Inhibition of hibernation by exercise is not affected by intergeniculate leaflets lesion in hamsters

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Menet, Jérôme S., Patrick Vuillez, Michel Saboureaux, and Paul Pévet. Inhibition of hibernation by exercise is not affected by intergeniculate leaflets lesion in hamsters. Am J Physiol Regul Integr Comp Physiol 285: R690–R700, 2003. First published April 24, 2003; 10.1152/ajpregu.00068.2003.—The circadian clock of mammals, located in the suprachiasmatic nuclei (SCN) of the hypothalamus, has been demonstrated to integrate day length change from long (LP) to short photoperiod (SP). This photoperiodic change induces in Syrian hamsters a testicular regression through melatonin action, a phenomenon that is inhibited when hamsters have free access to a wheel. The intergeniculate leaflets (IGL), which modulate the integration of photoperiod by the SCN, are a key structure in the circadian system, conveying nonphotic information such as those induced by novelty-induced wheel running activity. We tested in hamsters transferred from LP to a cold SP the effects of wheel running activity on a photoperiod-dependent behavior, hibernation. Lesions of the IGL were done to test the role of this structure in the inhibition induced by exercise of photoperiod integration by the clock. We show that wheel running activity actually inhibits hibernation not only in sham-operated animals, but also in hamsters with a bilateral IGL lesion (IGL-X). In contrast, IGL-X hamsters without a wheel integrate slower to the SP but hibernate earlier compared with sham-operated animals. Moreover, some hibernation characteristics are affected by IGL lesion. Throughout the experiment at 7°C, IGL-X hamsters were in hypothermia during 18% of the experiment vs. 32% for sham-operated hamsters. Taken together, these data show that the IGL play a modulatory role in the integration of photoperiodic cues and modulate hibernation, but they are not implicated in the inhibition of hibernation induced by wheel running activity.

photoperiod; suprachiasmatic nuclei; wheel running activity; testis

ANIMALS HAVE DEVELOPED STRATEGIES to adapt their physiology and behavior to the seasons. In mammals, the duration of day length (i.e., photoperiod) is integrated by the main endogenous circadian clock, located in the suprachiasmatic nuclei (SCN) of the hypothalamus (59, 62, 63). Light signals perceived by the retina constitute the main synchronizer of the central clock to environmental cues. Photic information reaches the SCN via at least two neuronal pathways: one direct, the retinohypothalamic tract, and one indirect, via neuropeptide Y (NPY) fibers coming from a thalamic structure, the intergeniculate leaflets (IGL) (41–43).

Other (i.e., nonphotic) factors are also capable of modulating the light-dark synchronization and phase shifting the SCN (60). Nonphotic information reaches the SCN through at least two pathways. One involves serotonergic fibers coming from the median raphe nucleus, and the other one via the IGL, which receive serotonergic innervation from the dorsal raphe nucleus and project to the SCN (40). Therefore, the IGL can mediate both photic and nonphotic cues to the SCN. Thus the IGL constitute a key structure that can affect and modulate the SCN activity. For example, it was recently shown that lesion of IGL delays the integration of a photoperiodic change by the SCN (27, 39). One of the main nonphotic factors studied is the behavioral activation induced by wheel running activity. This so-called novelty-induced wheel running activity during the subjective day phase shifts the clock (50, 65). Wheel running-induced activity for 2 h/day can entrain circadian rhythms in hamsters exposed to constant darkness (50). These effects of behavioral activation that phase shift circadian rhythms involve the IGL. Indeed, IGL lesion blocks activity-induced phase shifts in circadian activity rhythms in golden hamsters (29, 65).

In golden hamsters, decrease of day length is paired to a lengthening of melatonin secretion by the pineal, which induces testicular regression (for review, see Refs. 36, 44, 53). This testicular regression associated with exposure to a short photoperiod (SP) can be, however, reversed, reduced, or delayed when hamsters are given access to a wheel (18, 19). Therefore, we hypothesized that wheel running activity may inhibit the integration of photoperiodic change by the SCN via the IGL. It is also well known that Syrian hamsters hibernate when exposed to a cold SP (i.e., with low ambient temperature). Thus entry into hibernation and its prerequisite, testicular regression, constitute two indexes of SP integration (for the Syrian hamster: 30, 31; for the Turkish hamster: 22; for the European hamster: 6, 14, 20). Inasmuch as inhibiting effect of wheel running exercise on SP-induced testicular regression is often not complete (19; J. S. Menet, unpub-
lished observation), we tested our hypothesis on the hibernation cycle. To characterize hibernation processes, temperature was continuously recorded. These temperature data permitted us to follow the lengthening of the body temperature peak after the transfer to SP and, thus, to follow the photoperiodic integration by the SCN.

MATERIALS AND METHODS

Animals

Male Syrian hamsters (Mesocricetus auratus) were obtained from Harlan France (Ganat, France). They were housed five per cage and maintained at 22 ± 1°C under a 14:10-h light-dark (LD) cycle (lights on at 0400). A constant dim red light was on throughout the experiment. Food (UAR 105, U.A.R., Villemoisson-sur-orge, France) and water were available ad libitum.

All experiments were performed in accordance with National Institutes of Health Principles of Laboratory Animal Care (NIH publication No., 86-105, U.A.R., Villemoison-sur-orge, France) and water were provided. Three-month-old hamsters (n = 28) were bilaterally IGL-lesioned (IGL-X) or sham operated. One week later, they were implanted with a transmitter for measurement of body temperature (Tb). Data were collected for 1 wk under the LD 10:14 condition at 22°C. Then, on the same day, light condition was changed from long photoperiod (LP) to SP (LD 10:14, lights on at 0800), ambient temperature was decreased to 7°C, and one-half of the hamster cages were equipped with a 17-cm running wheel. This day was considered day 0 of SP. Hamsters were maintained in this condition for 18 wk.

After 12 wk in SP at 7°C, testis length and width were measured in the animals that showed at least one hibernation bout. These measurements, which were done at room temperature, were not conducted in nonhibernating animals to avoid disturbance that could have delayed a late entry in hibernation.

Eighteen weeks after transfer to SP at 7°C, animals were killed. Testis, seminal vesicles, and epididymal white adipose tissue (EWAT) were removed and weighed. Brains were removed and processed for NPY immunohistochemistry to check the quality of IGL lesion.

Surgery

IGL lesion. Hamsters were anesthetized during the light phase with intraperitoneal injections of zoletil (80 mg/kg; Virbac, Carros, France) and rompun (10 mg/kg; Bayer Pharma, Puteaux, France). They were placed in a Kopf stereotaxic instrument with the incisor bar set at −2 mm. IGL lesions were made bilaterally with a thermic electrode by heating to 80°C for 1 min with a lesion-generator system (Radionics model RFG-4A, Burlington, VT) at three rostrocaudal levels with reference to bregma: −1.1, −1.6, −2.1 mm posterior to bregma; ±3.1, ±3.3. ±3.1 mm laterally to bregma; and −4.3, −4.6, −5.1 mm ventral to the dura, respectively. In sham-operated groups, the electrode was lowered only 2 mm above each lesion level and no heat was delivered.

After the surgery procedure, hamsters were placed individually in a new cage at 22 ± 1°C.

Transmitter implantation. One week after IGL lesion or sham operation, hamsters were implanted with radiotransmitters for the telemetric recording of Tb. Under halothane anesthesia, transmitters (model VM-FH-LT, Mini-Mitter, Sunriver, OR) were placed into the peritoneal cavity via a single midline incision. The wound was sutured and hamsters were then sent back to the experimental room. Radiofrequency signals from the implanted transmitters were averaged every 5 min by receivers placed under each animal's cage and collected by an automated computer program (Dataquest, St. Paul, MN).

Testes measurement. Twelve weeks after transfer to SP at 7°C, hamsters that had hibernated were anesthetized with halothane (Laboratoires Belamont, Paris, France) at 22°C during the light phase. Incisions of skin and abdominal muscles were done, and the left testis was taken out of the body. Testis length (L) and width (W) were measured with a caliper (accuracy ±0.1 mm). The gonad was then replaced in the scrotum. Peritoneum and abdominal muscles were closed with sterile sutures, skin with suture clips, and the wound was treated with chlorhexidine (exoseptoplix, Laboratoires Diepha, Courbevoie, France). Hamsters were then replaced at 22°C for 24 h after surgery.

The determination of testis volume was made using equation of an ovoid: V = 1/6π* L* W². Testicular index was calculated as: It = (L* W²)/body mass.

Body Mass and Food Consumption

Measurement of body mass was done on weeks 0, 1, 2, 4, 6, 8, 10, 14, and 18 of SP with an accuracy of ±0.1 g. If hamsters were in hypothermia on the day of measurement, body mass was measured during subsequent arousal bout to avoid disturbance by manipulation.

Food consumption was measured on weeks 1, 2, 4, 6, 8, 10, 14, and 18 of SP. Briefly, an initial quantity of food was given to hamsters, and on the measurement day, remaining food was taken and weighed. Difference was reported as food consumption per day.

Death

After 18 wk in SP at 7°C, hamsters were deeply anesthetized with 6% pentobarbital sodium (150 mg/kg ip; Sanofi, Libourne, France). Paired testes, seminal vesicles, and EWAT were removed and weighed. Hamsters were then perfused transcardially with 100–150 ml 0.9% NaCl followed with 250–300 ml of freshly prepared 4% paraformaldehyde in phosphate buffer at 0.1 M (pH 7.4). Brains were removed, postfixed during 4–5 h at 4°C, and finally rinsed into PBS at 0.1 M at 4°C until the NPY immunohistochemistry procedure.

Immunohistochemistry

Fifty-micrometer coronal sections of the SCN and IGL were prepared on a vibratome and rinsed with PBS, and sections were used to test the quality of IGL lesions using NPY immunoreactivity detection. Sections were incubated overnight at 4°C with the primary antibody in PBS containing 0.5% Triton X-100 [rabbit anti-NPY, 1/6,000, gift from Prof. Buijs, Institute for Brain Research, Amsterdam, The Netherlands (4)]. Thereafter, sections were incubated 1 h 15 min with biotinylated secondary antibody in PBS-0.5% Triton and 1% of appropriate normal serum (Vector) at room temperature (1/500, goat anti-rabbit), followed by ABC reagent (Vector) in PBS-0.5% Triton for 1 h. Peroxidase activity was detected using 0.0125% diaminobenzidine (Sigma) in Tris at 0.05 M (pH 7.6) containing 0.0075% H2O2. Sections were mounted, dehydrated, and placed under a coverslip for microscopic analysis.

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Data Analysis

Wheel running activity analysis. Number of wheel running revolutions was collected every 5 min by an automated computer system (Dataquest). A first analysis was done by comparison of means of revolution number per day over 1 wk in the IGL-X group vs. sham-operated group and after number of weeks spent in SP.

A second analysis was done on actograms. On each one, an eye-fitted line marking offset of activity was drawn just after transfer to SP at 7°C. Integration of the SP was considered as complete when the fitted line crosses the line of dark offset (8 h in SP).

Tb analysis. Tb was recorded every 5 min. For analysis of hibernation criteria, hamsters were considered in hypothermia when Tb was under 30°C. In some cases, Tb decreased below 30°C only for several minutes and did not stay at a stable value close to ambient temperature. These events were not considered as hibernation bouts and thus do not appear in results analysis. Therefore, hamsters were considered in hibernation only when their Tb decreased to under 30°C and stayed at ~8-9°C over several hours. The time of entry or end of a hibernation bout was the time when the first or last Tb value was under 30°C. The duration of a bout was the difference between these two values.

Tb was also analyzed to observe effect of IGL lesion on lengthening of nocturnal Tb peak after the transfer to SP at 7°C. Analyzed Tb rhythms corresponded to the mean of temperature values during 3 consecutive days. We considered a control in LP (3, 2, and 1 days before transfer), SP5 (4, 5, and 6 days after transfer), SP10 (9, 10, and 11 days), SP20 (19, 20, and 21 days), and SP30 (29, 30, and 31 days). For statistical analysis, each individual pattern of temperature rhythm was smoothed using a least-squares regression (SigmaPlot, Chicago, IL) to a logistic peak with the following equation:

\[ y = y_0 + \frac{y_{\text{max}}}{[1 + \exp^{(\text{slope1} \cdot (\phi_1 - x))}] + [1 + \exp^{(\text{slope2} \cdot (\phi_1 - d))}]} \]

where \( y \) is Tb, \( y_0 \) is rest Tb, \( y_{\text{max}} \) is peak amplitude (above rest Tb), \( \phi_1 \) is time of half amplitude on increase, and \( d \) is duration of the peak (delay between times of half amplitude on increase and decrease). Values for rest temperature, half increase and half decrease of the peak, and amplitude of Tb peak (maximum of Tb subtracted from the rest Tb) were extracted and pooled according to groups.

Statistical Analysis

ANOVA with or without repeated measures or Student’s t-test was performed using Minitab (Minitab, State College, PA). For ANOVA, when significant F ratios were obtained (\( P < 0.05 \)), post hoc pairwise comparisons were conducted using the Tukey’s test. Values are reported as means ± SE.

RESULTS

Analysis of IGL Lesion

All IGL lesions resulted in the disappearance of NPY neurons along the entire rostrocaudal extent of the IGL (Fig. 1). Moreover, these lesions induced a sharp decrease of NPY immunoreactivity in the SCN (Fig. 1F), and, as described before, only scarce fibers persisted in the ventral SCN (38, 48). Most IGL lesions extended into the ventral and dorsal lateral geniculate nuclei. Nevertheless, damage to the surrounding area, such as the hippocampus, was negligible.

Hibernation

Most hamsters without a wheel, independently of the lesion, entered into hibernation during the 18th wk of the experiment (Table 1). Indeed, in both sham-operated and IGL-X groups without a wheel (\( n = 6 \) and \( n = 8 \), respectively), only one animal failed to hibernate. In contrast, no hamster with an access to a wheel showed hypothermia bouts (5 sham-operated and 9 IGL-X hamsters).

Two representative temperograms (1 for a sham-operated hamster, 1 for an IGL-X hamster) are presented in Fig. 2. Analysis of hibernation parameters revealed differences between sham-operated and IGL-X hamsters (Table 2). Bilateral IGL lesion advanced the day of entry into hibernation (51 ± 4 days vs. 70 ± 6 days; \( P < 0.05 \)) and advanced the day of the last recorded hypothermia bout (101 ± 10.3 vs. 125.7 ± 0.2 days; \( P < 0.05 \)). All hibernating sham-operated hamsters were still in hibernation process at the end of the 18 wk of experiment. Total time spent in hypothermia (Tb < 30°C) during the 126 days of the experiment was significantly reduced in IGL-X compared with sham-operated animals (536 ± 144 vs. 977 ± 127 h, corresponding to 17.7 ± 4.8 vs. 32.3 ± 4.2%; \( P < 0.05 \)). Number of hibernation bouts was decreased in IGL-X hamsters compared with the sham-operated hamsters, with a borderline difference (9.6 ± 2.2 vs. 15.2 ± 1.2; \( P = 0.052 \)). Mean duration of the bouts was not affected by the IGL lesion (57 ± 3 h in IGL-X hamsters vs. 69 ± 7 h in sham-operated hamsters; \( P = 0.2 \)).

No difference was observed either for the time of entry in hypothermia (Fig. 3; IGL-X: 2 h 25 min ± 37 min vs. sham operated: 1 h 35 min ± 40 min; light off at 1800; \( P > 0.05 \)), or for the time of end of hypothermia (IGL-X: 01 h 02 min ± 01 h 03 min vs. sham operated: 23 h 41 min ± 41 min; lights off at 1800; \( P > 0.05 \)). Even if these mean times of entry and ends of hypothermia are quite close, we observed that entries into hypothermia were linked to the LD cycle, whereas arousal onsets were not. Indeed, most entries took place between 2000 and 1000 (93.5% for IGL-X, 94.3% for sham operated), whereas the ends of hibernation bouts were randomly distributed (58.6% for IGL-X and 54.1% of total bouts ended during these 14 h).

Testes, Seminal Vesicles, and EWAT Weights

All data are shown in Table 3.

Because testes measurements after 12 wk of SP exposure were done only in hibernating animals, one sham-operated hamster and one IGL-X hamster without a wheel were excluded from the data analysis. When compared between 12 and 18 wk, both testicular volume and index were higher at the end of the experiment in hamsters without a wheel (testis volume: \( F = 9.59 \); \( P < 0.01 \); testis index: \( F = 11.64 \); \( P < 0.01 \)). Moreover, a significant effect of IGL lesion was observed for both parameters (testis volume: \( F = 8.37 \); \( P < 0.01 \); testis index: \( F = 7.85 \); \( P < 0.05 \)), due to higher values of testis volume and index in IGL-X animals compared with sham-operated animals.
At the end of the experiment (i.e., after 18 wk in cold SP), a strong effect of wheel running activity was observed, with higher testes mass, as well as bigger testicular volume and index in hamsters with vs. without a wheel (testes mass: $F_{1,1005} = 104.21$, $P < 0.001$). These values were similar to those of sexually active hamsters exposed to long photoperiod (data not shown). Furthermore, a significant lesion × time interaction was observed (testes mass: $F_{1,1005} = 7.39$, $P < 0.05$) due to the higher testes mass in IGL-X hamsters without a wheel compared with sham-operated animals without a wheel.

Seminal vesicles and EWAT masses were also higher in hamsters with a wheel ($F_{1,1005} = 27.62$, $P < 0.001$, and $F_{1,1005} = 8.57$, $P < 0.01$, respectively) and, as for testes, values were similar to those of hamsters exposed to LP. However, no wheel × lesion interaction was observed for either seminal vesicles weight ($F_{1,1005} = 1.11$, $P = 0.30$) or for EWAT weight ($F_{1,1005} = 0.66$, $P = 0.43$).

### Wheel Running Activity

Two representative actograms (1 from a sham-operated hamster, 1 from an IGL-X hamster) are presented in Fig. 4. Lengthening of wheel running activity just after transfer from LD 14:10 to cold LD 10:14 was evaluated with an eye-fitted line marking offset of activity. Analysis of the number of days necessary for hamsters to run until the end of the 14 h of darkness revealed that IGL lesion delayed lengthening of the
C. Indeed, some weeks after the transfer to the cold SP, onset of activity was delayed for some hours (~6 h) independently of the lesion. This delay was observed in most hamsters (3/5 sham operated, 8/9 IGL-X). In all cases, however, lengthening of wheel running activity induced by the photoperiodic change was complete. Moreover, wheel running activity became fragmented in all hamsters between 7 and 13 wk after transfer to cold SP. Finally, at the end of the experiment and for a majority of hamsters, pattern of locomotor activity became more typical, with an activity onset occurring a few minutes after the dark onset and most of wheel running activity at the beginning of the night.

The number of wheel revolutions during the night changed according to the time spent in SP (Fig. 5). Locomotor activity was high for the first 7–9 wk and thereafter decreased by ~60% (P < 0.01). Wheel running activity was decreased by IGL lesion (P < 0.01). However, IGL lesion did not significantly affect the time-induced decrease of wheel activity that occurred after 9 wk (time spent in SP × lesion interaction, F = 3.71, P = 0.055).

Temperature

Temperature rhythms are presented in Fig. 6. No difference was observed between groups in LP, but rhythms were greatly affected after the transfer to a cold SP.

First of all, transfer to SP induced a lengthening of the temperature peak (Fig. 6, A and B; F = 29.65; P < 0.001). This lengthening was delayed in IGL-X hamsters (interaction effect of lesion and time in SP; F = 5.54, P < 0.001). After 20 days in SP, a difference could no longer be observed between groups. Moreover, time of half increase of temperature peak was delayed in hamsters with a wheel (Fig. 6, A and B; interaction effect of wheel and time in SP; F = 11.35, P < 0.001).

A strong effect of wheel running activity could also be observed on the minima (P = 260.5; P < 0.001) and maxima (P = 573.5; P < 0.001) of Tb rhythms (Fig. 6, A and C). Indeed, minima and maxima of Tb were both decreased by 10.2% immediately after the photoperiodic change in hamsters without a wheel, whereas they remained unchanged in hamsters with a wheel.

Finally, whereas amplitude did not change after the transfer to SP for all IGL-X hamsters and for sham-operated animals without a wheel, an increase in am-

Table 2. Parameters of hypothermia bouts in hamsters without a wheel and being bilaterally IGL-X or sham operated

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham Operated (n = 5)</th>
<th>IGL-X (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days of first hypothermia bout</td>
<td>70 ± 6</td>
<td>51 ± 4*</td>
</tr>
<tr>
<td>Days of last hypothermia bout</td>
<td>126 ± 0.2</td>
<td>101 ± 10.3*</td>
</tr>
<tr>
<td>Time spent in hypothermia, h</td>
<td>977 ± 127</td>
<td>536 ± 144*</td>
</tr>
<tr>
<td>Percentage of time spent in hypothermia</td>
<td>32.3 ± 4.2</td>
<td>17.7 ± 4.8*</td>
</tr>
<tr>
<td>Number of hibernation bouts</td>
<td>15.2 ± 1.2</td>
<td>9.6 ± 2.2*P = 0.052)</td>
</tr>
<tr>
<td>Duration of bouts, h:min</td>
<td>69:15 ± 7:18</td>
<td>57:19 ± 3:16</td>
</tr>
<tr>
<td>Hour of entry in hypothermia, h:min</td>
<td>01:35 ± 00:40</td>
<td>02:25 ± 00:37</td>
</tr>
<tr>
<td>Hour of end of hypothermia, h:min</td>
<td>23:41 ± 00:41</td>
<td>01:02 ± 01:03</td>
</tr>
</tbody>
</table>

Values are means ± SE. See MATERIALS AND METHODS for details. *P < 0.05.
plitude was observed for sham-operated animals with a wheel 20 and 30 days after the transfer, as revealed by the interaction effect between lesion, wheel, and time factors ($F = 2.56; P < 0.05$).

**Body Mass and Food Consumption**

Hamster body mass at the beginning of the experiment ranged from 90 to 110 g. Results are presented as body mass changes (Fig. 7A). Wheel running activity affected strongly body mass change ($F = 402.93; P < 0.001$) and hamsters with a wheel increased their body mass by 40 g (increase of $45\%$). Hamsters without a wheel did not increase their body mass, and a small decrease could even be observed at the end of the experiment (weeks 14 and 18).

Food consumption was also strongly increased in hamsters with a wheel (Fig. 7B, $F = 385.98; P < 0.001$). In hamsters without a wheel, food consumption was stable during the first 6–8 wk and decreased thereafter. Moreover, whereas food consumption continued to decrease until the end of the experiment in sham-operated group, the food consumption of IGL-X hamsters stabilized between weeks 10 and 14 and even re-increased. Indeed, no statistical difference was observed in food consumption between week 18 and the first weeks of the experiment. In hamsters with a wheel, food consumption increased after the transfer to cold SP by $\sim 20–25\%$ and then remained stable until the end of the experiment.

**DISCUSSION**

In Syrian hamster, wheel running activity is known to be able to prevent SP-induced gonadal atrophy (18, 19). In the current study, we demonstrate that, in hamsters exposed to SP and cold temperature, free access to a wheel not only prevents testicular regression but also inhibits the hibernation process. Free access to a wheel in Syrian hamster can thus block the photoperiodic response, at least for the physiological parameters studied. The mechanisms and structures

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Table 3. Testis volume, testicular index, testes mass, seminal vesicles mass, and EWAT mass of hamsters 12 or 18 wk after the transfer to cold SP

<table>
<thead>
<tr>
<th></th>
<th>Hamsters Without a Wheel (Hibernating)</th>
<th>Hamsters With a Wheel (Nonhibernating)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Sham operated ($n = 5$)</td>
<td>IGL-X ($n = 7$)</td>
</tr>
<tr>
<td>Testis volume after 12 wk in SP at 7°C</td>
<td>0.59±0.06</td>
<td>1.04±0.19</td>
</tr>
<tr>
<td>Testis volume after 18 wk in SP at 7°C</td>
<td>1.07±0.12</td>
<td>1.51±0.11</td>
</tr>
<tr>
<td>Testicular index after 18 wk in SP at 7°C</td>
<td>11.0±1.2</td>
<td>19.2±3.4</td>
</tr>
<tr>
<td>Testicular index after 18 wk in SP at 7°C</td>
<td>20.8±2.1</td>
<td>27.5±1.8</td>
</tr>
<tr>
<td>Seminal vesicles mass after 18 wk in SP at 7°C</td>
<td>0.26±0.04</td>
<td>0.63±0.11</td>
</tr>
<tr>
<td>EWAT mass after 18 wk in SP at 7°C</td>
<td>0.61±0.13</td>
<td>0.92±0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE. Analysis was done between hamsters that had hibernated (i.e., 5 sham-operated and 7 IGL-X hamsters without a wheel) and hamsters that had free access to a wheel (5 sham operated and 9 IGL-X). Because testis size was only measured in hibernating hamsters, no data of testis volume and testicular index are available for nonhibernating animals 12 wk after the transfer to SP. EWAT, epididymal white adipose tissue.
involved are largely unknown. Wheel running activity could indeed prevent the integration of the photoperiodic message or it could act independently by a direct activation of the reproductive axis and/or a direct inhibition of the hibernation.

In accordance with the first hypothesis, the circadian clock, which is involved in the integration of the photoperiodic message, can be shifted by nonphotic factors such as novelty-induced wheel running activity. Wheel running activity information is known to affect the clock through two important nervous pathways: NPY fibers coming from the IGL (23, 24) and 5-hydroxytryptamine (5-HT) fibers coming from the median raphe nuclei (40). Some arguments favor a major role of the IGL in nonphotic phase shifting. Lesions of the IGL impair phase shifts induced by nonphotic factors such as voluntary wheel running (17) and forced running on treadmills in mice (37), response to Triazolam (9), and 8-OH-DPAT injections in Syrian hamsters (56). Moreover, several effects of nonphotic factors can be mimicked by injections of serotonin agonists in the SCN both in vivo (10, 11, 16, 61) and in vitro (for review, see Ref. 49). Finally, locomotor activity also increases serotonin content in the SCN (57) and novelty-induced wheel running activity induces serotonin release in the SCN (15).

Despite all these arguments suggesting a direct effect of exercise on the SCN, our data on Tb rhythms as well as on activity rhythms do not fit with this hypothesis. In all hamsters, both rhythms, which are directly under SCN control (1, 34, 51), lengthen as expected after the transfer from LP to SP conditions, whether the animals have access to a wheel or not. Because physiological rhythms are differently controlled by several distinct, dedicated SCN output pathways (for review, see Ref. 32, 54), we cannot not exclude that wheel running information can affect the clock through 5-HT fibers originating from the median raphe. These serotonergic fibers are also of importance in mediating nonphotic phase shifts, because their destruction in the SCN by 5,7-dihydroxytryptamine impairs phase shifts induced by nonphotic factors such as voluntary wheel running (17) and forced running on treadmills in mice (37), response to Triazolam (9), and 8-OH-DPAT injections in Syrian hamsters (56). Moreover, several effects of nonphotic factors can be mimicked by injections of serotonin agonists in the SCN both in vivo (10, 11, 16, 61) and in vitro (for review, see Ref. 49). Finally, locomotor activity also increases serotonin content in the SCN (57) and novelty-induced wheel running activity induces serotonin release in the SCN (15).

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In accordance with the first hypothesis, the circadian clock, which is involved in the integration of the photoperiodic message, can be shifted by nonphotic factors such as novelty-induced wheel running activity. Wheel running activity information is known to affect the clock through two important nervous pathways: NPY fibers coming from the IGL (23, 24) and 5-hydroxytryptamine (5-HT) fibers coming from the median raphe nuclei (40). Some arguments favor a major role of the IGL in nonphotic phase shifting. Lesions of the IGL impair phase shifts induced by nonphotic factors such as voluntary wheel running (17) and forced running on treadmills in mice (37). In rats, IGL lesion also blocks the period shortening induced by wheel running activity (33) and the phase-advancing properties of a timed caloric restriction in long days (7). In addition, IGL neurons are activated when hamsters run in a novel wheel (28). Pretreatment with NPY antisera markedly attenuates phase advances induced by novelty-induced wheel running in hamsters (3) and several effects of nonphotic factors (such as behavioral activation) could be mimicked by NPY injections in the SCN in vivo (26) as in vitro (2). Despite this strong influence of the IGL on SCN-driven rhythms, the present work reports no obvious effect of the IGL lesion in the mediation of the inhibiting message of wheel running activity on either testicular regression or hibernation. This finding raises the possibility that such an effect of wheel running activity on either reproduction or hibernation is mediated by the serotonergic fibers. Indeed, even in the absence of NPY afferences to the SCN, running activity can still have the ability to act on the clock through 5-HT fibers originating from the median raphe. These serotonergic fibers are also of importance in mediating nonphotic phase shifts, because their destruction in the SCN by 5,7-dihydroxytryptamine impairs phase shifts induced by nonphotic factors such as voluntary wheel running (17) and forced running on treadmills in mice (37), response to Triazolam (9), and 8-OH-DPAT injections in Syrian hamsters (56). Moreover, several effects of nonphotic factors can be mimicked by injections of serotonin agonists in the SCN both in vivo (10, 11, 16, 61) and in vitro (for review, see Ref. 49). Finally, locomotor activity also increases serotonin content in the SCN (57) and novelty-induced wheel running activity induces serotonin release in the SCN (15).

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![Fig. 5. Number of wheel revolutions per day in sham-operated or IGL-X hamsters exposed for 18 wk to a cold LD 10:14 cycle. Number of wheel revolutions, which was averaged weekly, decreased 8 wk after the transfer to the cold SP.](image-url)
running would disturb some rhythms while not affecting others. Nevertheless, because rhythms are lengthened after the transfer to SP, the inhibition by wheel access of SP-induced gonadal atrophy and hibernation cycle does not appear as the consequence of a disturbed integration of the photoperiodic message at the level of the SCN. The running activity might act downstream of the central clock on not-yet-identified structures. As the photoperiodic inhibition of sexual activity is required for animals to hibernate (see references in the introduction and also 45, 46), the effect of free access to a wheel on the sexual activity could be sufficient to explain the observed blockage of entry into hibernation. However, as demonstrated in the present experimental conditions, wheel running activity strongly impacts disturbed Tb rhythms, suggesting that it could also act on the hibernation physiology independently of reproduction function. Transfer to cold SP indeed induces a 1°C decrease of rest and maximal Tb, which is not observed when hamsters have free access to a wheel. This difference of TB average between hamsters with or without a wheel, previously described under

Fig. 6. Body temperature rhythms in sham-operated and IGL-X hamsters with or without free access to a wheel. A: body temperature rhythms in hamsters in long photoperiod (LP) and 5, 10, 20, and 30 days after the transfer from LD 14:10 to LD 10:14 (LP, SP5, SP10, SP20, SP30, respectively). B: times of half increase and half decrease of temperature rhythms presented in A. C: minima, maxima, and amplitudes of temperature rhythms. Gray areas correspond to the night of respective photoperiods.

A

B

C

Fig. 7. Body mass change (A) and daily food consumption (B) in hamsters exposed for 18 wk to a cold LD 10:14 cycle. Body mass as well as daily food consumption were strongly increased in hamsters transferred to the cold SP with a running wheel, whether they were IGL-X or sham operated.
normal temperature (8), could be the consequence of an increased metabolic rate, as suggested by the increase in food consumption and body mass by >40%. This increased metabolic rate could contribute to counteract some physiological effects induced by an SP exposure on testicular regression and hibernation. Indeed, several experiments buttressing up the metabolic hypothesis of reproduction showed that reproductive function is inhibited whatever the photoperiod in the case of metabolic shortage (for review, see Ref. 55). For example, glucose privation induces torpor in Djungarian hamsters (13). In our paradigm, it is likely that wheel running activity in SP, which induces an increase of food consumption and body mass, also increases as a consequence of metabolic rate in hamsters. This higher energy metabolism could be responsible, at least in part, for the increase by ~1°C of Tb.

Another aspect of the present work has to be considered. IGL lesion delays the lengthening of Tb peak as well as the wheel running activity pattern, both of which were induced by transfer to SP. The results confirm previous works that demonstrated that IGL lesion delays SP integration (27, 39). The IGL are thus involved in the building of a photoperiodic message by the central clock. As previously described at ambient temperature (29, 33, 35, 38, 65), we observed that IGL lesion also affects locomotor activity by decreasing the amount of wheel running activity. This effect, observed here at a cold ambient temperature, is in accordance with the involvement of the IGL in the mediation of nonphotic behavioral cues to the central clock.

IGL lesion, which does not prevent exercise-induced blockage of hibernation, however, deeply disturbs the hibernation process itself. Several parameters, such as time of entry and end of hibernation and total time spent in hypothermia, are impaired by IGL destruction. To our knowledge, these data constitute the first demonstration of an IGL role in hibernation physiology. Time of end of the hibernation cycle is well known to be dependent on sexual activity (6, 22, 30). This may explain the advance of arousal observed for IGL-X hamsters. Indeed, these animals present a testicular recrudescence earlier compared with sham-operated animals. Because some IGL neurons directly innervate hypothalamic neuroendocrine dopaminergic neurons, including some connecting to gonadotropin-releasing hormone neurons in the anteroventral paraventricular nucleus (25), a bilateral IGL lesion might then disturb the hypothalamic-pituitary axis. Smale and Morin (58) showed that effects of IGL lesion on SP-induced testicular regression might be related to lesion-induced hippocampal damage. This is unlikely to be the case in the present study, because our IGL lesions were restricted to the lateral geniculate complex and poorly extended to the hippocampus. Another effect of IGL lesion on hibernation is a decrease in the time spent in hypothermia. Even if we cannot exclude a role of IGL-efferent hypothalamic neuroendocrine neurons, direct IGL connection to the SCN may be involved. Indeed, the SCN appear to be part of the mechanism that controls the duration of the hibernation season and the temporal structure of individual torpor bouts (12, 52). IGL lesion may disturb the functioning of the SCN and thus affect pattern of hibernation bouts, which are under SCN control.

In contrast to these effects of IGL lesion on the hibernation process, hours of entry in hypothermia and of end of hypothermia were not affected by IGL lesion. Previous studies showed that entry into hypothermia is linked to the LD, whereas arousal onset is not controlled by the circadian system (5, 21, 64, 66). The current study confirms these results, and entries in hypothermia are thus well controlled by the central circadian clock.

In conclusion, wheel running activity prevents occurrence of hypothermia bouts usually observed in golden hamsters exposed to a cold SP. IGL are not involved in this inhibition, although they modulate to some extent the integration of a photoperiodic change by the central clock. Whether wheel running activity inhibits the integration of SP by the SCN still remains an open question, even if rhythms driven by the circadian clock are lengthened after the transfer to SP. Some other experiments are, therefore, needed to completely decipher whether the inhibiting effects of exercise act directly on or downstream of the SCN.

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