Effects of Prolonged Acclimation to Intermediate Photoperiod and Photoschedule Reversal in Photosensitive Golden Hamsters

MAŁGORZATA JEFIMOW*, MICHAŁ S. WOJCIECHOWSKI, AND EUGENIA TĘGOWSKA
Department of Animal Physiology, Institute of General and Molecular Biology, N. Copernicus University, ul. Gagarina 9, PL 87-100 Toruń, Poland

ABSTRACT We investigated the effect of prolonged acclimation to 12 hr of light and photoschedule reversal during the time of photosensitivity in golden hamsters (Mesocricetus auratus). Before the experiments, animals were housed under natural photoperiod and then transferred to 12L:12D (light 12 hr:dark 12 hr) in autumn for 12 weeks. After 4 weeks of acclimation, photoschedule was reversed (12D:12L). First experiments were done after 4 weeks of acclimation to an ambient temperature ($T_a$) of 23°C and a 12L:12D photoschedule. We examined the daily variations in brown adipose tissue (BAT) capacity for nonshivering thermogenesis (NST). Noradrenaline (NA) injections were given every 4 hr while BAT temperature ($T_{BAT}$) and preferred ambient temperature ($PT_a$) were monitored continuously and simultaneously in a thermal gradient system. Then, we investigated the effect of light–dark cycle reversal on a daily rhythm of NST. The hamsters were acclimated to the photoschedule reversed by 12 hr and the same $T_a$. After 4 and 8 weeks of acclimation to a reversed photoschedule, the experiments were repeated. We found that the daily rhythm of the response to NA was entrained to the new light–dark cycle after 4 weeks of acclimation to a reversed photoschedule. Maximum effect of NA was always recorded during the light phase and in the latter part of the dark phase of the day. NA-induced increase in $T_{BAT}$ was correlated with the decrease in $PT_a$ and was also inversely correlated with pre-injection $T_{BAT}$. These data imply that the daily rhythm of the capacity for NST opposes the daily rhythm of body temperature ($T_b$). After 8 weeks of acclimation to the reversed photoschedule, the rhythmicity of the response to NA disappeared, and the daily fluctuations in $T_{BAT}$ were the smallest. This lack of rhythm may be a physiological adaptation to winter conditions when the daily amplitude of $T_b$ rhythm is markedly reduced and, as a consequence, NST capacity does not vary within the day. Moreover, after 8 weeks of acclimation to reversed photoschedule, NST capacity decreased while response to saline increased. During the experiments, hamsters were photosensitive and were changing to their winter status. However, because of the lack of cold during acclimation, the capacity for NST did not increase. Increased responsiveness to saline, indicating an increase in stress-induced thermogenesis, might be advantageous for “fight or flight” reaction. J. Exp. Zool. 303A:987–997, 2005. © 2005 Wiley-Liss, Inc.

Life at higher latitudes requires seasonal changes in physiology and behavior to cope with unfavorable environmental conditions during cold months on the one hand, and to breed with success at the appropriate time of year on the other (Goldman, 2001; Goldman et al., 2004). The Golden hamster is a highly photoperiodic mammal living in northern Syria (36–37°N) where winter is cold and wet whereas summer is hot and dry (Gattermann et al., 2001). Large seasonal variations in ambient temperature and photoperiod result in the seasonal changes in physiology and behavior. Day length is the environmental cue that synchronizes reproduction with the time of year. In spring when day length exceeds 12.5 hr, hamsters enter their breeding season, while in autumn, shortening photoperiod (for less than 12 hr) induces gonadal regression and breeding is terminated (Vitaterna and Turek, ’93; Gorman and Zucker, ’98; Goldman, 2001; Goldman et al.,

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*Correspondence to: Dr. Małgorzata Jefimow, Department of Animal Physiology, Institute of General and Molecular Biology, N. Copernicus University, ul. Gagarina 9, PL 87-100 Toruń, Poland. E-mail: jefimow@biol.uni.torun.pl
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spontaneous gonadal re-growth (Goldman et al.,
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experiments were done on golden hamsters in
summer status (Jefimow et al., 2005). The present
reversal during the time of photorefractoriness did
photoperiod (12 hr of light) and light–dark cycle
found that prolonged acclimation to the transient
photoperiod in autumn would not affect capacity for
NST if an ambient temperature ($T_b$) would be maintained at a constant level of
23°C. However, although photoperiod does not
influence capacity for NST, it significantly affects
daily amplitude of body temperature ($T_b$). In
golden hamsters housed under a short photoper-
iod, daily amplitude of $T_b$ rhythm is markedly
reduced and during hibernation $T_b$ rhythm is not
even detectable (Janský, ’89). In our experiments
on Siberian hamsters (Jefimow et al., 2005), we
found that NST capacity was inversely correlated
with $T_b$. On the basis of our hypothesis, we
therefore predicted that prolonged acclimation of
golden hamsters to 12 hr of light would result in
dampening of $T_b$ rhythm [measured as BAT
temperature ($T_{BAT}$) rhythm] and as a conse-
quence, daily variations in NST capacity would
also be reduced. In our experiments on Siberian
hamsters, animals that during acclimatization rely
almost solely on photoperiod, $T_b$ rhythm was the
first one that was re-entrained after photosche-
dule reversal. Re-entrainment of the NST rhythm
was delayed by a few weeks, relative to the $T_b$
rhythm (Jefimow et al., 2005). Since in golden
hamsters the most important signal for winter
acclimatization and the increase in NST capacity
is not photoperiod but ambient temperature
(Janský et al., ’84), we expected that NST rhythm
would be reversed simultaneously to the rhythm
of body temperature.

To test our predictions, we housed golden
hamsters under natural photoperiod and then
transferred them to 12L:12D in autumn for 12
weeks. After the first 4-week period of acclimation,
we reversed the photoschedule by 12 hr. We
measured daily variations in the NST capacity as
changes in BAT temperature ($T_{BAT}$) and preferred
ambient temperature ($PT_a$) in response to nor-
adrenaline (NA) injections administered every 4 hr.
Since NA activates NST and induces hyperthermia

The response to a prevailing photoperiod is also
influenced by photoperiodic history of animals
(Gorman and Zucker, ’98; Goldman et al., 2004).
The direction of change in photoperiod (from
winter to summer or vice versa) is of great
importance since transient day lengths in tempe-
rate zone occur twice a year, i.e., in spring and
autumn. Thus, photoperiodic memory ensures
physiological and behavioral changes towards the
summer or winter status even in the absence of
any environmental signals.

Decreasing day length and low ambient tem-
perature are the main exogenous cues indicating
forthcoming winter (Janský, ’84). In autumn,
hamsters begin to prepare for winter hibernation.
Capacity for nonshivering thermogenesis (NST), a
very important source of heat, is similar in
hamsters acclimated to short and long photoper-
iods (Viswanathan et al., ’86), but it is greatly
improved after acclimation to cold (Janský, ’73).
Thus, NST capacity is low during summer and
high during autumn and winter. Besides seasonal
changes, daily variations in NST capacity are also
recorded and depend on season (Jefimow et al.,
2004).

In the experiments on Siberian hamsters, we
found that prolonged acclimation to the transient
photoperiod (12 hr of light) and light–dark cycle
reversal during the time of photorefractoriness did
not prevent animals from the transition to the
summer status (Jefimow et al., 2005). The present
experiments were done on golden hamsters in
autumn, i.e., during the time of photosensitivity.
While photorefractoriness is driven endogenously,
photosensitivity is determined by day length
(Gorman and Zucker, ’98). In the natural envir-
onment, photosensitivity is restricted to autumn
when hamsters are sensitive to short days and
exhibit gonadal regression whereas photorefrac-
toriness occurs in early spring and is defined by
spontaneous gonadal re-growth (Goldman et al.,
2004). The concept of these two physiological
states: photosensitivity and photorefractoriness,
originates from the studies on photoperiod and
reproduction; however, it is not restricted only to
reproduction but can also be applied to thermo-
regulation and behavior.

Based on the above facts, we hypothesized that
in the photosensitive golden hamsters, prolonged
acclimation to the transient photoperiod (12 hr of
light) would not affect total brown adipose tissue
(BAT) capacity for NST but would influence daily
variations in NST. Unlike in Siberian hamsters, in
golden hamsters cold is a fundamental prerequi-
site for winter acclimatization and seasonal
improvement of NST, while shortening photoper-
iod has only a minor effect (Janský et al., ’84).
That is why we predicted that prolonged exposure
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(Janský, ’73), the magnitude of changes in BAT temperature after NA would reflect the intensity of regulatory NST. When NST is activated, hamsters would prefer lower ambient temperatures to facilitate the maintenance of normothermic body temperature (Gordon, ’93).

**MATERIAL AND METHODS**

**Animals and housing**

Experiments were done on nine adult (5-month old) male golden hamsters (*Mesocricetus auratus*). Before the first experiments were started in September, the animals were housed individually under natural photoperiod (12.5L:11.5D in the middle of September) in standard laboratory cages (36 × 28 × 20 cm). Then, the hamsters were transferred to a climate chamber on a 12L:12D cycle (L:D cycle; lights on at 09:00), with an ambient temperature (*T_a*) of 23°C. Food and water were available ad libitum. After 4 weeks, the first set of measurements was made (L:D cycle). During the next 4 weeks, the hamsters were housed in the same climate chamber, but with a reversed photoschedule, i.e., 12D:12L (D:L cycle; lights on at 21:00 hr) at a *T_a* of 23°C. After 8 weeks under the reversed photoschedule, the animals were sampled again. Photoschedule reversal was accomplished by the prolongation of the light phase of the cycle, i.e., on the day of reversal, the hamsters experienced 24L:12D.

After acclimation to L:D cycle, six out of nine animals were used. After 4 weeks of acclimation to D:L cycle, three previously used hamsters were replaced with new ones that were acclimated simultaneously, but were not used in the first set of experiments. This replacement was applied to evaluate whether injections during the first experiment could affect the results of the second one. After 8 weeks of acclimation to D:L cycle, six hamsters used in the previous experiments were used again.

Before each set of experiments (every 4 weeks), the hamsters were weighed to ±0.1 g to follow possible seasonal changes of body mass (*m_b*).

**Measurements of *T_BAT* and *PT_a***

At least 3 days before the experiment, we implanted polyethylene cannula (0.8 mm in diameter, 6–8 cm in length, SIMS Portex Ltd., Hythe, UK) in each hamster under Sevorane anesthesia (Abbott Laboratories Ltd., Queensborough, UK). This 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 Inc., Clifton, NJ, USA).

After acclimation to L:D and D:L cycles, each animal was tested individually in a thermal gradient system that allowed for continuous and simultaneous measurements of preferred ambient temperature (*PT_a*) and BAT temperature (*T_BAT*). The thermal gradient consisted of a long aluminum 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. The system was heated at one end and cooled at the other, resulting in a range of temperatures increasing linearly from 5°C to 45°C. The position of each animal, and thus its selected temperature, was detected at 1 sec intervals by infrared photoemitter–photodetector pairs placed in each compartment. A narrow slit in the transparent lid allowed movement of the thermocouple that was suspended above the gradient by an elastic band. This design allowed hamsters to move freely inside the gradient without a load and permitted light entry. *T_BAT* and *PT_a* were automatically recorded at 1-sec intervals and saved on disk.

**Experimental design**

**Response to NA**

Each animal was placed in the thermal gradient box for 3 days. On the first day, the thermocouple wire was inserted into the cannula to a depth where large interscapular BAT deposits are present. Then the hamster was left undisturbed to enable habituation. On the next day, beginning at 09:00, NA ([(+)-Arterenol, Sigma-Adrich Chemie GmbH, Steinheim, Germany] at a dose of 0.6 mg kg⁻¹ or saline (in the same volume as NA) was injected subcutaneously every 4 hr. Saline injections were a control for NA; while NA activates NST, changes in body temperature after saline reflect stress of injection. Nighttime injections were given under dim red light. Half of the animals were injected with NA on the second day and then with saline on the third. The sequence of injections in the other half of animals was reversed (1st day—undisturbed, 2nd day—saline, 3rd day—NA). *T_BAT* and *PT_a* in saline and NA
injected hamsters were measured continuously. Temperatures before each injection were used to assess daily rhythms of $T_{BAT}$ and $PT_a$. After acclimation to L:D cycle, injections were given at: 09:00 (CT 0; circadian time 0; beginning of the light phase), 13:00 (CT 4), 17:00 (CT 8), 21:00 (CT 12), 01:00 (CT 16) and 05:00 (CT 20). After acclimation to the reversed photoschedule (i.e., after 4 and 8 weeks in D:L cycle), injections were given at: 09:00 (CT 12), 13:00 (CT 16), 17:00 (CT 20), 21:00 (CT 0), 01:00 (CT 4) and 05:00 (CT 8). While in the thermal gradient, hamsters were offered food and water ad libitum. Eight feeders were placed equidistantly along the gradient to avoid the influence of food searching on $PT_a$. If hamsters chewed their thermocouple lead wires, the break was repaired immediately. During lead repair, animals were handled for no more than 15 min.

**Statistical analysis**

All recorded data were plotted at 10 min intervals as mean ± SE. To assess daily rhythms of BAT temperature ($T_{BAT}$) and preferred ambient temperature ($PT_a$), data collected 30 min before each injection were analyzed. To assess effects of saline and NA on $T_{BAT}$ and $PT_a$, data collected 30 min before (as a reference) and 60 min after each injection were compared. Sequence of injections did not influence $T_{BAT}$ or $PT_a$ (two-way ANOVA, $P = 0.68$). There was no difference in the response to saline or NA by hamsters that were used for the first time or re-tested (two-way ANOVA, $P = 0.61$). Changes in $T_{BAT}$ and $PT_a$ induced by saline and NA are presented as the difference between mean $T_{BAT}$ or $PT_a$ recorded within 60 min after-, and 30 min before the injection ($\Delta T_{BAT}$; $\Delta PT_a$). To clarify the results, in the text below, $T_{BAT0}$ refers to BAT temperature before injection and $T_{BAT1}$ refers to BAT temperature after injection.

If hamsters chewed their lead shortly before or after injection, data collected for 1 hr after the repair were excluded from analysis. Changes in $T_{BAT0}$, $T_{BAT1}$ and $PT_a$ were analyzed using three-way ANOVA to investigate the influence of photoschedule, type of injection and time of day. When ANOVA showed significant effect of the analyzed factors, post hoc LSD test followed by a Bonferroni correction was used for comparisons of means. The Pearson correlation coefficient, $r$, was used to test for correspondence between $T_{BAT1}$ and $PT_a$ as well as between $T_{BAT0}$ and $T_{BAT1}$. Changes in body mass ($m_b$) were analyzed using one-way ANOVA followed by Tukey’s post hoc test.

Differences were considered statistically significant if $P<0.05$. All values are presented as mean ± SE.

Experiments were approved by the Local Committee for the Ethics in Animal Research.

**RESULTS**

**The effects of 4 weeks of acclimation to the reversed photoschedule**

**Brown adipose tissue temperature ($T_{BAT}$; Fig. 1)**

After 4 weeks of acclimation to a reversed photoschedule (D:L cycle), the daily rhythm of BAT temperature before injection ($T_{BAT0}$) was entrained to a new D:L cycle but mean $T_{BAT}$ was lower than in L:D cycle (three-way ANOVA: $F_{(3,114)} = 50.62, P < 0.0001$). In L:D and D:L cycles, $T_{BAT0}$ was similarly correlated with time of day (three-way ANOVA: $F_{(3,114)} = 3.80, P < 0.003$) and was slightly higher by night than by day. In L:D cycle, $T_{BAT0}$ ranged from 36.9 ± 0.2°C (CT 4) to 37.3 ± 0.1°C (CT 16), and after 4 weeks of acclimation to a reversed photoschedule, the highest $T_{BAT0}$ was recorded at the beginning of night (CT 12) and the lowest during the day (Fig. 1).

Independently of photoschedule, NA induced larger increase in BAT temperature ($T_{BAT1}$) than saline. The magnitude of changes in $T_{BAT1}$ was correlated with time of day and type of injection (three-way ANOVA: $F_{(3,112)} = 3.80, P < 0.05$). The pattern of daily variations in the response to NA was similar in L:D and D:L cycles (Fig. 1). The largest increase in $T_{BAT1}$ was recorded by day and at the end of the night, while the smallest in the first part of the night. In D:L cycle, daily variations in $T_{BAT1}$ after saline were not recorded but changes in $T_{BAT1}$ at CT 4 and CT 12 were smaller than in L:D cycle ($P<0.001$ and 0.02, respectively). On the contrary, the first saline injection at day (CT 0) induced larger increase in $T_{BAT1}$ in D:L than in L:D cycle ($P<0.001$).

**Preferred ambient temperature ($PT_a$; Fig. 2)**

$PT_a$ before injection was similar in L:D and D:L cycles. $PT_a$ was higher at the end of the light phase of the day than in the middle of the night, but these differences were not significant. After injection, $PT_a$ was correlated only with the type of
injection (three-way ANOVA: $F_{(1,113)} = 13.30$, $P < 0.0004$). Neither time of day nor photoschedule affected temperature selection. In NA-injected animals, decrease in $PT_a$ was much more pronounced than in hamsters injected with saline. After 4 weeks of acclimation to D:L cycle, small daily variations in $PT_a$ after NA were recorded (Fig. 2). Second NA injection at day (CT 4) induced larger decrease in $PT_a$ in D:L than in L:D cycle ($P < 0.05$). Changes in $PT_a$ after saline at the beginning of day (CT 0) and at the end of night (CT 20) were larger in D:L than in L:D cycle ($P < 0.02$ and 0.005, respectively).
Correlation between $T_{BAT0}$, $T_{BAT1}$ and $PT_a$ (Table 1)

In hamsters acclimated to L:D cycle, increase in BAT temperature after NA injection ($T_{BAT1}$) was inversely correlated with BAT temperature before injection $T_{BAT0}$ ($r = -0.50, P < 0.003$, Table 1). Such a correlation was not recorded in saline-injected hamsters. In D:L cycle, this correlation was very high in hamsters injected with NA ($r = -0.77, P < 0.001$) and only moderate in saline-injected ones ($r = -0.39, P < 0.03$). After NA, increase in $T_{BAT1}$ ($\Delta T_{BAT}$) was also highly correlated with decrease in preferred ambient temperature ($\Delta PT_a$), that is, the larger increase in $T_{BAT1}$ means the larger decrease in $PT_a$ ($r = 0.47, P < 0.001$ in L:D and $r = 0.49, P < 0.001$ in D:L cycle). In saline-injected hamsters acclimated to L:D cycle, there was no correlation between $\Delta T_{BAT}$ and $\Delta PT_a$ ($P = 0.43$) while in D:L cycle this correlation was small ($r = 0.20, P < 0.01$).

**The effects of 8 weeks of acclimation to the reversed photoschedule**

**Brown adipose tissue temperature ($T_{BAT}$; Fig. 1)**

After 8 weeks under the reversed photoschedule, we did not record a daily rhythm of BAT temperature ($T_{BAT0}$) and daily variations in the response to NA. Mean BAT temperature before injection was not correlated with time of day and type of injection, but it was lower than in animals acclimated to L:D cycle (three-way ANOVA: $F_{(1,108)} = 43.14, P < 0.0001$). Increase in $T_{BAT1}$ after NA and saline was similar, except for the first injection at night (CT 12) when saline induced even larger increase than NA ($P < 0.001$; Fig. 1). The increase in $T_{BAT1}$ after NA injections varied from $0.2 \pm 0.2^\circ C$ (CT 8) to $0.7 \pm 0.1^\circ C$ (CT 16), namely the effect of NA was smaller than in L:D cycle (three-way ANOVA: $F_{(1,102)} = 18.16, P < 0.0001$). Significant differences were recorded at CT 4 ($P < 0.01$), CT 8 ($P < 0.001$) and CT 20 ($P < 0.001$). On the contrary, the increase in $T_{BAT1}$ after saline was larger in D:L than in L:D cycle ($P < 0.001$), except for the injection at CT 4 and CT 8.

**Preferred ambient temperature ($PT_a$; Fig. 2)**

In hamsters acclimated to the reversed photoschedule for 8 weeks, preferred ambient temperature ($PT_a$) before injection was not correlated with time of day and type of injection but it was lower than in hamsters acclimated to L:D cycle (three-way ANOVA: $F_{(1,116)} = 32.89, P < 0.0001$). Changes in $PT_a$ after injection were significantly correlated with the type of injection and time of day (three-way ANOVA: $F_{(5,108)} = 6.62, P < 0.015$; Fig. 2). After NA, the largest changes were recorded after injection at CT 0, CT 4 and CT 16. However, the decrease in $PT_a$ after other NA injections (at CT 8, CT 12 and CT 20) was smaller than in L:D cycle ($P < 0.001, 0.01$ and 0.001, respectively). On the contrary, saline administration at the beginning of day (CT 0) and at the end of night (CT 20) in hamsters acclimated to D:L cycle for 8 weeks induced larger decrease in $PT_a$ than in animals acclimated to L:D cycle ($P < 0.001$).

**Correlation between $T_{BAT0}$, $T_{BAT1}$ and $PT_a$ (Table 1)**

After 8 weeks under a reversed photoschedule, NA induced increase in BAT temperature ($T_{BAT1}$) was inversely correlated with pre-injection level of $T_{BAT}$ ($T_{BAT0}$; $r = -0.38, P < 0.04$, Table 1). In saline-injected hamsters, this correlation was not significant ($r = -0.35, P = 0.055$). Small correlation between the increase in BAT temperature ($\Delta T_{BAT}$) and the decrease in preferred ambient temperature ($\Delta PT_a$) was also recorded after NA ($r = 0.22, P < 0.01$) and saline injections ($r = 0.29, P < 0.0001$).

**Influence of photoschedule and total time of acclimation to 12 hr of light on $T_{BAT1}$ and $PT_a$ after saline and noradrenaline injections (Fig. 3)**

During 12 weeks of exposure to 12 hr of light, response to NA decreased while the response to

**TABLE 1.** The increase in brown adipose tissue temperature ($T_{BAT}$) after noradrenaline (NA) and saline injections was inversely correlated with $T_{BAT}$ before injection in golden hamsters acclimated to L:D cycle and then after 4 and 8 weeks of acclimation to a reversed photoschedule (D:L 4 and D:L 8, respectively)

<table>
<thead>
<tr>
<th>Photoschedule</th>
<th>Injection</th>
<th>$r$</th>
<th>$P$</th>
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<tr>
<td>L:D</td>
<td>NA</td>
<td>-0.50</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>-0.25</td>
<td>ns (0.18)</td>
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<tr>
<td>D:L 4</td>
<td>NA</td>
<td>-0.77</td>
<td>0.001</td>
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<tr>
<td></td>
<td>Saline</td>
<td>-0.39</td>
<td>0.03</td>
</tr>
<tr>
<td>D:L 8</td>
<td>NA</td>
<td>-0.38</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Saline</td>
<td>-0.35</td>
<td>ns (0.055)</td>
</tr>
</tbody>
</table>

Only in saline-injected hamsters acclimated for 4 weeks to L:D cycle and for 8 weeks to D:L cycle, this correlation was not significant.
saline increased. Changes in mean BAT temperature after injection ($T_{BAT1}$; mean for all saline or NA injections) were significantly correlated with photoschedule and type of injection (two-way ANOVA: $F(2,189) = 11.56$, $P < 0.0001$). After 8 weeks of acclimation to D:L cycle, mean increase in $T_{BAT1}$ after NA was lower than in L:D cycle ($P < 0.001$) and than after 4 weeks in D:L cycle ($P < 0.001$). On the contrary, mean increase after saline was higher ($P < 0.001$; Fig. 3A). Mean decrease in preferred ambient temperature ($PT_a$) was also significantly correlated with photoschedule and type of injection (two-way ANOVA: $F(2,197) = 4.14$, $P < 0.02$). In NA-injected hamsters, mean decrease in $PT_a$ was smaller after 8 weeks in D:L cycle ($P < 0.03$) and after 4 weeks in D:L cycle ($P < 0.04$). In saline-injected hamsters acclimated to the reversed photoschedule for 8 weeks, mean decrease in $PT_a$ was larger than in animals acclimated to L:D cycle ($P < 0.001$; Fig. 3B).

Changes in body mass ($m_b$; Fig. 4)

Body mass significantly decreased with the time of acclimation (one-way ANOVA: $F(3,32) = 4.41$, $P < 0.02$; Fig. 4). Before acclimation to L:D cycle, hamsters weighed $90.7 \pm 2.1$ g. After 4 weeks of acclimation to L:D cycle and then after 4 weeks of acclimation to D:L cycle, $m_b$ was $88.7 \pm 3.0$ and $88.2 \pm 1.8$ g, respectively. After 8 weeks under the reversed photoschedule the hamsters weighed $82.6 \pm 2.1$ g and were lighter than before acclimation ($P < 0.01$).

**DISCUSSION**

The effects of photoperiod and photoperiodic memory on the annual rhythms in golden hamsters are usually studied in relation to reproduction (Sisk and Turek, ’83; Heideman and Bronson, ’93; Kirby et al., ’93; Vitaterna and Turek, ’93; Larkin et al., 2002). However, not only reproduction, but also behavior or thermoregulation undergo seasonal changes. The results of present experiments clearly show that golden hamsters, when photosensitive, but housed under the intermediate photoperiod (12 hr of light), continue to change toward their winter status. However, they need low ambient temperature to fully acclimate to winter condition and to increase capacity for NST.

In the absence of environmental cues or disturbances in the signals (in the present study:
12 weeks of acclimation to 12 hr of light and photoschedule reversal, respectively), the photoperiodic history of animals plays the most important role. Before the experiments, hamsters were housed under natural lighting conditions. They were transferred to the climate chamber in autumn (the middle of September) when natural L:D cycle was decreasing and a transient one, that is, 12.5L:11.5D. We hypothesized that prolonged acclimation to the transient photoperiod (12 hr of light) would not affect total BAT capacity for NST but would influence daily variations in NST. Regulatory NST in BAT is a very important source of heat in small mammals (Jansky, '73; Gordon, '93). Indeed, the decrease in mean BAT temperature ($T_{b}$), preferred ambient temperature ($PT_{a}$), and body mass that we recorded in the course of the experiment imply that golden hamsters partially changed to the winter status despite intermediate day length and photoschedule reversal. If golden hamsters are housed under short photoperiod, daily amplitude of the body temperature ($T_{b}$) rhythm is markedly reduced or even absent during hibernation (Jansky et al., '89). We predicted that prolonged acclimation to 12 hr of light would result in dampening of $T_{b}$ rhythm (measured as BAT temperature) because photoperiodic "memory" should indicate winter. Indeed, after 12 weeks of acclimation, a day–night difference in $T_{b}$ was the smallest. Thus, we recorded changes typical for a short photoperiod despite intermediate photoperiod and photoschedule reversal.

In addition, after acclimation to D:L cycle, mean $T_{b}$ and mean $PT_{a}$ before injections were lower than in L:D cycle, thereby suggesting lowering of the set point for $T_{b}$ regulation. Lowering of the set point during acclimatization to the winter conditions (short photoperiod and low $T_{a}$) would allow for energy savings (Heldmaier et al., '89). Significant decrease in body mass also indicates the process of acclimatization since golden hamsters lose body mass before hibernation (Lyman, '48). Denyes and Baumber ('64; in Pengelley, '67) found that prehibernation period was much shorter when hamsters were exposed to cold in winter than in summer. This early observation also confirms the importance of photoperiodic history in hamsters’ physiology and behavior.

We predicted that total capacity for NST would not change during acclimation because of relatively high ambient temperature ($T_{a} = 23^\circ C$). In the present experiments, acclimation to 12 hr of light (independently of photoschedule reversal) without cold exposure, did not induce increase in NST capacity, rather a decrease was recorded. This decrease seems to result from the housing conditions. In golden hamsters, cold is the most important factor for inducing seasonal improvement of NST and preparation for hibernation while short photoperiod (SP) has minor effect (Jansky et al., '84; Triandafillou et al., '84). Experiments done on golden hamsters acclimated to a $T_{a}$ of 24°C, showed that metabolic rate after NA administration (an indicator of NST efficiency) was similar in hamsters acclimated to long and short photoperiods (Viswanathan et al., '86). On the contrary, McElroy and Wade ('86) reported significant increase in NST capacity after acclimation to SP. These authors suggested that their discrepant observations could result from the fact that they used females which were more sensitive to changes in photoperiod, while in other studies, male hamsters were used.

After 8 weeks under a reversed photoschedule (D:L 8), increase in $T_{BAT}$ after injections was similar in NA and saline-injected hamsters. Moreover, mean increase in $T_{BAT}$ (mean for all injections) after NA administration was smaller than after acclimation to L:D and D:L—4 cycles while after saline, it was higher. It cannot be excluded that increase in the response to saline was mediated by alpha-adrenoceptors. In hamsters acclimated to cold, the number of alpha 1-adrenoceptors in BAT increases, and thus it is likely that these receptors mediate the thermogenic response in golden hamsters (Nedergaard et al., '96). Despite prolonged acclimation to 12 hr of light, photoperiodic “memory” could lead to an increase in sympathetic activity. As a result, a higher level of adrenaline released in response to the stress of saline injection could contribute to the increase in $T_{BAT}$, being a result of increased metabolism but not regulatory NST in BAT. Furthermore, saline-injected hamsters could prefer lower $T_{a}$'s due to vasoconstriction induced by adrenaline (Schmidt-Nielsen, '92). Considering lower set point for $T_{b}$ regulation, higher responsiveness to stress might be very important during “fight or flight” reaction. It could allow rapid elevation of $T_{b}$, e.g., during an approach by a predator and the necessity of fight or flight.

We predicted that NST rhythm would be related to body temperature ($T_{b}$) rhythm. Indeed, when daily rhythm of $T_{b}$ was recorded, the daily variations in the capacity for NST were also present. After 4 weeks of acclimation to L:D cycle, the largest effect of NA was recorded during the
day and in the latter part of the night. Similar rhythm of BAT capacity for NST was recorded in autumn, in golden hamsters housed under semi-natural conditions (Jefimow et al., 2004) and in Siberian hamsters acclimated to a short photoperiod (Jefimow et al., 2003). Strong inverse correlation between pre-injection $T_{BAT}$ and $T_{BAT}$ after NA administration, observed also in the Siberian hamsters in analogous set of experiments (Jefimow et al., 2005), indicates that the effect of exogenous NA is stronger when $T_{b}$ is kept at the lower level. Thus, the daily rhythm of NA-induced NST in BAT follows a daily rhythm of $T_{b}$ but opposes it in phase. Watts and Refinetti (’96) found that cold-induced thermogenesis in golden hamsters was higher when $T_{b}$ was low and suggested that their autonomic thermoregulatory system was more sensitive to cold at that time, i.e., when $T_{b}$ was low. Such a pattern of reaction can also prevent overheating when $T_{b}$ is high. Another possible explanation of daily rhythmicity in the response to NA is that when $T_{b}$ is high, NST is already activated and thus exogenous NA has a smaller effect due to the smaller number of available adrenergic receptors (Haim and Zisapel, ’99; Jefimow et al., 2003). Furthermore, daily rhythm of heat loss (Aschoff, ’81) can contribute to a daily rhythm of NST. During the dark phase of the 24 hr cycle, that is, during active cycle phase, heat loss might be higher due to selection of lower $T_{a}$, increased blood flow to periphery or less compact insulation (McNab, 2002).

After NA injection, $PT_{a}$ did not show daily variations. However, high correlation between $\Delta T_{BAT}$ and $\Delta PT_{a}$ indicates that hamsters preferred lower $T_{a}$ when increase in $T_{BAT}$ was higher. Such cold-seeking behavior indicates that increase in $T_{BAT}$ after NA injection is a forced hyperthermia and it is not related to changes in set point for $T_{b}$ regulation (Gordon, ’93).

After 8 weeks of acclimation to a reversed photoschedule, the daily rhythm of NST disappeared. It seems that lack of rhythmicity of the BAT capacity for NST reflects physiological adaptation to a short photoperiod. Under a short photoperiod, day–night difference in $T_{b}$ is absent although ultradian variations are large (Jansky et al., ’89). Because $T_{b}$ rhythm does not exist, NST rhythm is not expressed. This observation would also confirm the hypothesis that NST rhythm is related to the rhythm of body temperature. Lack of NST rhythmicity was recorded in root voles, which do not exhibit daily rhythm of $T_{b}$ (Gębczyński and Taylor, ’04).

We predicted that NST rhythm would be reversed simultaneously to the $T_{b}$ rhythm because golden hamsters are less sensitive to changes in photoperiod than Siberian hamsters (Janský et al., ’84; Ruf et al., ’93). In the Siberian hamsters, 4 weeks of acclimation to a reversed photoschedule was sufficient for re-entrainment of the $T_{b}$ rhythm but not for the rhythm of NST that was regained 4 weeks later. Indeed, in the golden hamsters after 4 weeks of acclimation to a reversed photoschedule, both rhythms: $T_{b}$ and NST were re-entrained. The largest effect of exogenous NA was again recorded during day and at the end of night. Inverse correlation between pre-injection $T_{BAT}$ and $T_{BAT}$ after NA injection as well as correlation between $\Delta T_{BAT}$ and $\Delta PT_{a}$ were also significant. Simultaneous re-entrainment of $T_{b}$ and NST rhythms confirms that during acclimatization, golden hamsters rely on changes in photoperiod to a lesser extent than Siberian hamsters.

In conclusion, the results of present experiments confirm that golden hamsters possess photoperiodic “memory” and, when photosensitive, can change to winter status despite prolonged acclimation to transient photoperiod and the reversal of photoschedule. After 8 weeks of acclimation to a reversed photoschedule, $T_{b}$ and NST rhythms disappeared and NST capacity was the lowest. We conclude that these changes result from the photoperiodic history of hamsters. Increase in total capacity for NST could not be recorded because of relatively high ambient temperature during acclimation. The results of the present experiments also confirm our prediction that BAT capacity for NST has a daily rhythm that opposes daily rhythm of body temperature. As in Siberian hamsters, NA induces the largest effect when $T_{b}$ is low. However, unlike in Siberian hamsters, in golden hamsters after 4 weeks of acclimation to a reversed photoschedule the daily rhythm of NST is fully re-entrained. Proper adjustment to light–dark cycle ensures proper energy balance and thermal homeostasis, which is a prerequisite for winter survival. When $T_{b}$ is low, high NST capacity could enable its elevation in a short time, e.g., during a sudden decrease of $T_{a}$ during the resting phase of the day.

Taken together, photoperiodic history of golden hamsters induced a change into the winter status because prolonged acclimation to intermediate photoperiod had started in autumn, during the time of photosensitivity. In the Siberian hamsters, this history induced a change into the summer status because acclimation started in spring. This
photoperiodic history seems not to be restricted to the taxa that during acclimatization rely mainly on photoperiod (like Siberian hamsters) and may play a very important role in all wild-living animals studied in the laboratory. The results of experiments on Siberian and golden hamsters indicate that photoperiodic “memory” is independent of the time of the year; however, it may require additional factors (e.g., ambient temperature) to lead to the full acclimatization of animals.

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