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

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Abbreviated title for the running head: Hibernation, exercise and intergeniculate leaflets

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Keywords: photoperiod, suprachiasmatic nuclei, wheel-running activity, testis

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Abstract

The circadian clock of mammals, located in the 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 hamster a testicular regression through melatonin action, a phenomenon that is inhibited when hamsters have a 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. Then, we have tested, in hamsters transferred from LP to a cold SP, the effects of wheel-running activity on a photoperiod-depandan behavior, the 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 the SP but hibernate earlier compared to Sham 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 hamsters. Taken together, these data show that the IGL play a modulatory role in the integration of photoperiodic cues, modulate hibernation but are not implicated in the inhibition of hibernation induced by wheel running activity.
Introduction

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 synchronisation 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 implies 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 which can affect and modulate the SCN activity. For example, it has been 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 hours per day can entrain circadian rhythms in hamsters exposed to constant darkness (50). These effects of behavioral activation which 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, 36, 44, 53). This testicular
regression associated with exposure to a SP can be however reversed, reduced or delayed when hamsters are given access to a wheel (18, 19). Therefore, we have hypothetized 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). Since inhibiting effect of wheel-running exercise on SP-induced testicular regression is often not complete (19; JS Menet, unpublished observation), we have tested our hypothesis on the hibernation cycle. To characterize hibernation processes, temperature was continuously recorded. These temperature data permitted to follow the lengthening of body temperature peak after the transfer in SP, and so 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 5 per cage and maintained at 22 ±1 °C under a 14:10 light-dark (LD) cycle (lights on at 04h00). A constant dim red light was on throughout the experiment. Food (UAR 105, U.A.R., Villemoison-sur-orge, France) and water were available *ad libitum*. All experiments were performed in accordance with National Institute of Health “Principles of laboratory animal Care” (NIH publication No., 86-23, revised 1985) as well as in accordance with the French law.

**General experimental procedure**

Three months old hamsters (n=28) were bilaterally IGL-lesioned or sham operated. One week later, they were implanted with a transmitter for measurement of body temperature (*T*<sub>b</sub>). Data were collected for one week under the LD 14:10 condition at 22 °C. Then, on the same day, light condition was changed from LP to SP (LD 10:14, lights on at 08h00), ambient temperature was decreased to 7 ± 1 °C and half of the hamster cages were equipped with a 17 cm running wheel. This day was considered as day 0 of SP. Hamsters were maintained in this condition during 18 weeks.

After 12 weeks 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 non-hibernating animals in order to avoid disturbance that could have delayed a late entry in hibernation.
Eighteen weeks after transfer in 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 quality of IGL lesion.

**Surgery**

*Intergeniculate leaflets lesion*

Hamsters were anesthetized during the light phase with i.p. 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 during 1 min, with a lesion generator system (Radionics model RFG-4A, INC. Burlington, USA) at 3 rostro-caudal 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 at 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 $T_b$. 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 set 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 hours after surgery.

Determination of testis volume was made using equation of an ovoid: \( V = \frac{1}{6}\pi LW^2 \). Testicular index was calculated as: \( It = \frac{LW^2}{\text{body mass}} \).

**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 a accuracy of ± 0.1 g. If hamsters were in hypothermia on the day of measurement, body mass was measured during subsequent arousal bout, in order to avoid disturbance of manipulation. Food consumption was measured on weeks 1, 2, 4, 6, 8, 10, 14 and 18 of SP. Briefly, a 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.
Sacrifice

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

Immunohistochemistry

Fifty micrometers coronal sections of the SCN and IGL were prepared on a vibratome, rinsed into 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, 1/6000, gift from Prof Buijs, Institute for Brain Research, Amsterdam, Netherlands (4)). Thereafter, sections were incubated 1h15 min with biotinylated secondary antibody in PBS triton 0.5 % and 1 % of appropriate normal serum (Vector, Burlingame, USA) at room temperature (1/500, goat anti-rabbit), followed by ABC reagent (Vector) in PBS triton 0.5 % for 1h. Peroxidase activity was detected using 0.0125 % di-amino-benzidine (Sigma, St Louis, USA) in Tris 0.05M (pH=7.6) containing 0.0075 % H₂O₂. Sections were mounted, dehydrated and coverslipped for microscopic analysis.
Data analysis

Wheel running activity analysis

Number of wheel-running revolutions was collected every 5 min by an automated computer system (Dataquest, St Paul, MN).

A first analysis was done by comparison of means of revolutions number per day during a week, in IGL-X group vs. Sham-operated group and following 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 (08h00 in SP).

Body temperature analysis

Body temperature was recorded every 5 min. For analysis of hibernation criteria, hamsters were considered in hypothermia when \( T_b \) was under 30 °C. In some cases, \( T_b \) 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 into hibernation only when their \( T_b \) decreased under 30 °C and stay at approximately 8-9 °C during several hours. The time of entry or end of an hibernation bout was the time when the first or last \( T_b \) value was under 30 °C. The duration of a bout was the difference between these 2 values.

\( T_b \) was also analyzed in order to observe effect of IGL lesion on lengthening of nocturnal body temperature peak after the transfer to SP at 7 °C. Analyzed \( T_b \) 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:

\[ y = y_0 + \frac{y_{\text{max}}}{(1 + \exp(slope_1 \times (\phi - x))) \times (1 + \exp(slope_2 \times (x - \phi - d)))} \]

where \( y = T_b \), \( y_0 = \text{rest } T_b \), \( y_{\text{max}} = \text{peak amplitude (above rest } T_b) \); \( slope_1 = \text{ascending slope} \); \( slope_2 = \text{descending slope} \); \( \phi = \text{time of half amplitude on increase} \); \( d = \text{duration of the peak (delay between times of half amplitude on increase and decrease)} \). Values for rest temperature, half-increase and half-decrease of the peak, and amplitude of \( T_b \) peak (maximum of \( T_b \) subtracted to the rest \( T_b \)) were extracted and pooled according to groups.

**Statistical analysis**

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

Analysis of intergeniculate leaflets lesion

All IGL lesions resulted in the disappearance of NPY neurons along the entire rostro-caudal 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 of IGL lesions stretched a little out the ventral and dorsal lateral geniculate nuclei. Nevertheless, damage to the surrounding area as hippocampus was negligible.

Hibernation

Most hamsters without a wheel, independently of the lesion, entered into hibernation during the 18 weeks 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 constrast, no hamster with an access to a wheel showed hypothermia bouts (5 Sham and 9 IGL-X hamsters).

Two representative temperograms (one for a Sham hamster, one for an IGL-X hamster) are presented in Fig. 2. Analysis of hibernation parameters revealed differences between Sham 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 days vs. 125.7 ±0.2 days; P < 0.05). All hibernating Sham hamsters were still in hibernation process at the end of the 18 weeks of experiment. Total time
spent in hypothermia ($T_b < 30 \, ^\circ C$) during the 126 days of the experiment was significantly reduced in IGL-X compared to Sham animals (536 ±144 hours vs. 977 ±127 hours, 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 to the Sham, 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 hours in IGL-X hamsters vs. 69 ±7 hours in Sham-operated hamsters; $P = 0.2$).

No difference was observed either for the time of entry in hypothermia (Fig. 3; IGL-X : 02h25min ± 00h37 vs. Sham : 01h35min ± 00h40; light off at 18h; $P > 0.05$), or for the time of end of hypothermia (IGL-X : 01h02min ± 01h03 vs. Sham : 23h41min ± 00h41; light off at 18h; $P > 0.05$). Even if these mean time of entry and end of hypothermia are quite close, we observed that entries into hypothermia were linked to the light/dark-cycle while arousal onset were not. Indeed, most of entries took place between 20h00 and 10h00 (93.5 % for IGL-X, 94.3% for Sham), whereas the ends of hibernation bouts were randomly distributed (58.6 % for IGL-X and 54.1 % of total bouts ended during these 14 hours).

**Testes, seminal vesicles and epididymal white adipose tissue weights**

All data are shown in table 3. Since testes measurements after 12 weeks of SP exposure were done only in hibernating animals, one Sham hamster and one IGL-X hamster without a wheel were excluded from the data analysis. When compared between 12 and 18 weeks, 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 : $F = 7.85$; $P <$
due to higher values of testis volume and index in IGL-X animals when compared to Sham animals.

At the end of the experiment (i.e. after 18 weeks 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 = 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 = 7.39; P < 0.05), due to the higher testes mass in IGL-X hamsters without a wheel compared to Sham animals without a wheel.

Seminal vesicles and EWAT masses were also higher in hamsters with a wheel (F = 27.62; P < 0.001 and F = 8.57; P < 0.01, respectively) and, as for testes, values were similar to those of hamsters exposed to LP. However, a wheel × lesion interaction was observed neither for seminal vesicles weight (F = 1.11, P = 0.30), nor for EWAT weight (F = 0.66, P = 0.43).

Wheel-running activity

Two representative actograms (one from a Sham hamster, one 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 hours of darkness revealed that IGL lesion delayed lengthening of the nocturnal pattern of wheel-running activity (IGL-X : 25.9 ±3.9 days, Sham : 6.0 ±1.4; P < 0.001).

Moreover, wheel-running activity differed from typical locomotor activity pattern in hamsters under LP cycle at 22°. Indeed, some weeks after the transfer to SP, onset of activity was delayed for some hours (approximately 6 hours) independently of the lesion. This delay was
observed in most hamsters (3/5 Sham, 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 weeks 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 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 weeks, and thereafter decreased by about 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 weeks (time spent in SP × lesion interaction, F = 3.71, P = 0.055).

Temperature

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

First of all, transfer to SP induced a lengthening of the temperature peak (Fig. 6A, Fig. 6B; 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. 6A, Fig. 6B; 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 (F = 260.5; P < 0.001) and maxima (F = 573.5; P < 0.001) of body temperature rhythms (Fig. 6A, Fig. 6C). Indeed, minima and maxima of T_b were both decreased by about 1 °C 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 animals without a wheel, an increase in amplitude was observed for Sham 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**

Hamsters body mass at the beginning of the experiment ranged from 90 to 110 grs. 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 approximately 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 weeks, and decreased thereafter. Moreover, while food consumption continued to decrease until the end of experiment in Sham group, IGL-X hamsters stabilized it between weeks 10-14 and even re-increased it. 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 about 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 the short-photoperiod induced gonadal atrophy (18, 19). In the present work, we demonstrate that, in hamster exposed to SP and cold temperature, free access to a wheel not only prevents testicular regression but also inhibits 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 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 2 important nervous pathways: NPY fibers coming from the IGL (23, 24) and 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 resulting from novelty-induced wheel running in hamsters (29, 65) and forced running on treadmills in mice (37). In rats, IGL lesion also block the period shortening induced by wheel-running activity (33) and the phase-advancing properties of a timed caloric restriction in LD (7). In addition, IGL neurons are activated when hamsters run in a novel wheel (28). Pretreatment with NPY antiserum markedly attenuates phase advances induced by novelty-induced wheel-running in hamsters (3) and several effects of nonphotic factors (like 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
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, since their destruction in the SCN by 5,7-dihydroxytryptamine impairs phase shifts induced by nonphotic factors like 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, 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 body temperature 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. Since physiological rhythms are differently controlled by several distinct dedicated SCN output pathways (for review, 32, 54), we cannot not exclude that wheel-running would disturb some rhythms while being inefficient on others. Nevertheless, since 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 non-yet identified structures. As the photoperiodic inhibition of sexual activity is required for animal 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 on disturb body temperature 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 body temperature which is not observed when hamsters have free access to a wheel. This difference of body temperature average between hamsters with or without a wheel, previously described under normal temperature (8), could be the consequence of an increased metabolic rate, as suggested by the increase in food consumption and body mass by more than 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, 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 metabolic rate in hamsters. This higher energy metabolism could be responsible, at least in part, for the increase by about 1 °C of body temperature.

Another aspect of the present work has to be considered. IGL lesion delays the lengthening of body temperature peak as well as this of wheel-running activity pattern, which both 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 to Sham-operated animals. Since some IGL neurons directly innervate hypothalamic neuroendocrine dopaminergic neurons, including some connecting to GnRH neurons in the anteroventral paraventricular nucleus (25), a bilateral IGL lesion might then disturb the hypothalamo-pituitary axis. Smale and Morin (58) had shown 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 since our IGL lesions were restricted to the lateral geniculate complex, and poorly extended to the hippocampus. An other effect of IGL lesion on hibernation is a decrease in the time spent in hypothermia. Even if we can not 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 the SCN, and thus affect pattern of hibernation bouts which are under SCN control.

In contrast to these effects of IGL lesion on hibernation process, hours of entry in hypothermia and of end of hypothermia were not affected by IGL lesion. Previous papers showed that entry into hypothermia is linked to the light/dark-cycle while arousal onset is not
controlled by the circadian system (5, 21, 66, 64). The present study confirms these results and entries in hypothermia are thus well controlled by the central circadian clock. In 1989, Pickard (47) has shown that the phase angle of entrainment changed significantly following IGL ablation in golden hamster. Indeed, onset of locomotor activity was delayed in IGL-X animals when compared to Sham-operated hamsters. These results, in relation to the present one, suggests that clock-dependant mechanisms which regulate the entrainment differ from those regulating the entry in hypothermia.

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 even though they modulate to some extent the integration of a photoperiodic change by the central clock. If 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.
References


Acknowledgments

Authors are very grateful to André Malan for his help with statistical analysis and his comments. We also thank Etienne Challet and Hugues Dardente for helpful discussions and correction of the manuscript, as well as Daniel Bonn and Aurore Senser for taking care of the animals.
Figures legend

Figure 1

*Neuropeptide Y immunohistochemistry on coronal sections of hamster brain (Sham-operated hamster : A-C; IGL-lesioned hamster : D-F), at the level of the IGL (A, B, D and E) and of the SCN (C and F).*

A, D : coronal sections at low magnification. IGL lesion extends to the lateral geniculate complex, but damage to the hippocampus are negligible. Scale bar = 2 mm.

B, E : higher magnification of A and D, respectively, at the level of the IGL. NPY immunolabbeling in Sham-operated hamster (B) is restricted to a leaflet (arrow) corresponding to the IGL, located between the dorsal (above) and the ventral (below) lateral geniculate nucleus. Scale bar = 500 µm.

C, F : NPY immunoreactivity in the hamster SCN and paraventricular hypothalamic nucleus. In Sham-operated hamster (C), NPY fibers are organized in a dense plexus in the ventral part of the SCN. Bilateral IGL lesion induces a sharp decrease of NPY immunoreactivity in the SCN, but not in the paraventricular hypothalamic nucleus. Scale bar = 500 µm.

Figure 2

*Records of body temperature of a Sham-operated hamster and a IGL-X hamster without a wheel, plotted in double representation.*
Sham-operated and IGL-X hamsters were transferred from LD14:10 to LD10:14 at 7°C. They did not have access to a running-wheel. Body temperature was plotted throughout the experiment only when it was up to 35°C. Thus, empty lines correspond to hypothermia bouts. Hibernation process was affected by IGL lesion.

**Figure 3**

*Hours of entry (A) and end (B) of hypothermia bouts in both IGL-X (filled circle) and Sham-operated hamsters (open circle) without a wheel.*

Time of entry and end of hypothermia bouts (n = 78 for Sham; n=70 for IGL-X) were pooled. Then, number of bouts was calculated in a one hour scale over the 24 hours, and finally reported as percentage per 24 hours. Hour of entry in hypothermia was affected by the light/dark cycle whereas exit from hypothermia was not. No effect of IGL lesion was observed.

**Figure 4**

*Representative double-plotted actograms of a Sham-operated hamster and a IGL-X hamster.*

Cages were equipped with a wheel when the animals were transferred from a LD14:10 cycle to a cold LD10:14 cycle. Black lines represent the offset and onset of the light phase. Gray lines correspond to an eye-fitted line marking offset of wheel-running activity that was drawn just after transfer to SP at 7 °C. Integration of the SP was considered complete when this eye-
fitted line crosses the line of dark offset (08h00 in SP). This integration of SP was delayed in IGL-X hamsters.

**Figure 5**

*Number of wheel revolution per day in Sham or IGL-X hamsters exposed for 18 weeks to a cold LD10:14 cycle.*

Number of wheel revolution, which was weekly averaged, decreased 8 weeks after the transfer to the cold SP.

**Figure 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 LP, and 5, 10, 20 and 30 days after the transfer from LD14:10 to LD10:14 (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.

**Figure 7**
Body mass change (A) and daily food consumption (B) in hamsters exposed for 18 weeks to a cold LD10:14 cycle.

Body mass (A) as well as daily food consumption (B) were strongly increased in hamsters transferred to the cold SP with a running wheel, whatever they were IGL lesionned or Sham-operated.
Table 1: Number of hamsters that showed at least one hypothermia bout during the 18 weeks of cold SP exposure.

<table>
<thead>
<tr>
<th></th>
<th>Number of hibernating hamsters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham</td>
<td>5/6</td>
</tr>
<tr>
<td>IGL-X</td>
<td>7/8</td>
</tr>
<tr>
<td>Sham + wheel</td>
<td>0/5</td>
</tr>
<tr>
<td>IGL-X + wheel</td>
<td>0/9</td>
</tr>
</tbody>
</table>

Hamsters were IGL-lesioned (IGL-X) or Sham-operated before the transfer to cold SP, and some cages were equipped with a wheel (+wheel groups) on the day of the transfer.
Table 2: Parameters of hypothermia bouts in hamsters without a wheel and being bilaterally IGL lesioned (IGL-X) or sham-operated (Sham).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sham (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 ± SEM. See Methods for details. * P<0.05.
Table 3: Testis volume, testicular index, testes mass, seminal vesicles mass and epididimal white adipose tissue (EWAT) mass of hamsters 12 or 18 weeks after the transfer to cold SP.

<table>
<thead>
<tr>
<th></th>
<th>Hamsters without a wheel (hibernating)</th>
<th>Hamsters with a wheel (non hibernating)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham (n=5)</td>
<td>IGL-X (n=7)</td>
</tr>
<tr>
<td>Testis volume after 12 weeks 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 weeks in SP at 7°C</td>
<td>1.07 ± 0.12</td>
<td>1.51 ± 0.11</td>
</tr>
<tr>
<td>Testis mass after 18 weeks in SP at 7°C</td>
<td>1.55 ± 0.16</td>
<td>2.30 ± 0.22</td>
</tr>
<tr>
<td>Testicular index after 12 weeks 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 weeks 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 weeks 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 weeks in SP at 7°C</td>
<td>0.61 ± 0.13</td>
<td>0.92 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SEM. Analysis was done between hamsters which had hibernated (i.e. 5 Sham and 7 IGL-X hamsters without a wheel) and hamsters which had free access to a wheel (5 Sham and 9 IGL-X).

Because testis size was only measured in hibernating hamsters, no data of testis volume and testicular index is available for non-hibernating animals 12 weeks after the transfer in SP.
Figure 2
Figure 3

**A**

- **Y-axis**: Hour of entry in hypothermia (% per 24 h)
- **X-axis**: Zeitgeber Time

**B**

- **Y-axis**: Hour of end of hypothermia (% per 24 h)
- **X-axis**: Zeitgeber Time
Figure 4
Figure 5

![Graph showing number of wheel revolutions over weeks for different groups.](image)
Figure 6
Figure 7