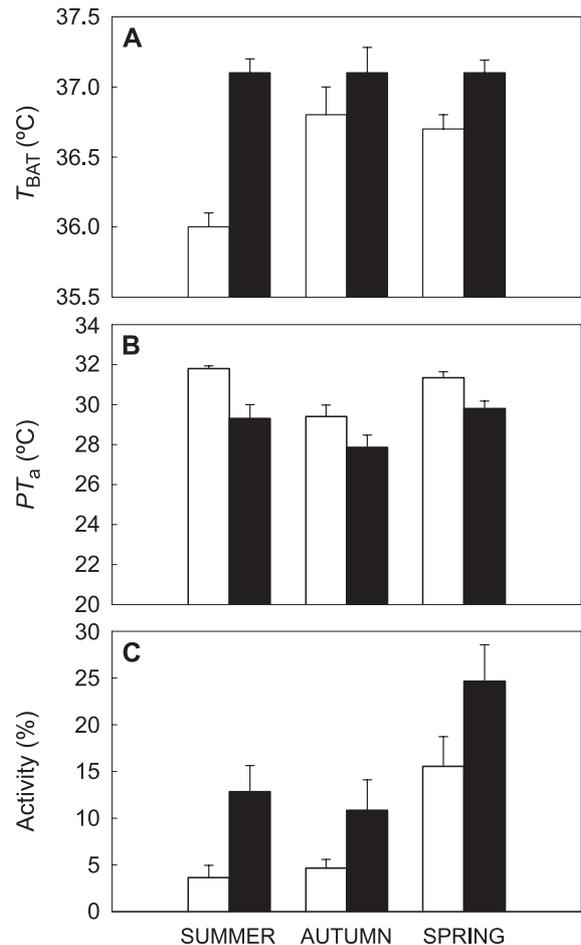


Fig. 3. Mean (\pm S.E.) body temperature (T_{BAT} ; A), preferred ambient temperature (PT_a ; B) and activity (C) of golden hamsters during day (open bars) and night (black bars) recorded in summer, autumn and spring experiments.

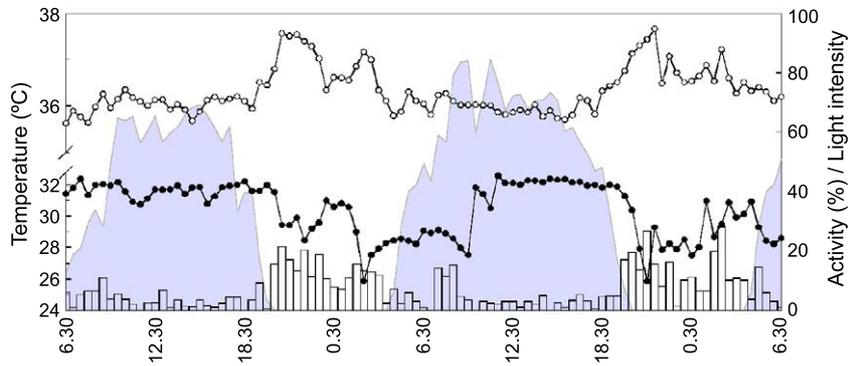


Fig. 4. Forty-eight-hour recording of body temperature (T_{BAT} , ○), preferred ambient temperature (PT_a , ●) and activity (bars) of golden hamsters during summer experiments. Values are mean for six hamsters, and standard errors are not presented for clarity. Each point is the average of the previous 30 min. Shaded area shows light intensity.

T_s at the time of experiments are presented in Fig. 2. In summer and spring, the differences between mean T_a by day and by night were much larger than in autumn. The lowest T_a was recorded before sunrise. During spring and summer, T_a started to increase with the beginning of the light phase of day, reaching its maximum a few hours before dusk. In autumn, T_a started to increase a few hours after dawn, and it was highest at the end of day. T_s was relatively constant during the 24-h cycle, and, in autumn, this temperature was always higher than T_a . Light phase of the day lasted for 16.8 h in July and 15.5 h in August. In autumn, it was much shorter (8.4 h in November and 7.7 h in December). Then, it gradually lengthened. Finally, in March, light phase of the day lasted for 11.9 and 13.5 h in April. Detailed data on air and soil temperatures and duration of the light phase of the day from May 2002 to April 2003 are presented in Table 1.

3.2. Seasonal changes in body mass (m_b)

Time of the year significantly affected mean body mass [$F(2,10)=11.33$, $P<0.003$]. Mean m_b of hamsters in summer was 122.4 ± 6.4 g. In autumn, it was lower by 17% (101.7 ± 5.8 g, $P<0.05$). The lowest m_b was recorded in spring (97.1 ± 4.3 g) when hamsters were significantly lighter than in summer ($P<0.02$).

3.3. Seasonal and daily changes in body temperature (T_{BAT})

Season and time of day significantly affected mean T_{BAT} [$F(2,10)=7.22$, $P<0.015$]. Mean T_{BAT} during day was significantly lower than mean T_{BAT} at night only in summer (36.0 ± 0.1 and 37.1 ± 0.1 °C, respectively; $P<0.001$). Mean T_{BAT} during day in summer was also lower than mean T_{BAT} during day in autumn ($P<0.01$) and spring ($P<0.03$; Fig. 3A). In summer, T_{BAT} increased rapidly at the beginning of the dark phase of the day, and then second peak was recorded in the latter part of the night (Fig. 4). Maximum daily variations in T_{BAT} were also dependent on season and time of day [$F(2,10)=5.23$, $P<0.03$]. During day, these variations were similar within all seasons, while, during night, they were larger in autumn (3.1 ± 0.1 °C, Fig. 5) than in summer (2.3 ± 0.1 °C, $P<0.01$, Fig. 4) and spring (2.3 ± 0.2 °C, $P<0.02$, Fig. 6).

3.4. Seasonal and daily changes in preferred ambient temperature (PT_a)

Season significantly affected mean PT_a [$F(2,10)=7.77$, $P<0.01$]. In autumn, golden hamsters preferred lower T_a than in summer ($P<0.02$) and spring ($P<0.02$; Fig. 3B). In all season, PT_a was higher during day than during night

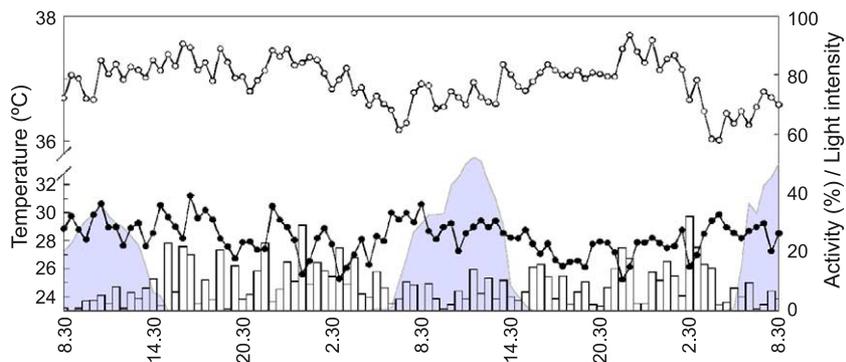


Fig. 5. Forty-eight-hour recording of body temperature (T_{BAT} , ○), preferred ambient temperature (PT_a , ●) and activity (bars) of golden hamsters during autumn experiments. Values are mean for six hamsters, and standard errors are not presented for clarity. Each point is the average of the previous 30 min. Shaded area shows light intensity.

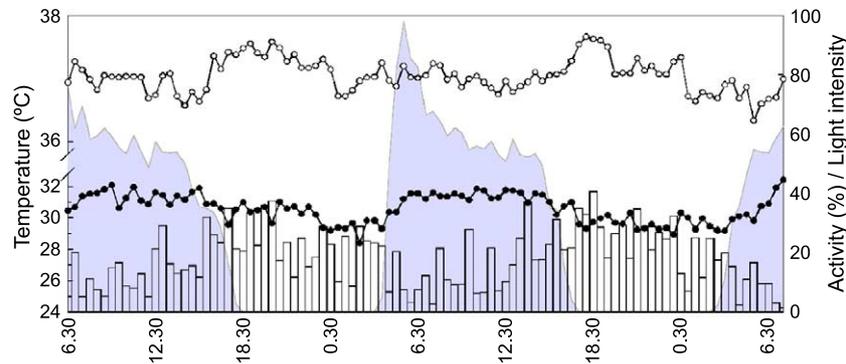


Fig. 6. Forty-eight-hour recording of body temperature (T_{BAT} , ○), preferred ambient temperature (PT_a , ●) and activity (bars) of golden hamsters during spring experiments. Values are mean for six hamsters, and standard errors are not presented for clarity. Each point is the average of the previous 30 min. Shaded area shows light intensity.

[$F(1,5)=44.89$, $P<0.002$]. The range of temperatures explored by hamsters was highest in autumn (Fig. 5) and least in spring ($P<0.002$; Fig. 6), and it was always larger during night than during day [$F(1,5)=30.98$, $P<0.003$].

In summer and autumn, significant inverse correlation between mean daily PT_a and T_{BAT} was recorded ($r=-0.60$, $P<0.01$); that is, the higher T_{BAT} means the lower PT_a .

3.5. Seasonal and daily changes in the locomotor activity

Golden hamsters were significantly more active in spring than in summer and autumn [$F(2,10)=9.53$, $P<0.005$; Fig.

3C]. Independently of season, activity was higher by night than by day [$F(1,5)=36.22$, $P<0.002$]. In summer, nighttime activity was 3.6-fold higher than daytime one and 2.4-fold in autumn. Despite the highest activity in spring, in this season, day–night difference was the smallest (Fig. 3C).

Only in summer T_{BAT} was correlated with activity ($r=0.60$, $P<0.005$), while activity and PT_a were inversely correlated in autumn ($r=-0.50$, $P<0.03$) and spring ($r=-0.62$, $P<0.002$).

3.6. Changes in body temperature (T_{BAT}), preferred ambient temperature (PT_a) and activity during light-to-dark and dark-to-light transitions

In summer, during light-to-dark transition, significant increase in T_{BAT} [$F(2,10)=6.25$, $P<0.02$] and activity [$F(2,10)=4.27$, $P<0.05$] was recorded, while PT_a lowered during this time [$F(2,10)=6.56$, $P<0.02$; Fig. 7]. In autumn, there were no significant changes in T_{BAT} , PT_a and locomotor activity during light-to-dark transition. In spring, significant increase in T_{BAT} was recorded again [$F(2,10)=6.25$, $P<0.02$]. Dark-to-light transition did not induce any significant changes in T_{BAT} , PT_a and activity in any season.

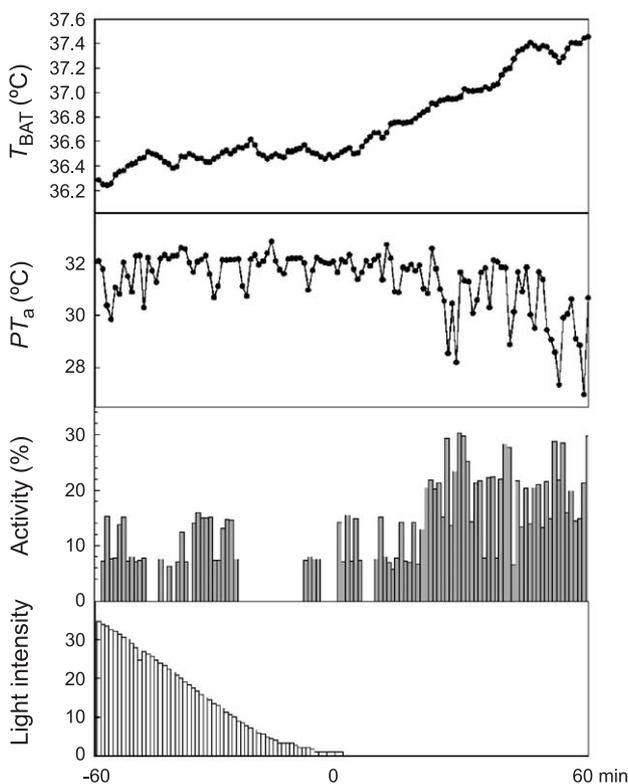


Fig. 7. Changes in body temperature (T_{BAT}), preferred ambient temperature (PT_a) and activity of golden hamsters during light-to-dark transition in summer. Values are mean for six hamsters, and standard errors are not presented for clarity. Each point is the average of the previous 1 min.

4. Discussion

Circadian rhythms are investigated mostly in animals maintained in strictly controlled environments to standardize the research protocol. However, experiments on animals living under natural or seminatural conditions can provide more realistic and comprehensive information about the role of the environmental factors that, acting in concert, may affect the physiological and behavioral responses of the animals in question. Until now, only scarce studies paid attention to changes in the daily rhythm of body temperature that occur during the transitional seasons (autumn and spring), between summer activity and winter inactivity, in hibernating animals continuously exposed to natural changes in the environment (Wollnik and Schmidt, 1995).

4.1. Environmental conditions

In the present experiments, laboratory golden hamsters were housed under seminatural outdoor conditions of Central Poland. The “hamster’s houses” were located at the depth of 60 cm. Recently, Gattermann et al. (2001) described burrow structures of wild golden hamsters. They found that the nest chambers were located at the same depth as in our experiments, namely, at 58.3 ± 12.7 cm. Furthermore, the size of these chambers was similar to the size of “hamster’s houses” that we used in our enclosures. In the natural environment, daily and seasonal fluctuations in soil (burrow) temperature are smaller than fluctuations in air temperature (Schmidt-Nielsen, 1964). Deep and branched burrows ensure proper microclimate and protect hamsters from excessive heat in summer and cold in winter. In the present study, during winter, when ambient temperature (T_a) was as low as -20 °C, mean T_s never dropped below 0 °C. Double-walled cages protected the hamsters from marked fluctuations in soil temperature (T_s) as well as temperatures below 0 °C (Table 1). On the contrary, in summer, when maximum T_a exceeded 34 °C, mean T_s at the depth of the “hamster’s house” was lower by about 10 °C. During the time of experiments, the daily variations in T_s did not exceed 0.5 °C. Similar short-term fluctuations in T_s were recorded under natural environment of golden hamsters (Gattermann et al., 2001). In addition, “hamster’s houses” were fully filled with hay and sawdust, providing large insulation. Similarly to natural burrows (Gattermann et al., 2001), the entrance to the tube connecting “hamster’s house” with the surface was plugged with a lump of soil, small stones and bedding materials. Thus, we may expect that the temperature of the nest was slightly higher in winter and lower in summer when compared to T_s at the same depth as the “hamster’s house.”

4.2. Body temperature (T_{BAT})

The level at which T_b is regulated is an outcome of heat loss and heat production. Both processes undergo daily variations, contributing to the daily rhythm of core body temperature [T_b , (Aschoff, 1981; Refinetti and Menaker, 1992; Brown and Refinetti, 1996)]. In the present experiments, body temperature was measured as the temperature of brown adipose tissue (T_{BAT}). In golden hamsters, brown adipose tissue (BAT) is a major thermoregulatory effector responsible for heat generation during nonshivering thermogenesis [NST, (Janský, 1973)]. BAT capacity for NST is increased towards winter to cope with low ambient temperatures and to ensure large amount of heat that is necessary during arousal from hibernation (Smalley and Dryer, 1963, 1967; Hayward and Lyman, 1967; Jefimow et al., 2004). During cold exposure, other heat source, namely, shivering thermogenesis, plays more important role in warm-acclimated than in cold-acclimated hamsters (McNab, 2002).

We predicted that T_{BAT} would be highest in autumn due to increased capacity for NST. Indeed, T_{BAT} was correlated with season and was highest in autumn and least in summer (Fig. 3A). In European hamsters (*Cricetus cricetus*), core body temperature (T_b) during euthermy is lower in winter than in summer (Wollnik and Schmidt, 1995), which may be related to the lowering of a set point for T_b regulation due to exposure to short photoperiod (Heldmaier et al., 1989). The results of present experiments do not contradict the hypothesis of lowering of a set point because we did not measure core T_b but temperature of brown adipose tissue. In summer, hamsters experienced neither low T_a nor short photoperiod (SP), and thus capacity for NST was lower. In autumn, T_{BAT} was much higher than in summer, which implies an increase in BAT efficiency (NST capacity). However, hamsters chose lower PT_a (Fig. 3A and B). Such preferences might result from higher T_{BAT} , but, assuming that core T_b was lowered in autumn, it can be suggested that set point for T_b regulation also could be lowered. In previous experiments done on golden hamsters housed under the same conditions, we found that the noradrenaline-induced NST was larger in autumn than in summer and spring (Jefimow et al., 2004). Short photoperiod and cold, i.e., environmental conditions prevailing in autumn, are the main cues for seasonal improvement of NST (Heldmaier et al., 1985, 1990; Heldmaier and Lynch, 1986). Thus, higher T_{BAT} level that we recorded in autumn is a result of increased capacity for NST.

In Djungarian hamsters, increased capacity for NST and lowered set point for T_b regulation can be accompanied by large ultradian variations in T_b (Heldmaier et al., 1982, 1989), which allows for energy savings additionally to daily torpor (Heldmaier et al., 1985). If golden hamsters are kept in the laboratory under SP and cold, they also display large ultradian variations in T_b (Janský, 1989). Both daily torpor and hibernation are used to reduce energy requirements during winter, and thus we can assume that, in both cases, large variations in T_b would allow for energy savings. In fact, in the present experiments, the largest difference (3.1 °C) between maximum and minimum T_{BAT} was recorded in autumn.

We predicted that the daily amplitude of T_{BAT} rhythm would be dampened towards winter. Indeed, daily amplitude of T_{BAT} rhythm was the largest in summer (1.1 ± 0.1 °C), while, in autumn, this amplitude declined to the non-significant level of 0.2 ± 0.1 °C. Seasonal reduction in the daily amplitude of T_b was recorded also in European hamsters housed under seminatural conditions. During normothermia, the daily rhythms of T_b were expressed in spring and summer but not in autumn and winter (Wollnik and Schmidt, 1995). The reduction in the daily amplitude of T_b can be a sign of preparation for hibernation, although existence of T_b rhythm during hibernation is controversial. It seems to be dependent on the level at which T_b is regulated (Barnes and Ritter, 1993; Zucker et al., 1993; Grahn et al., 1994; Waßmer and Wollnik, 1997; Florant et

al., 2000). In golden hamsters hibernating under short photoperiod (L8: D16) and cold (7 to 10 °C), when T_b was regulated at the level of about 12 °C, T_b rhythm was not recorded (Janský, 1989). In the present experiments, T_a in autumn was always lower than T_s and showed smaller daily fluctuations than in summer or spring, giving information about forthcoming winter. We conclude that the hamsters began to prepare for hibernation, and, as a result, T_{BAT} rhythm was dampened.

We predicted that the daily amplitude of T_{BAT} rhythm would be restored in spring. In fact, day–night difference in T_{BAT} in spring (0.4 ± 0.1 °C) was slightly higher than in autumn (0.2 ± 0.1 °C), but this difference was not significant. However, in spring, T_{BAT} increased significantly during light to dark transition, as in summer. During spring and summer, T_a after dusk was lower than T_s , while beginning of the light phase of day coincided with the increase in T_a , which finally, in the middle of day, exceeded T_s (Fig. 2). The hamsters could easily perceive these clear changes in the environment. In the laboratory experiments (L14: D10 that could correspond to early summer), the acrophase of the T_b rhythm occurs within 1 h after the lights are turned off (Refinetti, 1996a). Onset of the rise in T_b precedes activity onset by 1 h (DeCoursey et al., 1998). The increase in T_{BAT} during the first hour of darkness implies that T_{BAT} rhythm is restored gradually in spring and after hibernation hamsters return to their “summer status.” The rhythm of preferred ambient temperature (PT_a) seems to be secondary to the T_{BAT} rhythm because PT_a during light to dark transition in spring did not change significantly.

4.3. Preferred ambient temperature (PT_a) and activity

In the present study, we predicted that PT_a would follow seasonal changes in T_{BAT} . Indeed, mean PT_a was correlated with season. Although we did not measure thermal conductance, we can suppose that season also affected hamsters’ fur insulation and heat loss, contributing to the changes in selected temperatures. The lowest mean PT_a was recorded in autumn, i.e., when mean T_{BAT} was the highest. Within all seasons, PT_a during the day was significantly higher than at night. Except for the temperature preferred during nighttime in autumn, PT_a was near 30 °C, which corresponds to the thermoneutral zone of golden hamsters (Tęgowska and Gębczyński, 1975).

In summer and autumn, PT_a was inversely correlated with T_{BAT} . Refinetti (1995) found that, in a thermal gradient under L14: D10 photoperiod, daily rhythm of T_b coincided with activity and was 180° out of phase with the rhythm of PT_a . The opposition of these two rhythms was recorded also in other rodents, both diurnal and nocturnal (Refinetti, 1996b, 1998a,b). Such a preference for lower T_a during high phase of T_b may prevent an overheating (Gordon, 1994). However, in spring, there was no correlation between T_{BAT} and PT_a , but minimum PT_a at night was much higher than in autumn and summer (Figs. 4–6). The hamsters could prefer

higher T_a to reduce body–ambient temperature difference. Such reduction between T_b and T_a is crucial for energy savings (Studier, 1981; Webb et al., 1993). It can be suggested that, after hibernation period, adipose tissues, both white and brown, were depleted; and because body mass in spring was lower by 17% than in summer, the hamsters preferred higher T_a to reduce energy expenditure for thermoregulation.

A smaller range of T_a explored by hamsters during spring was not related to activity level (Fig. 3C). The increase in activity in spring can reflect changes in animal behaviour during a reproductive period. In European hamsters, temporal pattern of activity coincides with seasonal cycle of reproduction, namely, activity is highest in spring and early summer (Wollnik et al., 1991). In the natural environment, European hamsters are sexually active at the end of hibernation period, i.e., in March–April (Saboureau et al., 1999). In the present experiments, activity was higher by night than by day in all seasons, but, in spring, this difference was the smallest. Hamsters were very active throughout day and night, moving not only along the thermal gradient box but also changing their position within a small range of compartments. Although activity of golden hamsters predominates at night, short bursts of activity also occur during the light phase of the day (Song and Rusak, 2000). Under laboratory conditions (L14: D10), 80% of total activity occurs during darkness. Activity rhythms of laboratory and wild hamsters are similar (Weinert et al., 2001), thus high activity that we recorded in spring might be connected with reproductive period.

5. Summary

Current experiments confirmed our hypothesis that season strongly affects the daily rhythms of T_{BAT} , PT_a and activity in laboratory golden hamsters housed under semi-natural conditions.

The hamsters experienced natural seasonal changes in T_a and photoperiod. Daily changes in T_a as well as the difference between T_a and T_s provided important information about time of year. T_{BAT} was increased in autumn, indicating increased NST capacity in response to shortening photoperiod and lowering T_a , while the decrease in PT_a indicated the lowering of set point for T_b regulation. The decrease in the daily variations in T_{BAT} coupled with an increase in the maximum daily variations in T_{BAT} that were recorded in autumn might allow for energy savings and successful preparation for the hibernation season. Renewed increase in T_{BAT} during light-to-dark transition in spring indicates reestablishment of T_{BAT} daily rhythm.

In conclusion, the daily rhythms of T_{BAT} , PT_a and activity were adjusted during the course of the year to natural changes in both, photoperiod and ambient temperature. These rhythms have not been suppressed by long history of breeding in captivity and still can ensure proper

adjustment to the natural environment. In the present study, all hamsters exposed to outdoor conditions survived winter successfully. The climatic conditions in Poland are not the same as in Syria, however, animals can experience large daily and seasonal variations like in their natural habitat. Although ambient temperatures (T_a) are higher in Syria than in Poland, the pattern of seasonal changes is the same. In both regions, the lowest T_a is recorded from December to February and the highest from June to August. The difference between minimum and maximum monthly means during the year is also similar (23 and 24 °C; Table 1). Thus, the pattern of seasonal changes in the thermoregulation of golden hamsters that was recorded in the present experiments might be applied to wild-living hamsters.

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